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Synthesis, Molecular Modeling, and Selective Inhibitory Activity against Human Monoamine Oxidases of 3-Carboxamido-7-Substituted Coumarins†

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A large series of 3-carboxamido-7-substituted coumarins have been synthesized and tested in vitro for their human monoamine oxidase A and B (hMAO-A and hMAO-B) inhibitory activity. Taking into account all the relevant structural information on MAOs reported in the literature, we made some changes in the coumarin nucleus and examined with particular attention the effect on activity and selectivity of substituting at position 3 with *N*-aryl or *N*-alkyl carboxamide and at position 7 with a benzyloxy or a 4′-F-benzyloxy group. Some of the assayed compounds proved to be potent, selective inhibitors of hMAO-B with IC_{50} values in the micromolar range. To better understand the enzyme−inhibitor interaction and to explain the selectivity of the most active compounds toward hMAOs, molecular modeling studies were carried out on new, high resolution, hMAO-A and hMAO-B crystallographic structures.

Introduction

Monoamine oxidases (MAOs; EC 1.4.3.4)[a] are widespread enzymes responsible for the regulation and metabolism of major monoamine neurotransmitters (5-hydroxytryptamine (5-HT), norepinephrine, dopamine), modulating their concentrations in the brain and peripheral tissues. They are also involved in the biodegradation of exogenous amines such as tyramine and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). According to their substrate specificity, inhibitor sensitivity, and amino acid sequence, the following two isoforms were fully characterized:[1] hMAO-A and hMAO-B. Binda et al.[2,3] described the crystal structure of these two subtypes, highlighting the selective interaction between them and their ligands. This led to renewed interest in the rational design of potent, specific inhibitors with therapeutic potential and no undesirable side effects.[4] As mentioned above, hMAOs play an important role in the metabolism of several neurotransmitters and could be useful in the treatment of a number of psychiatric and neurological diseases. In fact, human MAO-B inhibitors are used alone or in combination in the therapy of Alzheimer's and Parkinson's diseases,[5] while human MAO-A inhibitors are antidepressants and antianxiety agents (Figure 1).[6] Furthermore, interest in selective inhibitors of hMAO-B has increased in recent years[7] due to the discovery of an age-related increase in hMAO-B expression after the 60th year of life, especially in glial cells.[8−10] MAO-B activity is believed to cause oxidative stress, during catalytic turnover, because of the production of hydrogen peroxide (H_2O_2) and other reactive oxygen species. Therefore, a selective hMAO-B inhibitor, insofar as it decreases the rate of oxidative deamination, could contribute to neuroprotection and prevent neuronal degeneration.[11]

In spite of such considerable progress in our understanding of the interactions of the two enzyme forms with their preferred substrates and inhibitors, no general rules are available for the design of potent, selective inhibitors of hMAOs. At the same time, a great deal of literature data on the importance of an acyl group in position C3 on the coumarin nucleus considers it crucial for a variety of pharmacological effects such as anticoagulant,[12] antineoplastic,[13] antimicrobial,[14,15] antidepressant,[16] radical scavenger,[17] anti-*Helicobacter pylori*,[18] antiinflammatory,[19] protease, and β-secretase inhibitory effects[20] and others.[21]

So far, among several MAO inhibitors, some (2*H*)-1-benzopyran-2-one derivatives (coumarins) have been screened for their good inhibitory activities (Figure 2).[22−28] Starting from these studies, our research group first investigated the MAO inhibitory activity of *N*,*N*′-bis[2-*oxo*-2*H*-benzopyran]-3-carboxamides.[29] The good results encouraged us to assay a large array of 3-carboxamido coumarins in order to evaluate the influence of a number of substitutions at this position as regards size, length, and lipophilic or electronic characteristics (H-bonding properties).

Chemistry. Most of the coumarin derivatives, (d) (Scheme 1), were synthesized starting from commercial substituted salicylaldehydes according to the pathway reported in Scheme 1 and are listed in Table 1. After Knoevenagel cyclization to ethyl esters of (2*H*)-1-benzopyran-2-one-3-carboxylic acids (a) (Scheme 1), the compounds bearing an OH group at position 7 were subsequently functionalized by benzylation in the presence of *N*,*N*′-dicyclohexyl-18-crown-6-ether, which by chelating potassium ion facilitated the nucleophilic attack to improve the yields of the related compounds (b) (Scheme 1). Hydrolysis with 10% sodium hydroxide and treatment of intermediates (a) and

† PDB ID of hMAO-A: 2BXR and 2Z5X; PDB ID of hMAO-B: 1GOS and 2BK3.

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[a] Abbreviations: MAO, monoamine oxidase; 5-HT (5-hydroxytryptamine); MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MC, Monte Carlo; PDB, Protein Data Bank. Abbreviations used for amino acids follow the rules of the IUPACIUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* **1972**, *247*, 977−983. Amino acid symbols denote L-configuration unless indicated.

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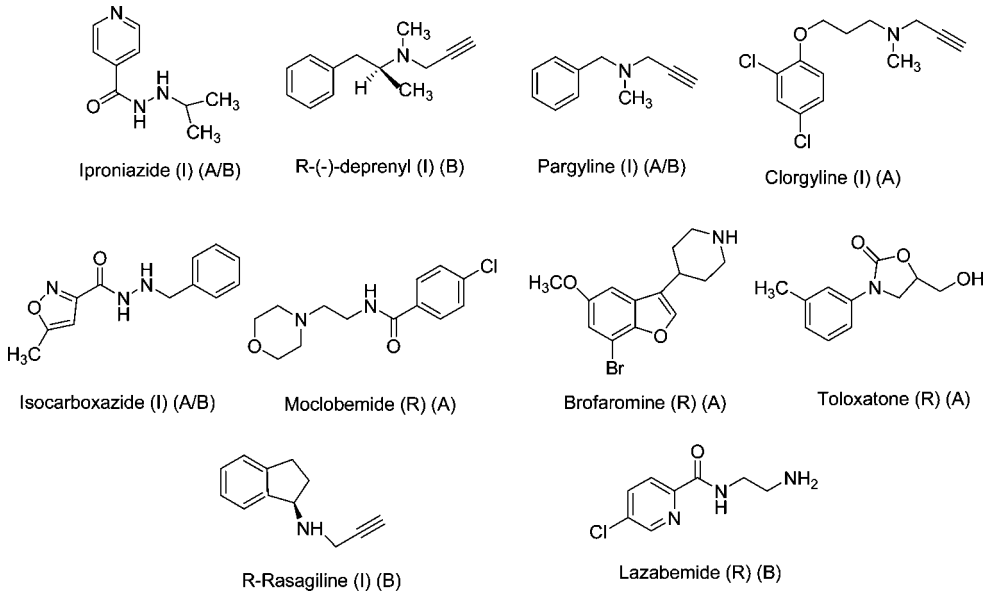


Figure 1. Irreversible (I), reversible (R), and selective hMAO-A and hMAO-B inhibitors.

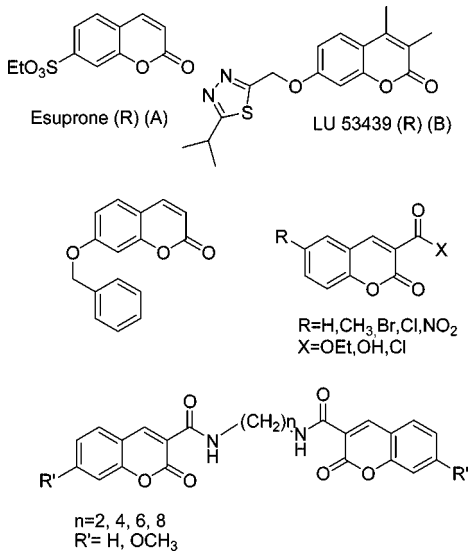
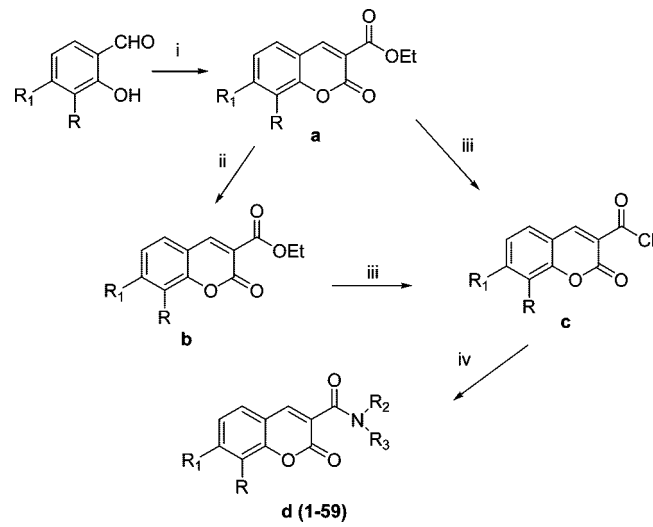


Figure 2. Structures of known coumarin-based MAO inhibitors.

Scheme 1[a]



[a] Reagents and conditions: (i) Diethyl malonate, piperidine (cat.). EtOH (ii) only when R_1 = OH, potassium carbonate, suitable benzyl bromide, *cis*-dicyclohexyl-18-crown-6, anhydrous acetone. (iii) (1) NaOH 10%, (2) HCl 3N, (3) $SOCl_2$, reflux. (iv) HNR^2R^3, suitable solvent.

(b) with thionyl chloride at reflux led to the desired derivatives (c) (Scheme 1). These are particularly reactive toward nucleophilic substitution with different amines, which were used in a double molar ratio in order to neutralize the excess of HCl.

Compounds **19** and **20** were prepared by alkaline hydrolysis and chlorination of compound **15** as reported in our previous article.[29]

Oxidation of compound **17** with oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) led to compound **21** in quantitative yield. Hydrolysis of arylmethyl ether **16** with hydroiodic acid 57% at reflux gave the desired compound **22**.

Biochemistry. The potential effects of the test drugs on hMAO activity were investigated by measuring their effects on the production of H_2O_2 from *p*-tyramine, using the Amplex Red MAO assay kit (Molecular Probes, Inc., Eugene, OR) and microsomal MAO isoforms prepared from insect cells (BTI-TN-5B1-4) infected with recombinant baculovirus containing cDNA inserts for hMAO-A or hMAO-B (Sigma-Aldrich Química S.A., Alcobendas, Spain).

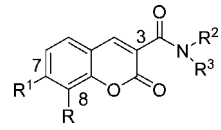
The production of H_2O_2 catalyzed by MAO isoforms can be detected using 10-acetyl-3,7-dihydroxyphenoxazine (Amplex Red reagent), a nonfluorescent and highly sensitive probe that reacts with H_2O_2 in the presence of horseradish peroxidase to produce a fluorescent product, resorufin. In this study, hMAO activity was evaluated using the above method following the general procedure previously described by us.[30] The test drugs (new compounds and reference inhibitors) themselves were unable to react directly with the Amplex Red reagent, which indicates that these drugs 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).

Most tested drugs inhibited this enzymatic control activity in a concentration-dependent way (see Table 1).

Results and Discussion

All synthesized compounds **1**−**59** were assayed for hMAO-A and hMAO-B inhibitory activity. By analyzing the data reported

Table 1. Structures and Biological Activity of Derivatives **1**−**59**



compd	R	R[1]	R[2]	R[3]	IC_{50}hMAO-A μM	IC_{50}hMAO-B μM	SI[f]	lit.
1	H	H	H	C_6H_{11}	*d*	0.50 ± 0.03	>200	31
2	H	H	H	*i*-C_3H_7	*d*	25.36 ± 1.88	>3.9	32
3	H	H	H	*i*-C_4H_9	*d*	3.27 ± 0.26	>31	33
4	H	H	H	CH_2CCH	*d*	*d*		
5	H	H	H	$CH_2CH_2NH_2$	*d*	7.68 ± 0.38	>13	34
6	H	H	H	Ph	13.12 ± 0.91[a]	0.76 ± 0.04	17	31
7	H	H	H	(2′-$C_6H_5CH_2$-Ph)	*d*	0.91 ± 0.03	>110	
8	H	H	H	(3′-CH_3-Ph)	14.50 ± 1.13[a]	0.60 ± 0.01	24	35
9	H	H	H	(3′-OCH_3-Ph)	*d*	0.64 ± 0.01	>156	36
10	H	H	H	(3′-F-Ph)	*d*	0.25 ± 0.01	>400	
11	H	H	H	(3′-CF_3-Ph)	*d*	0.25 ± 0.01	>400	
12	H	H	H	(4′-CH_3-Ph)	19.45 ± 1.53[a]	0.71 ± 0.01	27	31
13	H	H	H	(4′-C_2H_5-Ph)	15.67 ± 1.15[a]	0.89 ± 0.02	18	
14	H	H	H	(4′-i-C_3H_7-Ph)	15.14 ± 1.47[a]	0.65 ± 0.02	23	
15	H	H	H	(4′-COOEt-Ph)	15.98 ± 0.98	*d*	<0.16	19
16	H	H	H	(4′-OCH_3-Ph)	*d*	0.82 ± 0.01	>122	31
17	H	H	H	(4′- SCH_3-Ph)	14.76 ± 1.34[a]	2.84 ± 0.21	5.2	19
18	H	H	H	(4′-F-Ph)	13.06 ± 0.12[a]	0.067 ± 0.002	195	
19	H	H	H	(4′-COOH-Ph)	9.97 ± 0.87[a]	1.14 ± 0.09	8.7	19
20	H	H	H	(4′-COCl-Ph)	9.67 ± 0.29	*d*	<0.10	19
21	H	H	H	(4′-SO_2CH_3-Ph)	9.33 ± 0.73[a]	0.0014 ± 0.00012	6,664	
22	H	H	H	(4′-OH-Ph)	*d*	0.80 ± 0.01	>125	33
23	H	H	H	(2′-Cl-6′-CH_3-Ph)	*d*	19.07 ± 1.49	>5.2	
24	H	H	H	(2′,5′-CH_3-Ph)	15.95 ± 1.48[a]	22.45 ± 1.52	0.71	37
25	H	H	H	(2′,4′-CH_3-Ph)	12.32 ± 1.35	13.74 ± 1.32	0.89	
26	H	H	H	(2′,3′-CH_3-Ph)	20.63 ± 1.07	18.27 ± 1.57	1.1	
27	H	H	H	(2′,6′-CH_3-Ph)	9.79 ± 0.88[a]	1.29 ± 0.07	7.6	
28	H	H	H	(2′,6′-F-Ph)	12.76 ± 1.05[b]	9.23 ± 0.02	1.4	
29	H	H	H	(3′,4′-CH_3-Ph)	18.77 ± 1.47[a]	0.38 ± 0.02	49	38
30	H	H	H	(3′,4′-OCH_3-Ph)	9.45 ± 0.53[a]	0.86 ± 0.04	11	
31	H	H	H	(3′,5′-CH_3-Ph)	17.56 ± 1.60[a]	0.70 ± 0.01	25	
32	H	H	H	(3′,5′-OCH_3-Ph)	61.46 ± 6.13[a]	4.18 ± 0.12	15	
33	H	H	H	(2′,3′,4′,5′,6′-F-Ph)	*d*	0.46 ± 0.04	>217	19
34	H	H	H	(2′,3′,5′,6′-F-4′-CN-Ph)	16.04 ± 0.95[a]	0.86 ± 0.02	19	19
35	H	H	H	(2′,3′,5′,6′-F-pyridin-4′-yl)	11.43 ± 0.74[a]	0.58 ± 0.01	20	19
36	H	H	H	CH_2Ph	*d*	1.96 ± 0.15	>51	31
37	H	H	H	CH_2CH_2Ph	*d*	*d*		39
38	H	H	CH_3	Ph	*d*	8.89 ± 0.56	>11	
39	H	H	CH_3	CH_2Ph	*d*	*d*		
40	H	H	Ph	Ph	*d*	*d*		40
41	H	OCH_2Ph	H	C_6H_{11}	*d*	64.45 ± 5.98	>1.5	
42	H	OCH_2Ph	H	*i*-C_3H_7	63.52 ± 5.34	*d*	<0.63	
43	H	OCH_2Ph	H	*i*-C_4H_9	58.64 ± 5.09[a]	9.59 ± 0.45	6.1	
44	H	OCH_2Ph	H	(4′-*i*-C_3H_7-Ph)	60.93 ± 5.19[a]	3.69 ± 0.26	16	
45	H	OCH_2Ph	H	(2′,3′,5′,6′-F-4′-CN-Ph)	61.38 ± 4.93[a]	16.54 ± 0.87	3.7	
46	H	OCH_2Ph	H	(2′-$C_6H_5CH_2$-Ph)	51.89 ± 5.32	63.52 ± 4.64	0.82	
47	H	OCH_2Ph	H	(2′-CH_3-6′-Cl-Ph)	49.75 ± 2.36[b]	61.49 ± 3.47	0.81	
48	H	OCH_2Ph	H	(3′,4′-OCH_3-Ph)	*d*	4.39 ± 0.38	>23	
49	H	OCH_2Ph	H	(3′,5′-OCH_3-Ph)	52.89 ± 5.12	3.36 ± 0.09	16	
50	H	OCH_2Ph	H	CH_2Ph	*d*	0.99 ± 0.08	>101	
51	H	OCH_2Ph	H	NHPh	41.40 ± 2.45[a]	4.52 ± 0.33	9.2	
52	H	OCH_2(4′-F-Ph)	H	(2′,3′,5′,6′-F-4′-CN-Ph)	58.49 ± 4.33[a]	3.45 ± 0.28	17	
53	H	OCH_2(4′-F-Ph)	H	(2′-$C_6H_5CH_2$-Ph)	55.83 ± 3.68	*d*	<0.56	
54	H	OCH_2(4′-F-Ph)	H	(2′-CH_3-6′-Cl-Ph)	59.56 ± 5.29[b]	41.64 ± 2.95	1.4	
55	H	OCH_2(4′-F-Ph)	H	(3′,4′-OCH_3-Ph)	49.20 ± 2.35[a]	2.87 ± 0.25	17	
56	H	OCH_2(4′-F-Ph)	H	(3′,5′-OCH_3-Ph)	52.62 ± 3.83[a]	21.56 ± 2.18	2.4	
57	H	OCH_2(4′-F-Ph)	H	(4′-*i*-C_3H_7-Ph)	*d*	*d*		
58	H	OCH_2Ph	CH_3	Ph	*d*	2.37 ± 0.08	>42	
59	CH_3	OCH_2Ph	H	(3′,4′-OCH_3-Ph)	48.95 ± 5.18[a]	4.98 ± 0.35	9.8	
C[e]					0.004 ± 0.0002[a]	61.35 ± 1.13	0.000065	
D[e]					67.25 ± 1.02	0.020 ± 0.001	3,362	
I[e]					6.56 ± 0.76	7.54 ± 0.36	0.87	
M[e]					361.38 ± 19.37	*c*	<0.36	

Each IC_{50} value is the mean ± SEM from five experiments. Level of statistical significance: [a] $P < 0.01$. Each IC_{50} value is the mean ± SEM from five experiments. Level of statistical significance: [b] $P < 0.05$ versus the corresponding IC_{50} values obtained against hMAO-B, as determined by ANOVA/Dunnett's. [c] Inactive at 1 mM (highest concentration tested). [d] Inactive at 100 μM (highest concentration tested). [e] **C** = Clorgyline, **D** = *R*-(−)-deprenyl, **I** = Iproniazid, **M** = Moclobemide. [f] SI: MAO-B selectivity index = $IC_{50\ (MAO\text{-}A)}/IC_{50\ (MAO\text{-}B)}$.

in Table 1, it can be seen that most compounds show a selective inhibitory activity toward hMAO-B. Substitution at position 7 of the coumarin ring leads to compounds **41**−**58** with minor activity toward the two isoforms, and substitution of both amidic nitrogens (compounds **38**−**40** and **58**) appears to be unfavorable.

Inhibitory activity increases when the nitrogen is substituted with a phenyl group, and as regards compound **6** (IC_{50} against hMAO-B = 0.76 ± 0.04 μM), it can be seen that the presence of an electrondrawing group such as fluorine (compound **10**, IC_{50} against hMAO-B = 0.25 ± 0.01 μM) or trifluoromethyl

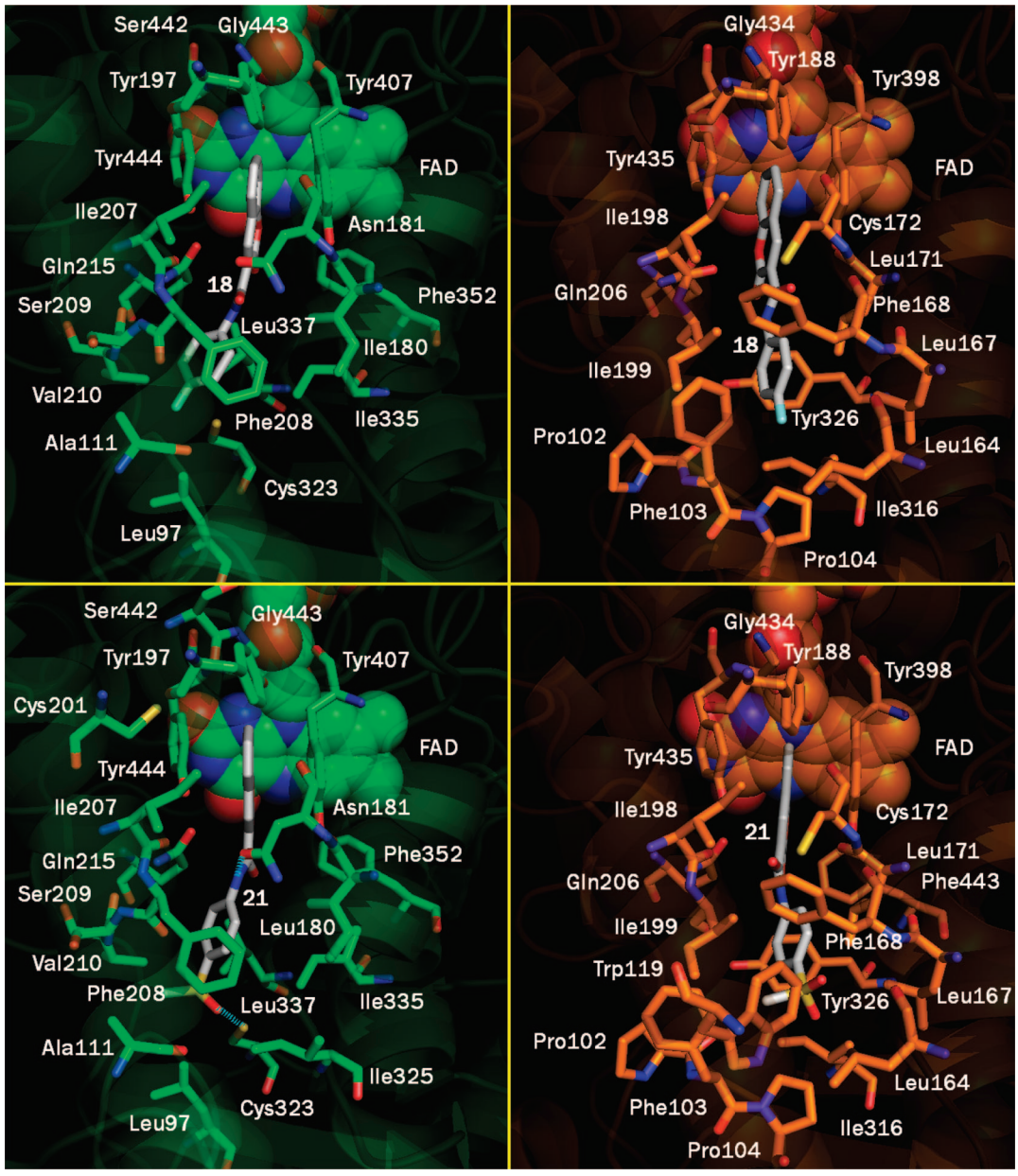


Figure 3. Most stable binding poses of compounds **18** (top) and**21** (bottom) with respect to hMAO-A (left side) and hMAO-B (right side). Ligands are shown in CPK colored sticks, interacting residues are colored in green (hMAO-A) or orange (hMAO-B), carbons in stick rendering. FAD is reported in spacefill with green (hMAO-A) or orange (hMAO-B) carbon atoms. Noninteracting aminoacids are in transparent green (hMAO-A) or orange (hMAO-B) cartoon notation. Inhibitor−enzyme hydrogen bonds are reported in cyan dashed lines.

(compound **11**, IC_{50} against hMAO-B = 0.25 ± 0.01 μM) is preferable for activity, whereas di- or poly substitution leads to poorly active compounds.

A fluorine atom present in 4′-position of the N-phenyl moiety improves activity compared to a fluorine atom present at the 3′-position, as observed in compounds **18** and **10** with IC_{50} = 0.067 ± 0.002 and 0.25 ± 0.01 μM, respectively. The most active and selective compound **21** has a methanesulfonyl group at the 4′-position of the *N*-phenyl substituent (IC_{50} = 0.0014 ± 0.00012 μM).

With the aim of carrying out a detailed analysis of the hMAOs binding modes of **18** and **21**, we graphically inspected the most stable configurations of all complexes of these compounds with both isoforms (Figure 3). Both ligands showed productive recognition of both hMAO-Aand hMAO-B. The coumarin moiety was always located in the known hMAOs binding clefts, leaving the lactone function close to the FAD cofactor. In all cases the coumarin ring was involved in stacking contacts to Tyr407 and Tyr444 of hMAO-A and to Tyr435 and Tyr398 of hMAO-B. As regards the number of interactions with other residues, **21** showed the most, followed by **18**. Remarkably **18** showed no hydrogen bonds with the targets, while **21** in hMAO-A did. This could partially explain the lower affinity shown by 18 for hMAO-A compared to the other modeled ligand, isoform selectivity could be due to the exclusive recognition in hMAO-B of an additional cleft (Phe103, Pro104, Trp119, Leu164, Leu167, Phe168) (Table 2).

Experimental Section

Chemistry. Unless otherwise noted, starting materials and reagents were obtained from commercial suppliers and were used without purification. Melting points (mp) were determined by the capillary method on an FP62 apparatus (Mettler-Toledo) and are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz on a

Table 2. List of hMAO-A and hMAO-B Corresponding Residues Recognized by **18** and **21**

corresponding residues		compd	
hMAO-A	hMAO-B	**18**	**21**
Leu97	Leu88	*a*	*a*
Ala111	Pro102	*ab*	*ab*
Phe112	Phe103	*b*	*b*
Pro113	Pro104	*b*	*b*
Trp128	Trp119		*b*
Phe173	Leu164	*b*	*b*
Leu176	Leu167	*b*	*b*
Phe177	Phe168	*b*	*b*
Ile180	Leu171	*ab*	*ab*
Asn181	Cys172	*ab*	*ab*$^+$
Tyr197	Tyr188	*ab*	*ab*
Cys201	Cys192		*a*
Ile207	Ile198	*ab*	*ab*
Phe208	Ile199	*ab*	*ab*
Ser209	Ser200	*a*	*a*
Val210	Thr201	*a*	*a*
Gln215	Gln206	*ab*	*ab*
Cys323	Thr314	*a*	*a*$^+$
Ile325	Ile316	*b*	*ab*
Ile335	Tyr326	*ab*	*ab*
Leu337	Leu328	*a*	*a*
Phe352	Phe343	*a*	*ab*
Tyr407	Tyr398	*ab*	*ab*
Ser442	Ser433		*a*
Gly443	Gly434	*ab*	*ab*
Tyr444	Tyr435	*ab*	*ab*
FAD	FAD	*ab*	*ab*

a = hMAO-A interaction. *b* = hMAO-B interaction. + = Hydrogen bond interaction.

Bruker spectrometer using DMSO-d_6 or $CDCl_3$ as solvent. Chemical shifts are expressed as δ units (ppm) relative to TMS. Coupling constants *J* are expressed in hertz (Hz). Elemental analyses for C, H, and N were determined with a Perkin-Elmer 240 B microanalyzer, and the analytical results were ≥95% purity for all compounds. All reactions were monitored by TLC performed on 0.2 mm thick silica gel plates (60 F_{254} Merck). Preparative flash column chromatography was carried out on silica gel (230−400 mesh, G60 Merck). Mass spectra (EI) were obtained with a Fisons QMD 1000 mass spectrometer (70 eV, 200 μA, ion source temperature 200 °C). The samples were introduced directly into the ion source. In the mass spectra, the fragment ion at *m/z* = 173, corresponding to the 3-acyl coumarin structure was always the most abundant observed.

The synthesis of some of these compounds has been described in previous references and was performed with slight changes. Their analytical and spectral data were in full agreement with those reported in the literature.

General Procedure for the Synthesis of Coumarin Derivatives 1−59. The starting coumarin-3-carboxylic acid ethyl ester (a) (Scheme 1) was prepared by Knoevenagel reaction between diethyl malonate (1 mmol) and the appropriate salicylaldehyde (1 mmol) with catalytic amounts of piperidine in ethanol. Then, if there was an OH group at position 7, etherification was performed by adding a suitable benzyl bromide (1 mmol) and potassium carbonate (1 mmol) in dry acetone, using *N*,*N*′-dicyclohexyl-18-crown-6-ether (1 mmol) as a chelating agent. All derivatives (a) and (b) (Scheme 1) were dissolved in 10% NaOH (50 mL) and 3 N HCl (50 mL) was added. The suspension was filtered and the solid refluxed under magnetic stirring with thionyl chloride (30 mL) to give the desired compound (c) (Scheme 1). Finally, a solution of the appropriate amine (2 mmol) in toluene or ethyl ether was added dropwise and stirred for 96 h at reflux. The organic layer was concentrated under vacuum, cooled to 4 °C, and filtered. The solid was purified by crystallization from ethanol or chromatography.

***N*-(Prop-2-ynyl)-2-*oxo*-2*H*-chromene-3-carboxamide (4).** Yield 58%; mp 182−183 °C. ^{1}H NMR ($CDCl_3$) 2.27 (s, 1H, CC*H*), 4.26 (s, 2H, NHCH_2), 7.38−7.43 (m, 2H, Ar*H*); 7.67−7.71 (m, 2H, Ar*H*), 8.96 (s, 1H, ArC*H*═), 9.03 (bs, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 228.

***N*-(2-Benzylphenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (7).** Yield 60%; mp 191−193 °C. ^{1}H NMR (DMSO-d_6) 4.04 (s, 2H, ArCH_2Ar), 7.19−7.22 (m, 5H, Ar*H*), 7.46−7.54 (m, 3H, Ar*H*), 7.77 (m, 2H, Ar*H*), 8.03−8.05 (m, 3H, Ar*H*), 8.97 (s, 1H, ArC*H*═), 10.64 (s, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 342.

***N*-(3-Fluorophenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (10).** Yield 73%; mp 242−243 °C. ^{1}H NMR (DMSO-d_6) 6.98−7.00 (m, 2H, Ar*H*), 7.43−7.49 (m, 2H, Ar*H*), 7.76−7.79 (m, 2H, Ar*H*), 8.00−8.01 (m, 2H, Ar*H*), 8.91 (s, 1H, ArC*H*═), 10.78 (s, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 284.

***N*-(3-Trifluoromethylphenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (11).** Yield 60%; mp 210−211 °C. ^{1}H NMR (DMSO-d_6) 7.51−7.64 (m, 3H, Ar*H*), 7.84−7.85 (m, 2H, Ar*H*), 8.03−8.05 (m, 1H, Ar*H*), 8.37−8.38 (m, 2H, Ar*H*), 9.07 (s, 1H, ArC*H*═), 11.12 (bs, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 334.

***N*-(4-Ethylphenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (13).** Yield 58%; mp 176−179 °C. ^{1}H NMR (DMSO-d_6) 1.16 (s, 3H, CH_2CH_3), 2.58 (s, 2H, ArCH_2CH_3), 7.23 (s, 2H, Ar*H*), 7.46 (s, 1H, Ar*H*), 7.55 (s, 1H, Ar*H*), 7.63 (s, 2H, Ar*H*), 7.77 (s, 1H, Ar*H*), 8.00 (s, 1H, Ar*H*), 8.91 (s, 1H, ArC*H*═), 10.59 (s, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 294.

***N*-(4-Isopropylphenyl)-2-*oxo*-2*H*-chromene-3-carboxamide)-amide (14).** Yield 68%; mp 160−165 °C. ^{1}H NMR (DMSO-d_6) 1.17−1.20 (d, *J* = 6.1 Hz, 6H, CH(CH_3)$_2$), 2.85−2.86 (m, 1H, ArC*H*(CH_3)$_2$), 7.23 (s, 2H, Ar*H*), 7.44 (s, 1H, Ar*H*), 7.55 (s, 1H, Ar*H*), 7.61 (s, 2H, Ar*H*), 7.76 (s, 1H, Ar*H*), 7.99 (s, 1H, Ar*H*), 8.90 (s, 1H, ArC*H*═), 10.59 (s, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 308.

***N*-(4-Fluorophenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (18).** Yield 59%; mp 251−252 °C. ^{1}H NMR ($CDCl_3$) 7.07−7.11 (t, 2H, Ar*H*), 7.43−7.48 (t, 2H, Ar*H*), 7.70−7.77 (m, 4H, Ar*H*), 9.03 (s, 1H, ArC*H*═), 10.83 (bs, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 284.

***N*-(4-Methanesulfonylphenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (21).** Compound **17** (1 mmol) was heated at 200 °C in methanol (50 mL). A solution of oxone (3 mmol) in H_2O (10 mL) was added dropwise followed by 24 h of stirring at room temperature. The suspension was filtered and the crude product was purified by crystallization (EtOH). Yield 99%; mp >280 °C. ^{1}H NMR ($CDCl_3$) 3.07 (s, 3H, SO_2CH_3), 7.42−7.49 (t, 2H, Ar*H*), 7.70−7.75 (t, 2H, Ar*H*), 7.93−7.96 (m, 4H, Ar*H*), 9.05 (s, 1H, ArC*H*═), 11.12 (bs, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 344.

***N*-(2-Chloro-6-methylphenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (23).** Yield 60%; mp 202−206 °C. ^{1}H NMR (DMSO-d_6) 2.22 (s, 3H, ArCH_3), 7.15−7.26 (m, 3H, Ar*H*), 7.34−7.38 (m, 2H, Ar*H*), 7.77 (s, 1H, Ar*H*), 8.01 (s, 1H, Ar*H*), 8.93 (s, 1H, ArC*H*═), 10.23 (bs, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 314.

***N*-(2,4-Dimethylphenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (25).** Yield 58%; mp 243−246 °C. ^{1}H NMR (DMSO-d_6) 2.22 (s, 3H, ArCH_3), 2.29 (s, 3H, ArCH_3), 7.05 (s, 1H, Ar*H*), 7.55−7.80 (m, 3H, Ar*H*), 8.01−8.04 (m, 3H, Ar*H*), 9.00 (s, 1H, ArC*H*═), 10.60 (bs, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 294.

***N*-(2,3-Dimethylphenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (26).** Yield 57%; mp 227−229 °C. ^{1}H NMR (DMSO-d_6) 2.21 (s, 3H, ArCH_3), 2.29 (s, 3H, ArCH_3), 7.00−7.03 (m, 2H, Ar*H*), 7.46−7.49 (m, 2H, Ar*H*), 7.79 (m, 1H, Ar*H*), 8.02−8.05 (m, 2H, Ar*H*), 9.03 (s, 1H, ArC*H*═), 10.56 (bs, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 294.

***N*-(2,6-Dimethylphenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (27).** Yield 53%; mp 223−225 °C. ^{1}H NMR ($CDCl_3$) 2.31 (s, 6H, 2ArCH_3), 7.26−7.27 (d, J_0 = 8.1 Hz, 1H, Ar*H*), 7.42−7.48 (m, 3H, Ar*H*), 7.71−7.75 (m, 3H, Ar*H*), 9.04 (s, 1H, ArC*H*═), 10.18 (bs, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 294.

***N*-(2,6-Difluorophenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (28).** Yield 54%; mp 169−171 °C. ^{1}H NMR ($CDCl_3$) 6.98 (s, 1H, Ar*H*), 7.26−7.33 (m, 3H, Ar*H*), 7.43 (s, 1H, Ar*H*), 7.52−7.57 (m, 3H, Ar*H*), 9.05 (s, 1H, ArC*H*═), 10.30 (bs, 1H, N*H*, D_2O exch). MS *m/z* [MH^+] = 302.

***N*-(3,4-Dimethoxyphenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (30).** Yield 77%; mp 215−217 °C. ^{1}H NMR (DMSO-d_6) 3.69 (s, 3H, ArOCH_3), 3.77 (s, 3H, ArOCH_3), 6.99 (m, 1H, Ar*H*),

7.45−7.59 (m, 3H, Ar*H*), 7.67−7.90 (m, 3H, Ar*H*), 8.97 (s, 1H, ArC*H*═), 10.25 (bs, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 326.

***N*-(3,5-Dimethylphenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (31).** Yield 52%; mp 205−207 °C. ^1H NMR (DMSO-d_6) 2.25 (s, 6H, 2 ArCH_3), 6.78 (s, 1H, Ar*H*), 7.29−7.36 (m, 2H, Ar*H*), 7.42−7.57 (m, 2H, Ar*H*), 7.75−7.99 (m, 2H, Ar*H*), 8.88 (s, 1H, ArC*H*═), 10.56 (bs, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 294.

***N*-(3,5-Dimethoxyphenyl)-2-*oxo*-2*H*-chromene-3-carboxamide (32).** Yield 55%; mp 207−209 °C. ^1H NMR ($CDCl_3$) 3.84 (s, 6H, 2ArOCH_3), 6.42 (s, 1H, Ar*H*), 6.99−7.00 (m, 2H, Ar*H*), 7.45−7.49 (m, 2H, Ar*H*), 7.79−7.81 (m, 2H, Ar*H*), 9.01 (s, 1H, ArC*H*═), 10.87 (bs, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 326.

***N*-Methyl-*N*-phenyl-2-*oxo*-2*H*-chromene-3-carboxamide (38).** Yield 65%; mp 138−140 °C. ^1H NMR ($CDCl_3$) 3.49 (s, 3H, NCH_3), 7.23−7.24 (m, 5H, Ar*H*), 7.41−7.42 (m, 2H, Ar*H*), 7.47−7.49 (m, 2H, Ar*H*), 7.72 (s, 1H, ArC*H*═); MS *m*/*z* [MH^+] = 280.

***N*-Methyl-*N*-benzyl-2-*oxo*-2*H*-chromene-3-carboxamide (39).** Yield 99%; mp 173−177 °C. ^1H NMR (DMSO-d_6) 2.50 (s, 3H, NCH_3), 4.08 (s, 2H, ArCH_2), 7.41−7.43 (m, 5H, Ar*H*), 7.53−7.55 (m, 4H, Ar*H*), 9.30 (s, 1H, ArC*H*═); MS *m*/*z* [MH^+] = 294.

***N*-Cyclohexyl-7-benzyloxy-2-*oxo*-2*H*-chromene-3-carboxamide (41).** Yield 99%; mp 198−200 °C. ^1H NMR (DMSO-d_6) 1.27−1.44 (m, 5H, cyclohexyl), 1.60−1.74 (m, 3H, cyclohexyl), 1.97−2.00 (m, 2H, cyclohexyl), 3.96−3.99 (m, 1H, cyclohexyl), 5.16 (s, 2H, ArCH_2), 6.93−7.01 (m, 2H, Ar*H*), 7.37−7.43 (m, 5H, Ar*H*), 7.58−7.60 (m, 1H, Ar*H*), 8.70 (s, 1H, ArC*H*═), 8.83 (s, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 378.

***N*-Isopropyl-7-benzyloxy-2-*oxo*-2*H*-chromene-3-carboxamide (42).** Yield 84%; mp 186−188 °C. ^1H NMR (DMSO-d_6) 1.26−1.28 (d, *J* = 6.2 Hz, 6H, CH(CH_3)$_2$), 4.24−4.26 (m, 1H, NHC*H*(CH_3)$_2$), 5.17 (s, 2H, ArCH_2), 6.93−7.01 (m, 2H, Ar*H*), 7.26−7.43 (m, 5H, Ar*H*), 7.60−7.62 (m, 1H, Ar*H*), 8.67 (s, 1H, ArC*H*═), 8.83 (s, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 338.

***N*-Isobutyl-7-benzyloxy-2-*oxo*-2*H*-chromene-3-carboxamide (43).** Yield 64%; mp 160−161 °C. ^1H NMR (DMSO-d_6) 0.98−1.00 (d, *J* = 6.0 Hz, 6H, CH(CH_3)$_2$), 1.93−1.95 (m, 1H, C*H*(CH_3)$_2$), 3.32−3.33 (m, NHCH_2CH), 5.17 (s, 2H, ArCH_2), 6.96−7.03 (m, 2H, Ar*H*), 7.27−7.43 (m, 5H, Ar*H*), 7.60−7.63 (m, 1H, Ar*H*), 8.84 (s, 1H, ArC*H*═), 8.99 (bs, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 352.

***N*-(4-Isopropylphenyl)-7-benzyloxy-2-*oxo*-2*H*-chromene-3-carboxamide (44).** Yield 69%; mp 275−278 °C. ^1H NMR (DMSO-d_6) 1.25−1.27 (d, *J* = 6.1 Hz, 6H, CH(CH_3)$_2$), 2.96−2.99 (m, 1H, ArC*H*(CH_3)$_2$), 5.19 (s, 2H, ArCH_2), 6.99−7.07 (m, 2H, Ar*H*), 7.25−7.27 (m, 2H, Ar*H*), 7.43−7.45 (m, 5H, Ar*H*), 7.63−7.65 (m, 3H, Ar*H*), 8.98 (s, 1H, ArC*H*═), 10.77 (s, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 414.

***N*-(4-Cyano-2,3,5,6-tetrafluorophenyl)-7-benzyloxy-2-*oxo*-2*H*-chromene-3-carboxamide (45).** Yield 72%; mp 242−245 °C. ^1H NMR (DMSO-d_6) 5.28 (s, 2H, ArCH_2), 7.12−7.24 (m, 2H, Ar*H*), 7.30−7.49 (m, 5H, Ar*H*), 7.98−8.00 (m, 1H, Ar*H*), 9.00 (s, 1H, ArC*H*═), 10.75 (bs, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 469.

***N*-(2-Benzylphenyl)-7-benzyloxy-2-*oxo*-2*H*-chromene-3-carboxamide (46).** Yield 61%; mp 207−209 °C. ^1H NMR (DMSO-d_6) 4.05 (s, 2H, ArCH_2Ar), 5.28 (s, 2H, ArCH_2), 7.03−7.29 (m, 8H, Ar*H*), 7.31−7.48 (m, 5H, Ar*H*), 7.98−8.03 (m, 4H, Ar*H*), 8.98 (s, 1H, ArC*H*═), 10.65 (s, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 462.

***N*-(2-Methyl-6-chlorophenyl)-7-benzyloxy-2-*oxo*-2*H*-chromene-3-carboxamide (47).** Yield 55%; mp 203−205 °C. ^1H NMR (DMSO-d_6) 2.23 (s, 3H, ArCH_3), 5.28 (s, 2H, ArCH_2), 7.05−7.07 (m, 1H, Ar*H*), 7.18−7.21 (m, 3H, Ar*H*), 7.39−7.46 (m, 3H, Ar*H*), 7.49−7.50 (m, 3H, Ar*H*), 7.98−8.00 (m, 1H, Ar*H*), 8.93 (s, 1H, ArC*H*═), 10.20 (s, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 420.

***N*-(3,4-Dimethoxyphenyl)-7-benzyloxy-2-*oxo*-2*H*-chromene-3-carboxamide (48).** Yield 62%; mp 183−185 °C. ^1H NMR (DMSO-d_6) 3.74 (s, 3H, ArOCH_3), 3.76 (s, 3H, ArOCH_3), 5.27 (s, 2H, ArCH_2), 6.90−6.93 (m, 2H, Ar*H*), 7.25−7.27 (m, 2H, Ar*H*), 7.30−7.39 (m, 3H, Ar*H*), 7.48−7.50 (m, 2H, Ar*H*), 7.98−8.01 (m, 2H, Ar*H*), 8.96 (s, 1H, ArC*H*═), 10.50 (s, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 432.

***N*-(3,5-Dimethoxyphenyl)-7-benzyloxy-2-*oxo*-2*H*-chromene-3-carboxamide (49).** Yield 65%; mp 242−243 °C. ^1H NMR (DMSO-d_6) 3.74 (s, 6H, 2ArOCH_3), 5.28 (s, 2H, ArCH_2), 6.29 (s, 1H, Ar*H*), 6.93−6.95 (m, 2H, Ar*H*), 7.24−7.30 (m, 3H, Ar*H*), 7.42−7.48 (m, 4H, Ar*H*), 7.98−8.00 (m, 1H, Ar*H*), 8.95 (s, 1H, ArC*H*═), 10.53 (s, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 432.

***N*-Benzyl-7-benzyloxy-2-*oxo*-2*H*-chromene-3-carboxamide (50).** Yield 58%; mp 183−184 °C. ^1H NMR ($CDCl_3$) 4.66−4,68 (m, 2H, NHCH_2), 5.17 (s, 2H, ArCH_2), 6.93 (s, 1H, Ar*H*), 7.01−7.04 (m, 1H, Ar*H*), 7.35−7.36 (m, 5H, Ar*H*), 7.42−7.44 (m, 5H, Ar*H*), 7.67−7.68 (m, 1H, Ar*H*), 8.88 (s, 1H, ArC*H*═), 9.29 (bs, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 386.

***N′*-Phenyl-7-benzyloxy-2-*oxo*-2*H*-chromene-3-carbohydrazide (51).** Yield 61%; mp 230−232 °C. ^1H NMR (DMSO-d_6) 5.25 (s, 2H, ArCH_2), 6.70−6.75 (m, 3H, Ar*H*), 6.83−6.85 (m, 2H, Ar*H*), 7.20−7.29 (m, 5H, Ar*H*), 7.32−7.37 (m, 3H, Ar*H*), 7.44 (s, 1H, ArC*H*═), 7.80 (bs, 1H, N*H*, D_2O exch), 8.55 (bs, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 387.

***N*-(4-Cyano-2,3,5,6-tetrafluorophenyl)-7-(4-fluorobenzyloxy)-2-*oxo*-2*H*-chromene-3-carboxamide (52).** Yield 56%; mp 237−238 °C. ^1H NMR (DMSO-d_6) 5.27 (s, 2H, ArCH_2), 7.12−7.15 (m, 1H, Ar*H*), 7.24−7.30 (m, 3H, Ar*H*), 7.52−7.53 (m, 2H, Ar*H*), 7.98−8.01 (m, 1H, Ar*H*), 8.99 (s, 1H, ArC*H*═), 10.72 (bs, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 487.

***N*-(2-Benzylphenyl)-7-(4-fluorobenzyloxy)-2-*oxo*-2*H*-chromene-3-carboxamide (53).** Yield 57%; mp 205−208 °C. ^1H NMR (DMSO-d_6) 4.04 (s, 2H, ArCH_2Ar), 5.26 (s, 2H, ArCH_2), 7.13−7.24 (m, 11H, Ar*H*), 7.26−7.28 (m, 1H, Ar*H*), 7.54−7.55 (m, 2H, Ar*H*), 7.98−8.02 (m, 2H, Ar*H*), 8.98 (s, 1H, ArC*H*═), 10.70 (bs, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 480.

***N*-(2-Methyl-6-chlorophenyl)-7-(4-fluorobenzyloxy)-2-*oxo*-2*H*-chromene-3-carboxamide (54).** Yield 59%; mp 236−238 °C. ^1H NMR (DMSO-d_6) 2.24 (s, 3H, ArCH_3), 5.27 (s, 2H, ArCH_2), 7.12−7.13 (m, 1H, Ar*H*), 7.20−7.25 (m, 5H, Ar*H*), 7.40−7.42 (m, 1H, Ar*H*), 7.53−7.55 (m, 2H, Ar*H*), 7.97−7.99 (m, 1H, Ar*H*), 8.99 (s, 1H, ArC*H*═), 10.19 (s, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 438.

***N*-(3,4-Dimethoxyphenyl)-7-(4-fluorobenzyloxy)-2-*oxo*-2*H*-chromene-3-carboxamide (55).** Yield 58%; mp 182−183 °C. ^1H NMR (DMSO-d_6) 3.74 (s, 3H, ArOCH_3), 3.76 (s, 3H, ArOCH_3), 5.24 (s, 2H, ArCH_2), 6.97−6.99 (m, 1H, Ar*H*), 7.12−7.20 (m, 5H, Ar*H*), 7.40−7.42 (m, 1H, Ar*H*), 7.52−7.54 (m, 2H, Ar*H*), 7.97−7.99 (m, 1H, Ar*H*), 8.96 (s, 1H, ArC*H*═), 10.49 (s, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 450.

***N*-(3,5-Dimethoxyphenyl)-7-(4-fluorobenzyloxy)-2-*oxo*-2*H*-chromene-3-carboxamide (56).** Yield 52%; mp 210−214 °C. ^1H NMR (DMSO-d_6) 3.74 (s, 6H, 2ArOCH_3), 5.25 (s, 2H, ArCH_2), 6.32 (s, 1H, Ar*H*), 6.94 (s, 2H, Ar*H*), 7.19−7.20 (m, 1H, Ar*H*), 7.26−7.27 (m, 3H, Ar*H*), 7.52−7.53 (m, 2H, Ar*H*), 7.97−7.99 (m, 1H, Ar*H*), 8.93 (s, 1H, ArC*H*═), 10.53 (bs, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 450.

***N*-(4-Isopropylphenyl)-7-(4-fluorobenzyloxy)-2-*oxo*-2*H*-chromene-3-carboxamide (57).** Yield 56%; mp 235−237 °C. ^1H NMR (DMSO-d_6) 1.25−1.27 (d, *J* = 6.1 Hz, 6H, CH(CH_3)$_2$), 2.96−2.99 (m, 1H, ArC*H*(CH_3)$_2$), 5.19 (s, 2H, ArCH_2), 6.99−7.07 (m, 2H, Ar*H*), 7.27−7.29 (m, 2H, Ar*H*), 7.43−7.45 (m, 4H, Ar*H*), 7.67−7.69 (m, 3H, Ar*H*), 8.98 (s, 1H, ArC*H*═), 10.77 (s, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 432.

***N*-Methyl-*N*-phenyl-7-benzyloxy-2-*oxo*-2*H*-chromene-3-carboxamide (58).** Yield 60%; mp 157−158 °C. ^1H NMR (DMSO-d_6) 3.33 (s, 3H, NCH_3), 5.17 (s, 2H, ArCH_2), 6.99−7.01 (m, 4H, Ar*H*), 7.24−7.46 (m, 8H, Ar*H*), 7.58−7.60 (m, 1H, Ar*H*), 8.04 (s, 1H, ArC*H*═); MS *m*/*z* [MH^+] = 386.

***N*-(3,4-Dimethoxyphenyl)-7-benzyloxy-8-methyl-2-*oxo*-2*H*-chromene-3-carboxamide (59).** Yield 59%; mp 220−222 °C. ^1H NMR (DMSO-d_6) 2.28 (s, 3H, ArCH_3), 3.74 (s, 3H, ArOCH_3), 3.76 (s, 3H, ArOCH_3), 5.32 (s, 2H, ArCH_2), 6.98−7.00 (m, 1H, Ar*H*), 7.24−7.50 (m, 8H, Ar*H*), 7.80−7.85 (m, 1H, Ar*H*), 8.80 (s, 1H, ArC*H*═), 10.50 (bs, 1H, N*H*, D_2O exch). MS *m*/*z* [MH^+] = 445.

Determination of hMAO Isoform Activity. The effects of the test compounds on the enzymatic activity of hMAO isoform were

evaluated by a fluorimetric method following the experimental protocol previously described by us.[30]

Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing the test drugs (new compounds or reference inhibitors) at various concentrations and adequate amounts of recombinant hMAO-A or hMAO-B required and adjusted to obtain in our experimental conditions the same reaction velocity, i.e., to oxidize (in the control group) the same concentration of substrate: 165 pmol of *p*-tyramine/min (hMAO-A: 1.1 μg protein; specific activity: 150 nmol of *p*-tyramine oxidized to *p*-hydroxyphenylacetaldehyde/min/mg protein; hMAO-B: 7.5 μg protein; specific activity: 22 nmol of *p*-tyramine transformed/min/mg protein) were incubated for 15 min at 37 °C in a flat-black-bottom 96-well microtest plate (BD Biosciences, Franklin Lakes, NJ) placed in the dark fluorimeter chamber. After this incubation period, the reaction was started by adding (final concentrations) 200 μM Amplex Red reagent, 1 U/mL horseradish peroxidase, and 1 mM *p*-tyramine. The production of H_2O_2 and, consequently, of resorufin, was quantified at 37 °C in a multidetection microplate fluorescence reader (FLX800, Bio-Tek Instruments, Inc., Winooski, VT) based on the fluorescence generated (excitation, 545 nm; emission, 590 nm) over a 15 min period, in which the fluorescence increased linearly.

Control experiments were carried out simultaneously by replacing the test drugs (new compounds and reference inhibitors) with appropriate dilutions of the vehicles. In addition, the possible capacity of the above test drugs to modify the fluorescence generated in the reaction mixture due to nonenzymatic inhibition (e.g., for directly reacting with Amplex Red reagent) was determined by adding these drugs to solutions containing only the Amplex Red reagent in a sodium phosphate buffer.

The specific fluorescence emission (used to obtain the final results) was calculated after subtraction of the background activity, which was determined from vials containing all components except the hMAO isoforms, which were replaced by a sodium phosphate buffer solution.

Molecular Modeling. As reported in our recent communications,[23,29] computational studies were performed in order to rationalize the recognition of the most potent inhibitors **18** and **21** with respect to human MAO-A and MAO-B. The new, high resolution, Protein Data Bank[41] (PDB) crystallographic structures were considered as receptor models of hMAO-A (PDB code 2Z5X[42]) and hMAO-B (PDB code 2BK3[43]), respectively.

The theoretical approach was carried out by following three main steps: (i) inhibitor conformational search, (ii) ligand flexible docking to hMAO-A and -B isoforms, (iii) full optimization of the previously generated complexes and their thermodynamic analysis.

Three-dimensional models of compounds **18** and **21** were built by means of the Maestro GUI,[44] and after preliminary optimization, they were submitted to 1000 steps of a Monte Carlo search as implemented in MacroModel version 7.2.[45] Energy evaluation of all generated conformers was performed using the AMBER* force field,[46] and water solvent effects were taken into account by using the GB/SA implicit model.[47] The Monte Carlo conformation ensembles population was computed at room temperature for each inhibitor using Boltzmann statistical analysis. The global minimum energy structure of all compounds reported a population larger than 89.0%. Consequently, we decided to use only these conformers for the docking simulations.

Both hMAO-A and -B crystallographic models required some graphical manipulation to be used in our studies. The cocrystallized ligands, harmine and farnesol, respectively for 2Z5X and 2BK3, were removed. FAD double bonds were fixed and hydrogen atoms were added onto both proteins and cofactors. According to the Glide[48] methodology, a regular box of about 110000 Å^3 centered on the cofactor N5 atom was considered as the enzyme active site for both hMAO-A and hMAO-B models. To partially take into account the induced fit phenomena, ligand global minimum energy structures were docked using the "flexible" algorithm. The Glide default scoring function was adopted to evaluate the binding mode of **18** and **21**, but no correlation was observed at this stage with respect to the experimental pIC_{50} data.

Table 3. Comparison between Theoretical Affinity and Experimental Inhibition Data[a]

	hMAO-A		hMAO-B	
compd	pIC_{50}	ΔG	pIC_{50}	ΔG
18	4.89	−35.80	7.18	−38.00
21	5.03	−37.70	8.85	−39.10

[a] ΔG are reported in kcal/mol. $pIC_{50} = -\log IC_{50}$.

To improve our binding models including enzyme flexibility, all generated complex configurations (10 and 8 for **18** and **21**, respectively) were submitted to energy minimization using the same force field and environment reported for the ligand Monte Carlo search. After full relaxation, the interaction energy of all complexes was computed according to the MOLINE method[49] and converted to average state equations (Table 3).

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Supporting Information Available: Chemistry and pharmacological studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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