

Homoisoflavonoids: Natural Scaffolds with Potent and Selective Monoamine Oxidase-B Inhibition Properties

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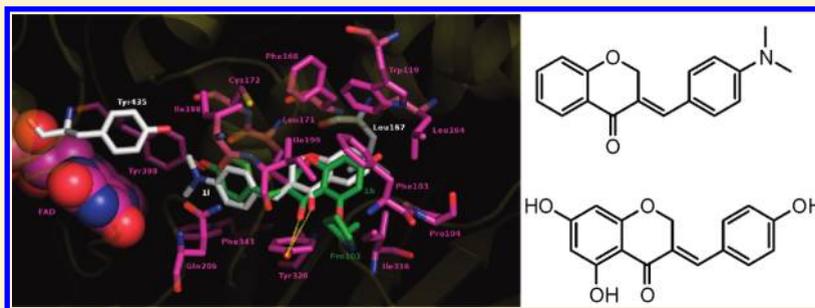
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S Supporting Information

ABSTRACT:



A series of homoisoflavonoids [(*E*)-3-benzylidenechroman-4-ones **1a–w**, 3-benzyl-4*H*-chromen-4-ones **2a–g**, and 3-benzylchroman-4-ones **3a–e**] have been synthesized and tested *in vitro* as inhibitors of human monoamine oxidase isoforms A and B (hMAO-A and hMAO-B). Most of the compounds were found to be potent and selective MAO-B inhibitors. In general, the (*E*)-3-benzylidenechroman-4-ones **1a–w** showed activities in the nano- or micromolar range coupled with high selectivity against hMAO-B. The reduction of the exocyclic double bond results in compounds **3a–e** selective against isoform B and active in the micromolar range. In contrast, the endocyclic migration of the double bond (compounds **2a–g**) generally produces the loss of the inhibitory activity or a marked reduction in potency. (*E*)-3-(4-(Dimethylamino)benzylidene)chroman-4-one (**11**) and (*E*)-5,7-dihydroxy-3-(4-hydroxybenzylidene)chroman-4-one (**1h**) were the most interesting compounds of the entire series of inhibitors, showing hMAO-B affinity better than the selective inhibitor selegiline. Molecular modeling studies have been carried out to explain the selectivity of the most active homoisoflavonoids **1h** and **11**.

INTRODUCTION

Monoamine oxidase (MAO) is an ubiquitous membrane-bound, flavin-containing enzyme, which is particularly abundant in the liver and brain.¹ MAO is located intracellularly in the mitochondrial outer membranes of neuronal, glial, and other cells and catalyzes the oxidative deamination of monoamine neurotransmitters and xenobiotic amines to the corresponding aldehydes with consumption of oxygen and production of hydrogen peroxide, thereby affecting the concentrations of neurotransmitter amines and many xenobiotic ones.^{2–4}

Currently, two MAO isoforms, encoded by separate genes sharing a common intron/exon organization,^{4–6} have been identified based on their differential substrate specificity and inhibitor sensitivity,^{7–9} tissue and cell distribution,¹⁰ and gene expression characteristics.^{11,12}

MAO-A preferentially deaminates serotonin, epinephrine, and norepinephrine and is selectively inhibited by low concentrations

of clorgyline, whereas MAO-B preferentially metabolizes β -phenylethylamine and benzylamine and is inhibited by selegiline.¹ Both isoenzymes deaminate dopamine, tyramine, and tryptamine.¹

Because of the important function played by MAO in the metabolism of neurotransmitters, MAO inhibitors can be useful in the treatment of many psychiatric and neurological diseases. In fact, MAO-A inhibitors act as antidepressant and anti-anxiety agents, whereas MAO-B inhibitors are used alone or in combination to treat Alzheimer's and Parkinson's diseases.¹³

Recognition of the importance of MAO as a drug target for treatment of neurological disorders has produced an enormous interest in the development of inhibitors of these enzymes. Various structural classes of potent MAO inhibitors, including

Received: October 21, 2010

Published: March 15, 2011

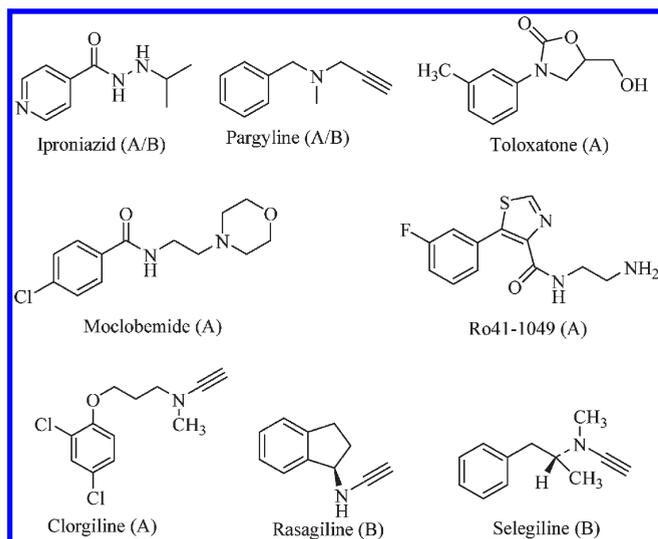


Figure 1. Some representative structures of nonselective and selective MAO inhibitors.

hydrazide, amide, thiazole, imidazole, oxazolidinone, oxadiazolone, diacylurea derivatives, etc., have been identified.^{14–27} In spite of considerable progress in understanding the interactions of the two enzyme forms with their corresponding substrates, no general rules are yet available for the rational design of potent and selective MAO inhibitors. There are many different structures of MAO inhibitors partly because the active sites of the MAO enzymes are ambiguous, which limits the design of potent selective MAO inhibitors. Figure 1 shows the chemical structures of some representative MAO inhibitors used in research or clinical practice.

The aim of the present study was the identification of novel potent MAO inhibitors that could serve as potential lead molecules for drug discovery.

Homoisoflavonoids constitute a small class of natural products prevalently isolated from the bulbs, rhizomes, or roots of several genera of *Hyacinthaceae* and *Caesalpinioideae*. These compounds are structurally related to the more widespread flavonoids and, according to their structural features, can be classified into three types: 3-benzylidenechroman-4-ones, 3-benzyl-4*H*-chromen-4-ones, and 3-benzylchroman-4-ones. Several natural and synthetic homoisoflavonoids, like the related flavonoids, were found to possess various biological properties such as antifungal,²⁸ antiviral,^{29–31} antimutagenic,^{32,33} antiproliferative,³⁴ antioxidant,^{35,36} antiallergic and antihistaminic,³⁷ anti-inflammatory,³⁸ and protein tyrosine kinase (PTK) inhibitor activities.³⁹ However, no data are available on the inhibitory activity of homoisoflavonoids on MAOs.

Recently, several substituted chalcones and flavanones were reported as potent and selective inhibitors of the B-isoform of human MAO (hMAO).^{40,41} The structural relationship of homoisoflavonoids to chalcones and flavanones prompted us to investigate their inhibitory activity against the A and B isoforms of hMAO. Initially, we tested the synthetic (*E*)-3-benzylidenechroman-4-ones **1a–g**, 3-benzyl-4-chromones **2a–g**, and 3-benzylchroman-4-ones **3a–e**, previously studied as anticoronavirals.^{29–31} Because all the (*E*)-3-benzylidenechroman-4-ones **1a–g**, which may be regarded as rigid analogues of chalcones, were potent and selective inhibitors of the B isoform of hMAO, we planned the synthesis of a larger panel

of (*E*)-3-benzylidenechroman-4-ones to further investigate the structure–activity relationships. The MAO recognition of the most active compounds, **1h** and **1i**, was investigated by docking experiments performed using available Protein Data Bank (PDB) structures as receptor models.

CHEMISTRY

The (*E*)-3-benzylidenechroman-4-ones **1a–w** were synthesized by acid-catalyzed condensation of the appropriate chroman-4-one with substituted benzaldehydes (Scheme 1).

In general, the target compounds were prepared in good yield using 85% phosphoric acid as the acid catalyst and heating the mixture at 80 °C for 6 h. Under these conditions, the reaction of chroman-4-one with *N*-(4-formylphenyl)acetamide gave a mixture of amide **1k** and the corresponding amine **1j**, which were easily separated by column chromatography. Starting from substituted chroman-4-ones or 2-substituted benzaldehydes, treatment with 85% phosphoric acid provided the corresponding chromanones (**1d**, **1e**, **1g**, **1i**, and **1s–v**) in very low yields. To obtain these compounds more efficiently, the condensations were carried out in dry ethyl alcohol saturated with dry hydrogen chloride. A single stereoisomer was obtained with both procedures. The ¹H NMR spectra allow the *E* configuration of the double bond to be assigned on the basis of the chemical shift of the olefinic proton, ranging from 7.6 to 7.9 ppm.

(*E*)-5,7-Dihydroxy-3-(4-hydroxybenzylidene)chroman-4-one (**1h**) was synthesized by saponification of the corresponding tribenzoate following published procedures.⁴²

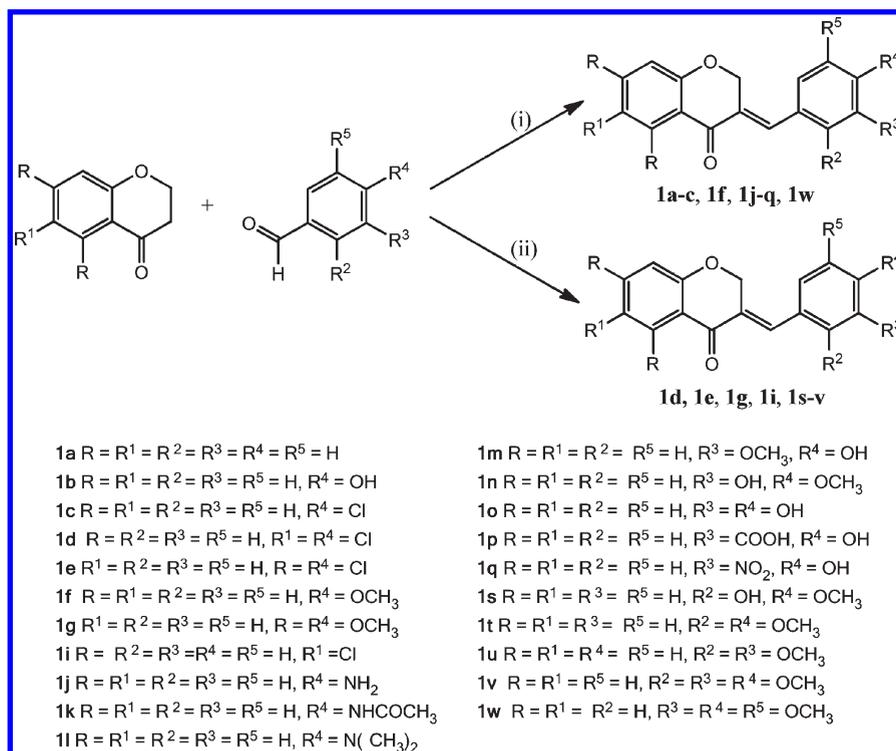
The reduction of (*E*)-3-(4-hydroxy-3-nitrobenzylidene)chroman-4-one (**1q**) with tin chloride in ethyl alcohol and concentrated hydrogen chloride provided the corresponding amino derivative (**1r**).

The (*E*)-3-benzylidenechroman-4-ones **1a–g** were converted into the corresponding 3-benzyl-4-chromones **2a–g** or 3-benzylchroman-4-ones **3a–e** according to procedures described previously.²⁹

BIOCHEMISTRY

The potential effects of the tested compounds on hMAO activity were investigated by measuring their effects on the production of hydrogen peroxide (H₂O₂) from *p*-tyramine, using the Amplex Red MAO assay kit (Molecular Probes, Inc., Eugene, Oregon, U.S.) and microsomal MAO isoforms prepared from insect cells (BTI-TN-SB1-4) infected with recombinant baculovirus containing cDNA inserts for hMAO-A or hMAO-B (Sigma-Aldrich Química S.A., Alcobendas, Spain). In this study hMAO activity was evaluated using the above method following the general procedure described previously by us.⁴³ The tested drugs (new compounds and reference inhibitors) themselves were unable to react directly with the Amplex Red reagent, which indicates that these compounds do not interfere with the measurements. On the other hand, in our experiments and under our experimental conditions, the control activity of hMAO-A and hMAO-B (using *p*-tyramine as a common substrate for both isoforms) was 165 ± 2 pmol of *p*-tyramine oxidized to *p*-hydroxyphenylacetaldehyde/min (*n* = 20).

The results of hMAO-A and hMAO-B inhibition studies are expressed as IC₅₀ and reported in Tables 1 and 2 together with the hMAO-B selectivity indexes (SI = IC₅₀ MAO-A/IC₅₀ MAO-B).

Scheme 1^a

^a Reagents and conditions: (i) 85% H₃PO₄, 80° C, 6 h; (ii) dry EtOH sat. HCl, room temp, 48 h.

In Table 1 are presented the data obtained for the (*E*)-3-benzylidenechroman-4-ones **1a–w**. Most of these homoisoflavonoids selectively inhibited the enzymatic activity of hMAO-B in the nanomolar or micromolar range. Derivatives **1h**, **1k**, **1o**, and **1r** were also able to inhibit hMAO-A in the micromolar range. Among these analogues, **1k** and **1r** were essentially nonselective (SI = 2 and 3, respectively) whereas **1h** and **1o** exhibited hMAO-B selectivity to different extents (SI = 1331 and 34, respectively). Only the acid **1p** was found to be completely inactive toward both isoforms up to the highest concentration tested (100 μM). (*E*)-3-[4-(Dimethylamino)benzylidene]chroman-4-one (**1l**) was the most potent and selective hMAO-B inhibitor identified in this study, exhibiting an IC₅₀ of 8.51 nM and SI of >11751. In comparison with the reference inhibitor selegiline, compound **1l** showed higher hMAO-B affinity and selectivity. The replacement of the dimethylamino group with the amino group resulted in compound **1j**, about 100-fold less potent and selective as a hMAO-B inhibitor (IC₅₀ = 879.18 nM, SI > 114) than **1l**. Interestingly, acetylation of the amino group (compound **1k**) produced a further decrease of hMAO-B inhibitory activity and a dramatic reduction of selectivity (IC₅₀ = 1.06 μM, SI = 2). (*E*)-5,7-Dihydroxy-3-(4-hydroxybenzylidene)chroman-4-one (**1h**) showed an affinity for hMAO-B (IC₅₀ = 8.61 nM) comparable to that for **1l**. Although **1h** was able to inhibit both hMAO isoforms, it still exhibited an excellent hMAO-B selectivity (SI = 1331). Although less active than **1h** and **1l**, (*E*)-5,7-dichloro-3-(4-chlorobenzylidene)chroman-4-one (**1e**) showed hMAO-B affinity and selectivity (IC₅₀ = 13.03 nM, SI > 7675) better than those of the reference compound, selegiline.

The results for inhibitory effects and selectivity of the 3-benzyl-4*H*-chromen-4-ones **2a–g** and 3-benzylchroman-4-ones **3a–e** on hMAO isoforms are reported in Table 2.

Several of these compounds (**2e**, **2f**, **3a**, and **3c–e**) showed selective hMAO-B inhibitory activities, whereas the 3-benzyl-4-chromen-4-ones **2a–d** and **2g** and the 3-benzylchroman-4-one **3b** were essentially inactive toward both isoforms up to the highest concentration tested (100 μM). Disappointingly, the endocyclic migration of the double bond produces a loss of the inhibitory activity (compounds **2a–d**, **2g**) or a marked reduction in potency (compounds **2e** and **2f**) with respect to the corresponding (*E*)-3-benzylidenechroman-4-ones **1a–g**. The reduction of the double bond results in compounds **3a–e**, generally more potent and selective than the corresponding 3-benzyl-4*H*-chromen-4-ones **2a–g**. The only exception was 5,7-dichloro-3-(4-chlorobenzyl)-4*H*-chromen-4-one (**2e**) that exhibited submicromolar potency toward hMAO-B, whereas the corresponding 3-benzylchroman-4-one (**3e**) was about 10-fold less active and less selective. However, all the 3-benzylchroman-4-ones **3a–e** were less potent hMAO-B inhibitors than the corresponding (*E*)-3-benzylidenechroman-4-ones **1a–e**.

Table 3 shows the results of the reversibility and irreversibility tests for the most effective compounds **1h** and **1l**. hMAO-A and hMAO-B inhibition was irreversible in the presence of **1h** as shown by the lack of enzyme activity restoration after repeated washing. Similar results were obtained for compound **1l** and selegiline against hMAO-B. On the contrary, significant recovery of hMAO-A activity was observed after repeated washing of moclobemide, indicating that this drug is a reversible inhibitor of hMAO-A.

DOCKING STUDIES

Docking studies were carried out to evaluate the binding modes of this class of homoisoflavonoids with respect to both

Table 1. IC₅₀ and SI for the Inhibitory Effects of (*E*)-3-Benzylidenechroman-4-ones **1a–w** and Reference Inhibitors on the Enzymatic Activity of Human Recombinant MAO Isoforms Expressed in Baculovirus Infected BTI Insect Cells^a

compd	R	R ¹	R ²	R ³	R ⁴	R ⁵	IC ₅₀		SI ^d
							MAO-A	MAO-B	
1a	H	H	H	H	H	H	<i>f</i>	479.70 ± 19.37 nM	>208 ^h
1b	H	H	H	H	OH	H	<i>f</i>	55.37 ± 2.18 nM	>1806 ^h
1c	H	H	H	H	Cl	H	<i>f</i>	154.23 ± 6.93 nM	>648 ^h
1d	H	Cl	H	H	Cl	H	<i>f</i>	31.82 ± 1.19 nM	>3143 ^h
1e	Cl	H	H	H	Cl	H	<i>f</i>	13.03 ± 0.79 nM	>7675 ^h
1f	H	H	H	H	OCH ₃	H	<i>f</i>	58.90 ± 2.09 nM	>1698 ^h
1g	OCH ₃	H	H	H	OCH ₃	H	<i>f</i>	1.57 ± 0.03 μM	>64 ^h
1h	OH	H	H	H	OH	H	11.46 ± 0.43 μM ^b	8.61 ± 0.12 nM	1331
1i	H	Cl	H	H	H	H	<i>f</i>	331.11 ± 9.63 nM	>302 ^h
1j	H	H	H	H	NH ₂	H	<i>f</i>	879.18 ± 45.31 nM	>114 ^h
1k	H	H	H	H	NHCOCH ₃	H	2.10 ± 0.11 μM ^c	1.06 ± 0.04 μM	2.0
1l	H	H	H	H	N(CH ₃) ₂	H	<i>f</i>	8.51 ± 0.32 nM	>11751 ^h
1m	H	H	H	OCH ₃	OH	H	<i>g</i>	104.34 ± 2.61 nM	>958 ^h
1n	H	H	H	OH	OCH ₃	H	<i>f</i>	476.51 ± 21.60 nM	>210 ^h
1o	H	H	H	OH	OH	H	4.74 ± 0.21 μM ^b	140.52 ± 4.71 nM	34
1p	H	H	H	COOH	OH	H	<i>f</i>	<i>f</i>	
1q	H	H	H	NO ₂	OH	H	<i>f</i>	490.84 ± 12.73 nM	>204 ^h
1r	H	H	H	NH ₂	OH	H	1.44 ± 0.51 μM ^b	483.88 ± 16.21 nM	3.0
1s	H	H	OH	H	OCH ₃	H	<i>g</i>	1.33 ± 0.07 μM	>75 ^h
1t	H	H	OCH ₃	H	OCH ₃	H	<i>f</i>	247.51 ± 6.31 nM	>404 ^h
1u	H	H	OCH ₃	OCH ₃	H	H	<i>f</i>	1.92 ± 0.08 μM	>52 ^h
1v	H	H	OCH ₃	OCH ₃	OCH ₃	H	<i>f</i>	1.31 ± 0.04 μM	>76 ^h
1w	H	H	H	OCH ₃	OCH ₃	OCH ₃	<i>f</i>	20.69 ± 0.37 μM	>4.8 ^h
clorgyline							4.46 ± 0.32 nM ^b	61.35 ± 1.13 μM	0.000073
selegiline							67.25 ± 1.02 μM ^b	19.60 ± 0.86 nM	3431
iproniazid							6.56 ± 0.76 μM	7.54 ± 0.36 μM	0.87
moclobemide							361 ± 19.37 μM	<i>e</i>	<0.36

^a All IC₅₀ values shown in this table are the mean ± SEM from five experiments. ^b Level of statistical significance: $P < 0.01$ versus the corresponding IC₅₀ values obtained against MAO-B, as determined by ANOVA/Dunnett's. ^c Level of statistical significance: $P < 0.05$ versus the corresponding IC₅₀ values obtained against MAO-B, as determined by ANOVA/Dunnett's. ^d SI: hMAO-B selectivity index = IC₅₀(hMAO-A)/IC₅₀(hMAO-B). ^e Inactive at 1 mM (highest concentration tested). ^f Inactive at 100 μM (highest concentration tested). ^g 100 μM inhibits the corresponding MAO activity by approximately 40–50%. At higher concentration the compounds precipitate. ^h Values obtained under the assumption that the corresponding IC₅₀ against MAO-B is the highest concentration tested (100 μM).

isoforms of human MAO. Crystallographic structures were selected from the PDB to build our theoretical receptors models (Experimental Section). Taking into account the experimental hMAO inhibition data, compounds **1h** and **1l** were chosen for our molecular modeling investigation. Although the irreversible inhibitors usually form a covalent bond with a residue on the enzyme, they may also act by other mechanisms.⁴⁴ The so-called tight-binding inhibitors, owing to the very low dissociation constant, may show kinetics similar to the kinetics of covalent irreversible inhibitors. In some cases, the inhibitors may rapidly bind to the enzyme in a low-affinity enzyme–inhibitor (EI) complex and then undergoes a slower rearrangement to a very tightly bound EI* complex. For instance, *R*-(–)-deprenyl, an irreversible MAO inhibitor, forms a noncovalent complex with MAO as an initial and reversible step. Afterward, the interaction of *R*-(–)-deprenyl with MAO leads to a reduction of the enzyme-bound FAD and concomitant oxidation of the inhibitor. This oxidized inhibitor is able to form a covalent bond at the N-5 position of FAD.⁴⁵ The initial noncovalent binding to MAO has

been also described for other MAO inhibitors (e.g., clorgyline derivatives).⁴⁶ Moreover, we have already docked noncovalent ligands^{40,41,47} showing an irreversible profile similar to **1h** and **1l**.

Both homoisoflavonoids were submitted to a conformational search revealing, not surprisingly, only two conformers within 10 kcal/mol from the global minimum energy. Using the AutoDock Vina method,⁴⁸ the most populated structures of **1h** and **1l** were submitted to flexible docking simulation with respect to both hMAO-A and hMAO-B receptor models (Experimental Section). The theoretical complexes were evaluated taking into account two interaction energy descriptors. Best interaction energy (IE) is the AutoDock Vina lowest ligand–target interaction energy. ΔG_{bind} is the Boltzmann averaged binding free energy computed with the entire complex ensemble generated by the docking program. In both cases, good qualitative agreements with experimental inhibition data have been obtained (Table 4).

The most stable binding modes of both homoisoflavonoids in the hMAO-A and -B active sites were graphically inspected (Figure 2).

Table 2. IC₅₀ and SI for the Inhibitory Effects of 3-Benzyl-4H-chromen-4-ones 2a–g and 3-Benzylchroman-4-ones 3a–e and Reference Inhibitors on the Enzymatic Activity of Human Recombinant MAO Isoforms Expressed in Baculovirus Infected BTI Insect Cells^a

compd	R	R ¹	R ²	R ³	R ⁴	R ⁵	IC ₅₀		SI ^c
							MAO-A	MAO-B	
2a	H	H	H	H	H	H	<i>e</i>	<i>e</i>	
2b	H	H	H	H	OH	H	<i>e</i>	<i>f</i>	
2c	H	H	H	H	Cl	H	<i>e</i>	<i>e</i>	
2d	H	Cl	H	H	Cl	H	<i>e</i>	<i>f</i>	
2e	Cl	H	H	H	Cl	H	<i>f</i>	438.60 ± 27.48 nM	>228 ^g
2f	H	H	H	H	OCH ₃	H	<i>e</i>	21.59 ± 1.06 μM	>4.6 ^g
2g	OCH ₃	H	H	H	OCH ₃	H	<i>e</i>	<i>f</i>	
3a	H	H	H	H	H	H	<i>e</i>	58.53 ± 2.24 μM	>1.7 ^g
3b	H	H	H	H	OH	H	<i>e</i>	<i>f</i>	
3c	H	H	H	H	Cl	H	<i>e</i>	7.37 ± 0.31 μM	>14 ^g
3d	H	Cl	H	H	Cl	H	<i>e</i>	3.07 ± 0.11 μM	>33 ^g
3e	Cl	H	H	H	Cl	H	<i>e</i>	4.59 ± 0.19 μM	>22 ^g
clorgyline							4.46 ± 0.32 nM ^b	61.35 ± 1.13 μM	0.000073
selegiline							67.25 ± 1.02 μM ^b	19.60 ± 0.86 nM	3431
iproniazid							6.56 ± 0.76 μM	7.54 ± 0.36 μM	0.87
moclobemide							361 ± 19.37 μM	<i>d</i>	<0.36

^a All IC₅₀ values shown in this table are the mean ± SEM from five experiments. ^b Level of statistical significance: $P < 0.01$ versus the corresponding IC₅₀ values obtained against MAO-B, as determined by ANOVA/Dunnett's. ^c SI: hMAO-B selectivity index = IC₅₀(hMAO-A)/IC₅₀(hMAO-B). ^d Inactive at 1 mM (highest concentration tested). ^e Inactive at 100 μM (highest concentration tested). ^f 100 μM inhibits the corresponding MAO activity by approximately 40–50%. At higher concentration the compounds precipitate. ^g Values obtained under the assumption that the corresponding IC₅₀ against MAO-B is the highest concentration tested (100 μM).

Table 3. Reversibility and Irreversibility of hMAO-B Inhibition of Derivatives **1h** and **1l**^a

compd	% hMAO-A inhibition	
	before washing	after repeated washing
1h (50 μM)	68.26 ± 4.34	61.76 ± 4.98
moclobemide (500 μM)	85.98 ± 4.03	11.45 ± 0.58 ^b

compd	% hMAO-B inhibition	
	before washing	after repeated washing
1h (10 nM)	48.66 ± 6.39	36.22 ± 4.26
1l (10 nM)	56.34 ± 5.37	58.67 ± 6.60
selegiline (20 nM)	53.28 ± 2.59	56.34 ± 2.01

^a Each value is the mean ± SEM from five experiments ($n = 5$). ^b Level of statistical significance: $P < 0.01$ versus the corresponding % MAO-A or MAO-B inhibition before washing, as determined by ANOVA/Dunnett's.

Docking simulations generated several low energy binding modes of **1h** and **1l** within both hMAO isoforms. These poses revealed the ability of these ligands to alternatively recognize the FAD with both benzyl and chromone rings (see Supporting Information). Interestingly, the hMAO-A best poses of the **1h** and **1l** chromone rings were located toward the enzymatic cofactor (Figure 2a). Most of the ligand–enzyme interactions appeared to be identical. The main difference between **1h** and **1l** hMAO-A recognition was due to the presence of a phenol OH in the former compound that established a hydrogen bond with FAD N5 atom. Moreover, **1h**, attracted by the FAD, resulted in

Table 4. Comparison between Experimental IC₅₀ Data and Theoretical Binding Affinities of **1h** and **1l** with Respect to hMAO-A and -B

parameter	hMAO-A		hMAO-B	
	1h	1l	1h	1l
IC ₅₀ ^a	11460.00	>100000.00	8.61	8.51
ΔG _{bind} ^b	−6.30	−1.88	−9.10	−9.35
best IE ^c	−7.00	−3.00	−9.50	−10.40

^a Experimental inhibition data (nM). ^b Free energy of binding (kcal/mol). ^c Best interaction energy (kcal/mol).

exclusive contact with Tyr69; conversely **1l** was involved in attractive hydrophobic contacts with Asn181 and Tyr444. The effects of these different interactions on binding energy are highlighted in Table 4.

The best poses of these compounds in the hMAO-B binding site were different from the A isoform. These configurations displayed the chromone ring far from the FAD cofactor. In both **1h** and **1l** hMAO-B complexes the interaction pattern was similar: one hydrogen bond between the carbonyl oxygen of the ligands and Tyr326-OH and several almost identical hydrophobic contacts. The slightly better interaction of **1l** can be attributed to its more hydrophobic nature compared with **1h**. Actually the chromone ring was surrounded by a large lipophilic cage (Phe103, Pro104, Trp119, Leu164, Leu167, Phe168, Leu171, Ile199, and Ile316) where **1h**, because of its hydrophilic substituents, was disfavored. Finally, the hMAO-B selectivity of both compounds can be attributed to (a) the larger set of residues interacting with hMAO-B and (b) the different hydrogen bond

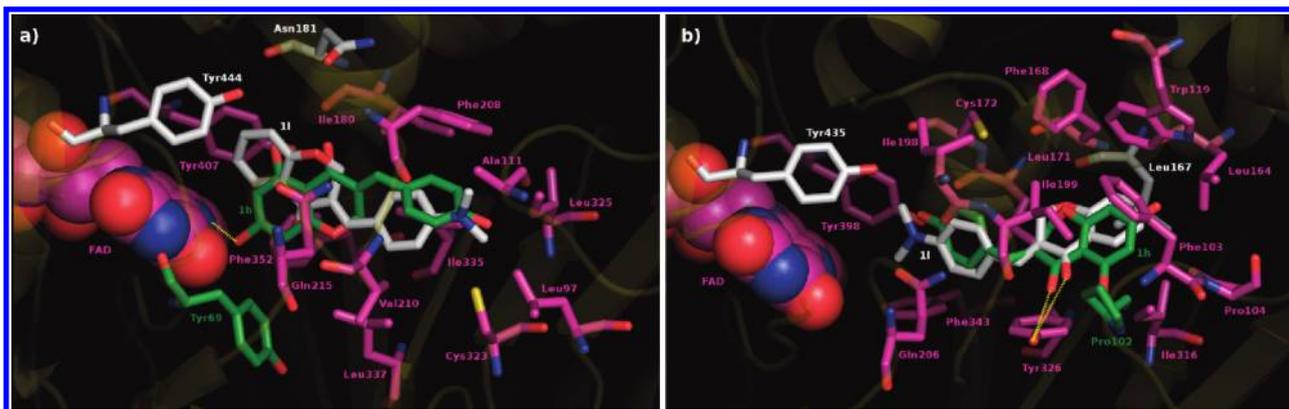


Figure 2. Most representative binding modes of **1h** (green) and **1l** (white) into (a) hMAO-A and (b) hMAO-B catalytic sites. Common **1h** and **1l** interacting residues are reported in magenta carbon atoms sticks. Exclusive **1h** or **1l** interacting residues are displayed according to the corresponding ligands. The FAD cofactor is depicted using the spacefill representation. The rest of the enzyme is shown in yellow, transparent cartoon. Yellow dotted lines indicate hydrogen bonds.

contributions, stronger in hMAO-B (Tyr326-OH \cdots O=**1h/1l**) than in hMAO-A (FAD-N5 \cdots HO-**1h**).

CONCLUSION

In the current study we have synthesized and evaluated a series of (*E*)-3-benzylidenechroman-4-ones **1a–w**, 3-benzyl-4*H*-chromen-4-ones **2a–g**, and 3-benzylchroman-4-ones **3a–e** as inhibitors of h-MAO isoforms A and B. In general, the active compounds showed potent and selective activity toward the B isoform. In particular, several (*E*)-3-benzylidenechroman-4-ones exhibited potencies in the nanomolar range. Two derivatives, **1h** and **1l**, were the most potent and selective hMAO-B inhibitors with higher potency than the reference inhibitor selegiline and, in the latter case, even higher selectivity. Molecular docking studies suggest that stronger enzyme–inhibitor hydrogen bonds and hydrophobic contacts in the hMAO-B active site can explain the selectivity of both inhibitors for this isoform.

EXPERIMENTAL SECTION

Chemistry. Chemicals were purchased from Sigma-Aldrich and used without further purification. Melting points were determined on a Stenford Research Systems OptiMelt (MPA-100) apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded in DMSO- d_6 , unless indicated otherwise, on a Bruker AM-400 spectrometer, using TMS as internal standard. IR spectra were recorded in KBr disks on an FT-IR PerkinElmer Spectrum 1000. All compounds were routinely checked by thin-layer chromatography (TLC), ^1H NMR, and ^{13}C NMR. TLC was performed on silica gel or aluminum oxide fluorescent coated plates (Merck, Kieselgel, or aluminum oxide 60 F254). Components were visualized by UV light. Compound purity was determined by elemental analysis and was confirmed to be $\geq 95\%$ for all compounds. (*E*)-3-Benzylidenechroman-4-ones (**1a–g**), 3-benzyl-4-chromones (**2a–g**), and 3-benzylchroman-4-ones (**3a–e**) were prepared as previously described by us.²⁹ (*E*)-5,7-Dihydroxy-3-(4-hydroxybenzylidene)chroman-4-one (**1h**) was obtained by saponification of the corresponding tribenzoate as previously reported.⁴²

General Procedure for the Synthesis of (*E*)-3-Benzylidenechroman-4-ones **1l–q and **1w**.** A mixture of the appropriate chroman-4-one (0.01 mol) and substituted benzaldehyde (0.01 mol) in 85% phosphoric acid (20 mL) was heated at 80 °C for 6 h. After cooling, the mixture was diluted with ice–water (and made alkaline for the

preparation of compound **1l**). The solid was filtered off, washed with water, and crystallized.

(*E*)-3-(4-(Dimethylamino)benzylidene)chroman-4-one (1l**).** Yield: 77%. Mp = 151–153 °C (lit. 150–153 °C)³⁴ from EtOH. The compound exhibited spectroscopic data identical to those previously reported.³⁴

(*E*)-3-(4-Hydroxy-3-methoxybenzylidene)chroman-4-one (1m**).** Yield: 71%. Mp = 130–131 °C (lit. 126–129 °C)⁴⁹ from ethyl alcohol. IR (KBr): 3200, 1655 cm^{-1} . ^1H NMR: δ (ppm) 9.75 (bs, 1H, OH), 7.88 (dd, 1H, H5, $J_{5-6} = 8.0$ Hz, $J_{5-7} = 1.6$ Hz), 7.71 (t, 1H, =CH, $J_{\text{all}} = 1.8$ Hz), 7.58 (ddd, 1H, H7, $J_{6-7} = 7.2$ Hz, $J_{7-8} = 8.4$ Hz, $J_{5-7} = 1.6$ Hz), 7.12 (ddd, 1H, H6, $J_{6-7} = 7.2$ Hz, $J_{5-6} = 8.0$ Hz, $J_{6-8} = 1.0$ Hz), 7.07 (d, 1H, H2', $J_{2'-6'} = 1.7$ Hz), 7.05 (dd, 1H, H8, $J_{7-8} = 8.4$ Hz, $J_{6-8} = 1.0$ Hz), 6.93 (dd, 1H, H6', $J_{2'-6'} = 1.7$ Hz), 6.90 (d, 1H, H5', $J_{5'-6'} = 8.4$ Hz), 5.47 (d, 2H, H2, $J_{\text{all}} = 1.7$ Hz), 3.84 (s, 3H, OCH₃). ^{13}C NMR: δ (ppm) 180.9, 160.4, 148.8, 147.6, 137.2, 135.8, 127.7, 127.1, 125.2, 124.4, 121.8, 121.6, 117.7, 115.6, 114.8, 67.5, 55.6.

(*E*)-3-(3-Hydroxy-4-methoxybenzylidene)chroman-4-one (1n**).** Yield: 78%. Mp = 192–193 °C from EtOH. IR (KBr): 3150, 1650 cm^{-1} . ^1H NMR: δ (ppm) 9.34 (bs, 1H, OH), 7.88 (dd, 1H, H5, $J_{5-6} = 7.9$ Hz, $J_{5-7} = 1.8$ Hz), 7.64 (t, 1H, =CH, $J_{\text{all}} = 1.8$ Hz), 7.58 (ddd, 1H, H7, $J_{6-7} = 7.2$ Hz, $J_{7-8} = 8.4$ Hz, $J_{5-7} = 1.8$ Hz), 7.13 (ddd, 1H, H6, $J_{6-7} = 7.2$ Hz, $J_{5-6} = 7.9$ Hz, $J_{6-8} = 1.0$ Hz), 7.07–7.03 (m, 2H, H8, H5'), 6.94–6.91 (m, 2H, H2', H6'), 5.43 (d, 2H, H2, $J_{\text{all}} = 1.8$ Hz), 3.85 (s, 3H, OCH₃). ^{13}C NMR: δ (ppm) 180.9, 160.4, 149.4, 146.5, 136.8, 135.9, 128.3, 127.1, 126.5, 122.9, 121.8, 121.5, 117.7, 117.2, 112.0, 67.4, 55.6.

(*E*)-3-(3,4-Dihydroxybenzylidene)chroman-4-one (1o**).** Yield: 54%. Mp = 227–230 °C (lit. 224–225 °C)⁴⁹ from EtOH. IR (KBr): 3400, 1640 cm^{-1} . ^1H NMR: δ (ppm) 9.50 (bs, 2H, 2OH), 7.87 (dd, 1H, H5, $J_{5-6} = 7.8$ Hz, $J_{5-7} = 1.7$ Hz), 7.61 (t, 1H, =CH, $J_{\text{all}} = 1.8$ Hz), 7.58 (ddd, 1H, H7, $J_{6-7} = 7.4$ Hz, $J_{7-8} = 8.4$ Hz, $J_{5-7} = 1.7$ Hz), 7.12 (ddd, 1H, H6, $J_{6-7} = 7.4$ Hz, $J_{5-6} = 7.8$ Hz, $J_{6-8} = 1.0$ Hz), 7.05 (dd, 1H, H8, $J_{7-8} = 8.4$ Hz, $J_{6-8} = 1.0$ Hz), 6.89–6.80 (m, 3H, H2', H5', H6'), 5.43 (d, 2H, H2, $J_{\text{all}} = 1.8$ Hz). ^{13}C NMR: δ (ppm) 180.9, 160.3, 147.8, 145.3, 137.2, 135.8, 127.3, 127.1, 125.2, 123.4, 121.7, 121.6, 117.7, 115.8, 67.5.

(*E*)-2-Hydroxy-5-[(4-oxochroman-3-ylidene)methyl]benzoic Acid (1p**).** Yield: 74%. Mp = 234–238 °C from EtOH. IR (KBr): 3500–2300, 1650, 1600 cm^{-1} . ^1H NMR: δ (ppm) 11.87 (bs, 1H, COOH), 7.87–7.80 (m, 2H, H5, H2'), 7.69 (bs, 1H, =CH), 7.63–7.54 (m, 2H, H6', H7), 7.12–7.01 (m, 3H, H5', H6, H8), 5.40 (d, 2H, H2, $J_{\text{all}} = 1.4$ Hz). ^{13}C NMR: δ (ppm) 180.9, 171.2, 162.0, 160.5, 137.4, 136.0, 135.6, 132.7, 129.4, 127.1, 125.0, 121.8, 121.4, 117.8, 117.7, 113.5, 67.2.

(E)-3-(4-Hydroxy-3-nitrobenzylidene)chroman-4-one (1q).

Yield: 77%. Mp = 203–205 °C from EtOAc. IR (KBr): 3240, 1655, 1530, 1300 cm⁻¹. ¹H NMR: δ (ppm) 11.65 (bs, 1H, OH), 7.94 (d, 1H, H2', J_{2'-6'} = 2.2 Hz), 7.88 (dd, 1H, H5, J₅₋₆ = 7.9 Hz, J₅₋₇ = 1.8 Hz), 7.70 (bs, 1H, =CH), 7.64 (dd, 1H, H6', J_{2'-6'} = 2.2 Hz, J_{5'-6'} = 8.7 Hz), 7.60 (ddd, 1H, H7, J₆₋₇ = 7.3 Hz, J₇₋₈ = 8.4 Hz, J₅₋₇ = 1.8 Hz), 7.23 (d, 1H, H5', J_{5'-6'} = 8.7 Hz), 7.13 (ddd, 1H, H6, J₆₋₇ = 7.3 Hz, J₅₋₆ = 7.9 Hz, J₆₋₈ = 0.9 Hz), 7.05 (dd, 1H, H8, J₇₋₈ = 8.4 Hz, J₆₋₈ = 0.9 Hz), 5.44 (d, 2H, H2, J_{all} = 1.8 Hz). ¹³C NMR: δ (ppm) 180.5, 160.5, 152.8, 137.2, 136.5, 135.1, 134.4, 130.2, 127.2, 127.1, 124.9, 121.9, 121.3, 119.3, 117.8, 67.2.

(E)-3-(3,4,5-Trimethoxybenzylidene)chroman-4-one (1w).

Yield: 63%. Mp = 105–106 °C (lit. 108–109 °C)⁵⁰ from EtOAc/petroleum ether. IR (KBr): 1660 cm⁻¹. ¹H NMR: δ (ppm) 7.90 (dd, 1H, H5, J₅₋₆ = 7.8 Hz, J₅₋₇ = 1.8 Hz), 7.73 (bs, 1H, =CH), 7.60 (ddd, 1H, H7, J₇₋₈ = 8.4 Hz, J₆₋₇ = 7.2 Hz, J₅₋₇ = 1.8 Hz), 7.14 (ddd, 1H, H6, J₆₋₇ = 7.2 Hz, J₅₋₆ = 7.8 Hz, J₆₋₈ = 1.0 Hz), 7.06 (dd, 1H, H8, J₇₋₈ = 8.4 Hz, J₆₋₈ = 1.0 Hz), 6.77 (s, 2H, H2', H6'), 5.50 (d, 2H, H2, J_{all} = 1.8 Hz), 3.84 (s, 6H, 2OCH₃), 3.74 (s, 3H, OCH₃). ¹³C NMR: δ (ppm) 181.0, 160.6, 152.8, 138.9, 136.9, 136.2, 130.0, 129.2, 127.2, 121.9, 121.5, 117.8, 108.0, 67.4, 60.1, 56.0.

Synthesis of (E)-3-(4-Aminobenzylidene)chroman-4-one (1j) and (E)-N-(4-[(4-Oxochroman-3-ylidene)methyl]phenyl)acetamide (1k). A mixture of chroman-4-one (0.01 mol) and 4-aminobenzaldehyde (0.01 mol) in 85% H₃PO₄ (20 mL) was heated at 80 °C for 6 h. After cooling, the mixture was diluted with ice-water and alkalized with 2 N NaOH. The solid was filtered off and washed with water. The obtained mixture of (E)-3-(4-aminobenzylidene)chroman-4-one (1j) and (E)-N-(4-[(4-oxochroman-3-ylidene)methyl]phenyl)acetamide (1k) was separated by column chromatography on silica gel, eluting with AcOEt/petroleum ether (1:1).

(E)-3-(4-Aminobenzylidene)chroman-4-one (1j).

Yield: 30%. Mp = 164–66 °C from EtOAc/petroleum ether. IR (KBr): 3430, 3340, 1650 cm⁻¹. ¹H NMR: δ (ppm) 7.85 (dd, 1H, H5, J₅₋₆ = 7.8 Hz, J₅₋₇ = 1.8 Hz), 7.62 (bs, 1H, =CH), 7.55 (ddd, 1H, H7, J₆₋₇ = 7.2 Hz, J₇₋₈ = 8.4 Hz, J₅₋₇ = 1.8 Hz), 7.21 (d, 2H, H2', H6', J_{2'-3'} = 8.6 Hz), 7.10 (ddd, 1H, H6, J₆₋₇ = 7.2 Hz, J₅₋₆ = 8.2 Hz, J₆₋₈ = 1.0 Hz), 7.03 (dd, 1H, H8, J₇₋₈ = 8.4 Hz, J₆₋₈ = 1.0 Hz), 6.66 (d, 2H, H3', H5', J_{2'-3'} = 8.6 Hz), 5.95 (bs, 2H, NH₂), 5.43 (d, 2H, H2, J_{all} = 1.8 Hz). ¹³C NMR: δ (ppm) 180.6, 160.2, 151.2, 137.8, 135.4, 133.0, 127.0, 124.6, 121.8, 121.6, 121.0, 117.6, 113.5, 67.8.

(E)-N-(4-[(4-Oxochroman-3-ylidene)methyl]phenyl)acetamide (1k).

Yield: 25%. Mp = 175–176 °C from EtOAc/petroleum ether. IR (KBr): 3300, 1664, 1660 cm⁻¹. ¹H NMR: δ (ppm) 10.23 (s, 1H, NH), 7.88 (dd, 1H, H5, J₅₋₆ = 7.8 Hz, J₅₋₇ = 1.6 Hz), 7.74–7.70 (m, 3H, H2', H6', =CH), 7.59 (ddd, 1H, H7, J₆₋₇ = 7.1 Hz, J₇₋₈ = 8.4 Hz, J₅₋₇ = 1.6 Hz), 7.44 (d, 2H, H3', H5', J_{2'-3'} = 8.6 Hz), 7.13 (ddd, 1H, H6, J₆₋₇ = 7.1 Hz, J₅₋₆ = 7.8 Hz, J₆₋₈ = 0.7 Hz), 7.06 (dd, 1H, H8, J₇₋₈ = 8.4 Hz, J₆₋₈ = 0.7 Hz), 5.45 (d, 2H, H2, J_{all} = 1.6 Hz), 2.09 (s, 3H, CH₃). ¹³C NMR: δ (ppm) 181.0, 168.7, 160.5, 140.8, 136.3, 136.0, 131.5, 129.1, 128.3, 127.2, 121.9, 121.5, 118.7, 117.8, 67.5, 24.1.

General Procedure for the Synthesis of (E)-3-Benzylidenechroman-4-ones 1i and 1s–v. A mixture of the appropriate chroman-4-one (0.01 mol) with substituted benzaldehyde (0.01 mol) in dry EtOH saturated with HCl (40 mL) was stirred at room temperature for 48 h. After this time, the mixture was diluted with ice-water and the solid filtered off and washed with water. The product was purified by column chromatography on silica gel or by crystallization.

(E)-3-Benzylidene-6-chlorochroman-4-one (1i).

Yield: 86%. Mp = 158–159 °C (lit. 148–150 °C)⁵¹ from EtOAc. IR (KBr): 1660 cm⁻¹. ¹H NMR (CDCl₃): δ (ppm) 7.98 (d, 1H, H5, J₅₋₇ = 2.6 Hz), 7.90 (bs, 1H, =CH), 7.49–7.37 (m, 4H, H7, H3'-H5'), 7.33–7.30 (m, 2H, H2', H6'), 6.93 (d, 1H, H8, J₇₋₈ = 8.8 Hz), 5.35 (d, 2H, H2, J_{all} = 1.8 Hz). ¹³C NMR (DMSO d₆): δ (ppm) 180.2, 159.2, 137.3, 135.7, 133.5, 130.3, 129.8, 128.8, 126.0, 125.9, 124.8, 122.4, 120.3, 67.5.

(E)-3-(2-Hydroxy-4-methoxybenzylidene)chroman-4-one

(1s). Purified by column chromatography, eluting with AcOEt/petroleum ether (1:5). Yield: 56%. Mp = 158–160 °C from EtOH. IR (KBr): 3124, 1655 cm⁻¹. ¹H NMR: δ (ppm) 10.34 (bs, 1H, OH), 7.89–7.86 (m, 2H, =CH, H5), 7.57 (ddd, 1H, H7, J₆₋₇ = 7.2 Hz, J₇₋₈ = 8.4 Hz, J₅₋₇ = 1.8 Hz), 7.12 (ddd, 1H, H6, J₆₋₇ = 7.2 Hz, J₅₋₆ = 7.9 Hz, J₆₋₈ = 1.0 Hz), 7.08 (d, 1H, H6', J_{5'-6'} = 8.2 Hz), 7.04 (dd, 1H, H8, J₇₋₈ = 8.4 Hz, J₆₋₈ = 1.0 Hz), 6.53–6.49 (m, 2H, H3', H5'), 5.33 (d, 2H, H2, J_{all} = 1.8 Hz), 3.77 (s, 3H, OCH₃). ¹³C NMR: δ (ppm) 181.2, 162.2, 160.5, 158.6, 135.8, 132.8, 131.7, 127.3, 127.1, 121.8, 121.7, 117.8, 114.0, 105.4, 101.1, 67.9, 55.2.

(E)-3-(2,4-Dimethoxybenzylidene)chroman-4-one (1t). Yield: 64%. Mp = 123–125 °C (lit. 133–135 °C)³⁴ from MeOH. The compound exhibited spectroscopic data identical to those previously reported.³⁴

(E)-3-(2,3-Dimethoxybenzylidene)chroman-4-one (1u).

Yield: 53%. Mp = 108–109 °C EtOAc/petroleum ether. IR (KBr): 1673 cm⁻¹. ¹H NMR: δ (ppm) 7.90 (dd, 1H, H5, J₅₋₆ = 7.8 Hz, J₅₋₇ = 1.7 Hz), 7.82 (bs, 1H, =CH), 7.61 (ddd, 1H, H7, J₇₋₈ = 8.9 Hz, J₆₋₇ = 7.2 Hz, J₅₋₇ = 1.7 Hz), 7.22–7.12 (m, 3H, H6, H5', H6'), 7.05 (d, 1H, H8, J₇₋₈ = 8.3 Hz), 6.83 (dd, 1H, H4', J_{4'-5'} = 6.9 Hz, J_{4'-6'} = 2.2 Hz), 5.29 (d, 2H, H2, J_{all} = 1.8 Hz), 3.86 (s, 3H, OCH₃), 3.74 (s, 3H, OCH₃). ¹³C NMR: δ (ppm) 181.2, 160.8, 152.5, 147.6, 136.3, 132.2, 131.3, 127.6, 127.3, 124.1, 122.0, 121.8, 121.5, 118.0, 114.8, 67.6, 60.6, 55.8.

(E)-3-(2,3,4-Trimethoxybenzylidene)chroman-4-one (1v).

Yield: 43%. Mp = 116–118 °C (lit. viscous oil)⁵⁰ from acetone. IR (KBr): 1668 cm⁻¹. ¹H NMR: δ (ppm) 7.89 (dd, 1H, H5, J₅₋₆ = 7.8 Hz, J₅₋₇ = 1.8 Hz), 7.79 (bs, 1H, =CH), 7.60 (ddd, 1H, H7, J₇₋₈ = 8.9 Hz, J₆₋₇ = 7.2 Hz, J₅₋₇ = 1.8 Hz), 7.13 (ddd, 1H, H6, J₆₋₇ = 7.2 Hz, J₅₋₆ = 7.8 Hz, J₆₋₈ = 1.0 Hz), 7.05 (dd, 1H, H8, J₇₋₈ = 8.9 Hz, J₆₋₈ = 1.0 Hz), 6.98 (s, 1H, H6', J_{2'-6'} = 8.7 Hz), 6.92 (s, 1H, H5', J_{2'-6'} = 8.7 Hz), 5.33 (d, 2H, H2, J_{all} = 1.8 Hz), 3.88 (s, 3H, OCH₃), 3.83 (s, 3H, OCH₃), 3.80 (s, 3H, OCH₃). ¹³C NMR: δ (ppm) 181.1, 160.6, 155.3, 152.7, 141.7, 136.0, 132.0, 129.5, 127.2, 125.6, 121.9, 121.6, 120.3, 117.8, 107.7, 67.7, 61.3, 60.5, 56.0.

Synthesis of (E)-3-(3-Amino-4-hydroxybenzylidene)chroman-4-one (1r).

Tin(II) chloride (0.03 mol) in EtOH (15 mL) was added to a suspension of (E)-3-(4-hydroxy-3-nitrobenzylidene)chroman-4-one (1q) (0.005 mol) in EtOH (35 mL) and concentrated HCl (75 mL). The mixture was refluxed for 2 h under stirring. After cooling, the mixture was neutralized with 2 N NaOH and the solid was filtered off. The product was purified by column chromatography on silica gel (AcOEt/petroleum ether 1:1). Yield: 46%. Mp = 192–195 °C from EtOAc/petroleum ether. IR (KBr): 3460, 3380, 1655 cm⁻¹. ¹H NMR: δ (ppm) 9.80 (bs, 1H, OH), 7.86 (d, 1H, H5, J₅₋₆ = 7.6 Hz), 7.59–7.55 (m, 2H, =CH, H7), 7.12 (t, 1H, H6, J₅₋₆ = J₆₋₇ = 7.6 Hz), 7.04 (d, 1H, H8, J₇₋₈ = 8.3 Hz), 6.78–6.75 (m, 2H, H2', H6'), 6.61 (d, 1H, H5', J_{5'-6'} = 7.9 Hz), 5.43 (d, 2H, H2, J_{all} = 1.5 Hz), 4.76 (bs, 2H, NH₂). ¹³C NMR: δ (ppm) 181.0, 160.4, 146.5, 137.9, 137.0, 135.7, 127.1, 126.8, 125.3, 121.8, 121.7, 120.9, 117.7, 115.9, 114.3, 67.7.

Pharmacological Studies. Determination of hMAO Isoform Activity. The effects of the test compounds on hMAO isoform enzymatic activity were evaluated by a fluorimetric method following the experimental protocol previously described.⁵²

Reversibility and Irreversibility Experiments. To evaluate whether some of the tested compounds (1h and 1i) are reversible or irreversible hMAO-B inhibitors, an effective centrifugation-ultrafiltration method (so-called repeated washing) was used.⁴⁰

Molecular Modeling. Compounds 1h and 1i were built by means of the Maestro GUI.⁵³ Conformational properties of both molecules have been investigated by means of 1000 steps of Monte Carlo (MC) search as implemented in MacroModel, version 7.2.⁵⁴ Each conformer was energy-minimized using the AMBER⁵⁵ force field in united atoms

notation. Water solvent effects have been taken into account by means of the implicit model GB/SA.⁵⁶ The global minimum energy structures of **1h** and **1l** were considered for the next docking simulations. Crystallographic structures, deposited into the Protein Data Bank (PDB)⁵⁷ with codes 2ZSX⁵⁸ and 2V60,⁵⁹ were considered, after graphical manipulation (fixing missing atoms and/or bond order, adding nonaliphatic H atoms), as receptor models of hMAO-A and hMAO-B, respectively. The cocrystallized ligands harmine and 7-(3-chlorobenzoyloxy)-4-carboxaldehyde-coumarin, respectively, for 2ZSX and 2V60, were removed, FAD double bonds were fixed, and hydrogen atoms were added onto both proteins and cofactors.

According to the AutoDock Vina⁴⁸ docking methodology, a regular box of 15 625 Å³, centered on the FAD NS, was considered as the active site in both hMAO-A and -B receptor models. The global minimum energy conformers of **1h** and **1l** were submitted to flexible docking simulations. The estimation of the ΔG_{bind} was carried out according to the following equation:

$$\Delta G_{\text{bind}} = \sum_{c=1}^n \frac{(\text{IE}_c)(P_c)}{100}$$

where ΔG_{bind} is the Boltzmann averaged binding free energy, c is the configuration of the ligand–target complex, n is the maximum number of configurations, IE_c is the AutoDock Vina interaction energy of the configuration c , and P_c is the Boltzmann population at 300 K for configuration c .

■ ASSOCIATED CONTENT

S Supporting Information. Elemental analysis results (C, H, N, Cl) of tested compounds and molecular modeling details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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■ ACKNOWLEDGMENT

We thank MURST (Italy) for financial support.

■ ABBREVIATIONS USED

MAO, monoamine oxidase; MAO-A, monoamine oxidase A; MAO-B, monoamine oxidase B; PTK, protein tyrosine kinase; hMAO, human MAO; PDB, Protein Data Bank

■ REFERENCES

- (1) Kalgutkar, A. S.; Dalvie, D. K.; Castagnoli, N.; Taylor, T. J. Interactions of nitrogen-containing xenobiotics with monoamine oxidase (MAO) isozymes A and B: SAR studies on MAO substrates and inhibitors. *Chem. Res. Toxicol.* **2001**, *14*, 1139–1162.
- (2) Haung, R. H.; Faulkner, R. The role of phospholipid in the multiple functional forms of brain monoamine oxidase. *J. Biol. Chem.* **1981**, *256*, 9211–9215.
- (3) Cohen, G.; Farooqui, R.; Kesler, N. Parkinson disease: a new link between monoamine oxidase and mitochondrial electron flow. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4890–4894.
- (4) Edmondson, D. E.; Mattevi, A.; Binda, C.; Li, M.; Hubálek, F. Structure and mechanism of monoamine oxidase. *Curr. Med. Chem.* **2004**, *11*, 1983–1993.

- (5) Shih, J. C.; Chen, K. Regulation of MAO-A and MAO-B gene expression. *Curr. Med. Chem.* **2004**, *11*, 1995–2005.
- (6) Shih, J. C.; Chen, K.; Ridd, M. J. Monoamine oxidase: from genes to behavior. *Annu. Rev. Neurosci.* **1999**, *22*, 197–217.
- (7) Youdim, M. B. H.; Finberg, J. P. M. New directions in monoamine oxidase A and B. Selective inhibitors and substrates. *Biochem. Pharmacol.* **1991**, *41* (2), 155–162.
- (8) Gottowik, J.; Cesura, A. M.; Malherbe, P.; Lang, G.; Prada, M. D. Characterisation of wild-type and mutant forms of human monoamine oxidase A and B expressed in a mammalian cell line. *FEBS Lett.* **1993**, *317*, 152–156.
- (9) Geha, R. M.; Rebrin, I.; Chen, K.; Shih, J. C. Substrate and inhibitor specificities for human monoamine oxidase A and B are influenced by a single amino acid. *J. Biol. Chem.* **2001**, *276*, 9877–9882.
- (10) Westlund, K. N.; Denney, R. M.; Kochersperger, L. M.; Rose, R. M. Distinct monoamine oxidase A and B populations in primate brain. *Science* **1985**, *230*, 181–183.
- (11) Bach, A. W. J.; Lan, N. C.; Johnson, D. L.; Abell, C. W.; Bembenek, M. E.; Kwan, S. W.; Seeburg, P. H.; Shih, J. C. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymic properties. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4934–4938.
- (12) Grimsby, J.; Chen, K.; Wang, L. J.; Lan, N. C.; Shih, J. C. Human monoamine oxidase A and B genes exhibit identical exon–intron organization. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 3637–3641.
- (13) Riederer, P.; Lachenmayer, L.; Laux, G. Clinical applications of MAO-inhibitors. *Curr. Med. Chem.* **2004**, *11*, 2033–2043.
- (14) Da Prada, M.; Kettler, R.; Keller, H. H.; Burkard, W. P.; Muggli-Maniglio, D.; Haefely, W. E. Neurochemical profile of moclobemide, a short-acting and reversible inhibitor of monoamine oxidase type A. *J. Pharmacol. Exp. Ther.* **1989**, *248*, 400–414.
- (15) Da Prada, M.; Kettler, R.; Keller, H. H.; Cesura, A. M.; Richards, J. G.; Saura, M. J.; Muggli-Maniglio, D.; Wyss, P. C.; Kyburz, E.; Imhof, R. From moclobemide to Ro 19-6327 and Ro 41-1049: the development of a new class of reversible, selective MAO-A and MAO-B inhibitors. *J. Neural Transm., Suppl.* **1990**, *29*, 279–292.
- (16) Moureau, F.; Wouters, J.; Vercauteren, D. P.; Collin, S.; Evraud, G.; Durant, F.; Ducrey, F.; Koenig, J. J.; Jarreau, F. X. A reversible monoamine oxidase inhibitor, toloxatone: structural and electronic properties. *Eur. J. Med. Chem.* **1992**, *27*, 939–948.
- (17) Mazouz, F.; Gueddari, S.; Burstein, C.; Mansuy, D.; Milcent, R. 5-[4-(Benzyloxy)phenyl]-1,3,4-oxadiazol-2(3H)-one derivatives and related analogs: new reversible, highly potent, and selective monoamine oxidase type B inhibitors. *J. Med. Chem.* **1993**, *36*, 1157–1167.
- (18) Löscher, W.; Lehmann, H.; Teschendorf, H. J.; Traut, M.; Gross, G. Inhibition of monoamine oxidase type A, but not type B, is an effective means of inducing anticonvulsant activity in the kindling model of epilepsy. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 984–992.
- (19) Chen, J. F.; Xu, K.; Petzer, J. P.; Staal, R.; Xu, Y. H.; Beilstein, M.; Sonsalla, P. K.; Castagnoli, K.; Castagnoli, N., Jr.; Schwarzschild, M. A. Neuroprotection by caffeine and A(2A) adenosine receptor inactivation in a model of Parkinson's disease. *J. Neurosci.* **2001**, *21*, RC143.
- (20) Khan, K. M.; Rahat, S.; Choudhary, M. I.; Atta-ur-Rahman; Ghani, U.; Perveen, S.; Khatoun, S.; Dar, A.; Malik, A. Synthesis and biological screening of 2-substituted 5,6-dihydro-5-oxo-4H-1,3,4-oxadiazine-4-propanenitriles and of their intermediates. *Helv. Chim. Acta* **2002**, *85*, 559–570.
- (21) Gokhan, N.; Yesilada, A.; Ucar, G.; Erol, K.; Bilgin, A. A. 1-N-Substituted thiocarbonyl-3-phenyl-5-thienyl-2-pyrazolines: synthesis and evaluation as MAO inhibitors. *Arch. Pharm.* **2003**, *336*, 362–371.
- (22) Chimentì, F.; Maccioni, E.; Secci, D.; Bolasco, A.; Chimentì, P.; Granese, A.; Turini, P.; Alcaro, S.; Ortuso, F.; Cirilli, R.; La Torre, F.; Cardia, M. C.; Distinto, S. Synthesis, molecular modeling studies, and selective inhibitory activity against monoamine oxidase of 1-thiocarbonyl-3,5-diaryl-4,5-dihydro-(1H)-pyrazole derivatives. *J. Med. Chem.* **2005**, *48*, 7113–7122.

- (23) Dar, A.; Khan, K. M.; Ateeq, H. S.; Khan, S.; Rahat, S.; Perveen, S.; Supuran, C. T. Inhibition of monoamine oxidase-A activity in rat brain by synthetic hydrazines: structure–activity relationship (SAR). *J. Enzyme Inhib. Med. Chem.* **2005**, *20*, 269–274.
- (24) Chimenti, F.; Secci, D.; Bolasco, A.; Chimenti, P.; Granese, A.; Carradori, S.; Befani, O.; Turini, P.; Alcaro, S.; Ortuso, F. Synthesis, molecular modeling studies, and selective inhibitory activity against monoamine oxidase of *N,N'*-bis[2-oxo-2*H*-benzopyran]-3-carboxamides. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4135–4140.
- (25) Vlok, N.; Malan, S. F.; Castagnoli, N.; Bergh, J. J.; Petzer, J. P. Inhibition of monoamine oxidase B by analogues of the adenosine A2A receptor antagonist (*E*)-8-(3-chlorostyryl)caffeine (CSC). *Bioorg. Med. Chem.* **2006**, *14*, 3512–3521.
- (26) Chimenti, F.; Maccioni, E.; Secci, D.; Bolasco, A.; Chimenti, P.; Granese, A.; Befani, O.; Turini, P.; Alcaro, S.; Ortuso, F.; Cardia, M. C.; Distinto, S. Selective inhibitory activity against MAO and molecular modeling studies of 2-thiazolylhydrazone derivatives. *J. Med. Chem.* **2007**, *50*, 707–712.
- (27) Van den Berg, D.; Zoellner, K. R.; Ogunrombi, M. O.; Malan, S. F.; Terre'Blanche, G.; Castagnoli, N.; Bergh, J. J.; Petzer, J. P. Inhibition of monoamine oxidase B by selected benzimidazole and caffeine analogues. *Bioorg. Med. Chem.* **2007**, *15*, 3692–3702.
- (28) Al Nakib, T.; Bezjak, V.; Meegan, M. J.; Chandry, R. Synthesis and antifungal activity of some 3-benzylidenechroman-4-ones, 3-benzylideneethiochroman-4-ones and 2-benzylidene-1-tetralones. *Eur. J. Med. Chem.* **1990**, *25*, 455–462.
- (29) Desideri, N.; Olivieri, S.; Stein, M. L.; Sgro, R.; Orsi, N.; Conti, C. Synthesis and anti-picornavirus activity of homo-isoflavonoids. *Antiviral Chem. Chemother.* **1997**, *8* (1997), 545–555.
- (30) Quaglia, M. G.; Desideri, N.; Bossu, E.; Sgro, R.; Conti, C. Enantioseparation and anti-rhinovirus activity of 3-benzylchroman-4-ones. *Chirality* **1999**, *11* (5/6), 495–500.
- (31) Tait, S.; Salvati, A. L.; Desideri, N.; Fiore, L. Antiviral activity of substituted homoisoflavonoids on enteroviruses. *Antiviral Res.* **2006**, *72* (3), 252–255.
- (32) Wall, M. E.; Wani, M. C.; Manikumar, G.; Taylor, H.; McGivney, R. Plant antimutagens, 6. intricatin and intricatinol, new antimutagenic homoisoflavonoids from *hoffmanosseggia intricata*. *J. Nat. Prod.* **1989**, *52*, 774–778.
- (33) Miadokova, E.; Masterova, I.; Vlckova, V.; Duhova, V.; Toth, J. Antimutagenic potential of homoisoflavonoids from *Muscari racemosum*. *J. Ethnopharmacol.* **2002**, *81*, 381–386.
- (34) Perjési, P.; Das, U.; De Clercq, E.; Balzarini, J.; Kawase, M.; Sakagami, H.; Stables, J. P.; Lorand, T.; Rozmer, Z.; Dimmock, J. R. Design, synthesis and antiproliferative activity of some 3-benzylidene-2,3-dihydro-1-benzopyran-4-ones which display selective toxicity for malignant cells. *Eur. J. Med. Chem.* **2008**, *43*, 839–845.
- (35) Siddaiah, V.; Rao, C. V.; Venkateswarlu, S.; Krishnaraju, A. V.; Subbaraju, G. V. Synthesis, stereochemical assignments, and biological activities of homoisoflavonoids. *Bioorg. Med. Chem.* **2006**, *14*, 2545–2551.
- (36) Siddaiah, V.; Maheswara, M.; Rao, C. V.; Venkateswarlu, S.; Subbaraju, G. V. Synthesis, structural revision, and antioxidant activities of antimutagenic homoisoflavonoids from *Hoffmanosseggia intricata*. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1288–1290.
- (37) Kirkiacharian, S.; Tongo, H. G.; Bastide, J.; Bastide, P.; Grenie, M. M. Synthèse et activités angioprotectrice, anti-allergique et antihistaminique de benzyl-3 chromones (homo-isoflavones). *Eur. J. Med. Chem.* **1989**, *24*, 541–546.
- (38) Hung, T. M.; Thu, C. V.; Dat, N. T.; Ryoo, S. W.; Lee, J. H.; Kim, J. C.; Na, M.; Jung, H. J.; Bae, K.; Min, B. S. Homoisoflavonoid derivatives from the roots of *Ophiopogon japonicus* and their in vitro anti-inflammation activity. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2412–2416.
- (39) Lin, L. G.; Xie, H.; Li, H. L.; Tong, L. J.; Tang, C. P.; Ke, C. Q.; Liu, Q. F.; Lin, L. P.; Geng, M. Y.; Jiang, H.; Zhao, W. M.; Ding, J.; Ye, Y. Naturally occurring homoisoflavonoids function as potent protein tyrosine kinase inhibitors by c-Src-based high-throughput screening. *J. Med. Chem.* **2008**, *51*, 4419–4429.
- (40) Chimenti, F.; Fioravanti, R.; Bolasco, A.; Chimenti, P.; Secci, D.; Rossi, F.; Yañez, M.; Orallo, F.; Ortuso, F.; Alcaro, S. Chalcones: a valid scaffold for monoamine oxidases inhibitors. *J. Med. Chem.* **2009**, *52*, 2818–2824.
- (41) Chimenti, F.; Fioravanti, R.; Bolasco, A.; Chimenti, P.; Secci, D.; Rossi, F.; Yañez, M.; Orallo, F.; Ortuso, F.; Alcaro, S.; Cirilli, R.; Ferretti, R.; Sanna, M. L. A new series of flavones, thioflavones, and flavanones as selective monoamine oxidases-B inhibitors. *Bioorg. Med. Chem.* **2010**, *18*, 1273–1279.
- (42) Krishnamurty, H. G.; Parkash, B.; Seshadri, T. R. Synthesis of eucomin, 4'-demethyleucomin and 5,7-di-O-methyleucomol. *Indian J. Chem.* **1974**, *12* (6), 554–556.
- (43) Yañez, M.; Fraiz, N.; Cano, E.; Orallo, F. Inhibitory effects of cis- and trans-resveratrol on noradrenaline and 5-hydroxytryptamine uptake and on monoamine oxidase activity. *Biochem. Biophys. Res. Commun.* **2006**, *344* (2), 688–695.
- (44) Silverman, R. B. Enzyme Inhibition. In *Wiley Encyclopedia of Chemical Biology*; John Wiley & Sons, Inc.: Hoboken, NJ, 2009; Vol. 1, pp 663–681.
- (45) Gerlach, M.; Riederer, P.; Youdim, M. B. The molecular pharmacology of L-deprenyl. *Eur. J. Pharmacol.* **1992**, *226*, 97–108.
- (46) O'Brien, E. M.; Tipton, K. F.; Meroni, M.; Dostert, P. Inhibition of monoamine oxidase by clorgyline analogs. *J. Neural Transm., Suppl.* **1994**, *41*, 295–305.
- (47) Chimenti, F.; Maccioni, E.; Secci, D.; Bolasco, A.; Chimenti, P.; Granese, A.; Carradori, S.; Alcaro, S.; Ortuso, O.; Yañez, M.; Orallo, F.; Cirilli, R.; Ferretti, R.; La Torre, F. Synthesis, stereochemical identification, and selective inhibitory activity against human monoamine oxidase-B of 2-methylcyclohexylidene-(4-arylthiazol-2-yl)hydrazones. *J. Med. Chem.* **2008**, *51*, 4874–4880.
- (48) Trott, O.; Olson, J. A. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010**, *31*, 455–461.
- (49) Pfeiffer, P.; Breit, E.; Hoyer, H. Oxy-benzyl-chromanone. XI. Miettelung zur brasilin- und haematoxylinfrage. *J. Prakt. Chem.* **1931**, *129*, 31–54.
- (50) Sathunuru, R.; Koh, B.; Zhang, H.; Biehl, E. One-pot synthesis of *trans*-7-aryl-6*H*-6a,7-dihydro[1]benzopyrano[3,4-*c*][1,5]benzothiazepines. *Heterocycles* **2005**, *65* (10), 2493–2503.
- (51) Bennett, P.; Donnelly, J. A.; Meaney, D. C.; O'Boyle, P. Stereochemistry of cyclopropyl ketones from the reaction of dimethylsulfoxonium methylide with 3-benzylidene-4-chromanones. *J. Chem. Soc., Perkin Trans. 1* **1972**, *12*, 1554–1559.
- (52) Chimenti, F.; Secci, D.; Bolasco, A.; Chimenti, P.; Bizzarri, B.; Granese, A.; Carradori, S.; Yañez, M.; Orallo, F.; Ortuso, F.; Alcaro, S. Synthesis, molecular modeling, and selective inhibitory activity against human monoamine oxidases of 3-carboxamido-7-substituted coumarins. *J. Med. Chem.* **2009**, *52* (7), 1935–1942.
- (53) *Maestro*, version 4.1; Schroedinger Inc.: Portland, OR, 1998–2001.
- (54) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel, an integrated software system for modeling organic and bioorganic molecules using molecular mechanics. *J. Comput. Chem.* **1990**, *11*, 440–467.
- (55) (a) McDonald, D. Q.; Still, W. C. AMBER torsional parameters for the peptide backbone. *Tetrahedron Lett.* **1992**, *33*, 7743–7746.
(b) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. A new force field for molecular mechanical simulation of nucleic acids and proteins. *J. Am. Chem. Soc.* **1984**, *106*, 765–784.
- (56) Hasel, W.; Hendrickson, T. F.; Still, W. C. A rapid approximation to the solvent accessible surface areas of atoms. *Tetrahedron Comput. Methodol.* **1988**, *1*, 103–116.
- (57) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235–242.

(58) Son, S. Y.; Ma, J.; Kondou, Y.; Yoshimura, M.; Yamashita, E.; Tsukihara, T. Structure of human monoamine oxidase A at 2.2-Å resolution: the control of opening the entry for substrates/inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 5739–5744.

(59) Binda, C.; Wang, J.; Pisani, L.; Caccia, C.; Carotti, A.; Salvati, P.; Edmondson, D. E.; Mattevi, A. Structures of human monoamine oxidase B complexes with selective noncovalent inhibitors: safinamide and coumarin analogs. *J. Med. Chem.* **2007**, *50*, 5848–5852.