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Synthesis and MAO-B Substrate Properties of 1-Methyl-4-heteroaryl-1,2,3,6-tetrahydropyridines

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Abstract—The parkinsonian inducing drug 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is bioactivated in a reaction catalyzed by the flavoenzyme monoamine oxidase B (MAO-B) to form the corresponding dihydropyridinium and subsequently pyridinium metabolites. As part of our ongoing studies to characterize the structural features responsible for this unexpected biotransformation, we have examined the MAO-B substrate properties of a variety of MPTP analogues bearing various heteroaryl groups at the 4-position of the tetrahydropyridinyl ring. The newly synthesized analogues are 4-(1-methylimidazol-2-yl)-, 4-(3-methylfuran-2-yl)-, 4-(3-methylthien-2-yl)-, 4-(3,4-dimethylpyrrol-1-yl)-, 4-(3-methylpyrrol-2-yl)-, and 4-(1,3-dimethylpyrrol-2-yl)-1-methyl-1,2,3,6-tetrahydropyridine. Except for the 4-(1-methylimidazol-2-yl) analogue, all compounds displayed good to excellent substrate properties. The 1-methyl-4-(3-methylfuran-2-yl) analogue is the most active member of this series with a k_{cat}/K_m value greater than 8,500 $min^{-1}mM^{-1}$. The results of these studies are discussed in terms of catalytic pathways proposed for MAO-B.
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Introduction

The monoamine oxidase B (MAO-B) generated pyridinium metabolite **5** mediates the nigrostriatal neurodegenerative properties of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**, Scheme 1), a compound that causes a parkinsonian syndrome in humans and subhuman primates.[1–3] The reaction pathway proceeds via the 1-methyl-2,3-dihydropyridinium intermediate ($MPDP^+$, **4**)[4] followed by autooxidation to **5**. This pyridinium metabolite is transported into the nigrostriatal nerve terminals by the dopamine transporter where it inhibits Complex 1 of the mitochondrial respiratory chain.[5] The substrate properties of **1** and related 6- and 5-membered[6] cyclic allylamine derivatives appear to be unique since no other class of cyclic amine has been reported that displays significant MAO substrate properties.

As illustrated with MPTP, the generally accepted mechanism for the MAO catalyzed α-carbon oxidation of amines proceeds by an initial single electron transfer (SET) step from the substrate nitrogen lone pair to the oxidized flavin FAD to generate an aminyl radical cation (**2**) and the flavin radical $FAD^{\cdot -}$. α-Carbon deprotonation of **2** yields radical **3**, which undergoes a second one electron oxidation to give the iminium metabolite **4** and the reduced flavin $FADH_2$ (Scheme 1). A primary deuterium isotope effect has been observed for the conversion of MPTP to $MPDP^+$.[7]

Results from a series of elegant studies by Silverman, which demonstrate that the MAO-B inactivating properties of cyclopropylamines can be rationalized by a process involving spontaneous ring opening of the SET generated cyclopropylaminyl radical cation followed by covalent bond formation between the resulting distonic primary carbon centered radical cation and an essential active site residue.[8–11] This behavior is consistent with estimates (10^{10} s^{-1} or greater) of the rates at which cyclopropylaminyl radicals and radical cations undergo ring opening.[12] The characterization of an active site *S*-3-hydroxypropylcysteinyl residue, in which the propyl carbon atoms were shown to be derived from the cyclopropyl group of racemic 1-cyclopropylamino-1-phenylethane, provides definitive evidence for the SET pathway.[11]

Also consistent with the SET pathway are the mechanism based inactivator properties of various 4-substituted 1-cyclopropyl-1,2,3,6-tetrahydropyridinyl derivatives including the *N*-cyclopropyl analogue **6** of MPTP.[13–15] The presumed pathway proceeds via SET to generate the cyclopropylaminyl radical cation **7**, which ring opens to the radical cation **8**, the putative bioalkylating species (Scheme 1). Somewhat unexpectedly, however, compounds structurally related to **6**, in which the phenyl group is replaced with a benzyl, phenoxy or 4-(1-methyl-2-pyrrolyl)

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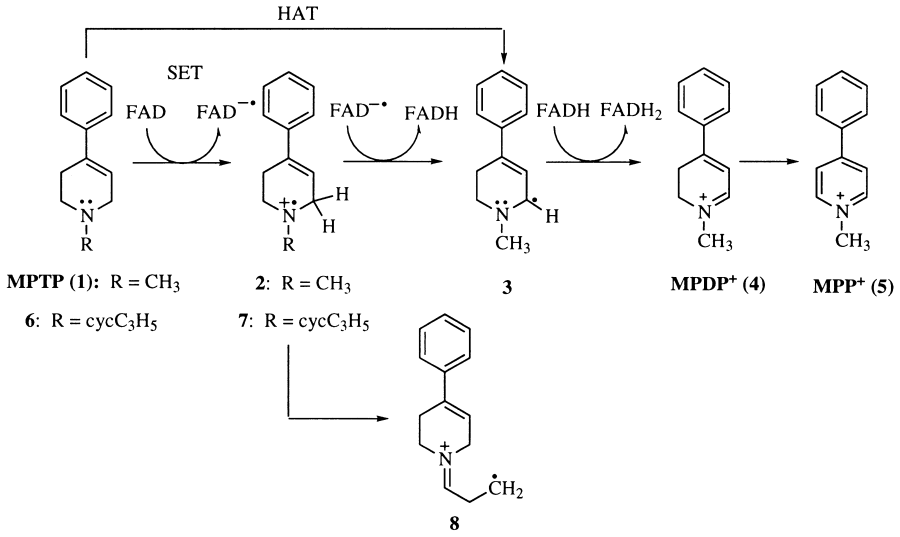


Scheme 1. Proposed SET pathway for the MAO-B catalyzed oxidation of MPTP (**1**) and its cyclopropyl analog **6**.

group, are weak inhibitors,[14,16] but good to excellent substrates,[13,16,17] for the ring α-carbon oxidation pathway catalyzed by MAO-B. When generated chemically, however, the cyclopropylaminyl radical cations derived from these substrate molecules do not undergo ring α-carbon oxidation but rather are converted to products that can be rationalized as being derived from ring opened species.[18] The substrate properties of these 1-cyclopropyltetrahydropyridinyl derivatives have prompted us to consider the possible contribution of a hydrogen atom transfer (HAT) pathway for MAO (Scheme 1), a pathway also proposed by others,[19] that would lead to the same intermediary carbon radical **3** without passing through the aminyl radical cation **2**.

As part of our previous studies on the MAO-B catalytic pathway related to this series of compounds, we have examined the effects of varying the C-4 substituent on the k_{cat}/K_m values of various 1-methyl-4-substituted-1,2,3,6-tetrahydropyridinyl derivatives. The results indicate that stabilization of the proposed radical intermediates, such as **3**, may be related to the efficiency of MAO-B catalysis. For example, the transformations of substrates bearing C-4 substituents that should stabilize the putative allylic radicals (**9–14** → **49–54**, Chart 1) occur more readily (k_{cat}/K_m = 155 to 4151 $min^{-1}mM^{-1}$),[13,20,21] than the transformations of compounds bearing more electronegative C-4 substituents [**15–17** → **55–57** (k_{cat}/K_m = 6 to 60 $min^{-1}mM^{-1}$)].[13] Steric effects, however, also may contribute to the substrate properties of these types of compounds. For example, the k_{cat}/K_m value for the (2-methyl)phenyl analogue **18** is over two times greater than that of MPTP[20] while the k_{cat}/K_m value for 1-methyl-4-(1-methyl-2-pyrrolyl)-1,2,3,6-tetrahydropyridine **12** is over 39 times greater than that of the corresponding N-H pyrrolyl analogue **19**.[22]

The present study was undertaken in an attempt to provide additional information on the stereoelectronic features that contribute to the unexpected substrate properties of 1,4-disubstituted-1,2,3,6-tetrahydropyridinyl derivatives and in particular to assess the influence of steric and electronic effects of the C-4 substituent by comparing the substrate properties of a series of 1-methyltetrahydropyridinyl derivatives bearing substituted and unsubstituted heteroaryl groups.

The structures of the compounds examined in this study (**1, 12–14**, and **19–25**) and their MAO-B generated

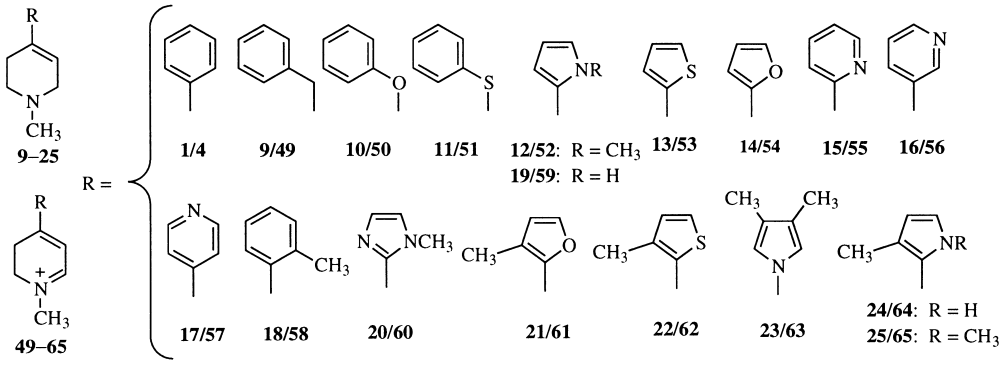
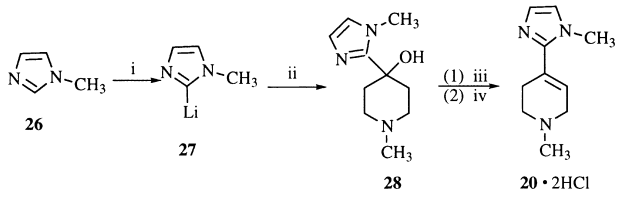


Chart 1. 1-Methyl-4-substituted-1,2,3,6-tetrahydropyridinyl derivatives discussed in the text.



Scheme 2. Synthetic pathway to the dihydrochloride salt of 1-methyl-4-(1-methylimidazol-2-yl)-1,2,3,6-tetrahydropyridine (**20**). *Reagents and conditions*: (i) *n*-BuLi, THF, rt; (ii) 1-methyl-4-piperidone, −78 °C; (iii) H_3PO_4:H_2O (4:1), 100 °C; (iv) HCl gas, Et_2O, rt.

dihydropyridinium metabolites are shown in Chart 1. The newly synthesized analogues are 1-methyl-4-(1-methylimidazol-2-yl)-1,2,3,6-tetrahydropyridine (**20**), 1-methyl-4-(2-methylfuran-2-yl)-1,2,3,6-tetrahydropyridine (**21**), 1-methyl-4-(3-methylthien-2-yl)-1,2,3,6-tetrahydropyridine (**22**), 1-methyl-4-(3,4-dimethylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (**23**), 1-methyl-4-(3-methylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (**24**), and 1-methyl-4-(1,3-dimethylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (**25**).

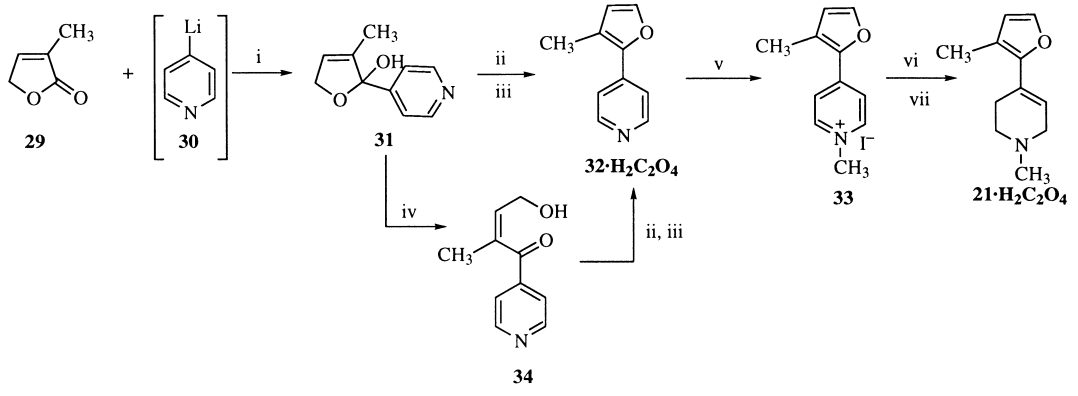
Results and Discussion

Chemistry

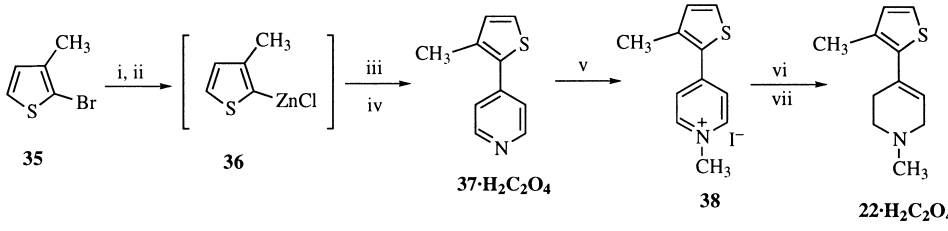
Compounds **21**–**25** (Chart 1) were prepared via $NaBH_4$ reduction of the corresponding pyridinium intermediates **33, 38, 42, 47**, and **48**, respectively (see below). The preparation of 1-methyl-4-(1-methylimidazol-2-yl)-1,2,3,6-tetrahydropyridine **20** required an alternative pathway that involved the acid catalyzed dehydration of the 4-(1-methylimidazol-2-yl)-4-piperidinol **28** which, in turn, was obtained by the addition of 1-methylimidazol-2-yllithium, prepared from 1-methylimidazole **26** and butyllthium, to 1-methyl-4-piperidone **27** (Scheme 2).

The route to the 4-(3-methyl-2-furanyl) analogue **21** (Scheme 3) was based on the observation reported by Kondo et al. that unsaturated 5-membered lactols are converted to the corresponding furanyl derivatives under acidic conditions.[23] The intermediate lactol **31** could be obtained by condensation of 3-methyl-(5*H*)-2-furanone (**29**) with 4-pyridinyllithium (**30**). Treatment of **31** with H_2SO_4 in THF gave **32**, which was purified as its oxalate salt. If the reaction mixture containing **31** was quenched under basic conditions, the ring opened product **34** was formed. Compound **34** underwent ring closure under acidic conditions to give the **32**. Methylation of **32** followed by $NaBH_4$ reduction of the resulting pyridinium species **33** yielded the 1,2,3,6-tetrahydropyridinyl product **21** that was purified as its oxalate salt.

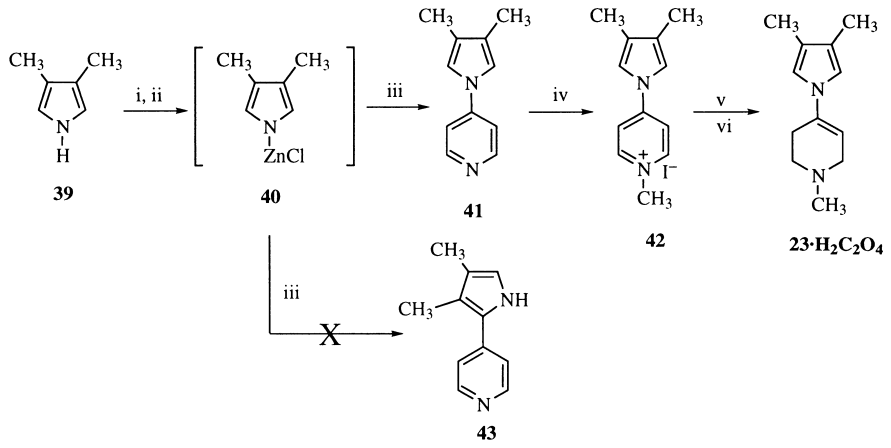
The syntheses of 4-(3-methyl-2-thienyl)pyridine (**22**) and 4-(3,4-dimethyl-1-pyrrolyl)pyridine (**23**) were achieved by cross-coupling reactions between 4-bromopyridine and the zinc salt **36** of 3-methyl-2-thiophene and the zinc salt **40** of 3,4-dimethyl-1-pyrrole[24] in the presence of $Pd(PPh_3)_4$ (Schemes 4 and 5). The reaction leading to **23** initially was designed to prepare the 4-(3,4-dimethylpyrrol-2-yl) analogue **43**. The N-coupled product **41**, however, was the only product isolated from the reaction mixture suggesting that the steric hindrance introduced by the methyl groups controls the regiochemistry of this reaction and prevents reaction at the electro-



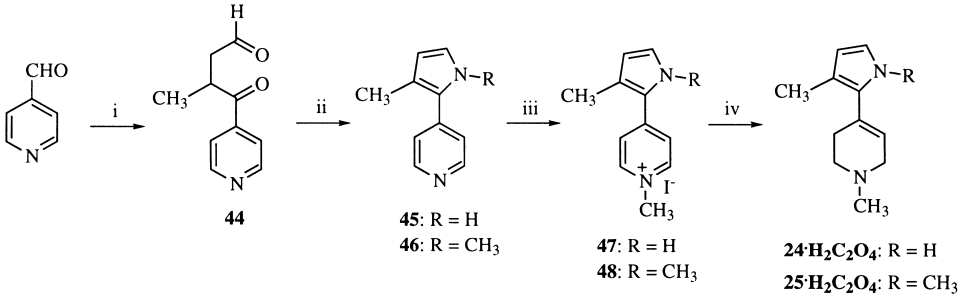
Scheme 3. Synthetic pathway for 1-methyl-4-(2-methylfuran-2-yl)-1,2,3,6-tetrahydropyridine (**21**). *Reagents and conditions*: (i) THF, −78 °C, 4 h, then rt, 24 h; (ii) H_2SO_4 in THF, −78 °C; (iii) $H_2C_2O_4$ in Et_2O; (iv) aq NaOH; (v) CH_3I, acetone; (vi) $NaBH_4$, CH_3OH; (vii) $H_2C_2O_4$ in Et_2O



Scheme 4. Synthetic pathway to 1-methyl-4-(3-methylthien-2-yl)-1,2,3,6-tetrahydropyridine (**22**). *Reagents and conditions*: (i) *n*-BuLi, THF, −78 °C; (ii) $ZnCl_2$, THF; (iii) 4-bromopyridine, $Pd(PPh_3)_4$, THF; (iv) $H_2C_2O_4$, Et_2O; (v) CH_3I, acetone; (vi) $NaBH_4$, MeOH; (vii) $H_2C_2O_4$, Et_2O.



Scheme 5. Synthetic pathway to 1-methyl-4-(3,4-dimethylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (**23**). *Reagents and conditions*: (i) *n*-BuLi, THF; (ii) $ZnCl_2$, THF; (iii) 4-bromopyridine, $Pd(PPh_3)_4$; (iv) Ch_3I, acetone; (v) $NaBH_4$, MeOH; (vi) $H_2C_2O_4$, Et_2O.



Scheme 6. Synthetic pathway to 1-methyl-4-(3-methylpyrrol-2-yl)- (**24**) and 1-methyl-4-(1,3-dimethylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (**25**). *Reagents and conditions*: (i) propenal, NaCN, DMF, 0 °C; (ii) NH_3 or CH_3NH_2, ethanol, reflux; (iii) CH_3I, acetone; (iv) $NaBH_4$, methanol; (v) $H_2C_2O_4$, Et_2O.

nically favored α-position.[25] The resulting 4-substituted pyridines, **37** and **41**, were converted to the required tetrahydropyridines by $NaBH_4$ reduction of the corresponding *N*-methylpyridinium intermediates **38** and **42**, respectively.

An alternative approach was developed to obtain the 2-pyrrolyl derivatives **24** and **25**, which involved the cyanide ion catalyzed Michael addition of aldehydes to an α,β-unsaturated carbonyl system followed by the Paar–Knorr cyclization of the resulting 1,4-dione with ammonia or primary amines.[26,27] The intermediate dione **44** was obtained from the reaction of 4-pyridinecarboxaldehyde with propenal. Treatment of **44** with ammonia or methylamine generated the corresponding pyridinylpyrroles **45** and **46**, which in turn gave the required pyridinium species **47** and **48** and tetrahydropyridines **24** and **25** as shown in Scheme 6.

Enzymology

The MAO-B (0.09 μM) substrate properties of the newly synthesized terahydropyridines **20**–**25** (500 μM) were examined using enzyme purified from beef liver. In each case, the time dependent increase of a chromophore consistent with that expected for the corresponding dihydropyridinium metabolite (**60**–**65**, repectively) was observed (see Chart 1 and Table 1). The enzyme kinetic parameters of the test compounds then were determined by estimating the initial rates of formation (first 120 s) of the dihydropyridinium metabolites at initial substrate concentrations that bracketed the K_m for that substrate. Since not all of the dihydropyridinium metabolites were available as synthetic standards, rates of product formation were approximated using ε values for the struc-

Table 1 Kinetic parameters for the MAO-B catalyzed oxidations of 1-methyl-4-heteroaryl-1,2,3,6-tetrahydropyridines and MPTP

C-4 Substituent	λ_{max} (nm)	K_{cat} $(min)^{-1}$	K_m (mM)	K_{cat}/K_m $(min^{-1}nM^{-1})$
C_6H_5(**1**)[a]	343	270	0.2	1350[13]
2-C_4(1-CH_3)H_3N(**12**)[b]	420	360	0.2	1800[13]
2-C_4H_3S (**13**)[c]	386	60	0.2	300[13]
2-C_4H_3O (**14**)[d]	384	31	0.2	155[13]
2-C_4H_3NH (**19**)[b]	424	85	1.8	46[22]
2-C_3H_2-1-CH_3(1,3)N (**20**)[b]	383	13	0.7	17
2-C_4(3-CH_3)H_2O (**21**)[d]	399	348	0.04	8620
2-C_4(3-CH_3)H_2S (**22**)[d]	400	337	0.05	6690
1-C_4(3,4-CH_3)$_2H_2N$ (**23**)[b]	381	390	0.2	1510
2-C_4(3-CH_3)H_3N (**24**)[b]	432	640	0.92	690
2-C_4(1,3-CH_3)$_2H_2N$ (**25**)[b]	436	430	0.3	1436

[a]Estimated using ε = 16,000 M^{-1}, the value for the perchlorate salt of the 1-methyl-4-phenyl-2,3-dihydropyridinium species.[13]
[b]Estimated using ε = 24,000 M^{-1}, the value for the perchlorate salt of the 1-methyl-4-(1-methylpyrrol-2-yl)-2,3-dihdropyridinium species.[13]
[c]Estimated using ε = 18,800 M^{-1}, the value for the perchlorate salt of the 1-methyl-4-(thien-2-yl)-2,3-dihydropyridinium species.[13]
[d]Estimated using ε = 23,000 M^{-1}, the value for the perchlorate salt of the 1-methyl-4-(2-furanyl)-2,3-dihydropyridinium species.[13]

turally related compounds identified in the Table 1 footnotes. The plots of the initial velocities versus substrate concentrations were linear in all cases as were the corresponding double-reciprocal plots from which k_{cat} and K_m were calculated (Table 1). The k_{cat}/K_m values used to estimate the relative substrate properties of this series of compounds, covered the range of 17 to 8620 $(min^{-1}\,mM)^{-1}$.

A comparison of the substrate properties of the *N*-methylpyrroyl analogue **12** with the corresponding *N*-methylimidazoyl analogue **20** suggests that compounds bearing electron donating heteroarene groups are better substrates than analogues bearing electron withdrawing heteroarene groups. Comparisons of the substrate properties of analogues **13** versus **22** and **14** versus **21**, however, emphasize the importance of steric effects on the rates of the MAO-B catalyzed oxidations within this series of compounds. The introduction of the methyl group at C-3 of the thienyl and furanyl groups both increases the k_{cat} values and decreases the K_m values. Consequently, the overall substrate properties, as measured by k_{cat}/K_m, increase by a factor of 22 for the thienyl compound and 55 for the furanyl compound. The slight bathochromic shifts in the λ_{max} values for the dihydropyridinium metabolites suggest increased electron delocalization of the heteroaryl electons into the 6-membered ring but it seems unlikely that such a nominal electronic effect could account for the dramatic increase in the substrate properties of the methylated analogues. Furthermore, comparison of the substrate properties of the NCH_3-2-pyrrolyl analogue **12** and the NH-2-pyrrolyl analogue **19** shows an analogous trend with the k_{cat}/K_m value for the methylated analogue being 39 times greater than that of the NH compound. In this case the λ_{max} value for the NH compound is at a longer wavelength than that of the NCH_3 compound.

Factors other than steric and electronic also may contribute to the interactions of these compounds with MAO-B. For example, the isomeric monomethyl-2-pyroylyl derivatives **12** (*N*-methyl) and **24** (3-methyl) have the same k_{cat} values but the K_m value for the NH compound **24** is over four times higher than for the NCH_3 compound **12**. This effect is also observed with analogue **19**, which has a high K_m value (1.8 mM) compared to the structurally similar compounds **12, 13, 14**, and **25** (K_m = 0.2, 0.2, 0.2, 0.3, respectively). In the case of **23**, the nitrogen atom is connected directly to the tetrahydropyridine. This compound, also lacking an NH group, exhibits the same substrate properties as those observed with analogue **25**. Consequently, in addition to favorable steric and electronic effects, unfavorable interactions with the NH group may influence the substrate properties of compounds in this series.

The results of these studies as well as those reported previously suggest that a variety of factors contribute to the MAO-B substrate properties of 4-substituted 1-methyl-1,2,3,6-tetrahydropyridinyl derivatives. Although, in general, electron donating substituents often improve the k_{cat}/K_m values, steric and, perhaps, polar factors appear to dominate the substrate interactions with the enzyme. Efforts to relate the results of these studies with the substrate properties of more traditional acyclic primary and secondary amines currently are under way.

Materials and Methods

Caution!

1,4-Disubstituted tetrahydropyridines such as MPTP (**1**) are known or potential nigrostriatal neurotoxins and should be handled using disposable gloves in a properly ventilated hood. Detailed procedures for the safe handling of MPTP have been reported.[28]

Chemistry

All reagents were obtained from commercial sources and were used directly. Synthetic reactions were carried out under a nitrogen atmosphere. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium benzophenone ketyl. Acetone was distilled from potassium carbonate. Melting points were determined using a Thoms–Hoover melting point apparatus and are uncorrected. UV-Vis spectra were recorded on a Beckman DU 7400 spectrophotometer and proton NMR spectra on a Bruker WP 360-MHz or a 270-MHz or a 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. The conventional symbols are used to describe spin multiplicities. Gas chromatography–electron ionization mass spectrometry (GC–EIMS) was performed on a Hewlett Packard 5890 GC fitted with an HP-1 capillary column (15 m×0.2 mm id, 0.33 mm film thickness) which was coupled to 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. High resolution-electron ionization mass spectrometry (HR-EIMS) and high resolution-chemical ionization mass spectrometry (HR-CIMS) were performed on a VG 7070 HF instrument. Elemental analyses, performed by Atlantic Microlab, Inc., Norcross, GA, were within 0.37% of the theoretical values calculated for C, H, and N although some compounds were obtained as partial hydrates (**38** and **45**) or contained excess oxalic acid (**21**, **23**, and **37**).

4-(1-Methylimidazol-2-yl)-4-piperidinol (28). To a solution of 1-methylimidazole **26** (4.1 mL, 50 mmol) in dry THF (50 mL) was added *n*-butyllithium (20 mL, 50 mmol) dropwise at room temperature under nitrogen. The solution was stirred at 40°C for 3 h. The temperature was then decreased to −78°C and 1-methyl-4-piperidone (5.7 g, 50 mmol) was added. This mixture was allowed to warm to room temperature slowly and stirred at this temperature for 6 h and for an additional 3 h at 40°C. The reaction was quenched with water (30 mL). The aqueous layer was extracted with ethyl acetate (4×40 mL). The combined organic extracts were dried over $MgSO_4$ and the solvent was removed in vacuo. The residue was purified by recrystallization from diethyl ether:methanol affording pure **28** (8.279 g, 84.8%): mp 151.5–152°C; GC (t_R = 7.98 min)–EIMS

m/*z* (%) 195 (4, $M^{\bullet+}$), 177 (61), 125 (100), 83 (98), 72 (70); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 6.82 (s, 1H, ArH), 6.78 (s, 1H, ArH), 3.84 (s, 3H, N′CH_3), 3.57 (s, 1H, OH), 2.63–2.68 (m, 2H, $NCHH_{eq}$), 2.42–2.50 (dt, 2H, $NCHH_{ax}$), 2.29 (s, 3H, NCH_3), 2.04–2.24 (m, 2H, NCH_2CHH_{eq}), 1.84–1.89 (d, 2H, NCH_2CHH_{ax}); ^{13}C NMR ($CDCl_3$, 360 MHz) δ 151.64, 125.44, 122.62, 67.79, 51.26, 46.14, 35.76, 34.65; Anal calcd for $C_{10}H_{17}N_3O$: C, 61.51; H, 8.78; N, 21.53. Found: C, 61.63; H, 8.77; N, 21.49.

1-Methyl-4-(1-methylimidazol-2-yl)-1,2,3,6-tetrahydropyridine dihydrochloride (20·2HCl). To **28** (1.757 g, 9 mmol) was added aqueous H_3PO_4 solution (85%; H_3PO_4:H_2O, 4:1). This mixture was stirred and heated at 100°C for 12 h. The solution was then cooled and the pH adjusted to 9 with saturated aq Na_2CO_3. The aqueous layer was extracted with ethyl acetate (4×40 mL. The extracts were dried over $MgSO_4$ and the solvent removed in vacuo. To the residue in dry diethyl ether (50 mL), was introduced dry HCl with stirring. After stirring for 3 h, the white solid was collected and recrystallized from methanol:diethyl ether to afford pure **20·2HCl** (2.07 g, 92%): mp 256°C (decomposed); GC (t_R = 7.84 min)–EIMS *m*/*z* (%) 177 (100, $M^{\bullet+}$), 162 (87), 133 (72), 120 (30), 107 (20), 94 (37); 1H NMR (CD_3OD, 270 MHz) δ 7.56–7.59 (dd, 2H, ArH), 6.53 (m, 1H, C-5), 4.16 (m, 2H, C-6), 3.91 (s, 3H, NCH_3), 3.71 (m, 2H, C-2), 3.00 (s, 3H, N′CH_3), 2.86 (m, 2H, C-3); ^{13}C NMR (CD_3OD, 360 MHz) δ 144.12, 132.11, 126.28, 122.03, 120.31, 52.76, 51.01, 43.22, 36.78, 25.71; UV (phosphate buffer, pH 7.4) λ_{max} 253 nm ($\varepsilon = 8,666\ M^{-1}$); Anal calcd for $C_{10}H_{17}Cl_2N_3$: C, 48.01; H, 6.85; N, 16.80. Found: C, 48.24; H, 6.82; N, 16.63.

Oxalate salt of 4-(3-methylfuran-2-yl)pyridine (32·$H_2C_2O_4$). Method 1: A solution of 4-bromopyridine (3.16 g, 20 mmol) in 50 mL of anhydrous diethyl ether at −78°C under nitrogen was treated dropwise with a hexane solution of *n*-butyllithium (8 mL, 20 mmol). A yellow precipitate was formed and 30 min later 3-methyl-2-(*5H*)-furanone (**29**, 1.95 mL, 20 mmol) was added. The mixture was stirred for 4 h at −78°C. The reaction mixture was stirred for an additional 24 h at room temperature. The temperature was lowered again to −78°C and a dilute H_2SO_4 solution in THF was added to adjust the pH to 2. After warming to room temperature, 50 mL of water was added and the aqueous layer (adjusted to pH 8) was extracted with diethyl ether (4×50 mL). The combined extracts were dried over $MgSO_4$ and the solvent was removed in vacuo. The resulting brown oil was chromatographed (silica gel, ethyl acetate:hexane, 10:1) to give **32** in 87% yield. Method 2: The same procedures are used except the reaction was quenched with aq NaOH at −78°C and the aqueous layer was extracted by ethyl acetate. After recrystallization from methanol:acetone:diethyl ether, **34** was obtained as a white solid in 78% yield. mp 152.5–153°C; GC (t_R = 7.14 min)–EIMS *m*/*z* (%) 177 (2, $M^{\bullet k+}$) 159 (6), 133 (13), 117 (7), 99(100), 79 (56); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.57–8.59 (dd, 2H, C-2 and C-6), 7.40–7.42 (dd, 2H, C-3 and C-5), 5.82 (m, 1H, C=CH), 4.71–4.87 (m, 2H, CH_2), 3.71 (s, 1H, OH), 1.62–1.65 (m, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 151.58, 149.30, 137.98, 123.35, 120.88, 109.26, 72.59, 11.00; Anal calcd for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.69; H, 6.26; N, 7.82. To **34** (30 mmol) in 30 mL THF and 10 mL H_2O, 1 mL concentrated H_2SO_4 was added slowly. After stirring overnight at room temperature the THF was removed in vacuo. Aqueous Na_2CO_3 was added to adjust the pH to 9. The mixture was extracted by diethyl ether and the combined extracts were dried over $MgSO_4$ and the solvent was removed in vacuo. The residue was chromatographed on silica gel to give **32** in 99% yield. This brown oil in anhydrous diethyl ether (50 mL) was treated with oxalic acid (2.16 g, 24 mmol) in 10 mL of anhydrous diethyl ether. The precipitate was recrystallized from methanol:diethyl ether to afford a white crystalline solid **32·$H_2C_2O_4$** in 90% yield. mp 165°C; GC (t_R = 10.74 min)–EIMS *m*/*z* (%) 159 (100, $M^{\bullet+}$), 130 (100), 103 (22), 77 (50); ^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.60–8.62 (d, 2H, C-2 and C-6), 7.79–7.80 (d, 1H, C′-5), 7.58–7.61 (dd, 1H, C-3 and C-5), 6.57–6.58 (d, 1H, C′-4), 2.3 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 161.24, 149.08, 145.00, 143.79, 137.43, 122.33, 118.67, 116.35, 11.76; Anal calcd for $C_{12}H_{11}NO_5{\cdot}0.5H_2C_2O_4$: C, 52.97; H, 4.10; N, 4.74. Found: C, 53.17; H, 4.08; N, 4.73.

Oxalate salt of 4-(3-methylthienyl-2-yl)pyridine (37·$H_2C_2O_4$). To a solution of 2-bromo-3-methylthiophene (**35**, 5 g, 27.4 mmol) in dry THF (25 mL) at −78°C under nitrogen, was added *n*-butyllithium in hexane (12 mL, 30 mmol) dropwise. The mixture was stirred at −78°C for 1.5 h and then was transferred to a solution of dry $ZnCl_2$ (30 mmol) in THF (15 mL). After stirring for 3 h at room temperature, the new mixture was transferred again to the mixture of 4-bromopyridine (4.725 g, 30 mmol) and *tetrakis*(triphenylphosphine) palladium (1 g, 3 mmol) in THF (20 mL) at 0°C. After stirring at 45°C for 2 days, water was added. The water layer at pH 8 was extracted with ethyl acetate (4×100 mL). The combined extracts were dried over $MgSO_4$ and the solvent was removed in vacuo. Column chromatography (silica gel, ethyl acetate) of the residue afforded **37** as a yellow oil. Treatment of this oil in dry diethyl ether with oxalic acid afforded a yellow precipatate that was recrystallized in methanol:diethyl ether to give the yellow oxalate salt **37** (4.45 g, 93%). mp 138–140°C; GC (t_R = 7.06 min)–EIMS *m*/*z* (%) 175 (100, $M^{\bullet+}$), 147 (24), 130 (13), 103 (5), 97 (39); 1H NMR (DMSO-d_6, 200 MHz) δ 8.60–8.65 (d, 2H, C-2 and C-6), 7.63–7.65 (d, 1H, C′-5), 7.50–7.57 (dd, 2H, C-3 and C-5), 7.04–7.09 (d, 1H, C′-4), 2.38 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 161.19, 149.36, 142.22, 136.18, 133.67, 132.33, 126.60, 122.56, 15.13; Anal calcd for $C_{12}H_{11}NO_4S{\cdot}0.23\ H_2C_2O_4$: C, 52.34; H, 4.04; N, 4.90. Found: C, 52.36; H, 4.14; N, 4.78.

4-(3,4-Dimethylpyrrol-2-yl)pyridine (41). A hexane solution of *n*-butyllithium (6 mL, 15 mmol) was added to a stirred solution of 3,4-dimethylpyrrole (**39**,[24] 1.43 g, 15 mmol) in 10 mL THF at room temperature under

nitrogen. The mixture was stirred for 1 h and then was transferred to a stirred solution of dry zinc chloride (2.72 g, 20 mmol) in 10 mL THF at room temperature. The mixture was stirred for 1.5 h after which the 3,4-dimethyl-1-pyrrolyl zinc chloride solution was transferred at 0°C to a flask containing a mixture of 4-bromopyridine (2.36 g, 15 mmol) and *tetrakis*(triphenylphosphine)palladium (0.75 g, 4.3 mol%) in dry THF (25 mL). The resulting reaction mixture was stirred for 3 days at room temperature and then was cooled and 50 mL of water added. The aqueous layer was saturated with NaCl and extracted with ethyl acetate (4×50 mL). The extracts were dried over $MgSO_4$ and the solvent was removed in vacuo. The residue was recrystallized in diethyl ether to afford **41** (1.03 g, 40%) as a white solid: mp 125–126°C; GC (t_R = 13.43 min)–EIMS *m*/*z* (%) 172 (76, $M^{\bullet+}$), 171 (100), 157 (76), 130 (6), 94 (6); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.51–8.53 (dd, 2H, C-2 and C-6), 7.17–7.19 (dd, 2H, C-3 and C-5), 6.94 (s, 2H, C′-2 and C′-5), 2.06 (s, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 150.99, 146.00, 123.00, 115.80, 112.05, 10.33; Anal calcd for $C_{11}H_{12}N_2$: C, 76.71; H, 7.02; N, 16.26. Found: C, 76.76; H, 7.07; N, 16.33.

4-(3-Methylpyrrol-2-yl)pyridine (45). A solution of 4-pyridinecarboxaldehyde (5.85 g, 0.05 mol) in anhydrous DMF (25 mL) was added dropwise to a mixture of potassium cyanide (0.26 g, 0.004 mol) and anhydrous DMF (25 mL) at 0°C under nitrogen. After 30 min the propenol (3.54 g, 0.05 mol) in 10 mL of anhydrous DMF was added dropwise. The mixture was stirred for 2 h at 0°C and then acetic acid (2.5 mL) was added, followed 10 min later, by ice water. The pH was adjusted to 9 and the mixture was extracted with ethyl acetate (4×150 mL). The organic solution was washed with dilute aq $NaHCO_3$, dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was chromatographed (silica gel, ethyl acetate:hexane, 10:2) to give **44** in 40% yield. GC (t_R = 6.59 min)–EIMS *m*/*z* (%) 177 (1, $M^{\bullet+}$), 149 (3), 135 (54), 106 (100), 78 (90), 51 (72); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 9.76 (s, 1H, CHO), 8.76–8.81 (dd, 2H, C-2 and C-6), 7.74–7.79 (dd, 2H, C-3 and C-5), 3.85–3.94 (m, 1H, C*H*CH_3), 3.16–3.24 (dd, 1H, CH_1H_2), 2.64–2.70 (dd, 1H, CH_1H_2), 1.20–1.27 (d, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 360 MHz) δ 202.20, 200.05, 150.96, 142.21, 121.56, 47.18, 35.50, 17.44; HR-CIMS. calcd for $C_{10}H_{11}NO_2H^+$: 178.0868038. Found: 178.087585.

A solution of 30% NH_4OH (6.8 g, 0.12 mol) was added to the solution of **44** (10 mmol) in ethanol (75 mL). The mixture was heated and stirred for 3 h at 80°C. The solvent was removed in vacuo and the residue in 50 mL water was extracted with ethyl acetate (4×50 mL). The combined extracts were washed with 150 mL 5% $NaHCO_3$, dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was chromatographed (silica gel, ethyl acetate:methanol, 10:1) to give **45** (1.03 g, 65%) as a white solid: mp 137.5–138.5°C; GC (t_R = 7.68 min)–EIMS *m*/*z* (%) 158 (93, $M^{\bullet+}$), 157 (100), 130 (28), 103 (86), 80 (20); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.52–8.55 (dd, 2H, C-2 and C-6), 7.33–7.34 (dd, 2H, C-3 and C-5), 6.86–6.88 (t, 1H, C′-5), 6.18 (t, 1H, C′-4), 2.35 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 360 MHz) δ 150.07, 145.08, 140.77, 125.16, 120.45, 119.72, 113.41, 13.30; Anal calcd for $C_{10}H_{10}N_2$·0.16 H_2O: C, 75.01; H, 6.46; N, 17.38. Found: C, 75.01; H, 6.41; N, 17.39.

4-(1,3-Dimethylpyrrol-2-yl)pyridine (46). To the solution of **44** (10 mmol) in methanol (20 mL), was added a solution of methylamine (20 mmol) in methanol. This solution was stirred at room temperature for 24 h. The solvent was removed in vacuo and the residue in 20 mL water was extracted with diethyl ether (4×30 mL). The extracts were dried over $MgSO_4$ and the solvent was removed in vacuo. The residue was chromatographed (silica gel, ethyl acetate:methanol, 10:1) to give **46** as a yellow oil (1.211 g, 70%): GC (t_R = 7.20 min)–EIMS *m*/*z* (%) 172 (93, $M^{\bullet+}$), 171 (100), 156 (20), 130 (15), 103 (8), 94 (41); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.62–8.66 (dd, 2H, C-2 and C-6), 7.18–7.22 (dd, 2H, C-3 and C-5), 6.68–6.69 (d, 1H, C′-5), 6.09–6.10 (d, 1H, C′-4), 3.58 (s, 3H, N-CH_3), 2.13 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 149.76, 140.71, 128.57, 124.44, 124.02, 110.17, 35.38, 12.50; HR-EIMS calcd for $C_{11}H_{12}N_2$: 172.1000485. Found: 172.100327.

General procedure for the synthesis of the *N*-methyl-4-substituted pyridinium iodides. A mixture of iodomethane (8 mmol) and 4-substituted pyridine (2 mmol) in anhydrous acetone (10 mL) was stirred at room temperature overnight. After filtration, the precipitate was recrystallized from the appropriate solvent.

1-Methyl-4-(3-methylfuran-2-yl)pyridinium iodide (33) was recrystallized from methanol in 97% yield; mp 203–203.5°C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.82–8.87 (d, 2H, C-2 and C-6), 8.11–8.15 (d, 2H, C-3 and C-5) 8.08–8.10 (d, 1H, C′-5), 6.79–6.80 (d, 1H, C′-4), 4.27 (s, 3H, NCH_3), 2.46 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 147.86, 145.56, 143.70, 143.50, 130.20, 120.68, 118.47, 47.50, 12.95; Anal. calcd for $C_{11}H_{12}INO$: C, 43.88; H, 4.02; N, 4.65. Found: C, 43.71; H, 3.97; N, 4.53.

1-Methyl-4-(3-methylthienyl-2-yl)pyridium iodide (38) was recrystallized from methanol:diethyl ether in 67% yield. mp 194°C (decomposed); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.87–8.91 (d, 2H, C-2 and C-6), 8.16–8.20 (d, 2H, C-3 and C-5), 7.93–7.98 (d, 1H, C′-5), 7.21–7.22 (d, 1H, C′-4), 4.30 (s, 3H, NCH_3), 2.52 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 148.77, 145.64, 145.19, 141.39, 133.62, 131.20, 124.35, 46.92, 15.93; Anal calcd $C_{11}H_{12}INS$·0.7 H_2O: C, 40.06; H, 4.09; N, 4.25. Found: C, 40.02; H, 3.72; N, 4.29.

4-(3,4-Dimethylpyrrol-1-yl)-1-methylpyridinium iodide (42) was recrystallized from methanol in 90% yield. mp 239–239.5°C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.79–8.81 (d, 2H, C-2 and C-6), 8.10–8.12 (d, 2H, C-3 and C-5), 7.58 (s, 2H, C′-2 and C′-5), 4.14 (s, 3H, NCH_3), 2.01 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 149.60, 146.13, 125.81, 117.12, 112.92, 45.88, 9.92; Anal calcd for $C_{12}H_{15}IN_2$: C, 45.88; H, 4.81; N, 8.92. Found: C, 45.72; H, 4.86; N, 8.81.

1-Methyl-4-(3-methylpyrrol-2-yl)pyridinium iodide (47). was recrystallized from methanol and acetone in 84% yield. mp 244–245°C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.64–8.67 (dd, 2H, C-2 and C-6), 7.91–7.94 (dd, 2H, C-3 and C-5), 7.25–7.28 (t, 1H, C′-5), 6.25 (t, 1H, C′-4), 4.14 (s, 3H, NCH_3), 2.40 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 145.43, 144.18, 127.73, 126.07, 122.56, 119.09, 115.02, 45.99, 14.44; Anal calcd $C_{11}H_{13}IN_2$: C, 44.02; H, 4.36; N, 9.33. Found: C, 43.93; H, 4.38; N, 9.26.

4-(1,3-Dimethylpyrrol-2-yl)-1-methylpyridinium iodide (48). was recrystallized from methanol in 96% yield. mp 274°C (decomposed); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.79–8.82 (d, 2H, C-2 and C-6), 7.95–7.99 (d, 2H, C-3 and C-5), 7.14–7.15 (d, 1H, C′-5), 6.14–6.16 (d, 1H, C′-4), 4.26 (s, 3H, NCH_3), 3.74 (s, 3H, N'CH_3), 2.21 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 146.12, 144.23, 129.71, 125.60, 125.22, 124.43, 111.53, 46.54, 35.85, 13.03; Anal calcd for $C_{12}H_{15}IN_2$: C, 45.88; H, 4.81; N, 8.92. Found: C, 45.77; H, 4.86; N, 8.81.

General procedures for the synthesis of the oxalate salts of *N*-methyl-4-substituted-1,2,3,6-tetrahydropyridine. Sodium borohydride (4 mmol) was added in portions to a stirred solution of the appropriate *N*-methyl-4-substituted pyridinium iodide (2 mmol) in 30–150 mL of methanol at 0°C. The mixture was stirred for an additional 1 h at room temperature and the solvent was subsequently removed in vacuo. The residue in 15 mL of water was extracted with diethyl ether (4×30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to 15% of the original volume. Treatment with oxalic acid (2.4 mmol) in 10 mL of diethyl ether precipitated the crude oxalate salt which was recrystallized from the appropriate solvent.

1-Methyl-4-(3-methylfuran-2-yl)-1,2,3,6-tetrahydropyridine oxalate (21·$H_2C_2O_4$) was recrystallized from ethanol in 66% yield; mp 157–157.5°C; GC (t_R = 11.31 min)–EIMS *m*/*z* (%) 177 (100, $M^{\bullet+}$), 176 (76), 162 (13), 148 (22), 134 (20), 119 (28), 105 (20), 95 (30), 83 (41); ^{1}H NMR (DMSO-d_6, 270 MHz) δ 7.53 (d, 1H, C′-5), 6.37–6.38 (d, 1H, C′-4), 5.92 (b, 1H, C-5), 3.73 (d, 2H, C-6), 3.24–3.28 (t, 2H, C-2), 2.77 (s, 3H, NCH_3), 2.70 (m, 2H, C-3), 2.1 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 164.67, 147.04, 140.98, 126.13, 116.73, 115.35, 115.29, 50.94, 49.19, 41.82, 22.98, 11.49; UV (phosphate buffer, pH 7.4) λ_{max} 264 nm (ε = 13,204 M^{-1}). Anal calcd for $C_{13}H_{17}NO_5$·0.083 $H_2C_2O_4$: C, 57.56; H, 6.30; N, 5.10. Found: C, 57.60; H, 6.35; N, 5.17.

1-Methyl-4-(3-methylthienyl-2-yl)-1,2,3,6-tetrahydropyridine oxalate (22·$H_2C_2O_4$) was recrystallized from methanol:diethyl ether in 98% yield. mp 139–140°C; GC (t_R = 7.57 min)–EIMS *m*/*z* (%) 193 (100, $M^{\bullet+}$), 192 (72), 178 (13), 164 (13), 150 (13), 135 (24), 111 (22), 94 (20); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.38–7.40 (d, 1H, C′-5), 6.90–6.92 (d, 1H, C′-4), 5.83 (b, 1H, C-5), 3.75–3.77 (d, 1H, C-6), 3.28–3.32 (t, 2H, C-2), 2.79 (s, 3H, NCH_3), 2.67 (b, 2H, C-3), 2.24 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 164.11, 136.35, 133.39, 131.48, 129.10, 123.23, 119.42, 51.36, 49.65, 41.97, 27.17, 15.30; UV (phosphate buffer, pH 7.4) λ_{max} 264 nm (ε = 14,211 M^{-1}). Anal calcd for $C_{13}H_{17}NO_4S$: C, 55.11; H, 6.05; N, 4.94. Found: C, 54.84; H, 6.04; N, 5.00.

4-(3,4-Dimethylpyrrol-1-yl)-1-methyl-1,2,3,6-tetrahydropyridine oxalate (23·$H_2C_2O_4$) was recrystallized from ethanol in 69% yield. mp 152–153°C; GC (t_R = 13.87 min)–EIMS *m*/*z* (%) 190 (30, $M^{\bullet+}$), 189 (26), 175 (2), 146 (22), 132 (15), 108 (11), 96 (100); ^{1}H NMR (DMSO-d_6, 270 MHz) δ 6.82 (s, 2H, C′-2 and C′-5), 5.60 (b, 1H, C-5), 3.65 (s, 2H, C-6), 3.26–3.30 (t, 2H, C-2), 2.7 (s, 3H, NCH_3), 1.90 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 164.58, 132.89, 119.16, 115.30, 100.44, 50.14, 49.17, 41.65, 23.22, 9.90; Anal calcd for $C_{14}H_{20}N_2O_4$: C, 59.98; H, 7.19; N, 9.99. Found: C, 60.04; H, 7.23; N, 10.07.

1-Methyl-4-(3-methylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine oxalate (24·$H_2C_2O_4$) was recrystallized from methanol:diethyl ether in 56% yield. mp 170°C (decomposed); GC (t_R = 7.60 min)–EIMS *m*/*z* (%) 176 (91, $M^{\bullet+}$), 175 (67), 161 (41), 132 (46), 118 (78), 94 (100); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 6.61–6.63 (t, ^{1}H, C′-5), 5.89 (b, 1H, C′-4), 5.68 (b, 1H, C-5), 3.74 (s, 2H, C-6), 3.27–3.30 (t, 2H, C-2), 2.79 (s, 3H, NCH_3), 2.69 (m, 2H, C-3), 2.11 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 164.64, 127.86, 126.10, 117.03, 115.54, 112.07, 111.37, 51.15, 49.45, 41.77, 24.24, 13.49; Anal calcd for $C_{13}H_{18}N_2O_4$: C, 58.63; H, 6.81; N, 10.52. Found: C, 58.46; H, 6.86; N, 10.43.

4-(1,3-Dimethylpyrrol-2-yl)-1-methyl-1,2,3,6-tetrahydropyridine oxalate (25·$H_2C_2O_4$) was recrystallized from methanol:diethyl ether in 62% yield. mp 144°C (decomposed); GC (t_R = 7.10 min)–EIMS *m*/*z* (%) 190 (91, $M^{\bullet+}$), 175 (35), 161 (33), 147 (59), 132 (100), 108 (80), 94 (80), 70 (69); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 6.57–6.58 (d, 1H, C′-5), 5.8 (d, 1H, C′-4), 5.60 (s, 1H, C-5), 3.76 (d, 2H, C-6), 3.59 (s, 3H, NCH_3), 3.28–3.34 (t, 2H, C-2), 2.81 (s, 3H, N′CH_3), 1.94 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 mHz) δ 164.58, 132.89, 119.16, 115.30, 100.44, 50.14, 49.17, 41.65, 23.22, 9.90; Anal calcd for $C_{14}H_{20}N_2O_4$: C, 59.98; H, 7.19; N, 9.99. Found: C, 59.88; H, 7.23; N, 9.97.

Enzyme studies. MAO-B was isolated from bovine liver mitochondria according to the method of Salach and Weyler.[29] The activity was determined spectrophotometrically at 30°C on a Beckman 7400 series spectrophotometer using 5 mM MPTP as a substrate and recording initial rates (120 s) of formation of the dihydropyridinium metabolite (λ_{max} = 343 nm, ε = 16,000 M^{-1}) as described previously.[30] The final enzyme concentration was calculated to be 9 nmol mL^{-1}.

All enzyme assays were performed at 37°C on a Beckman DU 7400 spectrophotometer. The substrate properties of each test compound (50–1000 μM) were first examined by recording repeated scans (300 to 500 nm) in the presence of MAO-B. For kinetic studies, initial rates of oxidation of the tetrahydropyridinyl analogues were determined at four substrate concentrations. Solutions (ranging from 25 to 1400 μM) of the substrates were

prepared in 100 mM sodium phosphate buffer (pH 7.4). A 480–495 μL aliquot of each solution was added to the sample cuvette, which was placed in the spectrophotometer and maintained at 37°C. After a 3 min equilibration period, 5 μL of the MAO-B enzyme preparation was added (final MAO-B concentration was 0.09 μM). For compound **20**, 20 μL MAO-B enzyme-B preparation was added (final MAO-B enzyme concentration was 0.36 μM). The rates of oxidation of each substrate were estimated by monitoring the absorbance of the corresponding dihydropyridium metabolite every 5 s for 2 min (for compound **20**, the time is 5 min). The K_m and k_{cat} values were calculated from Lineweaver–Burk double-reciprocal plots. Duplicate analyses gave k_{cat}/K_m values that differed less than 6.5%.

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