Assessment of Structural Requirements for the Monoamine Oxidase-B-Catalyzed Oxidation of 1,4-Disubstituted-1,2,3,6-tetrahydropyridine Derivatives Related to the Neurotoxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

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The monoamine oxidase B (MAO-B) substrate properties and distance measurements along the N_1-C_4 axis of 38 1,4-disubstituted-1,2,3,6-tetrahydropyridine derivatives, including seven newly synthesized MPTP analogs, were used to define the maximum size that can be accommodated by the MAO-B active site. Only those compounds measuring less than 12 Å displayed significant MAO-B substrate properties. The behavior of various 4-substituted-1-cyclopropyltetrahydropyridine analogs also is discussed in terms of this N_1-C_4 distance parameter in an effort to understand factors which contribute to their substrate vs inactivator properties. We conclude that this distance parameter will predict the majority of substrates vs nonsubstrates with this class of compound.

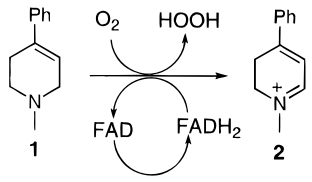
Introduction

The flavoenzymes monoamine oxidase A and B (MAO-A and MAO-B) catalyze the oxidative deamination of several primary and secondary amines, including the neurotransmitters serotonin and dopamine, and selected tertiary amines.[1] Of special toxicological interest is a variety of 1,4-disubstituted-1,2,3,6-tetrahydropyridines, including the parkinsonian-inducing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**), which are good to excellent substrates or inactivators of MAO-A and/or MAO-B. The overall catalytic pathway (Scheme 1) involves the 2-electron C_6 (allylic) α-carbon oxidation of the substrate molecule to yield the corresponding dihydropyridinium species **2**, a reaction which is coupled to the reduction of oxidized flavin (FAD) to reduced flavin ($FADH_2$). $FADH_2$ subsequently is oxidized back to FAD by dioxygen (O_2).[2]

Molecular modeling studies on MPTP analogs have identified lipophilicity as a contributing parameter to good MAO-A and MAO-B substrate behavior and also have provided some approximate estimates of the size and shape of molecules that display substrate properties.[3,4] Two features of the active sites of these enzymes are pockets that accommodate the tetrahydropyridine (THP) ring and its C_4 substituent. Both flexible (one or more sp^3-hybridized atoms) and constrained (aryl) C_4 substituents may be well tolerated by both MAO-A and MAO-B,[5] although the MAO-A active site appears to accommodate bulkier groups at C_4 better than does the active site of MAO-B.[3,6] Only substitutions at the N_1 and C_4 positions of the THP ring are tolerated,[7] and apparently, only small substituents can be accommodated at the N_1 position. On the basis of results with a limited set of compounds, Efange suggested a maximum distance of 10.23 Å between the N-methyl carbon and the farthest carbon on the C_4 substituent.[4]

The studies described in the present paper were undertaken in an effort to define more accurately the maximum distance along the N_1-C_4 axis [d $(Å)_{max}$] of the THP ring that can be accommodated by the MAO-B active site.[8] During the course of these studies, inconsistencies in the MAO-B kinetic parameters reported for several compounds were identified.[3,7,9] In order to resolve these conflicts and to add to the data base used to define [d $(Å)_{max}$], we have synthesized and characterized the substrate properties of several *p*-phenyl-substituted MPTP derivatives.

Scheme 1. MAO-B-Catalyzed Oxidation of MPTP (**1**) to Its Dihydropyridinium Metabolite (**2**)



Results

We have focused our analysis specifically on the estimated length of the N_1-C_4 axis which is defined as the distance between the two most distant atoms which fall on a line drawn through N_1 and C_4 (Figure 1). At the outset we encountered conflicting literature reports concerning the MAO-B substrate properties of the 4-(4-methylphenyl) (**3**), 4-(4-chlorophenyl) (**4**), and 1-ethyl (**5**) analogs of MPTP. An early report[10] concluded that the 4-methylphenyl analog **3** was not metabolized by MAO-B. Even though the poor substrate properties of **3** were later confirmed,[11] subsequent studies from the same laboratory reported a $k_{cat}/K_M = 345$ min^{-1} mM^{-1} (over 60% the activity of MPTP) for this compound.[12] Various authors have incorporated the poor[7,13] or good[3,4,14] substrate properties of **3** in the development of their structure−activity relationship (SAR) and quantitative SAR (QSAR) relationships. The 4-chlorophenyl derivative **4** also has been described as a poor (7%, 12% activity relative to MPTP)[7,15−17] or moderate[9] MAO-B substrate. Excellent substrate properties of **4** ($k_{cat}/K_M = 595$ min^{-1} mM^{-1}) were reported later[12] and used to develop QSAR relationships.[3] Finally, the *N*-ethyl derivative **5** was described as a poor substrate relative to MPTP (7%,[10] 13%,[11] "poor substrate"[18]). Subse-

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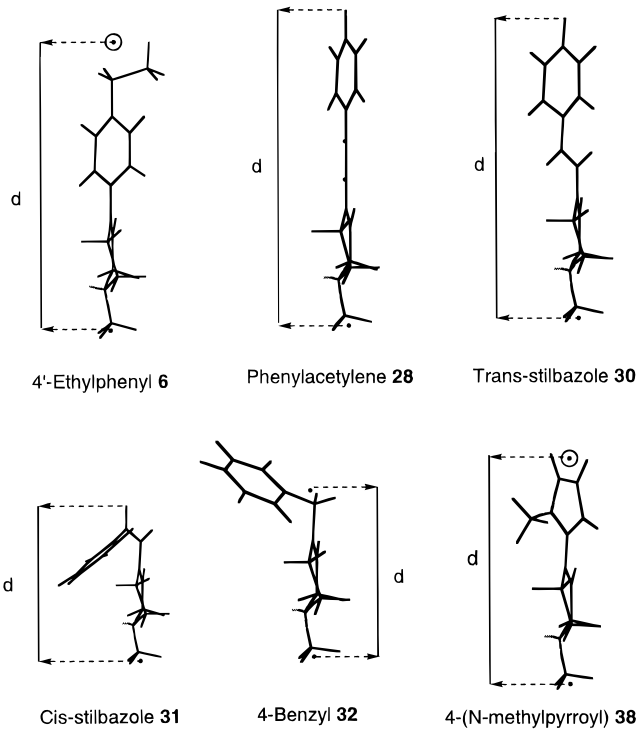


Figure 1. MM2 views of a few selected 1,4-disubstituted 1,2,3,6-tetrahydropyridine derivatives showing the distance [*d* (Å)].

quently, Youngster et al. reported a k_{cat}/K_M of 200 min^{-1} mM^{-1} for **6**,[12] but the compound was later described as a "poor substrate" in a QSAR study.[7] Unfortunately, the synthesis and chemical characteristics of many of the compounds for which enzyme kinetic data have been published[12,14] have been reported in a thesis which does not include complete microanalytical and spectroscopic details.[19]

As the biological activities of several of these compounds were of particular relevance to the present study, we undertook the synthesis and full chemical characterization of the 4-chlorophenyl (**4**), 4-methylphenyl (**3**), and *N*-ethyl (**5**) derivatives. We also studied the following additional analogs: 4-(4-ethylphenyl) (**6**), 4-(4-bromophenyl) (**7**), 4-(4-hydroxyphenyl) (**8**), and 4-(4-methoxyphenyl) (**9**), for which no definitive enzyme kinetic data could be found in the literature.[20,21] Compounds **3**, **4**, and **6**−**9** were prepared by nucleophilic addition of the corresponding *para*-substituted phenyllithiated reagent to 1-methyl-4-piperidone (**10**) followed by dehydration of the resulting piperidinols **11**−**16** in acidic medium (Scheme 2).[5,15,22] The 4-hydroxyphenyl analog **8** was prepared after protection of the 4-bromophenol as its *tert*-butyldimethylsilyl ether.[23] The protecting group was cleaved during the acidic dehydration step.[24] Compound **5** was obtained by reduction of the corresponding pyridinium species in protic solvent.[25,26] All compounds were obtained as their crystalline oxalate salts which exhibited the expected elemental values and spectroscopic properties.

The substrate properties of the synthesized compounds were evaluated with purified MAO-B at 30 °C (Table 1). The k_{cat}/K_M values of the 4-methylphenyl (345 min^{-1} mM^{-1}) and 4-chlorophenyl (595 min^{-1} mM^{-1}) analogs **3** and **4**, respectively, compared favorably with the 'excellent substrate'[12,14] rather than the poor substrate[10,11,15,16] properties reported for these two compounds. On the other hand, the 1-ethyl-4-phenyl analog **5** was found to be a poor substrate (k_{cat}/K_M = 68 min^{-1} mM^{-1}), as reported in some papers[10,11,15] and in contrast with the good substrate properties for this compound reported in other papers.[12,14] The 4-bromophenyl analog **7** was an excellent substrate, while the 4-ethylphenyl (**6**) and 4-methoxyphenyl (**9**) analogs were poor MAO-B substrates. The 4-hydroxyphenyl analog **8** exhibited no substrate activity, as might be anticipated from results of earlier in vivo studies.[20] For comparison purposes, the MAO-B substrate properties of the 11 MPTP analogs bearing a substituent at the *para*-position of the phenyl group and the 1-ethyl-4-phenyl analog **5** examined in this study are reported in Table 1.

Scheme 2. Synthesis of 4′-Substituted MPTP Analogs

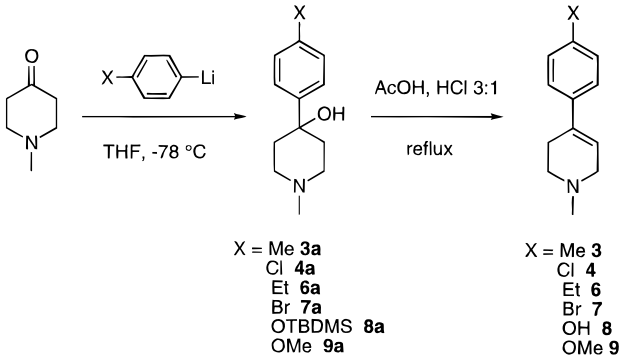


Table 1. MAO-B Substrate Properties of *N*-R_1-4′-R_2-Substituted MPTP Derivatives

	R_1	R_2	k_{cat}[a]	K_M	k_{cat}/K_M	source
1	Me	H	204	0.39	523	12
3	Me	Me	175	0.50	349	*c*
			169	0.49	345	12
4	Me	Cl	97	0.18	551	*c*
			119	0.20	595	12
5	Et	H	74	1.09	68	*c*
			34	0.17	200	12
6	Me	Et	85	1.89	45	*c*
7	Me	Br	71	0.14	493	*c*
8	Me	OH		inactive		*c*
9	Me	OMe	68	0.94	73	*c*
17	Me	NH_2	*b*	*b*	54	14
18	Me	NO_2	*b*	*b*	16	14
19	Me	Ph		inactive		27
20	Me	F	93	0.22	423	12

[a] Units: k_{cat} in min^{-1}, K_M in mM, k_{cat}/K_M in min^{-1} mM. [b] Not reported. [c] Determined in this study.

The values for the distance measurements of the N_1−C_4 axis for the various tetrahydropyridine derivatives were estimated with the Mac Mimic-MM2 molecular modeling program (Version 91). With the purpose of accounting for the possible rotation of substituents such as C_4′-ethyl and measuring the actual steric hindrance of such groups relative to a methyl group, a cylindrical volume was defined around the N_1−C_4 axis. The diameter of this cylinder was defined as the carbon frame width of the tetrahydropyridine ring (C_3−C_5 distance). Atoms that did not fall in the cyclindrical volume were not included in the calculated distance *d* (Å). Virtual atoms were simulated on the N_1−C_4 axis, at the farthest position occupied by the substituents at both N_1 and C_4 (see Figure 1). van der Waals volumes also were not taken into account in these measurements.

In addition to C_4- and N_1-substituted MPTP analogs, compounds representing the various classes of C_4-

Table 2. Maximum Distance [*d* (Å)] Along the N_1-C_4 Axis and the Corresponding Kinetic Data [k_{cat}/K_M (min^{-1} μM^{-1})] of a Series of 1,4-Disubstituted-1,2,3,6-Tetrahydropyridine Derivatives[a]

X	N_1 substituent	C4 substituent	*d* (Å)	I_1	k_{cat}/K_M	I_2	ref
1	Me	Ph	10.07	+	523[b]	+	12
					1431[c]		28
3	Me	4′-Me-Ph	10.86	+	349[b]	+	*d*
4	Me	4′-Cl-Ph	10.80	+	551[b]	+	*d*
5	Et	Ph	11.30	+	68[b]	+	*d*
6	Me	4′-Et-Ph	12.09	−	45[b]	−	*d*
7	Me	4′-Br-Ph	10.84	+	493[b]	+	*d*
8	Me	4′-OH-Ph	10.66	+	4[b]	−	*d*
9	Me	4′-OMe-Ph	11.87	+	73[b]	+	*d*
17	Me	4′-NH_2-Ph	10.78	+	54[b]	+	14
18	Me	4′-NO_2-Ph	10.95	+	16[b]	−	14
19	Me	4′-Ph-Ph	14.27	−	NS[b]	−	27
20	Me	4′-F-Ph	10.28	+	423[b]	+	12
21	H	4′-Cl-Ph	9.86	+	382[b]	+	29
22	allyl	Ph	12.32	−	NS[c]	−	30
23	cyclopentyl	Ph	12.84	−	NS[c]	−	30
24	cyclobutyl	Ph	12.15	−	NS[c]	−	30
25	*n*-Pr	Ph	12.15	−	4[c]	−	30
26	H	Ph	9.25	+	319[b]	+	29
27	Me	acetylenyl	8.40	+	790[c]	+	30
28	Me	Ph-acetylenyl	12.68	−	NS[c]	−	30
29	Me	vinyl	7.96	+	2370[c]	+	30
30	Me	*trans*-Ph-vinyl	12.16	−	19[b]	−	31
31	Me	*cis*-Ph-vinyl	7.96	+	912[b]	+	31
32	Me	benzyl	6.72	+	3580[c]	+	32
33	Me	phenoxy	6.60	+	4151[c]	+	28
34	Me	CO_2Et	8.08	+	433[b]	+	29
35	Me	$OCONMe_2$	7.93	+	57[b]	+	33
36	Me	OCONMePh	7.93	+	67[b]	+	33
37	Me	*t*-Bu	7.82	+	92[b]	+	12
38	Me	*N*-Me-pyrrolyl	9.09	+	347[b]	+	14
39	Me	thien-2-yl	9.37	+	55[b]	+	5
40	Me	cyclohexyl	10.02	+	688[b]	+	12
41	Me	phenylethyl	7.82	+	1130[c]	+	32
42	Me	cyclopropyl	7.90	+	974[c]	+	34

[a] I_1 is set to + for compounds $d \leq 12$ Å that are predicted to be substrates and − for compounds $d > 12$ Å that are predicted to be nonsubstrates. I_2 corresponds to the experimentally determined substrate properties and was fixed to + for a substrate and − for a nonsubstrate (k_{cat}/K_M < 10%). [b] Measured at 30 °C. [c] Measured at 37 °C. [d] Determined in this study. NS: no MAO-B substrate activity demonstrated.

substituted tetrahydropyridine derivatives reported in the literature were included without exception. The following four classes of *N*-methyltetrahydropyridine derivatives were identified (Figure 1): 4-aryl, 4-ethynyl, 4-ethenyl and 4-aryloxy, and 4-arylmethyl, plus a fifth, miscellaneous group. The results (Table 2) compare the *d* (Å) values of 34 compounds with the literature (or newly estimated) k_{cat}/K_M values for those compounds which are substrates for MAO-B. Enzyme activity was based on k_{cat}/K_M values as used in similar SAR analyses.[3,5]

A comparison of the k_{cat}/K_M values with the *d* (Å) values of the 34 compounds listed in Table 1 leads us to propose a maximum *d* (Å) value [d (Å)$_{max}$] of 12 Å for MAO-B substrates. In order to relate more easily the predicted vs the experimental values, an indicator (I_1) was assigned to each compound to designate its predicted substrate (+) or nonsubstrate (−) behavior. A second indicator (I_2) was assigned to each compound to correspond to the experimental result, and then I_1 and I_2 were compared. Those compounds with a k_{cat}/K_M value less than 10% of the k_{cat}/K_M value for MPTP (<52 min^{-1} mM^{-1} at 30 °C and <143 min^{-1} mM^{-1} at 37 °C) were considered nonsubstrates. Under these conditions, only two compounds, the C_4-hydroxyphenyl (**8**) and C_4-nitrophenyl (**18**) derivatives, of the 34 compounds did not show a correspondence between I_1 and I_2.

In an attempt to investigate the utility of this proposal, the following series of tetrahydropyridine derivatives were examined using the same I_1 and I_2 descriptors:[35] (1) 14 4-phenyltetrahydropyridine derivatives bearing F, Cl, Br, OCH_3, CH_3, CH_3CH_2, and CH_3-CH_2CH_2 *ortho*- or *meta*-substituents;[12,14] (2) 19 4-(aryloxy)tetrahydropyridine derivatives including α- and β-naphthoxy analogs and various phenoxy derivatives bearing Cl, Me, OCH_3, NO_2, C_6H_5, $CH_3(CH_2)_3$, and $C(CH_3)_3$ *ortho*-, *meta*-, and *para*-substituents;[6,28] (3) 12 4-benzyltetrahydropyridine derivatives bearing F, I, OCH_3, and CH_3 *ortho*-, *meta*-, and *para*-substituents plus other arylmethyl derivatives;[32] (4) 15 4-*cis*- and 4-*trans*-arylethenyl derivatives substituted in various positions with OCH_3, Br, F, CH_3, and NMe_2;[21,31] (5) 7 4-(arylalkyl)tetrahydropyridine derivatives including various thienyl and naphthyl groups;[5,32] and (6) 4 *N*-alkyl-4-aryltetrahydropyridine derivatives.[3,15] Of the total of 69 compounds, only 7 compounds failed to match the I_1 vs I_2 relationship. In regard to the wide range of polarity, molecular volumes, and substituent orientation, such a result points out the importance of the parameter d (Å)$_{max}$ and makes it a useful determinant for predicting the substrate properties within this series. Misfits generally were associated with very bulky C_4 substituents such as naphthyl and phenanthryl and with stilbazole derivatives.

We have been particularly interested in the interactions of *N*-cyclopropyl derivatives with MAO-B. Indeed, in contrast with their expected inactivator properties,[36,37] certain analogs proved to be either mixed

Table 3. MAO-B Biological Properties of C_4-Substituted *N*-Cyclopropyl Derivatives[27,39,40]

C_4 substituent	*d* (Å)	k_{cat}/K_M	inact
43 phenyl	11.20	NS[a]	+
44 4-methoxyphenyl	12.97	NS	+
45 4-chlorophenyl	11.82	NS	+
46 2-methylphenyl	11.20	630	−
47 α-naphthyl	11.20	NS	+
48 benzyl	7.92	1538	+
49 phenoxy	7.80	1650	−
50 4-nitrophenoxy	7.80	150	−
51 4-chlorophenoxy	7.80	136	−
52 thiophenoxy	7.84	1900	−
53 1-methylpyrrol-2-yl	10.29	413	+
54 2-furanyl	10.25	NS	+
55 2-thienyl	10.57	NS	+

[a] NS: no substrate activity demonstrated.

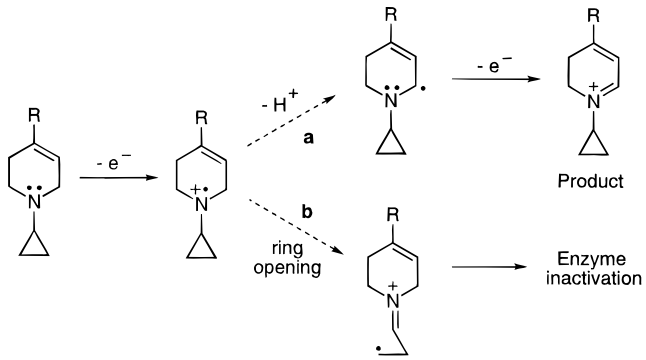
inactivator/substrates[38,39] or good to excellent substrates.[40] We considered the relationship between *d* (Å) and the biological activity of the *N*-cyclopropyltetrahydropyridines that have been reported in the literature. The results (Table 3) led to the identification of two sets of compounds—those with *d* (Å) of about 8 Å, all of which exhibit substrate properties, and those with *d* (Å) of about 11 Å, which display inactivator properties. The small size of the data set prevents an accurate estimation of the critical size that might be associated with the substrate vs inactivator properties of this series of compounds.

Discussion

These studies were undertaken to evaluate the N_1-C_4 distance parameter as a prediction of the MAO-B substrate properties of various 1,4-disubstituted-tetrahydropyridine derivatives. On the basis of the results summarized above, we propose the maximum length [d $(Å)_{max}$] of 12 Å for those derivatives that will display MAO-B substrate properties. This empirical analysis makes no attempt to predict k_{cat}/K_M values, which would require the evaluation of a variety of parameters such as lipophilicity, molecular volume, and electron distribution. Because of their linearity along the N_1-C_4 axis, the *para*-substituted 4-phenyl MPTP analogs appeared to be particularly useful in this exercise. Unfortunately, conflicting literature reports concerning the substrate properties of several members of this series, some of which had never been fully characterized, complicated our efforts. In an effort to resolve the contradictory literature reports and to better substantiate our analyses, seven compounds with *d* (Å) values ranging from 10.66 to 12.09 Å were synthesized and characterized and their MAO-B substrate properties determined. Enzyme kinetic data obtained using the test of determination of H_2O_2 after 1 h of incubation appeared to be the source of many discrepancies. Data obtained by direct determination of the oxidation product by spectroscopic means and using purified enzyme seem to be more reliable and reproducible.

Based on an initial series of 34 1,4-disubstituted-tetrahydropyridine derivatives, the value of d $(Å)_{max}$ of 12 Å has been identified as the distance between the two farthest atoms along the N_1-C_4 axis of compounds that will be MAO-B substrates. The utility of this value to predict whether or not a compound will be an MAO-B substrate was challenged with a second set of 69 tetrahydropyridine derivatives. In total, 97 of the 103 compounds examined fit the prediction. From previous QSAR studies on MPTP derivatives, lipophilicity has been recognized as a very important determinant for MAO-B activity. Consequently, the poor substrate properties of the 4-*p*-hydroxy, -amino, and -nitro derivatives **8**, **17**, and **18**, respectively, and the 4-carbamoyloxy derivatives **35** and **36** may partially be explained in terms of unfavored polar interactions within the active site. Nevertheless, other polar derivatives (e.g., the (nitrophenoxy)-1-methyl-1,2,3,6-tetrahydropyridine derivatives)[6] show good to excellent MAO-B substrate properties suggesting that favorable electrostatic interactions also may play a role.

Scheme 3. *N*-Cyclopropyl Derivatives' Reactivity: (a) Pathway Leading to Substrate and (b) Pathway Leading to Ring Opening and Inactivation



The unexpected substrate properties of certain *N*-cyclopropyl derivatives have been puzzling since, on the basis of Silverman's work on cyclopropylamines, one would predict that those derivatives should behave as mechanism-based inactivators. The catalytic pathway for MAO-B has been proposed to proceed via a single-electron transfer (SET) step to generate a cyclopropylaminyl radical cation that undergoes ring opening. The resulting primary carbon-centered radical is thought to react with and inactivate the enzyme (Scheme 3).[41,42] The ring-opening reaction requires the half-filled p-orbital on nitrogen to overlap with the π-type orbitals of the cyclopropyl C−C bond. The cyclopropane can adopt either a coplanar "bisected conformation", where the p-orbital on nitrogen can overlap the π-type orbitals of the cyclopropyl ring, or a perpendicular conformation in which orbital overlap is minimal (Figure 2).[43] In the case of cyclopropylaryl derivatives (phenyl, naphthyl, phenanthryl), ring opening of the radical cations does not occur when the cyclopropyl group is forced out of the bisected conformation by steric interactions. On the other hand, under the same reaction conditions, whenever the cyclopropyl can adopt a bisected conformation, ring opening takes place. The kinetics of the reaction depend on how easily the cyclopropyl group can adopt a bisected conformation ($k_{phenyl} > k_{naphthyl}$).[44,45]

Similar types of reactivity and constraints imposed by the active site might occur for the cyclopropyltetrahydropyridinyl system. From Table 3, it appears that compounds with small *d* (Å) values exhibit substrate properties while those with larger *d* (Å) values exhibit inactivator properties. If one assumes that the SET pathway takes place, a partitioning between substrate and inactivator properties might occur after generation of the aminyl radical cation (Scheme 3). The experimental data suggest that when the molecule is long

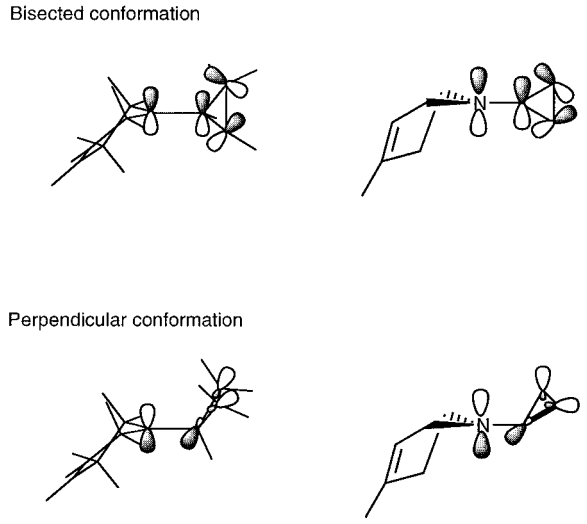


Figure 2. Comparison of bisected and perpendicular conformations of the cyclopropyl group relative to the tetrahydropyridine ring (for both conformations, the first view was imported from MacMimic).

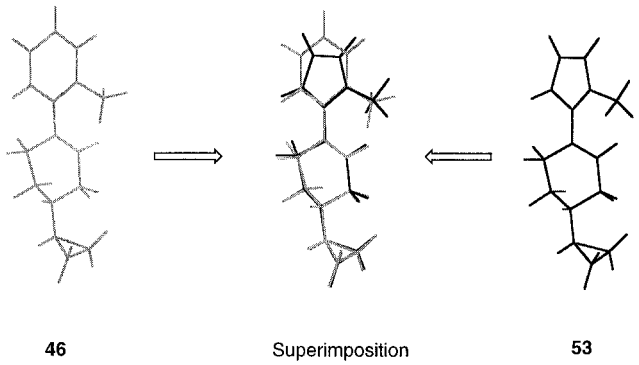


Figure 3. Superimposition of 1-cyclopropyl-4-(2-methylphenyl)-1,2,3,6-tetrahydropyridine (**46**) and 1-cyclopropyl-4-(1-methyl-2-pyrrolyl)-1,2,3,6-tetrahydropyridine (**53**) (Mac Mimic-MM2 view).

(≈11 Å), the cyclopropyl group could adopt an appropriate position relative to the nitrogen for ring opening to occur. With shorter molecules (≈8 Å), the relative orientation would not be favorable for ring opening and the partitioning would thus evolve in favor of substrate properties.

Two compounds, the 4-(2-methylphenyl) derivative **46** and the 4-(1-methyl-2-pyrrolyl) derivative **53**, however, do not fit in this analysis. On the basis of their size, they both are expected to exhibit inactivator properties, while they experimentally both show good substrate properties.[40,39] Superimposition of the two compounds reveals that the *o*-methyl group of both **46** and **53** point in the same direction (Figure 3). In the *N*-methyl series, substituents in this same region also lead to improved MAO-B substrate properties.[14] Substituents pointing into this region might orientate the tetrahydropyridine moiety in a specific arrangement which modifies the catalytic properties of the molecule and confers high substrate activity. Syntheses of new *ortho*-substituted C_4-aryl derivatives are currently in progress to probe this proposal.

Conclusion

The maximum size of MPTP-type derivatives that can be accommodated by the MAO-B active site along the N_1-C_4 axis was determined to be 12 Å. A simple correlation between the size of the compounds and their substrate properties helps to predict the behavior of various compounds and yields a primary determinant for the design of new compounds. This relationship was established without consideration of electronic and lipophilicity characteristics of the C_4 substituent, but those QSAR parameters remain very important to predict relative substrate activities. *N*-Cyclopropyl derivatives were proposed to partition between substrate and inactivator behavior on the basis of their size. Synthesis of new compounds is in progress to further explore the effect of $C_{2'}$ substituents on this partitioning.

Experimental Section

CAUTION: MPTP and its derivatives are potential neurotoxins and must be handled with care, in a well-ventilated hood.[46]

Enzymology. MAO-B was purified from beef liver using procedures previously reported (method described by Salach with minor modifications). We purchased phospholipase A from Sigma (St. Louis, MO) and did not subject the preparation to the glucose gradient purification step. Kinetic studies were carried out at 30 °C using a Beckman DU-7400 spectrophotometer. Solutions of the test compounds (final volume 500 μL, final concentrations 150−3000 μM) in 0.1 M sodium phosphate buffer (pH = 7.4) were incubated in the presence of 0.09 μM MAO-B. The rates of oxidation were obtained by monitoring the increment in the absorbance of the dihydropyridinium chromophore (343 nm) over a 2 min time period. Repeated determinations on MPTP performed prior to the study confirmed the reliability of the kinetic data. The k_{cat} and K_M values were derived from Lineweaver−Burke double-reciprocal plots ($r^2 \geq 0.99$). All enzyme assays were performed in triplicate.

Distance Measurements. All compounds were drawn using MacMimic software. Measurements refer to the distance between the two farthest atoms falling in a cylindrical volume around the line N_1-C_4. The diameter of the cylinder considered is that of the tetrahydropyridine ring width (distance C_3-C_5). Virtual atoms were positioned along the N_1-C_4 axis at both N_1 and C_4 positions. For methyl groups, a virtual center was simulated in the middle of the three hydrogen atoms. For ethyl and propyl groups, the virtual center was created using the most extended conformation. For arylmethyl and aryloxy derivatives, the carbon of the aromatic ring bonded to the methylene or the oxygen was chosen (assumption justified by the examination of the minimized conformations of such compounds). Distances do not account for van der Waals volumes.

Chemistry. Reagents and starting materials were obtained from commercial suppliers and used without further purification. Tetrahydrofuran was distilled from sodium/benzophenone ketyl, and acetone was distilled over K_2CO_3. All reactions were conducted using flame-dried glassware under an atmosphere of dry nitrogen. Melting points were performed on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA. Proton and carbon spectra were recorded on a Bruker WP 270-MHz spectrometer. Exponential function (LB = 0.1−0.2) was applied to the FID to obtain integrals and Gaussian function (LB = −1, GB = 0.25) to record coupling constants. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane ($\delta = 0$). Spin multiplicities are given as s (singlet), bs (broad singlet), d (doublet), t (triplet), or m (multiplet). Coupling constant (*J*) values are given in hertz (Hz). Electron ionization mass spectrometry (EIMS) was performed on a Hewlett Packard 5870 mass-selective detector. Data were acquired using an HP 5970 Chemstation. Normalized peak heights are reported as a percentage of the base peak. UV−vis absorption spectra were recorded on a Beckman DU Series 7400 spectrophotometer.

General Procedure. Briefly, compounds **3**, **4**, and **6**−**9** were classically prepared by nucleophilic addition of the

4-substituted phenyl-lithiated reagent (1 equiv) on the 1-methyl-4-piperidone **10** (1 equiv), in anhydrous THF (10 mL/mmol of compound) at −78 °C.[15] Compound **8** was prepared by addition of the lithiated reagent derived from 4-bromo(*tert*-butyldimethylsilyl)phenol.[23] After 3−5 h reaction at −78 °C, water was added and the mixture extracted with Et_2O. After drying over $MgSO_4$ and evaporation of the organic solvent under reduced pressure, the piperidinols **11**−**16** were directly dehydrated in acidic medium (acetic acid/HCl, 3:1) to afford the tetrahydropyridines which were crystallized as their oxalate salts. The 1-ethyl-4-phenyl-1,2,3,6-tetrahydropyridine (**5**) was obtained by ethylation of 4-phenylpyridine with ethyl iodide (2.5 equiv) in anhydrous acetone, followed by reduction of the corresponding pyridinium with $NaBH_4$ in methanol,[25] and crystallized as its oxalate salt.

Oxalate salt of 1-methyl-4-(4-methylphenyl)-1,2,3,6-tetrahydropyridine (3): obtained in 71% yield after recrystallization from methanol; mp 185−186 °C; ^{1}H NMR (DMSO-d_6) δ 7.37 (2H, d like, J = 8.2 Hz), 7.17 (2H, d like, J = 8.2 Hz), 6.12 (1H, bs), 3.78 (2H, bd, J = 2.5 Hz), 3.33 (2H, t, J = 6.0 Hz), 2.80 (3H, s), 2.72 (2H, bm), 2.30 (3H, s); ^{13}C NMR (DMSO-d_6) δ 164.8, 137.2, 135.6, 133.6, 129.1, 124,6, 115.9, 51.3, 49.6, 41.8, 23.8, 20.7; MS (*m*/*z*, rel int) 187 (100), 186 (74), 158 (25), 144 (21), 129 (72), 115 (33), 105 (23), 96 (56), 82 (18); UV (nm, MeOH) 209, 249. Anal. ($C_{15}H_{19}NO_4$) C, H, N.

Oxalate salt of 1-methyl-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine (4): obtained in 66% yield after recrystallization from methanol; mp 178−179 °C; ^{1}H NMR (DMSO-d_6) δ 7.49 (2H, m), 7.41 (2H, m), 6.21 (1H, m), 3.75 (2H, bd, J = 3.2 Hz), 3.31 (2H, t, J = 6.0 Hz), 2.78 (3H, s), 2.72 (2H, bm); ^{13}C NMR (DMSO-d_6) δ 165.0, 137.7, 133.1, 132.8, 128.9, 127.0, 118.3, 51.8, 49.9, 42.3, 24.3; MS (*m*/*z*, rel int) 209 (12), 208 (13), 207 (36), 206 (28), 178 (10), 149 (4), 129 (41), 115 (9), 96 (32), 82 (12), 42 (100); UV (nm, MeOH) 209, 250. Anal. ($C_{14}H_{16}ClNO_4$) C, H, N.

Oxalate salt of 1-ethyl-4-phenyl-1,2,3,6-tetrahydropyridine (5): obtained in 82% yield after recrystallization from methanol; mp 172−173 °C; ^{1}H NMR (DMSO-d_6) δ 7.47 (2H, m), 7.38 (2H, m), 7.32 (1H, tt, J = 2.8, 7.2 Hz), 6.19 (1H, m), 3.79 (2H, bd, J = 2.8 Hz), 3.35 (2H, t, J = 6.0 Hz), 3.14 (2H, q, J = 7.2 Hz), 2.75 (2H, bm), 1.25 (3H, t, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ 164.9, 138.9, 134.6, 129.0, 128.3, 125.3, 117.4, 50.5, 48.8, 48.1, 24.4, 9.8; MS (*m*/*z*, rel int) 187 (100), 186 (44), 158 (13), 129 (38), 115 (41), 110 (31), 91 (27), 77 (15), 56 (32); UV (nm, MeOH) 210, 248. Anal. ($C_{15}H_{19}NO_4$) C, H, N.

Oxalate salt of 1-methyl-4-(4-ethylphenyl)-1,2,3,6-tetrahydropyridine (6): obtained in 73% yield after recrystallization from methanol; mp 179−181 °C; ^{1}H NMR (DMSO-d_6) δ 7.36 (2H, m, d like, J = 8.0 Hz), 7.20 (2H, m, d like, J = 8.0 Hz), 6.10 (1H, bs), 3.75 (2H, bd, J = 3.2 Hz), 3.31 (2H, t, J = 6.4 Hz), 2.78 (3H, s), 2.71 (2H, bm), 2.58 (2H, q, J = 7.6 Hz), 1.16 (3H, t, J = 7.6 Hz); ^{13}C NMR (DMSO-d_6) δ 165.0, 143.9, 136.4, 134.2, 128.3, 125.2, 116.4, 51.9, 50.1, 42.3, 28.2, 24.4, 15.9; MS (*m*/*z*, rel int) 201 (100), 200 (78), 186 (16), 172 (26), 129 (70), 115 (35), 96 (57); UV (nm, MeOH) 209, 249. Anal. ($C_{16}H_{21}NO_4$) C, H, N.

Oxalate salt of 1-methyl-4-(4-bromophenyl)-1,2,3,6-tetrahydropyridine (7): obtained in 58% yield after recrystallization from methanol; mp 203 °C; ^{1}H NMR (DMSO-d_6) δ 7.50 (2H, m), 7.38 (2H, m), 6.11 (1H, bs), 3.78 (2H, dd, J = 2.4, 3.6 Hz), 3.36 (2H, t, J = 6.0 Hz), 2.83 (3H, s), 2.71 (2H, m); ^{13}C NMR (DMSO-d_6) δ 164.9, 138.0, 133.7, 131.8, 127.5, 121.6, 117.4, 52.0, 50.4, 42.3, 24.1; MS (*m*/*z*, rel int) 253 (74), 252 (60), 251 (76), 250 (54), 224 (12), 222 (13), 172 (24), 171 (22), 129 (100), 115 (21), 96 (76); UV (nm, MeOH) 210, 253. Anal. ($C_{14}H_{16}BrNO_4$) C, H, N.

Oxalate salt of 1-methyl-4-(4-hydroxyphenyl)-1,2,3,6-tetrahydropyridine (8): obtained in 67% yield after recrystallization from methanol/water; mp 224−225 °C; ^{1}H NMR (DMSO-d_6) δ 7.30 (2H, m), 6.76 (2H, m), 5.99 (1H, bm), 3.75 (2H, bs), 3.32 (2H, t, J = 6.0 Hz), 2.80 (3H, s), 2.69 (2H, m); ^{13}C NMR (DMSO-d_6) δ 165.2, 157.8, 133.8, 129.5, 126.4, 115.7, 114.1, 51.8, 50.1, 42.1, 40.6, 24.3; MS (*m*/*z*, rel int) 189 (100), 188 (75), 160 (29), 145 (24), 131 (24), 96 (32), 94 (21); UV (nm, MeOH) 209, 259. Anal. ($C_{14}H_{17}NO_5$) C, H, N.

Oxalate salt of 1-methyl-4-(4-methoxyphenyl)-1,2,3,6-tetrahydropyridine (9): obtained in 70% yield after recrystallization from methanol; mp 182−183 °C; ^{1}H NMR (DMSO-d_6) δ 7.40 (2H, m), 6.92 (2H, m), 6.06 (1H, bm), 3.74 (3H, s), 3.73 (2H, bs), 3.31 (2H, t, J = 6.0 Hz), 2.78 (3H, s), 2.69 (2H, m); ^{13}C NMR (DMSO-d_6) δ 164.9, 159.4, 133.6, 131.3, 115.3, 114.3, 55.6, 51.9, 50.2, 42.3, 24.4; MS (*m*/*z*, rel int) 203 (100), 202 (83), 188 (24), 179 (21), 160 (21), 145 (23), 121 (20), 115 (23), 96 (29), 94 (24); UV (nm, MeOH) 209, 258. Anal. ($C_{15}H_{19}NO_5$) C, H, N.

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Supporting Information Available: Kinetic data of the compounds used for this study (3 pages). Ordering information is found on any current masthead page.

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