Brief Articles

Structures of Human Monoamine Oxidase B Complexes with Selective Noncovalent Inhibitors: Safinamide and Coumarin Analogs

Claudia Binda,[†] Jin Wang,[‡] Leonardo Pisani,[||] Carla Caccia,[§] Angelo Carotti,[||] Patricia Salvati,[§] Dale E. Edmondson,*[,‡] and Andrea Mattevi*[,†]

Department of Genetics and Microbiology, University of Pavia, via Ferrata 1, Pavia, 27100 Italy, Department of Biochemistry, Emory University, 1510 Clifton Road, Atlanta, Georgia 30322, Newron Pharmaceuticals Spa, Bresso (MI), Italy, Dipartimento Farmaco-Chimico, University of Bari, via Orabona 4, Bari, 70125 Italy

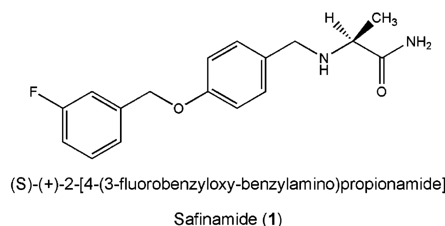
Received June 11, 2007

Structures of human monoamine oxidase B (MAO B) in complex with safinamide and two coumarin derivatives, all sharing a common benzyloxy substituent, were determined by X-ray crystallography. These compounds competitively inhibit MAO B with K_i values in the 0.1−0.5 μM range that are 30−700-fold lower than those observed with MAO A. The inhibitors bind noncovalently to MAO B, occupying both the entrance and the substrate cavities and showing a similarly oriented benzyloxy substituent.

Introduction

Monoamine oxidases A and B (MAO A and MAO B)[a] are FAD-dependent enzymes responsible for the metabolism of neurotransmitters such as dopamine, serotonin, adrenaline, and noradrenaline and for the inactivation of exogenous arylalkyl amines.[1] Both enzymes are bound to the outer mitochondrial membrane and catalyze the oxidative deamination of their substrates. Although they share 70% sequence identity, MAO A and MAO B exhibit different substrate and inhibitor specificities; serotonin is preferentially metabolized by MAO A and dopamine by MAO B, whereas selegiline (*l*-deprenyl) and clorgyline selectively inhibit MAO B and MAO A, respectively. Due to their central role in neurotransmitters metabolism, these enzymes represent attractive drug targets in the pharmacological therapy of neurodegenerative diseases and depression.[2−5] MAO B-selective inhibitors, such as selegiline, co-administrated with levo-dopa represent an effective treatment for relieving symptoms resulting from the loss of dopaminergic neurons in Parkinson's disease.[6] A recently developed MAO B inhibitor, rasagiline, has improved efficacy and is advantageous in being metabolized to nontoxic metabolites.[7] Like selegiline, rasagiline is an acetylenic compound that forms an irreversible flavin N(5) flavocyanine covalent adduct.[8,9]

Safinamide, **1** (Figure 1), is a new molecule currently in phase III clinical trials for the treatment of Parkinson's disease.[10] Safinamide has a novel mode of action as a dopamine modulator (comprising both selective and reversible MAO B inhibition



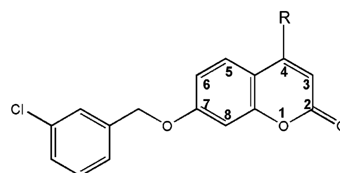


Figure 1. Chemical structures of the inhibitors used in this study.

and also blockade of dopamine reuptake) complemented by an effect on the glutamate pathway.[10] Safinamide was shown to be a potent (IC_{50} = 0.08 μM), selective, and reversible MAO B inhibitor in human brain and represents a promising compound that may be subject of further structure-based optimization. In an effort to characterize the mode of MAO inhibition by safinamide, we have carried out a biochemical and structural investigation by using the recombinant human enzymes. We have extended this analysis to two coumarin derivatives that share a common benzyloxy substituent with safinamide: the 4-(methlylamino)methyl coumarin derivative **2** and its corresponding 4-carboxaldehyde congener **3** (Figure 1). Compound **2** has been recently characterized, both in vitro and in vivo, as a strong, short-acting, reversible, and selective MAO B inhibitor with potential in the therapy of Parkinson's disease.[11,12]

Results

Chemistry. Safinamide (**1**) was kindly provided by Newron Pharmaceuticals, whereas 7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin (**2**) and 7-(3-chlorobenzyloxy)-4-car-

* To whom correspondence should be addressed. Tel.: +39-0382-985534 (A.M.); +1-404-727-5972 (D.E.E.). Fax: +39-0382-528496 (A.M.); +1-404-727-2738 (D.E.E.). E-mail: mattevi@ipvgen.unipv.it (A.M.); deedmon@emory.edu (D.E.E.).

[†] University of Pavia.

[‡] Emory University.

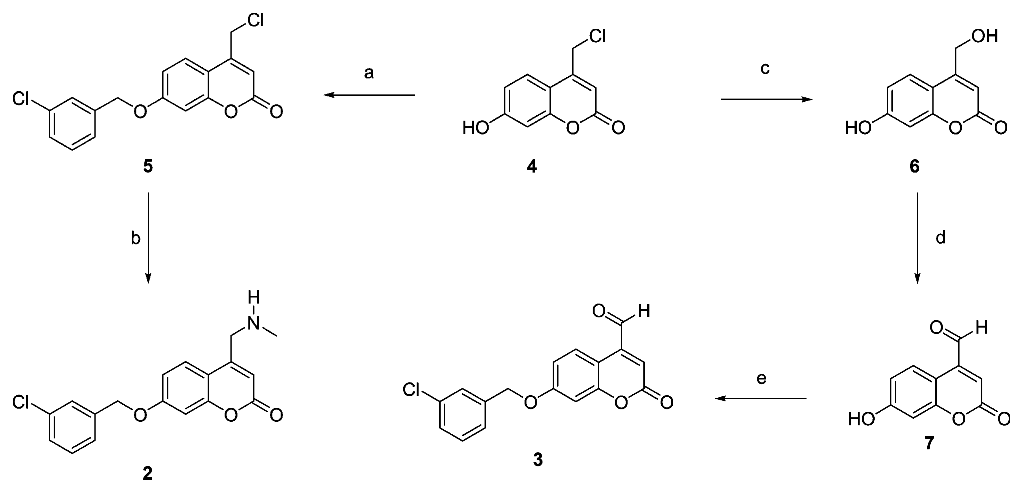
[§] Newron Pharmaceuticals Spa.

[||] University of Bari.

[a] Abbreviations: MAO, monoamine oxidase. Data deposition: Coordinates have been deposited in the PDB with ID codes 2V5Z, 2V61, and 2V60 for MAO B in complex with safinamide, 7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin, and 7-(3-chlorobenzyloxy)-4-carboxaldehyde-coumarin, respectively.

10.1021/jm070677y CCC: $37.00 © 2007 American Chemical Society
Published on Web 10/04/2007

Scheme 1. Synthesis of Compounds 7-(3-Chlorobenzyloxy)-4-(methylamino)methyl-coumarin (**2**) and 7-(3-Chlorobenzyloxy)-4-carboxaldehyde-coumarin (**3**)[a]



[a] Reagents and conditions: (a) and (e) 3-chlorobenzyl bromide, K_2CO_3, ethanol, reflux; (b) 2.0 M CH_3NH_2 in THF, 55 °C; (c) H_2O, 150 °C, MW; (d) MnO_2, dry THF, rt.

Table 1. Inhibition Constants (K_i, μM)[a] of the Investigated Inhibitors for Human MAO A and B

	MAO A	MAO B
safinamide (**1**)	365.0 ± 18.7	0.45 ± 0.13
7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin (**2**)	15.7 ± 2.0	0.10 ± 0.02
7-(3-chlorobenzyloxy)-4-carboxaldehyde-coumarin (**3**)	11.0 ± 0.5	0.40 ± 0.02

[a] MAO A and B activities were measured at 25 °C by kynuramine and benzylamine assays, respectively, in 50 mM potassium phosphate buffer pH 7.5, 0.5% (w/v) reduced Triton X-100.

boxaldehyde-coumarin (**3**) were synthesized from 4-chloromethyl-7-hydroxycoumarin (**4**; Scheme 1).

Binding Affinity to Human MAOs. Inhibitors were assayed for their inhibitory activity using recombinant human MAO A and MAO B. Initial velocity data were best fit to the equation describing competitive inhibition (Table 1). Safinamide binds to human MAO B with a K_i of 0.5 μM, which is 700-fold lower than that found for human MAO A under identical conditions. These data reflect the IC_{50} values measured on human and rat brain mitochondria,[10] which revealed an even higher isoform selectivity that may be related to the different experimental conditions (mitochondrial preparations vs purified recombinant enzymes). Measurement of the UV/vis spectrum of human MAO B incubated at different times with safinamide revealed no spectral perturbations, suggesting that this inhibitor does not form any covalent adduct with the flavin cofactor.

Coumarins bearing a benzyloxy substituent on position 7 were shown to be effective MAO inhibitors.[12−15] Further investigation of these derivatives revealed that a methylamino-methyl group at position 4 improves solubility and the pharmacokinetic properties (Carotti, unpublished observations). We found that 7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin (**2**) is a much stronger inhibitor of human MAO B (K_i = 0.1 μM) than of MAO A (K_i = 15.7 μM, Table 1). The 4-carboxaldehyde analog (**3**) shows a similar behavior, although it is slightly less isoform-selective. Similarly to safinamide, both these coumarin compounds do not perturb the UV/vis spectral properties of the enzyme-bound flavin, indicating that they bind noncovalently to MAOs.

Structures of Inhibitor Binding to Human MAO B. Human MAO B was cocrystallized with the investigated compounds,

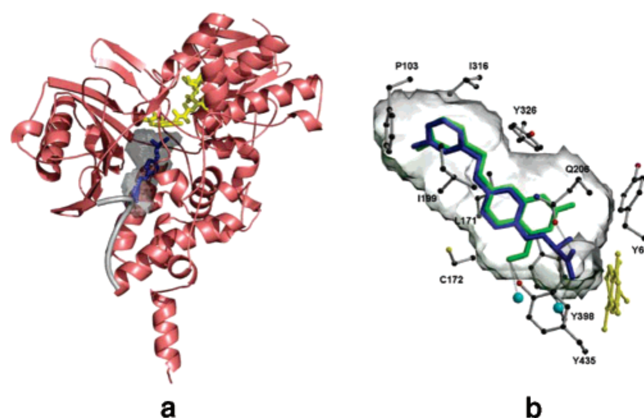


Figure 2. Structure of human MAO B in complex with the investigated inhibitors. (a) Ribbon diagram of MAO B overall structure. The FAD cofactor and safinamide are highlighted in yellow and blue stick representation, respectively. The semi-transparent surface represents the active site cavity, whose inner part (dark gray) forms the substrate binding site whereas the outward-facing space (light gray) corresponds to entrance cavity. Loop formed by residues 99−112, which admits ligands into the active site, is highlighted as gray thick ribbon. (b) Safinamide (blue) and 7-(3-chlorobenzyloxy)-4-carboxaldehyde-coumarin (green) bound in the MAO B cavity. The structure is rotated approximately 90° with respect to Figure 2a. The active site residues and the flavin are drawn in gray and yellow, respectively. Hydrogen bonds established by the two inhibitors with protein residues and water molecules (cyan spheres) are shown as dashed lines.

and the three-dimensional structures of the complexes were determined at high resolution (Figure 2a, Table 2). Human MAO B is dimeric, with each monomer consisting of a globular domain anchored to the membrane through a C-terminal helix.[16] Inhibitor binding does not induce any significant conformational change in the overall structure (Supporting Information). In each case, the inhibitor molecule could be unambiguously modeled in the electron density map (Figures S1 and S2), showing that all three ligands do not form a covalent adduct with the flavin cofactor (Figure 2b). The MAO B active site consists of two cavities, the substrate cavity in front of the flavin and the entrance cavity located underneath the protein surface and closed by the loop formed by residues 99−112 (Figure 2a). Safinamide binds to human MAO B in an extended conformation occupying both cavities (Figure 2b). The 3-fluorobenzyloxy moiety is located in the entrance cavity space, whereas the other aromatic

Table 2. Crystallographic Data Collection and Refinement Statistics

	safinamide (**1**)	7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin (**2**)	7-(3-chlorobenzyloxy)-4-carboxaldehyde-coumarin (**3**)
space group	*C*222	*C*222	*C*222
unit cell (Å)	$a = 132.5$, $b = 223.6$, $c = 86.6$	$a = 132.0$, $b = 222.5$, $c = 86.2$	$a = 130.7$, $b = 224.4$, $c = 86.8$
resolution (Å)	1.6	1.7	2.0
$R_{sym}{}^{a,b}$ (%)	7.7 (46.6)	6.7 (22.1)	12.8 (55.3)
completeness^{b} (%)	97.9 (98.8)	99.6 (99.4)	99.3 (99.3)
unique reflections	164 454	138 335	85 456
redundancy	3.1	3.4	3.6
I/σ^{b}	7.1 (1.5)	7.6 (3.4)	4.5 (1.1)
No. of atoms protein/ligand/water^{c}	8017/1 × 22/717	8017/2 × 23/921	8017/2 × 22/756
avg B value for ligand atoms (Å^2)	17.5	26.7	36.3
$R_{cryst}{}^{c}$ (%)	20.8	17.2	17.2
$R_{free}{}^{c}$ (%)	22.7	20.1	22.1
Rms bond length (Å)	0.007	0.011	0.017
Rms bond angles (°)	1.06	1.29	1.55

[a] $R_{sym} = \sum|I_i - \langle I \rangle|/\sum I_i$, where I_i is the intensity of *i*th observation and $\langle I \rangle$ is the mean intensity of the reflection. [b] Values in parentheses are for reflections in the highest resolution shell. [c] $R_{cryst} = \sum|F_{obs} - F_{calcd}|/\sum F_{obs}$, where F_{obs} and F_{calcd} are the observed and calculated structure factor amplitudes, repectively. R_{cryst} and R_{free} were calculated using the working and test sets, respectively.

ring occupies the substrate cavity with the primary amide group oriented toward the flavin cofactor (Figure 3a). Three ordered water molecules are present in the active site, as found in other MAO B-inhibitor complexes.[8] The amide group of safinamide is engaged in two hydrogen bonds with Gln206 and an ordered water molecule (Figure 3a). The mode of binding of the two coumarins (**2** and **3**) resembles that of safinamide (Figures 3b,c). Their benzyloxy group occupies the entrance cavity space, whereas the coumarin moiety binds in the substrate cavity with the pyran ring oxygen pointing toward Tyr326 at the top of the cavity. The substituents at position 4 that distinguish the two inhibitors (Figure 1) are proximal to the Tyr398–Tyr435 aromatic pair (Figures 3b,c). In the 7-(3-chlorobenzyloxy)-4-carboxaldehyde-coumarin (**3**) complex, the 4-aldehyde oxygen is H-bonded to the hydroxyl group of Tyr435 and to a water molecule in front of the flavin. In the 7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin (**2**) complex, no clear electron density was observed for the terminal methyl group of the 4-(methylamino)methyl substituent (Figure S2). This observation raised the hypothesis that, during crystal growth, 7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin might slowly undergo an enzymatic oxidation at its secondary amino group, generating a product equivalent to the carboxaldehyde derivative. However, this possibility has been ruled out by testing 7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin as substrate for human MAO B (data not shown). No enzymatic activity has been detected even upon prolonged time assays and no flavin reduction was observed on its anaerobic incubation with purified MAO B. These data rule out the catalytic oxidation of the secondary amine moiety by the enzyme and suggest multiple conformations of the terminal methyl group of the 4-(methylamino)methyl moiety as the reason for not observing clear electron density attributable to this group.

Discussion

Safinamide (**1**) and the related coumarin derivatives (**2** and **3**) represent new MAO inhibitors with promising therapeutic properties. On comparison of their interactions with purified recombinant human MAO A and MAO B, we found that safinamide exhibits a >700-fold selectivity for human MAO B. 7-(3-Chlorobenzyloxy)-4-(methylamino)methyl-coumarin and 7-(3-chlorobenzyloxy)-4-carboxaldehyde-coumarin showed tighter binding to human MAO B than to human MAO A, although their isoform selectivity is less pronounced than that of safinamide.

The crystal structures of human MAO B in complex with the three inhibitors revealed that they all bind noncovalently to the enzyme, which represents a desirable property to minimize toxic side effects since de novo protein synthesis is not required for the recovery of enzymatic activity. Safinamide and the coumarin derivatives bind to human MAO B traversing the active site cavities in their entire length, which contributes to the high selectivity of these inhibitors because the active site of human MAO A does not have this bipartite cavity.[17] Moreover, unlike the other cavity-spanning MAO B specific inhibitor,[18] they have polar substituents that orient their binding mode to the hydrophilic space in front of the flavin to establish H-bond interactions both with conserved water molecules and with protein residues. These H-bonds were not found in the structures of other MAO B complexes with reversible inhibitors, where binding interactions were generally limited to van der Waals or hydrophobic contacts.[18] Inspection of the structures reveals that a niche of the substrate cavity lined by Tyr60, Tyr326, and Gln206 remains unoccupied in the safinamide complex (Figure 3a), whereas it is filled by the pyran ring of the coumarin derivatives (Figures 2, 3b,c). Conversely, safinamide occupies the hydrophilic part of the cavity with its propionamide group extending more toward the flavin and replacing two water molecules found in the complexes with the coumarin compounds (Figure 3).

In conclusion, the most relevant features that distinguish these compounds from the other clinically used MAO inhibitors are their potential multiple-target mechanism of neuroprotection[10,19] and, relatively to MAO inhibition, their high isoform selectivities and the noncovalent manner of binding. Our data pave the way to further optimization strategies, particularly in view of the multiple mechanism of action of these inhibitors.

Experimental Section

Chemistry. Starting materials, reagents, and analytical grade solvents were purchased from Sigma-Aldrich (Europe). Chromatographic separations were performed on silica gel (15–40 mesh,

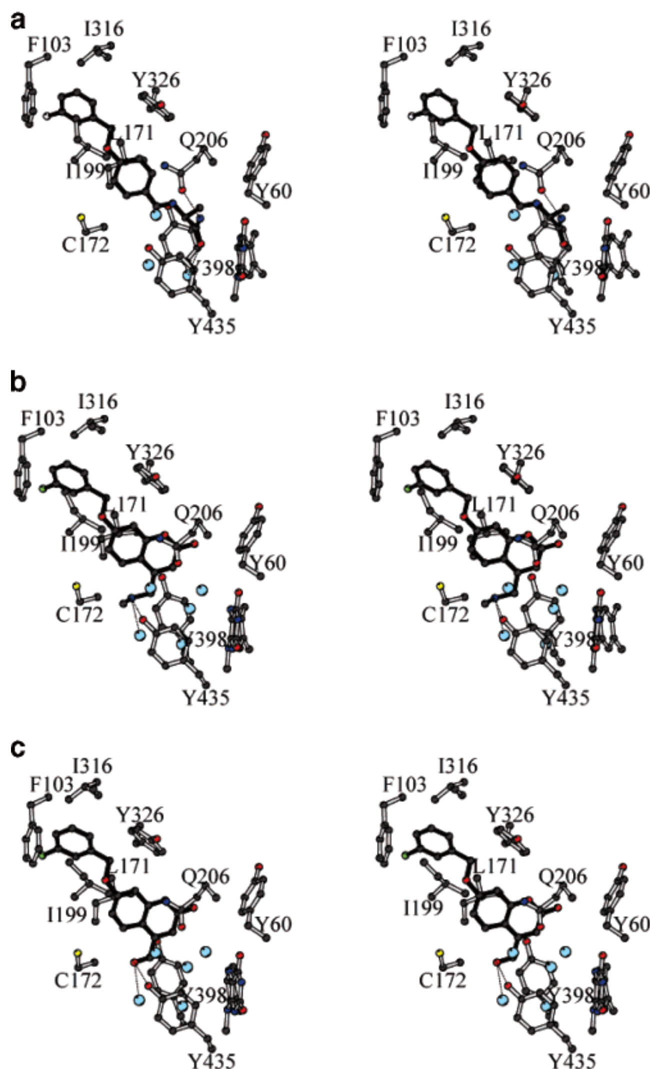


Figure 3. Stereoplots of the analyzed inhibitors bound to MAO B active site. (a) Safinamide (**1**) complex. (b) 7-(3-Chlorobenzyloxy)-4-(methylamino)methyl-coumarin (**2**) complex. (c) 7-(3-Chlorobenzyloxy)-4-carboxaldehyde-coumarin (**3**) complex. Color code and orientation are as in Figure 2b. Active site residues and the inhibitor are drawn in gray and black, respectively. Water molecules are shown as cyan spheres. Dashed lines show hydrogen bonds between the inhibitor and protein residues. The terminal methyl group of the 4-(methylamino)-methyl substituent of compound **2** is not visible in the electron density, most likely reflecting a disordered conformation. The position of the terminal methyl group displayed in Figure 3b is purely indicative and shown to facilitate the correlation of the three-dimensional structure with the chemical formula depicted in Figure 1.

Merck) by flash methodologies. Microwave reactions were performed in a Milestone MicroSynth apparatus. Melting points (mp) were determined only for the final products by the capillary method on a Stuart Scientific SMP3 electrothermal apparatus and are uncorrected. Elemental analysis (C, H, N) were determined only on the target final products on an Europea 3000 analyzer and were within ±0.4% of the calculated values. ^{1}H NMR spectra were recorded at 300 MHz on a Varian Mercury 300 instrument. Chemical shifts are expressed in δ (ppm) and coupling constants J in hertz (Hz). The following abbreviations were used: br (broad signal), s (singlet), d (doublet), t (triplet), dd (double doublet), m (multiplet). Signals due to OH and NH protons were detected by deuterium exchange with D_2O. Synthesis of compounds **2** and **3** described below refers to Scheme 1.

4-(Chloromethyl)-7-hydroxy-2*H*-chromen-2-one (4). The title compound was prepared according to literature[20] with slight modifications. Resorcinol (10 g, 91 mmol) was dissolved in 100 mL of sulfuric acid 96% at 0 °C. Ethyl 4-chloroacetoacetate (10 mL, 74 mmol) was slowly added, and the mixture was stirred at room temperature for 24 h. The reaction mixture was then poured onto ice water (500 g), and the solid residue was filtered and washed with water, yielding the desired coumarin as white solid (12.2 g, 78% yield). MS (EI) m/z 210 (M$^+$); ^{1}H NMR (acetone-d_6, 300 MHz) δ 9.50 (s, 1H, dis. with D_2O), 7.73 (d, J = 8.8 Hz, 1H), 6.91 (dd, J_1 = 2.5 Hz, J_2 = 8.8 Hz, 1H), 6.80 (d, J = 2.5 Hz, 1H), 6.40 (s, 1H), 4.92 (s, 2H).

7-[(3-Chlorobenzyl)oxy]-4-(chloromethyl)-2*H*-chromen-2-one (5). 4-(Chloromethyl)-7-hydroxy-2*H*-chromen-2-one (**4**; 1.0 g, 4.8 mmol), anhydrous K_2CO_3 (0.66 g, 4.8 mmol), and 0.94 mL (7.2 mmol) of 3-chlorobenzyl bromide were heated in refluxing absolute ethanol (32 mL) for 2 h. The reaction mixture was cooled to room temperature, and the inorganic precipitate was filtered off. After solvent evaporation, the crude residue was treated with ethyl ether and filtered giving a solid with a level of purity that allowed its use as such in the next steps (1.3 g, 78% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.58 (d, J = 8.8 Hz, 1H), 7.43 (br s, 1H), 7.37−7.27 (m, 3H), 6.96 (dd, J_1 = 2.5 Hz, J_2 = 8.8 Hz, 1H), 6.88 (d, J = 2.5 Hz, 1H), 6.41 (s, 1H), 5.11 (s, 2H), 4.62 (s, 2H).

7-[(3-Chlorobenzyl)oxy]-4-[(methylamino)methyl]-2*H*-chromen-2-one (2) Methanesulfonate. Compound **5** (0.80 g, 2.50 mmol) and a 2.0 M solution of *N*-methylamine in THF (25.0 mL, 50 mmol) were stirred at 55 °C under argon for 5 h. The mixture was cooled to room temperature, and the inorganic precipitate was filtered off. The solvent was evaporated and the resulting solid was purified by column chromatography using AcOEt as eluent affording the title compound as a free base (0.25 g, 28% yield). To a solution of this free base (0.18 g, 0.55 mmol, in 3 mL of dry THF) was added methanesulphonic acid (0.040 mL, 0.6 mmol). The resulting white solid was filtered, washed with dry THF, and crystallized from absolute ethanol (0.22 g, 95% yield). MS (ESI) m/z 330 (M + H)$^+$; mp 213−215 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.01 (s, 2H, dis. with D_2O), 7.77 (d, J = 8.8 Hz, 1H), 7.54 (s, 1H), 7.44−7.37 (m, 3H), 7.14 (d, J = 2.5 Hz, 1H), 7.10 (dd, J_1 = 2.5 Hz, J_2 = 8.8 Hz, 1H), 6.41 (s, 1H), 5.27 (s, 2H), 4.44 (s, 2H), 2.71 (s, 3H), 2.31 (s, 3H); Anal. ($C_{19}H_{20}ClNO_6S$) C, H, N.

7-Hydroxy-4-(hydroxymethyl)-2*H*-chromen-2-one (6). A Pyrex vessel was charged with a magnetic stirring bar, 1.0 g (4.8 mmol) of **4**, and 50 mL of water. Then it was introduced in the microwave reactor and irradiated for 15 min at 150 °C, with a heating ramp of 5 min. Then the mixture was cooled to room temperature and the product was crystallized from absolute water (0.77 g, 84% yield). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.49 (s, 1H, dis. with D_2O), 7.49 (d, J = 8.5 Hz, 1H), 6.74 (dd, J_1 = 2.4 Hz, J_2 = 8.5 Hz, 1H), 6.69 (d, J = 2.4 Hz, 1H), 6.20 (s, 1H), 5.56 (t, J = 4.9 Hz, 1H, dis. with D_2O), 4.67 (d, J = 4.9 Hz, 2H).

7-Hydroxy-2-oxo-2*H*-chromene-4-carboxaldehyde (7). MnO_2 (1.7 g, 20 mmol) was added to a solution of 0.77 g (4.0 mmol) of **6** in 30 mL of dry THF. The mixture was stirred at rt for 24 h and then filtered over celite flowed by a washing with hot ethanol. The combined solvents were concentrated to dryness and the resulting crude solid was purified by column chromatography using as a mixture of chloroform/methanol 9.5/0.5 (v/v) as eluent (0.52 g, 68% yield). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.67 (br s, dis. with D_2O, 1H), 10.05 (s, 1H), 8.29−8.26 (m, 1H), 6.87−6.80 (m, 2H), 6.68 (s, 1H).

7-[(3-Chlorobenzyl)oxy]-2-oxo-2*H*-chromene-4-carboxaldehyde (3). Intermediate **7** (0.19 g, 1.0 mmol) was dissolved in 10 mL of absolute ethanol and anhydrous K_2CO_3 (0.14 g, 1.0 mmol) and 3-chlorobenzyl bromide (0.26 mL, 2.0 mmol) were added. The mixture was refluxed for 2 h. The inorganic precipitate was filtered off, and the solution was evaporated under vacuum affording an oily residue that was purified by flash chromatography ($CHCl_3$/petroleum ether 7/3 v/v as the eluent) to give a pure yellow solid that was crystallized from $CHCl_3$/hexane (0.18 g, 56% yield). Mp 145−147 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.09 (s, 1H), 8.38 (d, J = 8.8 Hz, 1H), 7.54 (s, 1H), 7.44−7.39 (m, 3H), 7.16−7.15 (m, 1H), 7.12−7.07 (m, 1H), 6.99−6.98 (m, 1H), 5.24 (s, 2H); Anal. ($C_{17}H_{11}ClO_4$) C, H.

Enzymatic and Crystallographic Studies. Activity and inhibitory assays as well as crystallographic studies were performed according to published procedures[18] and are described in Supporting Information.

Acknowledgment. This work was supported by grants from the National Institute of General Medical Sciences (GM-29433) and MIUR (FIRB and COFIN06). C.B. is supported by an Investigator Fellowship from Collegio Ghislieri, Pavia, Italy. Ms. M. Aldeco provided excellent technical assistance with this project.

Supporting Information Available: Elemental analysis of compounds **2** and **3** and enzymatic and crystallographic methods. This material is available free of charge via the Internet at: http://pubs.acs.org.

References

(1) Shih, J. C.; Chen, K.; Ridd, M. J. Monoamine Oxidase: From Genes to Behaviour. *Annu. Rev. Neurosci.* **1999**, *22*, 197−217.
(2) Cesura, A. M.; Pletscher, A. The New Generation of Monoamine Oxidase Inhibitors. *Prog. Drug Res.* **1992**, *38*, 171−297.
(3) Yamada, M.; Yasuhara, H. Clinical Pharmacology of MAO Inhibitors: Safety and Future. *Neurotoxicology* **2004**, *25*, 215−221.
(4) Fariello, R. G.; Lieberman, A. Present and Future Approaches to Parkinson Disease: From Molecular Insights to New Therapeutic Avenues. *Neurology* **2006**, *67*, S1−S4.
(5) Youdim, M. B.; Edmondson, D. E.; Tipton, K. F. The Therapeutic Potential of Monoamine Oxidase Inhibitors. *Nat. Rev. Neurosci.* **2006**, *7*, 295−309.
(6) Olanow, C. W. Rationale for Considering that Propargylamines Might Be Neuroprotective in Parkinson's Disease. *Neurology* **2006**, *66*, S69−S79.
(7) Rascol, O.; Brooks, D. J.; Melamed, E.; Oertel, W.; Poewe, W.; Stocchi, F.; Tolosa, E. LARGO Study Group. Rasagiline as an Adjunct to Levodopa in Patients with Parkinson's Disease and Motor Fluctuations (LARGO, Lasting Effect in Adjunct Therapy with Rasagiline Given Once Daily, Study): A Randomised, Double-Blind, Parallel-Group Trial. *Lancet* **2005**, *365*, 947−954.
(8) Binda, C.; Hubalek, F.; Li, M.; Herzig, Y.; Sterling, J.; Edmondson, D. E.; Mattevi, A. Crystal Structures of MAO B in Complex with Four Inhibitors of the *N*-Propargylaminoindan Class. *J. Med. Chem.* **2004**, *47*, 1767−1774.
(9) Hubalek, F.; Binda, C.; Li, M.; Herzig, Y.; Sterling, J.; Youdim, M. B.; Mattevi, A.; Edmondson, D. E. Inactivation of Purified Human Recombinant Monoamine Oxidases A and B by Rasagiline and Its Analogues. *J. Med. Chem.* **2004**, *47*, 1760−1766.
(10) Caccia, C.; Maj, R.; Calabresi, M.; Maestroni, S.; Faravelli, L.; Curatolo, L.; Salvati, P.; Fariello, R. G. Safinamide: From Molecular Targets to a New Anti-Parkinson Drug. *Neurology* **2006**, *67*, S18−S23.
(11) Carotti, A.; Melloni, P.; Thaler, F.; Caccia, C.; Maestroni, S.; Salvati, P. Substituted Aminoalkyl and Amidoalkyl Benzopyran Derivatives. WO 2006/102958 A1.
(12) Catto, M.; Nicolotti, O.; Leonetti, F.; Carotti, A.; Favia, A. D.; Soto-Otero, R.; Mendez-Alvarez, E.; Carotti, A. Structural Insights into Monoamine Oxidase Inhibitory Potency and Selectivity of 7-Substituted Coumarins from Ligand- and Target-Based Approaches. *J. Med. Chem.* **2006**, *49*, 4912−4925.
(13) Novaroli, L.; Daina, A.; Favre, E.; Bravo, J.; Carotti, A.; Leonetti, F.; Catto, M.; Carrupt, P. A.; Reist, M. Impact of Species-Dependent Differences on Screening, Design, and Development of MAO-B Inhibitors. *J. Med. Chem.* **2006**, *49*, 6264−6272.
(14) Carotti, A.; Altomare, C.; Catto, M.; Gnerre, C.; Summo, L.; De Marco, A.; Rose, S.; Jenner, P.; Testa, B. Lipophilicity Plays a Major Role in Modulating the Inhibition of Monoamine Oxidase B by 7-Substituted Coumarins. *Chem. Biodiversity* **2006**, *3*, 134−149.
(15) Gnerre, C.; Catto, M.; Leonetti, F.; Weber, P.; Carrupt, P. A.; Altomare, C.; Carotti, A.; Testa, B. Inhibition of Monoamine Oxidases by Functionalized Coumarin Derivatives: Biological Activities, QSARs, and 3D-QSARs. *J. Med. Chem.* **2000**, *43*, 4747−4758.
(16) Binda, C.; Newton-Vinson, P.; Hubalek, F.; Edmondson, D. E.; Mattevi, A. Structure of Human Monoamine Oxidase B, A Drug Target for the Treatment of Neurological Disorders. *Nat. Struct. Biol.* **2002**, *9*, 22−26.
(17) De Colibus, L.; Li, M.; Binda, C.; Lustig, A.; Edmondson, D. E.; Mattevi, A. Three-Dimensional Structure of Human Monoamine Oxidase A (MAO A): Relation to the Structures of Rat MAO A and Human MAO B. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12684−12689.
(18) Binda, C.; Li, M.; Hubalek, F.; Restelli, N.; Edmondson, D. E.; Mattevi, A. Insights into the Mode of Inhibition of Human Mitochondrial Monoamine Oxidase B from High-resolution Crystal Structures. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9750−9755.
(19) Bruhlmann, C.; Ooms, F.; Carrupt, P. A.; Testa, B.; Catto, M.; Leonetti, F.; Altomare, C.; Carotti, A. Coumarin Derivatives as Dual Inhibitors of Acetylcholinesterase and Monoamine Oxidase. *J. Med. Chem.* **2001**, *44*, 3195−3198.
(20) Conconi, M. T.; Guiotto, A.; Manzini, P.; Chilin, A.; Bassani, V.; Parnigotto, P. P. 4-Hydroxymethyltetrahydro- and 4-Hydroxymethylbenzopsoralen. Synthesis and Biological Activity. *Il Farmaco* **1995**, *50*, 125−130.

JM070677Y