Notes

4-Substituted Cubylcarbinylamines: A New Class of Mechanism-Based Monoamine Oxidase B Inactivators

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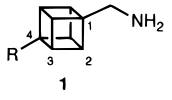
Cubylcarbinylamine (**1a**), (4-cyclopropylcubyl)carbinylamine (**1b**), and (4-phenylcubyl)carbinylamine (**1c**) were synthesized and shown to be time-dependent, irreversible inactivators of monoamine oxidase B (MAO B). Substrate protects the enzyme from inactivation, but β-mercaptoethanol does not, suggesting that these compounds are mechanism-based inactivators. All three compounds were also substrates for MAO B with partition ratios ranging from 152 to 536. The 4-substituted analogues were more potent inactivators than the unsubstituted analogue, indicating a benefit to 4-substitution in this class of inactivators.

Introduction

Monoamine oxidase (EC 1.4.3.4; MAO), a flavin-dependent enzyme located in the outer membrane of the mitochondria, is responsible for the degradation of various neurotransmitters and xenobiotic amines.[1] Two major isozymes of this enzyme are known:[2] MAO A selectively degrades serotonin and norepinephrine and MAO B is selective for 2-phenylethylamine and benzylamine. Compounds that selectively inhibit MAO A exhibit antidepressant activity; selective MAO B inhibitors are used clinically as adjuncts in the treatment of Parkinson's disease.[3]

We have reported a variety of structures based on the hypothesis that MAO proceeds by a single-electron transfer mechanism (Scheme 1).[4] In a mechanism-based approach, new structures are designed to test the mechanism, and this often provides unusual molecules that can be used for further design of new selective inhibitors. Our goal has not been to prepare selective MAO inhibitors but rather to design new classes of inhibitors as lead compounds for future selective inhibitor design.

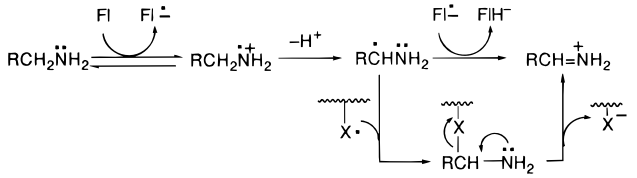
One of the unusual compounds that we reported a a probe for the mechanism of MAO is (aminomethyl)-cubane (**1a**).[5] This compound is a mechanism-based inactivator and substrate of MAO B, the first cubane-containing molecule demonstrated to be an inactivator for any enzyme. Here we show that 4-substituted cubane-containing analogues (**1b**,**c**) also are substrates and inactivators of MAO B.



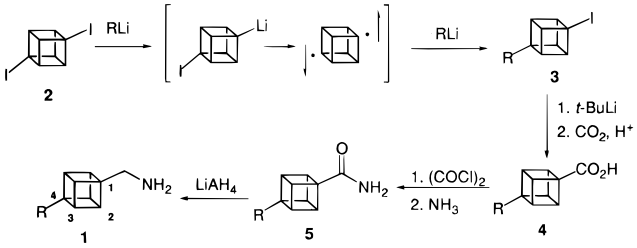
1

a. R = H
b. R = cyclopropyl
c. R = phenyl

Scheme 1



Scheme 2



Results and Discussion

Synthesis. The syntheses of compounds **1a**−**c** are summarized in Scheme 2. 4-Substituted cubane 1-iodides (**3**) were made from 1,4-diiodocubane (**2**) via the 1,4-cubanediyl intermediate.[6−9] Unlike many known 1,4-diradicals,[10] cubane-1,4-diyl is singlet because there is a strong through-bond interaction between the cubyl 1- and 4-positions. The cubane iodides were transformed into the cubylcarbinylamines (**1**) by standard procedures via the carboxylic acid (**4**) and the amide (**5**). All reactions proceeded in good yields.

Enzymology. All of the cubylcarbinylamines (**1a**−**c**) are time-dependent irreversible MAO B inactivators and substrates (Table 1; see Figure 1 for representative kinetic plots for **1b**). The substrate activity of the compounds is consistent with the increased activity of arylalkylamine substrates. The kinetic constants were determined by the method of Kitz and Wilson.[11] Inactivation of MAO B by cubylcarbinylamines **1a**−**c** was protected by a 10-fold excess of the substrate 2-phenylethylamine, indicating that inactivation occurred at the active site. The addition of β-mercaptoethanol to the

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Table 1. Kinetic Constants for the Inactivation of Monoamine Oxidase B by Cubylcarbinylamines

compd	K_I (mM)	k_{inact} (min^{-1})	k_{inact}/K_I (min^{-1} mM^{-1})	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1} mM^{-1})	partition ratio
1a[a]	0.17 ± 0.05	0.033 ± 0.004	0.19 ± 0.05	0.14 ± 0.01	5.0 ± 0.2	36 ± 3	152
1b	0.26 ± 0.04	0.28 ± 0.01	1.1 ± 0.2	1.54 ± 0.10	150 ± 9	100 ± 6	536
1c	0.12 ± 0.02	0.75 ± 0.06	6.3 ± 1.0	1.01 ± 0.07	208 ± 10	210 ± 15	277

[a] From ref 5.

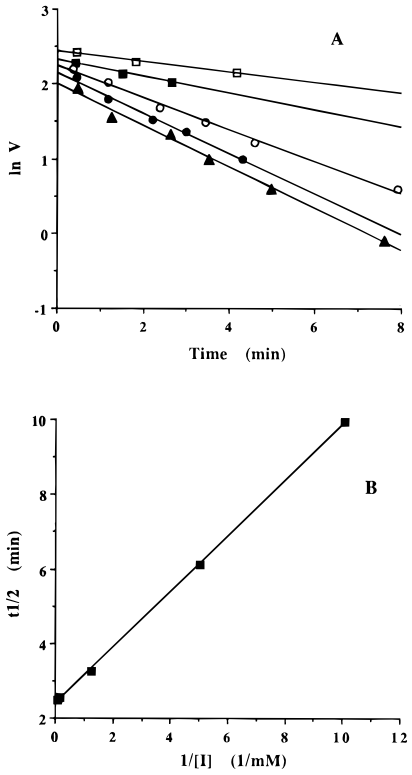
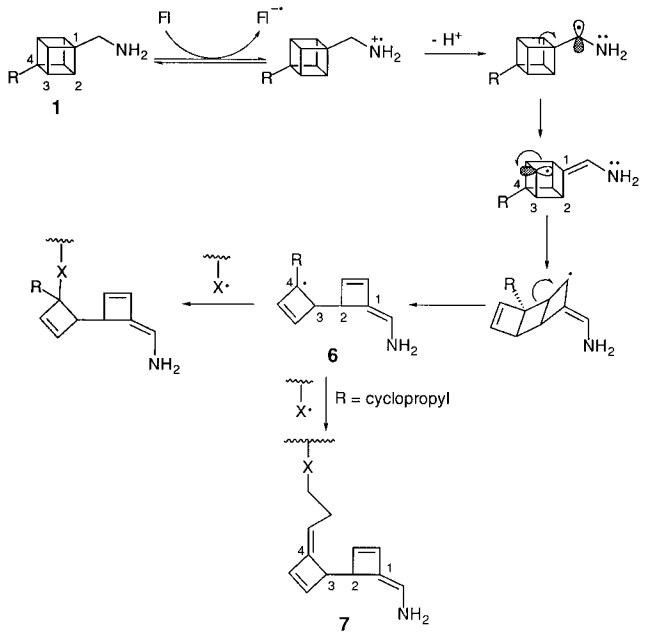


Figure 1. (A) Time-dependent inactivation of monoamine oxidase by 0.10 (□), 0.20 (■), 0.80 (○), 6.36 (●), and 12.73 (▲) mM (4-cyclopropylcubyl)carbinylamine (**1b**). See the Experimental Section for details. (B) Kitz and Wilson replot[11] of the data in part A.

inactivation buffer had no effect on the rate of inactivation, suggesting that the reactive species does not escape the active site prior to inactivation. Enzyme activity did not return, even after exhaustive dialysis, indicating an irreversible inactivation process. The efficiency of the inactivators, as measured by the k_{inact}/K_I values, indicates that it is favorable to have substituents at the 4-position. The inactivation mechanism is not completely understood; however, on the basis of metabolite studies and model reaction studies,[5,12,13] it is believed that cubane ring opening reactions are responsible for the enzyme inactivations (Scheme 3). Opening of the cubane ring to a cyclooctatrienyl radical instead of to **6** is not predicted from orbital symmetry arguments and was not observed in model reactions.[13] Since the cubylcarbinyl radical ring opening is expected to generate a radical center at the C-4 carbon atom, substitution at the C-4 position of cubanes would eventually form substituted radicals such as **6**. These substituted radicals would be stabilized, and the driving force for the third ring-opening reaction would be larger. Since the third bond cleavage is the slowest of the three,[13] this would suggest that the rates of ring-opening reactions of 4-substituted cubylcarbinyl radicals should be faster than those of the unsubstituted cubylcarbinyl radical, and 4-substituted analogues (**1b**,**c**) would be more efficient inactivators than the unsubstituted analogue

Scheme 3



(**1a**). This appears to be the case (Table 1). Whereas the K_I for the unsubstituted analogue (**1a**) and for the 4-phenyl-substituted analogue (**1c**) are essentially the same, the k_{inact} value of the 4-phenyl analogue is 23 times greater than that of the unsubstituted compound; the 4-cyclopropyl analogue has an intermediate value. This order of increased k_{inact} values, however, also follows the order of substrate activities (k_{cat}), so it is not yet clear if there is something intrinsic to the structures or if the radical stabilization effect is more important. The fact that the K_I values for **1b** and **1c** differ from their K_m values suggests that either the inactivation pathway diverges from the turnover pathway after E·I complex formation, as is shown in Scheme 3, or two different E·I complexes form, one that leads to product and the other that leads to inactivation. More analogues need to be synthesized to determine if the proposed increased stabilization at the 4-position is responsible for the increased inactivator efficiency.

Experimental Section

Chemistry. All reagents are from Aldrich and were used without further purification. Cubanecarboxylic acid (**4a**) was prepared as previously reported.[14] NMR spectra were run in chloroform-*d* at ambient probe temperature. ^{1}H NMR spectra are at 400 MHz and were referenced to an internal standard of tetramethylsilane; ^{13}C NMR spectra are at 100.6 MHz and were referenced to the main peak of the solvent.

4-Phenylcubyl Iodide (3c). Phenyllithium (12 mL, 0.022 mol, 1.8 M in cyclohexane/diethyl ether, 7:3) was added dropwise to a cold and magnetically stirred suspension of 1,4-diiodocubane[6] (3.56 g, 0.01 mol) in ether (80 mL). The mixture was then stirred at room temperature for 1 h during which time it turned to a clear light yellow-brown solution. More phenyllithium (6 mL, 0.011 mol) was added, and the solution was stirred for 10 min. The solution was cooled (0 °C) and the reaction quenched with methanol (20 mL). The organic

layer was washed with water and brine, dried (Na_2SO_4), and concentrated to dryness to give 4-phenylcubyl iodide (3.06 g, 100%) as a light yellow solid which was used in the next step without further purification: mp 77−79 °C; ^{1}H-NMR δ 4.32 (s, 6 H), 7.09−7.19 (m, 5 H) ppm; ^{13}C-NMR δ 39.0, 52.0, 54.3, 60.5, 124.7, 126.3, 128.5, 141.5 ppm.

4-Phenylcubanecarboxylic Acid (4c). A solution of 4-phenylcubyl iodide (3.06 g, 0.01 mol) in dry THF (160 mL) was cooled to −78 °C. *tert*-Butyllithium in pentane (1.7 M, 11.8 mL, 20 mmol) was added dropwise, and the resulting pale yellow solution was stirred at −78 °C for 30 min. Dry CO_2 was bubbled through for 10 min, and the reaction mixture was allowed to warm up to room temperature while CO_2 passed through for 1 h. The reaction mixture was mixed with 50 mL of aqueous HCl (2 M) and was extracted with chloroform. The combined organic solution was washed with aqueous HCl (2 M) and dried over Na_2SO_4. Removal of the solvents gave 4-phenylcubanecarboxylic acid as a colorless solid in 71% yield: mp 178−179 °C; ^{1}H-NMR δ 4.19 (m, 3 H), 4.30 (m, 3 H), two sets of multiple peaks centered at 7.22 and 7.37 (5 H) ppm; ^{13}C-NMR δ 46.1, 48.7, 56.1, 60.2, 124.7, 126.2, 128.5, 141.8, 178.3 ppm.

4-Cyclopropylcubanecarboxylic Acid (4b). The same procedure was used as described for **4c** except substituting 4-cyclopropylcubyl iodide.[9] Recrystallization of the crude product in hexane gave pure 4-cyclopropylcubanecarboxylic acid as colorless plates in 62% yield: mp 155−156 °C; ^{1}H-NMR ($CDCl_3$) δ 0.18 and 0.43 (m, 4 H), 1.00 (m, 1 H), 3.65 (m, 3 H), 4.08 (m, 3 H); ^{13}C-NMR ($CDCl_3$) δ 0.1, 11.7, 45.0, 45.8, 56.3, 60.4, 178.1 ppm. Anal. ($C_{12}H_{12}O_2$) H; C: calcd, 76.57; found, 76.12.

General Procedure for the Synthesis of Cubanecarboxamides (5a−c). The cubanecarboxylic acid was dissolved in oxalyl chloride (*ca.* 10 mL/g) and was stirred at room temperature for 20 min. The excess oxalyl chloride was removed under vacuum. The residue was dissolved in dry CH_2Cl_2 (*ca.* 40 mL/g) in a round bottom flask equipped with a cold finger filled with dry ice/acetone, and NH_3 gas was condensed into the flask. After being stirred for 20 min, the resulting suspension was allowed to warm to room temperature, and the excess NH_3 was slowly evaporated. The reaction mixture was mixed with water and extracted with $CHCl_3$, and the combined organic solution was dried over Na_2SO_4. Removal of solvents gave the cubanecarboxamides as white solids.

5a (89% yield): mp 209−212 °C; ^{1}H NMR δ 4.01 (m, 4 H), 4.22 (m, 3 H), 5.41 (br, 2 H) ppm; ^{13}C-NMR δ 44.8, 47.9, 49.5, 57.1, 174.9 ppm.

5b (96% yield): mp 225 °C dec. The crude product was used for the next step without purification. The sample for elemental analysis was obtained by recrystallization of the crude product in EtOAc and $CHCl_3$: ^{1}H-NMR ($CDCl_3$) δ 0.18 and 0.43 (m, 4 H), 1.02 (m, 1 H), 3.63 (m, 3 H), 4.00 (m, 3 H), 5.40 (br 2 H); ^{13}C-NMR (DMSO-d_6) δ 0.0, 11.7, 43.6, 44.8, 57.7, 59.2, 173.3 ppm. Anal. ($C_{12}H_{13}ON$) H, N; C: calcd, 76.98; found, 76.38.

5c (80% yield): mp 196−197 °C; ^{1}H-NMR δ 4.17 (m, 3 H), 4.22 (m, 3 H), 7.21−7.36 (5 H), 5.45 (br, 2 H) ppm; ^{13}C-NMR δ 46.1, 48.3, 57.9, 60.3, 124.7, 126.2, 128.5, 141.9, 174.4 ppm.

(4-Phenylcubyl)carbinylamine Hydrochloride Salt (1c·HCl). The procedure described below was used also for **1a** and **1b**. $LiAlH_4$ (0.64 g, 11 mmol) was added slowly to a suspension of 4-phenylcubanecarboxamide (0.81 g, 3.6 mmol) in THF (80 mL) at 0 °C. The mixture was heated to reflux for 15 h and then it was cooled to room temperature. The excess $LiAlH_4$ was destroyed at 0 °C by dropwise addition of aqueous NaOH (saturated), and then the mixture was stirred at room temperature for 1 h. The granular precipitate was filtered off and was washed with CH_2Cl_2, and the filtrate was dried (Na_2SO_4). Evaporation of the solvents gave (4-phenylcubyl)carbinylamine as a pale yellow oil (0.62 g, 82%): ^{1}H-NMR δ 2.93 (s, 2 H) 3.81 (m, 3 H), 4.02 (m, 3 H), 7.21−7.35 (m, 5 H) ppm. The oil was dissolved in dry ether (50 mL), and HCl gas was bubbled through the solution. The resulting precipitate was collected by filtration and was recrystallized from methanol to give 0.5 g (69%) of (4-phenylcubyl)carbinylamine hydrochloride salt (**1c**·HCl) as colorless crystals: mp 203−204 °C dec; ^{1}H-NMR (D_2O) δ 3.10 (s, 2 H) 3.75 (m, 3 H), 3.88 (m, 3 H) ppm; ^{13}C-NMR (D_2O) δ 41.1, 43.3, 47.4, 55.2, 60.1, 124.6, 126.2, 128.8, 142.9 ppm. Anal. ($C_{15}H_{16}ClN$) C, H, Cl, N.

(4-Cyclopropylcubyl)carbinylamine Hydrochloride Salt (1b·HCl). This compound was synthesized in a 78% yield in the same way as was 1c·HCl described above: mp 230 °C dec; ^{1}H NMR (CD_3OD) δ 0.18−0.43 (m, 4 H), 1.02 (m, 1 H), 3.13 (s, 2 H), 3.58 (m, 3 H), 3.77 (m, 3 H); ^{13}C-NMR (CD_3OD) δ 0.3, 12.8, 42.6, 44.6, 45.1, 57.1, 62.2 ppm. Anal. ($C_{12}H_{16}NCl$) C, H, Cl, N.

Cubylcarbinylamine Hydrochloride (1a·HCl). This was synthesized in an 81% yield from the corresponding amide in the same way as was 1c·HCl: mp 275 °C dec; ^{1}H-NMR (D_2O) δ 3.00 (s, 2 H) 3.73 (m, 6 H), 3.82 (m, 1 H) ppm; ^{13}C-NMR (D_2O) δ 41.1, 43.8, 46.6, 48.2, 54.0 ppm. Anal. ($C_9H_{12}ClN$) C, H, Cl, N.

Enzyme and Assays. Mitochondrial MAO (EC 1.4.3.4) was isolated[15] as previously reported. MAO was assayed by a modification of the procedure of Tabor et al.[16] A typical assay would be the addition of a solution of MAO (5 μL) to 0.495 mL of a 2 mM benzylamine solution in 20 mM Tris·HCl buffer, pH 9.0 at 25 °C. The change in absorbance at 250 nm was observed with time.

Substrate Activity of the Cubylcarbinylamine Hydrochlorides. Kinetic constants (K_m and k_{cat}) were determined by measuring the amount of hydrogen peroxide that was formed with time as described previously.[17]

Time-Dependent Inactivation Experiments (General Methods). Solutions (200 μL each) of cubylcarbinylamine hydrochlorides of various concentrations in 100 mM Tris buffer, pH 9.0 containing 4% v/v DMSO (freshly distilled), were preincubated at 25 °C. To these solutions was added MAO B (20 μL of 2.6 mg/mL). After being mixed, the samples were incubated at 25 °C, periodically agitated, and assayed for MAO activity by removing 10 μL of the mixture and adding it to 490 μL of a 1.0 mM benzylamine solution in 100 mM Tris buffer, pH 9.0. The enzyme activity thus determined was corrected against a control containing no inactivator (control activity set to 100%). Kinetic constants (K_I and k_{inact}) were determined as described by Kitz and Wilson.[11] The control enzyme also contained 4% DMSO (it was shown that there are no adverse effects on activity up to 10% DMSO). After inactivation each enzyme solution was dialyzed against 100 mM Tris-HCl buffer, pH 9.0, for 48 h to determine reversibility.

Effects of β-Mercaptoethanol and 2-Phenylethylamine on the Rate of Inactivation of MAO B by Cubylcarbinylamines Hydrochloride. The following solutions (200 μL each) were prepared in 100 mM Tris buffer, pH 9.0, containing 4% v/v DMSO and were preincubated at 25 °C: (1) (4-phenylcubyl)carbinylamine hydrochloride (0.16 mM); (2) (4-phenylcubyl)carbinylamine hydrochloride (0.16 mM) and β-mercaptoethanol (2 mM); (3) (4-cyclopropylcubyl)carbinylamine hydrochloride (0.22 mM); (4) (4-cyclopropylcubyl)carbinylamine hydrochloride (0.22 mM) and β-mercaptoethanol (2 mM); and (4) a control with only the buffer. To these solutions was added MAO B (20 μL of 2.6 mg/mL). The mixtures were incubated at 25 °C, and MAO activity was assayed as described above. The same experiments were repeated except the 2 mM β-mercaptoethanol was replaced with 2.4 mM 2-phenylethylamine.

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