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

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Abstract—Six analogues of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP, (**1**)] bearing various heteroaryl groups at C-4 were synthesized and examined for their monoamine oxidase B substrate properties. The C-4 substituents include the 1-ethylpyrrol-2-yl, 1-propylpyrrol-2-yl, 1-isopropylpyrrol-2-yl, 1-cyclopropylpyrrol-2-yl, 3-ethylfuran-2-yl and 3-ethylthien-2-yl groups. The results provide information concerning steric and polar interactions between the C-4 substituent and the active site of MAO-B that are transmitted to the position of oxidation at C-6 of the tetrahydropyridinyl moiety.

Introduction

The flavoenzyme monoamine oxidase B (MAO-B) catalyzes the α-carbon oxidative deamination of a variety of acyclic amines including the biogenic amine neurotransmitters.[1–4] Some cyclic tertiary amines, such as the parkinsonian inducing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP (**1**)], are also good MAO-B substrates.[5–8] This bioactivation reaction generates the corresponding dihydropyridinium intermediate **2** which subsequently is oxidized to the pyridinium metabolite MPP^+ (**3**), the ultimate toxin that mediates the neurodegeneration of dopaminergic nigrostriatal neurons (Scheme 1).[9–12]

Extensive structure–activity relationship studies on the biotransformation of 1-methyl-4-aryl-1,2,3,6-tetrahydropyridinyl derivatives (structures **a**, Chart 1) to yield the corresponding dihydropyridinium metabolites (structures **b**, Chart 1) have been conducted in attempts to understand the unique features of these cyclic tertiary allylamines that lead to their unexpected MAO-B substrate properties and neurotoxicity.[8,13–16] Youngster et al. examined changes in the MAO-B substrate properties of MPTP analogues bearing substituents on the phenyl ring.[8,17–19] Introducing an *o*-methyl substituent (**4a**, $k_{cat}/K_m = 1275\ min^{-1}\ mM^{-1}$) leads to an increase in k_{cat}/K_m relative to MPTP (523) whereas larger alkyl substituents [*o*-ethylphenyl (**5a**, 295), *o*-propylphenyl (**6a**, 86), *o*-isopropylphenyl (**7a**, 51)] are poorer substrates, suggesting specific steric constraints in the active site.[8] The observation that the k_{cat}/K_m values for the *o*-chlorophenyl analogue (**8a**, 1353) and *o*-methoxyphenyl analogue (**9a**, 233) are similar to the values for the *o*-methylphenyl (**4a**) and *o*-ethylphenyl (**5a**) analogues, respectively, argues that electronic factors may not play a significant role in determining the substrate properties in this series of compounds. In general, the neurotoxicity of these analogues correlated well with their MAO-B substrate properties.[17–19]

We have observed similar effects with a series of 1-methyl-4-heteroaryl-1,2,3,6-tetrahydropyridinyl analogues (Chart 1).[13,20,21] For example, replacement of hydrogen with a methyl group on an atom one bond away from the point of attachment of a 2-pyrrolyl (**10a** versus **11a**), 2-furanyl (**16a** versus **17a**) or 2-thienyl (**19a** versus **20a**) system leads to dramatic increases (20 to 55 times) in k_{cat}/K_m values. In order to gain a better understanding of the influence of such steric factors on substrate activity, we have extended this investigation to additional 1-methyl-4-heteroaryl-1,2,3,6-tetrahydropyridinyl derivatives. The structures of the compounds examined in this study and their MAO-B generated dihydropyridinium metabolites are shown in Chart 1. The newly synthesized analogues are 1-methyl-4-(1-ethylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (**12a**), 1-methyl-4-(1-propylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (**13a**), 1-methyl-4-(1-isopropyl-pyrrol-2-yl)-1,2,3,6-tetrahydropyridine (**14a**), 1-methyl-4-(1-cyclopropylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (**15a**),

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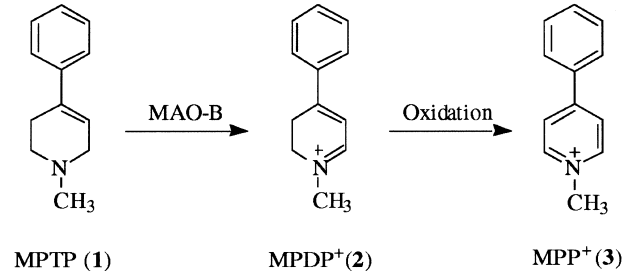
1-methyl-4-(3-ethylfuran-2-yl)-1,2,3,6-tetrahydropyridine (**18a**) and 1-methyl-4-(3-ethylthien-2-yl)-1,2,3,6-tetrahydropyridine (**21a**).

Results and Discussion

Chemistry

Syntheses of the target compounds proceeded via the corresponding 1,4-dicarbonyl precursors which were prepared as shown in Scheme 2. Treatment of 4-bromopyridine (**22**) with *n*-butyllithium at −78°C in dry diethyl ether followed by reaction of the resulting lithiated intermediate with γ-butyrolactone and α-ethyl-γ-butyrolactone,[22,23] yielded the corresponding γ-hydroxyalkylketones **24** and **25**, respectively. These intermediates were oxidized by the complex of chromium trioxide with pyridine in dichloromethane to give 4-oxo-4-(4-pyridyl)butanal (**26**) and 3-ethyl-4-oxo-4-(4-pyridyl)butanal (**27**), respectively.[24] The ^{1}H NMR and ^{13}C NMR spectra indicate that **24** and **25** exist in equilibria with their corresponding enol forms at room temperature. These mixtures were used directly in the subsequent conversions.



Scheme 1. MAO-B catalyzed oxidation of MPTP.

The general synthetic pathways for the preparation of the 4-(1-alkylpyrrol-2-yl)pyridines, 4-(3-ethylfuran-2-yl)pyridine and 4-(3-ethylthien-2-yl)pyridine are given in Schemes 3 and 4. Parr–Knorr cyclization[25–27] of 4-oxo-4-(4-pyridyl)butanal (**26**) with the appropriate amine furnished the desired 4-(1-alkylpyrrol-2-yl)pyridines **28–31**. Treatment of **28–31** with iodomethane gave the corresponding *N*-methylpyridinium iodides **32–35**. These intermediates subsequently were reduced with $NaBH_4$ to give the desired 1,2,3,6-tetrahydropyridines **12–15** which were characterized as their oxalate salts (Scheme 3).

Treatment of 3-ethyl-4-oxo-4-(4-pyridyl)butanal (**27**) with sulfuric acid or phosphorus pentasulfide[28] afforded 4-(3-ethylfuran-2-yl)pyridine (**36**) or 4-(3-ethylthien-2-yl)pyridine (**37**), respectively. Methylation of these pyridines with iodomethane gave the corresponding *N*-methylpyridinium iodides **38** and **39**. Treatment with NaBH4 converted the pyridinium intermediates to the desired 1,2,3,6-tetrahydropyridines **18** and **21**, which were characterized as their oxalate salts (Scheme 4).

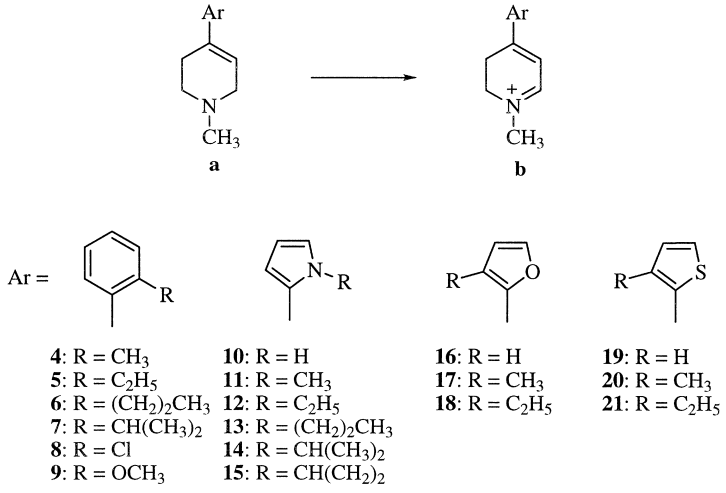
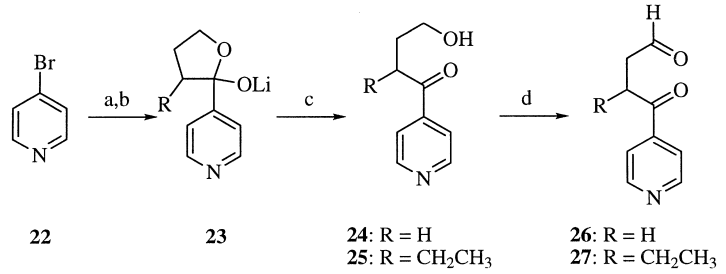


Chart 1. Structures of 1-methyl-4-aryl-1,2,3,6-tetrahyropyridines (**a**) and 1-methyl-4-aryl-2,3-dihydropyridinium species (**b**) discussed in the text.

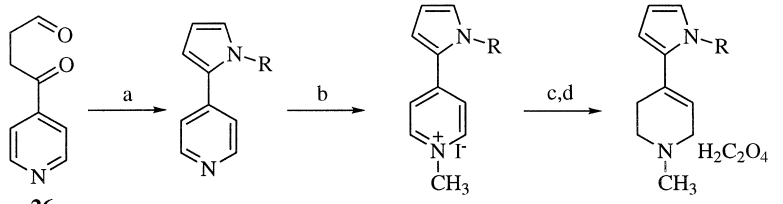


Scheme 2. The synthesis of 1,4-dicarbonyl precursors **26** and **27**. Reagents and conditions: (a) *n*-BuLi, Et_2O, −78°C, 1 h; (b) γ-butyrolactone or α-ethyl-γ-butyrolactone, −78°C, 3 h, then room temperature 1 day; (c) aq Na_2CO_3 solution, room temprature; (d) CrO_3/pyridine, CH_2Cl_2, room temperature.

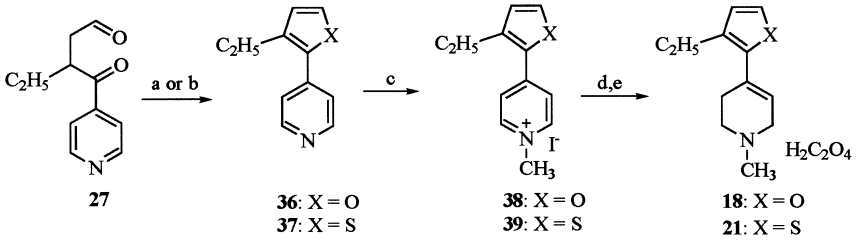
Enzymology

The substrate properties of the newly synthesized tetrahydropyridines **12**–**15**, **18** and **21** (50–500 μM) were examined using MAO-B (0.09 μM) purified from beef liver.[13] In each case the time dependent increase of a chromophore consistent with that expected for the corresponding dihydropyridinium metabolite was observed (see Chart 1 and Table 1). The enzyme kinetic parameters of the test compounds were then 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 structurally 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 substrate properties of this series of compounds ranged from 47 to 11,000 (min^{-1} mM^{-1}).

Arguments supporting both a hydrogen atom transfer pathway (HAT: **A** → **C** → **D**)[5–7] and a single electron transfer pathway (SET: **A** → **B** → **C** → **D**)[4,8–10] have been advanced to characterize MAO-B catalysis (Scheme 5). Since a normal kinetic deuterium isotope effect is observed on the MAO-B catalyzed oxidation of MPTP to $MPDP^+$ (Scheme 1), the rate determining step for this transformation must involve carbon–hydrogen bond cleavage. Consequently, resonance stabilization of **C** via conjugation with the C-4 substituent



Scheme 3. Synthesis of 1-methyl-4-(1-alkylpyrrol-2-yl)-1,2,3,6-tetrahydropyridines (**12**–**15**). Reagents and conditions: (a) RNH_2, CH_3OH, room temperature, 12 h ; (b) CH_3I, room temperature; (c) $NaBH_4$, CH_3OH, 0°C; (d) $H_2C_2O_4$, Et_2O, room temperature.

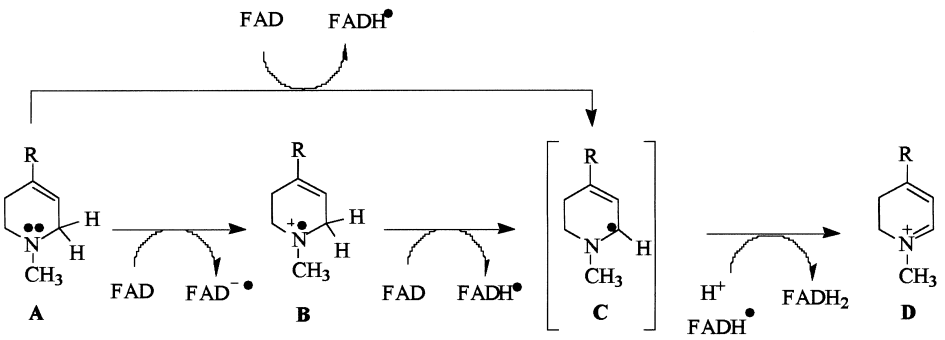


Scheme 4. Synthesis of 1-methyl-4-(3-ethylfuran-2-yl)-1,2,3,6-tetrahydropyridine (**18**) and 1-methyl-4-(3-ethylthien-2-yl)-1,2,3,6-tetrahydropyridine (**21**). Reagents and conditions: (a) H_2SO_4, H_2O/THF, 55°C; (b) P_2S_5, toluene, 100°C; (c) CH_3I, room temperature; (d) $NaBH_4$, methanol, 0°C; (e) $H_2C_2O_4$, Et_2O, room temperature.

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

C-4 substituent	λ_{max} (nm)[d]	log *P*	k_{cat} $(min)^{-1}$	K_m (mM)	k_{cat}/K_m (min^{-1} mM^{-1})
2-C_4HNH (**10a**)[a]	424	1.27	85	1.80	47[20]
2-C_4H_3NCH$_3$ (**11a**)[a]	420	1.73	360	0.20	1800[13]
2-C_4H_3NC$_2$H$_5$ (**12a**)[a]	420	2.26	360	0.16	2200
2-C_4H_3NC$_3$H$_7$ (**13a**)[a]	421	2.79	209	0.38	557
2-C_4H_3NCH(CH$_3$)$_2$ (**14a**)[a]	422	2.61	121	1.28	94
2-C_4H_3NCH(CH$_2$)$_2$ (**15a**)[a]	420	1.62	418	0.14	2878
2-C_4H_4O (**16a**)[b]	384	1.90	31	0.20	155[13]
2-C_4H_2(3-CH$_3$)O (**17a**)[b]	399	2.36	348	0.04	8600[21]
2-C_4H_2(3-C$_2$H$_5$)O (**18a**)[b]	399	2.89	336	0.03	11,000
2-C_4H_4S (**19a**)[c]	386	2.40	60	0.20	300[13]
2-C_4H_2(3-CH$_3$)S(**20a**)[c]	400	2.87	337	0.05	6690[21]
2-C_4H_2(3-C$_2$H$_5$)S (**21a**)[c]	393	3.40	222	0.04	4892

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



Scheme 5. Proposed pathways for the MAO-B catalyzed oxidation of MPTP and analogues.

could lead to a decrease in $\Delta G^{\dagger}$ and increase in k_{cat}. As a possible indication of such stabilization, we have compared the λ_{max} values and the relative rates of formation for each of the dihydropyridinium metabolites. As shown in Table 1, no meaningful correlation exists between these two parameters. This analysis prompted a closer consideration of polar and/or steric interactions as potentially important factors influencing the MAO-B substrate properties of these tetrahydropyridinyl derivatives.

The k_{cat}/K_m values of the 4-pyrrol-2-yl analogues bearing a methyl (**11a**), ethyl (**12a**) or cyclopropyl (**14**) group on nitrogen are much larger than that observed with the NH analogue **10a**. These values follow the same trend observed with the corresponding log *P* values. A similar, but less dramatic, correlation between log *P* and k_{cat}/K_m is observed with the pyrrolyl (**10a**), furanyl (**16a**) and thienyl (**19a**) set of analogues. These results suggest that the region of the active site occupied by the phenyl group of MPTP is a liphophilic pocket that can accommodate small substituents on the nitrogen atom of the pyrrolyl moiety. Larger substituents, however, are not well tolerated since the *N*-propyl (**14a**) and *N*-isopropyl (**15a**) analogues are poorer MAO-B substrates than the *N*-methyl, *N*-ethyl and *N*-cyclopropyl analogues even though their log *P* values are higher.

The substrate properties of the furanyl and thienyl analogues are even more dramatically affected by the introduction of a methyl or ethyl group at C-3. The k_{cat}/K_m values of the 3-methyl- and 3-ethylfuranyl analogues **17a** and **18a** were estimated to be 8620 and 11,000 min^{-1} mM^{-1}. These compounds are 50 and 70 times more active substrates than the unsubstituted analogue **16a**. The very low K_m values for **17a** and **18a** must reflect particularly favorable interactions with a lipophilic domain present in the active site of the enzyme. Similar results were observed with 3-methyl (**20a**) and 3-ethyl (**21a**) thienyl analogues.

These SAR results are analogous to those reported for a related series of 4-heteroaryl MPTP analogues[21] and also to those generated with a series of MPTP analogues in which the *ortho*-position of the phenyl ring was substituted with various alkyl groups.[8] Attempts currently are underway to construct a molecular model of the active site of MAO-B that will accommodate all of the available SAR results derived from 1,4-disubstituted tetrahydropyridinyl derivatives.

Experimental

Caution! 1,4-Disubstituted tetrahydropyridines such as MPTP 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.[29]

Chemistry

All reagents were obtained from commercial sources and were used directly. Melting points (mp) were determined using a Thomas–Hoover melting point apparatus and are uncorrected. UV–vis spectra were recorded on a Beckman 7400 spectrophotometer. Proton NMR spectra were recorded on a Bruker WP 360-, 270- or 200-MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. The following abbreviations are used to describe spin multiplicities when appropriate: b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets. 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 i.d., 0.33 mm film thickness) which was coupled to a Hewlett Packard 5870 mass-selective detector. Data were acquired using an HP 5970 chemstation. The temperature program is 45°C for 2 min and then with the ramp of 25°C/min for 11.8 min. Normalized peak heights are reported as a percentage of the base peak. High resolution–chemical ionization mass spectrometry (HR–CIMS) and high resolution–electron ionization mass spectrometry (HR–EIMS) were performed on a VG 7070 HF instrument. Elemental analyses, performed by Atlantic Microlab, Inc., Norcross, GA, were within 0.17% of the theoretical values calculated for C, H and N.

4-Oxo-4-pyridin-4-yl-butyralcohol (24). To 4-bromopyridine (3.95 g, 25 mol) in anhydrous diethyl ether (50 mL) at −78°C was added *n*-butyllithium (25 mmol) in hexane dropwise. The mixture was stirred at −78°C for 1 h. Butyrolactone (2.2 g, 25 mmol) was added dropwise to the mixture at −78°C. The mixture was stirred at this temperature for 4 h and then at room temperature for another 6 h. The reaction mixture was quenched with dilute Na_2CO_3 aqueous solution (30 mL). The aqueous layer was extracted by ethyl acetate (40 mL). The extracts were dried over $MgSO_4$

and the solvent was removed in vacuo. The residue was purified by column (silica gel, ethyl acetate) to afford **24** in 94.5% yield. See the Results and Discussion. GC(t_R = 6.65 min)–EIMS *m*/*z* (%) 165 (10, $M^{\cdot+}$), 147 (3), 134 (5), 121 (100), 106 (100), 87 (10), 78 (98), 51 (72); HR–CIMS calcd for $C_9H_{11}NO_2H^+$: 166.0868. Found: 166.0862.

3-Ethyl-4-oxo-4-pyridin-4-yl-butyralcohol (25). This was prepared with 4-bromopyridine and α-ethyl-γ-butyrolactone[22] using the same method. The crude product was recrystallized in dichloromethane:diethyl ether in 53.5% yield. See Results and Discussion. GC (t_R = 7.63 min)–EIMS *m*/*z* (%) 192 (4, $M^{\cdot+}$), 160 (3), 149 (66), 124 (28), 106 (100), 78 (86), 51 (92). Anal. calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.27; H, 7.84; N, 7.16.

4-Oxo-4-pyridin-4-yl-butyraldehyde (26). To a stirred solution of pyridine (20 g, 240 mmol) in anhydrous dichloromethane (300 mL), at room temperature, was added chromium trioxide (12.0 g, 120 mmol) in portions. The deep burgundy solution was stirred for 30 min. A solution of **24** (20 mmol) in 20 mL of dichloromethane was added in one portion. The mixture was stirred for 12 h at room temperature. The solution was decanted from the residue and the residue was washed with dichloromethane (2×200 mL). The solvent was removed in vacuo and the residue was purified by column (silica gel, ethyl acetate). **26** was obtained in 65% yield. GC (t_R = 6.25 min)–EIMS *m*/*z* (%) 163 (1, $M^{\cdot+}$), 135 (44), 121 (34), 106 (76), 78 (100), 51 (80); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 9.89 (s, 1H, CHO), 8.81–8.83 (dd, 2H, C-2 and C-6), 7.75–7.77 (dd, 2H, C-3 and C-5), 3.29–3.32 (t, 2H, 3-CH_2), 2.96–3.00 (t, 2H, 2-CH_2); ^{13}C NMR ($CDCl_3$, 360 MHz) δ 200.00, 197.56, 151.12, 142.36, 121.11, 37.46, 31.30; HR–CIMS calcd for $C_9H_9NO_2H^+$: 164.0712. Found: 164.0714.

3-Ethyl-4-oxo-4-pyridin-4-yl-butyraldehyde (27). This was prepared using the same methods as **26** in 59.7% yield. GC (t_R = 7.26 min)–EIMS *m*/z (%) 191 (1, $M^{\cdot+}$), 163 (2), 149 (34), 134 (12), 106 (100), 78 (74), 51 (67); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 9.78 (s, 1H, CHO), 8.81–8.82 (dd, 2H, C-2 and C-6), 7.76–7.77 (dd, 2H, C-3 and C-5), 3.78–3.86 (m, 1H, CH), 3.18–3.26 (dd, 1H, 2-CHH), 2.70–2.76 (dd, 1H, 2-CHH), 1.68–1.78 (m, 1H, 1′-CHH), 1.50–1.60 (m, 1H, 1′-CHH), 0.84–0.92 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 360 MHz) δ 202.23, 200.30, 151.00, 142.89, 121.45, 45.08, 41.67, 25.02, 11.42; HR–EIMS calcd for $C_{11}H_{13}NO_2H^+$: 192.1024. Found: 192.1022.

General procedures for the synthesis of the oxalate salts of 4-(1-alkylpyrrol-2-yl)pyridine

To 4-oxo-4-pyridinylbutanal (20 mmol) in 40 mL methanol was added the corresponding primary amines (4 equiv.). The mixture was stirred at room temperature for 1 day. The solvent was removed in vacuo. After chromatography (silica gel, ethyl acetate), a yellow oil of 4-(1-alkylpyrrol-2-yl)pyridine was obtained. To this yellow oil (5 mmol) in dry diethyl ether (15 mL) was added the oxalic acid (6 mmol) in 5 mL diethyl ether. The mixture was stirred for 4 h and the precipitate was filtered and recrystallized in methanol/diethyl ether.

Oxalate salt of 4-(1-ethylpyrrol-2-yl)pyridine (28·$H_2C_2O_4$). This was obtained in 93% yield. Mp 142.5–143°C; GC (t_R = 7.10 min)–EIMS *m*/*z* (%) 172 (100, $M^{\cdot+}$), 157 (39), 144 (22), 130 (22), 117 (22), 104 (9), 89 (35); ^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.56–8.59 (dd, 2H, C-6 and C-2), 7.47–7.50 (dd, 2H, C-3 and C-5), 7.08–7.10 (dd, 1H, C′-5), 6.47–6.50 (dd, 1H, C′-4), 6.16–6.19 (dd, 1H, C′-3), 4.06–4.17 (q, 2H, CH_2), 1.10–1.29 (t, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 162.03, 147.63, 141.87, 129.40, 126.32, 121.59, 112.60, 108.78, 42.19, 16.43. Anal. calcd for $C_{13}H_{14}N_2O_4$·0.58$H_2C_2O_4$: C, 54.06; H, 4.86; N, 8.90. Found: C, 54.01; H, 5.03; N, 8.90.

Oxalate salt of 4-(1-propylpyrrol-2-yl)pyridine (29·$H_2C_2O_4$). This was obtained in 90% yield. Mp 115–117°C; GC (t_R = 7.45 min)–EIMS *m*/*z* (%) 186 (72, $M^{\cdot+}$), 157 (100), 144 (30), 130 (22), 117 (20), 104 (11), 89 (28); ^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.55–8.58 (dd, 2H, C-2 and C-6), 7.47–7.50 (dd, 2H, C-3 and C-5), 7.06–7.08 (dd, 1H, C′-5), 6.47–6.49 (dd, 1H, C′-4), 6.14–6.18 (dd, 1H, C′-3), 4.02–4.10 (t, 2H, CH_2N), 1.53–1.64 (m, 2H, CH_2), 0.70–0.77 (t, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 162.03, 147.82, 141.90, 129.66, 127.10, 121.59, 112.61, 108.49, 48.96, 24.02, 10.70. Anal. calcd for $C_{14}H_{16}N_2O_4$·0.58$H_2C_2O_4$: C, 55.40; H, 5.26; N, 8.52. Found: C, 55.37; H, 5.46; N, 8.62.

4-(1-Isopropylpyrrol-2-yl)pyridine (30). This was obtained in 87% yield. GC (t_R = 6.78 min)–EIMS *m*/*z* (%) 186 (56, $M^{\cdot+}$), 171 (10), 144 (100), 117 (21), 89 (18); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 8.59–8.61 (dd, 2H, C-2 and C-6), 7.26–7.27 (dd, 2H, C-3 and C-5), 6.98 (dd, 1H, C′-5), 6.28 (m, 2H, C′-3 and C′-4), 4.52–4.59 (m, 1H, CH), 1.43−1.44 (d, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 360 MHz) δ 150.13, 141.32, 131.34, 123.13, 119.88, 110.59, 109.20, 47.78, 24.31; HR–EIMS calcd for $C_{12}H_{14}N_2$: 186.1157. Found: 186.1152.

Oxalate salt of 4-(1-cyclopropylpyrrol-2-yl)pyridine (31·$H_2C_2O_4$). This was obtained in 97% yield. Mp 150.5–151.5°C; GC (t_R = 7.87 min)−EIMS *m*/*z* (%) 184 (100, $M^{\cdot+}$), 169 (29), 156 (22), 142 (11), 130 (13), 116 (23), 106 (33), 89 (39); ^{1}H NMR (DMSO-d_6, 360 MHz) δ 8.55–8.57 (dd, 2H, C-2 and C-6), 7.74–7.77 (dd, 2H, C-3 and C-5), 7.07 (dd, 1H, C′-5), 6.61–6.63 (dd, 1H, C′-4), 6.11–6.14 (dd, 1H, C′-3), 3.73–3.74 (m, 1H, NCH), 0.95–1.0 (m, 2H, CH_2), 0.81–0.85 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 162.03, 146.99, 141.82, 130.48, 127.20, 121.10, 112.80, 108.37, 30.04, 8.41. Anal. calcd for $C_{14}H_{14}N_2O_4$·0.38 H_2O: C, 59.83; H, 5.29; N, 9.97. Found: C, 59.85; H, 5.15; N, 9.73.

Oxalate salt of 4-(3-ethylfuran-2-yl)pyridine (36·$H_2C_2O_4$). To 3-ethyl-4-oxo-4-(4-pyridyl)butanal **27** (1.00 g, 5.23 mmol) in 20 mL THF and 10 mL H_2O was added 5 mL H_2SO_4 dropwise. The mixture was stirred for 15 h at 55°C. THF was evaporated in vacuo. The remaining solution was basified and extracted with diethyl ether (4×40 mL). The extracts were dried over $MgSO_4$ and the solvent was removed in vacuo. The crude product was chromatographed (silica gel, 10:1.5 ethyl acetate: hexane) to give **36** (0.875 g, 96.6%). Treatment of **36** in dry diethyl ether with oxalic acid (1.2 equiv) afforded a

precipitate, which was recrystallized in methanol:diethyl ether to give a yellow solid **36·$H_2C_2O_4$** in 97% yield. Mp 170–171°C (decomposed); GC (t_R = 6.85 min)–EIMS *m/z* (%) 173 (100, $M^{\cdot+}$), 158 (87), 144 (11), 130 (93), 115 (13), 103 (26), 89 (11); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.61–8.63 (dd, 2H, C-2 and C-6), 7.82 (d, 1H, C′-5), 7.57–7.60 (dd, 2H, C-3 and C-5), 6.65 (d, 1H, C′-4), 2.69–2.81 (q, 2H, CH_2), 1.18–1.26 (t, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 161.40, 148.56, 144.21, 143.96, 138.70, 128.86, 118.76, 114.24, 18.67, 13.75. Anal. calcd for $C_{13}H_{13}O_5N\cdot 0.45H_2C_2O_4$: C, 54.93; H, 4.61; N, 4.59. Found: C, 54.95; H, 4.76; N, 4.58.

1-Methyl-4-(3-ethylfuran-2-yl)pyridinium iodide (38). To 4-(3-ethylfuran-2-yl)pyridine (0.484 g, 2.8 mmol) in 5 mL dry acetone was added methyl iodide (5 equiv). The solution was stirred overnight. After filtration, the crude product was recrystallized from methanol:diethyl ether to afford a yellow solid **38** (0.37 g, 42%). Mp 162.5—163.5°C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.82–8.85 (dd, 2H, C-2 and C-6), 8.11–8.14 (m, 3H, C-3, C-5 and C′-5), 6.85 (d, 1H, C′-4), 4.28 (s, 3H, NCH_3), 2.82–2.94 (q, 2H, CH_2), 1.21–1.29 (t, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 147.61, 145.28, 143.03, 142.80, 135.77, 120.30, 115.66, 46.84, 19.13, 13.41. Anal. calcd for $C_{12}H_{14}INO$: C, 45.73; H, 4.48; N, 4.44. Found: C, 45.56; H, 4.54; N, 4.36.

1-Methyl-4-(3-ethylthien-2-yl)pyridine (37). To 3-ethyl-4-oxo-4-(4-pyridyl)butanal **27** (0.955 g, 5 mmol) in dry toluene (20 mL) was added powdered phosphorus pentasulfide (5.5 g, 25 mmol). The mixture was stirred for 2 h at 100°C under N_2. The mixture was cooled and treated with water (25 mL) and the aqueous layer was adjusted to pH 8. The aqueous layer was extracted with ether (3×40 mL). The combined organic layer was washed with dilute Na_2CO_3 solution, dried over $MgSO_4$ and evaporated. The residue was separated through column (silica gel, ethyl acetate:hexane, 10:2). 0.284 g yellow oil was obtained in 30% yield. GC (t_R = 6.98 min)–EIMS *m/z* (%) 189 (71, $M^{\cdot+}$), 174 (100), 147 (20), 130 (15), 115 (6), 102 (7); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 8.58–8.62 (dd, 2H, C-3 and C-5), 7.34–7.36 (m, 3H, C-2, C-6 and C′-5), 7.03–7.04 (d, 1H, C′-4), 2.72–2.78 (q, 2H, CH_2), 1.24–1.30 (t, 3H, CH_3); ^{13}C NMR ($CDCL_3$, 360 MHz) δ 150.00, 142.50, 142.07, 134.30, 129.85, 125.66, 123.34, 22.169, 15.28; HR–EIMS calcd for $C_{11}H_{11}NS$: 189.0612. Found: 189.0613.

1-Methyl-4-(3-ethylfuran-2-yl)pyridinium iodide (39). To 4-(3-ethylthien-2-yl)pyridine (0.19 g, 1 mmol) in 5 mL dry acetone was added methyl iodide (5 equiv). The solution was stirred overnight. After filtration, the crude product was recrystallized from methanol:diethyl ether to afford a yellow solid **39** in 45% yield. Mp 164–165°C; ^{1}H NMR (DMSO-d_6, 360 MHz) δ 8.89–8.90 (dd, 2H, C-2 and C-6), 8.13–8.15 (dd, 2H, C-3 and C-5), 7.97–7.98 (d, 1H, C′-5), 7.30 (d, 1H, C′-4), 4.31 (s, 3H, NCH_3), 2.84–2.88 (q, 2H, CH_2), 1.22–1.27 (t, 3H, CH3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 148.86, 147.12, 145.30, 131.56, 131.36, 130.65, 124.90, 46.98, 22.17, 14.55. Anal. calcd for $C_{12}H_{14}INS$: C, 43.52; H, 4.26; N, 4.23. Found: C, 43.55; H, 4.31; N, 4.17.

General procedures for the synthesis of oxalate salt of 1-methyl-4-(1-alkylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine

To 4-(alkylpyrrol-2-yl)pyridine (5 mmol) in dry acetone (10 mL) was added methyl iodide (6 equiv) at room temperature. The mixture was stirred overnight. The solvent was removed in vacuo. To the residue was added methanol (25 mL). Sodium borohydride (2.5 equiv) was added in portions to this stirred solution 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 was taken up in 15 mL of water and the aqueous solution 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 (1.2 equiv) in 10 mL of diethyl ether precipitated the crude oxalate salt, which was recrystallized from the appropriate solvent.

Oxalate salt of 1-methyl-4-(1-ethylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (12a·$H_2C_2O_4$). This was obtained in 80% yield. Mp 146.5–147.7°C; GC (t_R = 7.15 min)–EIMS *m/z* (%) 190 (100, $M^{\cdot+}$), 176 (16), 161 (63), 146 (29), 132 (83), 117 (43), 108 (55), 94 (96); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 6.82 (dd, 1H, C′-5), 6.06 (m, 1H, C′-3), 5.98–6.01 (m, 1H, C′-4), 5.65 (b, 1H, C-5), 3.90–4.0 (q, 2H, N′-CH_2), 3.75 (d, 2H, C-6), 3.26–3.32 (t, 2H, C-2), 2.80 (s, 3H, NCH_3), 2.60 (b, 2H, C-3), 1.22–1.29 (t, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 164.39, 131.22, 127.31, 122.89, 116.13, 108.18, 107.13, 51.19, 49.67, 41.76, 41.66, 26.18, 16.61. Anal. calcd for $C_{14}H_{20}N_2O_4\cdot 0.15H_2C_2O_4$: C, 58.46; H, 6.96; N, 9.54. Found: C, 58.49; H, 7.03; N, 9.65.

Oxalate salt of 1-methyl-4-(1-propylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (13a·$H_2C_2O_4$). This was obtained in 76% yield. Mp 177.5–178°C; GC (t_R = 7.49 min)–EIMS *m/z* (%) 204 (89, $M^{\cdot+}$), 189 (13), 175 (41), 161 (27), 146 (35), 132 (50), 117 (46), 104 (28), 94 (100); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 6.80–6.82 (dd, 1H, C′-5), 6.06–6.09 (m, 1H, C′-3), 5.98–6.01 (m, 1H, C′-4), 5.65 (b, 1H, C-5), 3.85–3.91 (t, 2H, N′CH_2), 3.75 (d, 2H, C-6), 3.28–3.32 (t, 2H, C-2), 2.80 (s, 3H, NCH_3), 2.60 (b, 2H, C-3), 1.55–1.72 (m, 2H, N′$CHCH_2$), 0.72–0.82 (t, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 164.39, 131.51, 127.45, 123.66, 116.38, 108.22, 106.89, 51.23, 49.71, 48.51, 41.83, 26.35, 24.08, 10.84. Anal. calcd for $C_{15}H_{22}N_2O_4$: C, 61.21; H, 7.53; N, 9.52. Found: C, 61.12; H, 7.49; N, 9.55.

Oxalate salt of 1-methyl-4-(1-isopropylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (14a·$H_2C_2O_4$). This was obtained in 91% yield. Mp 180–180.5°C; GC (t_R = 7.38 min)–EIMS *m/z* (%) 204 (100, $M^{\cdot+}$), 189 (20), 175 (24), 161 (39), 146 (46), 133 (17), 118 (30), 104 (14); ^{1}H NMR (DMSO-d_6, 270 MHz) δ 6.94 (s, 1H, C′-5), 6.02–6.03 (m, 1H, C′-4), 6.98 (m, 1H, C′-3), 5.60 (b, 1H, C-5), 4.43–4.48 (m, 1H, N′CH), 3.75 (d, 2H, C-6), 3.30 (t, 2H, C-2), 2.80 (s, 3H, NCH_3), 2.58 (b, 2H, C-3), 1.31–1.34 (d, 6H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 164.39, 132.03, 127.85, 118.41, 118.01, 107.53, 107.17, 51.26, 49.76, 46.71, 41.84, 26.99, 23.87. Anal. calcd for $C_{15}H_{22}N_2O_4\cdot 0.07H_2C_2O_4$: C, 60.50; H, 7.42; N, 9.32. Found: C, 60.50; H, 7.44; N, 9.25.

Oxalate salt of 1-methyl-4-(1-cyclopropylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (15a·$H_2C_2O_4$). This was obtained in 71% yield. Mp 183–183.5°C; GC (t_R = 8.05 min)–EIMS *m*/*z* (%) 202 (100, $M^{\cdot+}$), 187 (11), 173 (42), 159 (30), 144 (41), 130 (36), 118 (41), 94 (55); ^{1}H NMR (DMSO-d_6, 270 MHz) δ 6.80 (d, 1H, C′-5), 6.08 (m, 2H, C′-3 and C′-4), 5.93–5.95 (m, 1H, C-5), 3.77 (s, 2H, C-6), 3.38 (m, 1H, N′CH), 3.28–3.32 (t, 2H, C-2), 2.80 (s, 3H, NCH_3), 2.68 (b, 2H, C-3), 0.94-0.98 (m, 2H, CH_2CH_2), 0.86 (m, 2H, CH_2CH_2); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 164.39, 132.70, 126.76, 123.72, 115.25, 108.43, 106.63, 51.28, 49.68, 41.80, 29.96, 25.24, 8.25. Anal. calcd for $C_{15}H_{20}N_2O_4$: C, 61.63; H, 6.90; N, 9.58. Found: C, 61.53; H, 6.88; N, 9.66.

Oxalate salt of 1-methyl-4-(3-ethylfuran-2-yl)-1,2,3,6-tetrahydropyridine (18a·$H_2C_2O_4$). This was obtained in 86% yield. Mp 148.5–149°C; GC (t_R = 6.96 min)–EIMS *m*/*z* (%) 191 (26, $M^{\cdot+}$), 176 (6.5), 162 (6.5), 148 (3.3), 133 (15), 105 (20), 91 (14), 83 (45), 70 (100); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.57 (d, 1H, C′-5), 6.47–6.48 (d, 1H, C′-4), 5.92 (b, 1H, C-5), 3.76 (d, 2H, C-6), 3.26–3.29 (t, 2H, C-2), 2.79 (s, 3H, NCH_3), 2.71 (b, 2H, C-3), 2.50–2.51 (m, 2H, CH_2), 1.10–1.17 (t, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 164.32, 146.40, 141.24, 126.36, 123.20, 115.76, 113.11, 51.01, 49.25, 41.85, 23.09, 18.38, 14.34. Anal. calcd for $C_{14}H_{19}NO_5 \cdot 0.22H_2O$: C, 58.94; H, 6.87; N, 4.91. Found: C, 58.89; H, 6.78; N, 4.91.

Oxalate salt of 1-methyl-4-(3-ethylthien-2-yl)-1,2,3,6-tetrahydropyridine (21a·$H_2C_2O_4$). This was obtained in 90% yield. Mp 162–162.5°C, GC (t_R = 7.13 min)–EIMS *m*/*z* (%) 207 (41, $M^{\cdot+}$), 192 (13), 178 (13), 164 (6), 149 (41), 134 (25), 115 (11), 94 (21), 83 (51), 70 (100); ^{1}H NMR (DMSO-d_6, 360 MHz) δ 7.41–7.40 (d, 1H, C′-5), 6.99–6.98 (d, 1H, C′-4), 5.80 (b, 1H, C-5), 3.73 (d, 2H, C-6), 3.28 (b, 2H, C-2), 2.78 (s, 3H, NCH_3), 2.57–2.59 (m, 4H, CH_2 and C-3), 1.13–1.17 (t, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 360 MHz) δ 164.13, 140.07, 136.20, 129.67, 129.07, 123.93, 120.07, 51.60, 49.93, 28.00, 21.87, 15.47. Anal. calcd for $C_{14}H_{19}NO_4S$: C, 56.55; H, 6.44; N, 4.71. Found: C, 56.42; H, 6.45; N, 4.63.

Bioassay

Enzyme studies. MAO-B was isolated from bovine liver mitochondria according to the method of Salach and Weyler.[30] 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.[31] The enzyme concentration was calculated to be 9 nmol/mL.

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 (300–500 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 mL 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). The rates of oxidation of each substrate were estimated by monitoring the absorbance of the corresponding dihydropyridium metabolite every 5 s for 0.5 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 9.4%.

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