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Brief Article

Synthesis, Stereochemical Separation, and Biological Evaluation of Selective Inhibitors of Human MAO-B: 1-(4-Arylthiazol-2-yl)-2-(3-methylcyclohexylidene)hydrazines

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Novel 1-(4-arylthiazol-2-yl)-2-(3-methylcyclohexylidene)hydrazine derivatives have been investigated for their ability to inhibit selectively the activity of the human B isoform of monoamine oxidase. These compounds were obtained as racemates and (*R*)-enantiomers by a stereoconservative synthetic pattern in high yield and enantiomeric excess. The (*S*)-enantiomers of the most active derivatives have been separated by enantioselective HPLC. All compounds showed selective activity against hMAO-B with IC_{50} ranging between 21.90 and 0.018 μM.

Introduction

Monoamine oxidases A and B (EC 1.4.3.4) are outer mitochondrial membrane bound enzymes involved in the oxidative degradation of monoamine neurotransmitters and xenobiotic arylalkylamines to aldehydes by using O_2 as the electron acceptor. To complete the catalytic cycle, the reduced FAD[a] cofactor reacts with O_2 to generate oxidized flavin and H_2O_2.[1] All mammals contain MAO-A and MAO-B, which are localized in different tissues. Human MAO-A (hMAO-A) and human MAO-B (hMAO-B) are encoded by separate genes and consist of different amino acid sequences that are ~70% identical.[2] The A isoform preferentially metabolizes norepineprhine and 5-hydroxytryptamine and is inhibited by clorgyline, whereas the B isoform prefers benzylamine as substrate and is inhibited by (*R*)-deprenyl and rasagiline. Tyramine and dopamine are equally metabolized by both forms of the enzyme.[3,4] The recent three-dimensional structures of hMAO-A[5] and hMAO-B[6] with their corresponding inhibitors show overall similarity in their crystalline forms but differ in oligomeric states and active site structures. In fact, hMAO-B crystallizes as the dimer whereas hMAO-A crystallizes as the monomer. In detail, the active site of hMAO-B is characterized by a generally hydrophobic dipartite cavity with a substrate entrance cavity (~290 Å^3) connected to a larger substrate-binding cavity (~ 420 Å^3). Ile199 was identified as an important structural element because it acts as a gate that allows the accessibility to the second cavity. In the closed conformation, Ile199 physically separates the two cavities, whereas in the presence of bulky ligands it adopts an open conformation. Binding of clinical inhibitors such as rasagiline and (*R*)-deprenyl induces a midspan type of cavity pushing Ile199 into the open conformation. Furthermore, the observation that hMAO-B levels in humans increase 4- to 5-fold on aging[2,7] represents a rationale for the involvement of hMAO-B in age-related neurological disorders such as Parkinson's and Alzheimer's diseases.[8−10] Increased hMAO-B activity would be expected to diminish dopamine concentration and to release catalytic reaction products (H_2O_2). Dopamine has been involved in (*R*)-synuclein aggregation implicated in the etiology of Parkinson's disease, while high levels of H_2O_2 in the cell promote apoptotic signaling events and the development of Parkinson's disease. Inhibition of hMAO-B allows a sort of neuroprotection against this bioactivation. In fact, agents that specifically limit the activity of hMAO-B are likely to act as neuroprotectants.[11] Indeed, treatment of pre-Parkinson's patients with selective hMAO-B inhibitors has been shown to be effective in reducing the development of this neurodegeneration. A disadvantage of the first selective hMAO-B inhibitor, (*R*)-deprenyl, was its sympathomimetic effect related to its chemical structure because it is metabolized in vivo to methamphetamine compounds with sympathomimetic activity. One advantage of rasagiline, therefore, was that it was not an amphetamine derivative and showed no sympathomimetic activity.[12] Rasagiline [*N*-propargyl-1(*R*)-aminoindan] is currently under development for the treatment of Parkinson's disease. It has been shown to have neuroprotective properties and to be a potent, selective inhibitor of hMAO-B. The (*S*)-enantiomer is a much less potent (3 orders of magnitude) inhibitor of hMAO-B than

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[a] Abbreviations: MAO, monoamine oxidase; FAD, flavin adenine dinucleotide; IC_{50}, 50% inhibitory concentration; HPLC, high-performance liquid chromatography; CPSs, polysaccharide-based chiral stationary phases; 3-MCE, 3-methylcyclohexanone; 3-MCET, 3-methylcyclohexylidene thiosemicarbazone; K_m, Michaelis constant; V_{max}, maximum reaction velocity; BTI-TN-5B1-4, insect cells infected with recombinant baculovirus containing cDNA; PDB, Protein Data Bank; 2BXR, PDB code of hMAO-A; 1GOS, PDB code of hMAO-B; SEM, standard error of the mean; *P*, probability value; $pIC_{50} = -\log IC_{50}$. 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 otherwise indicated.

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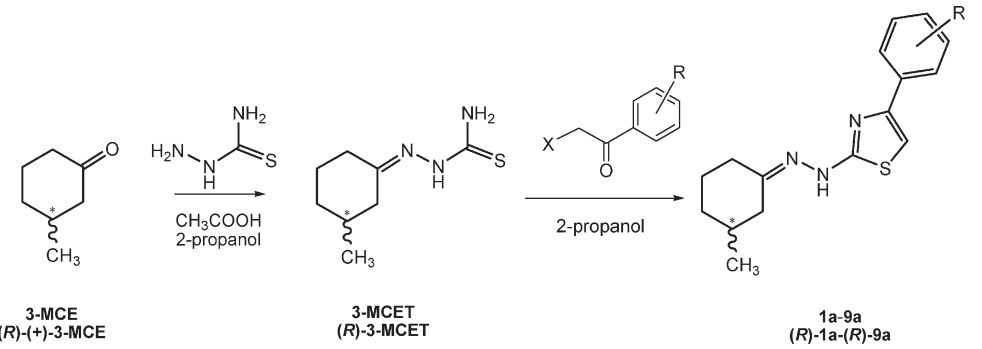
Scheme 1. Synthesis of Racemic Compounds (**1a**−**9a**) and Their Corresponding (*R*)-Enantiomers



the (*R*)-enantiomer, but it retains its neuroprotective properties. The inverted chirality leads to a different binding of the indan ring, probably resulting from steric hindrance.[13]

In the course of our research,[14] we have previously reported the synthesis and MAO inhibitory activity of different series of 1-(4-arylthiazol-2-yl)-2-(cyclohexylidene)hydrazines.[15−18] We also pointed out that the (*R*)-enantiomers of reported chiral derivatives (eutomers) were generally the most selective hMAO-B inhibitors. For this reason it was important to have the pure enantiomers of the most active compounds to evaluate the influence of stereochemistry on the biological activity. In this study, we present the synthesis of racemic 1-(4-arylthiazol-2-yl)-2-(3-methylcyclohexylidene)hydrazines (**1a**−**9a**) and their corresponding (*R*)-enantiomers and the enantioseparation of the (*S*)-homochiral forms. Indeed, owing to the presence of a stereogenic carbon on the cyclohexyl ring, these compounds exist as (*R*)- and (*S*)-enantiomers. The first step of the investigation of the impact of absolute stereochemistry on the activity and selectivity was to isolate sufficient quantities of the enantiomers of four of the most active compounds to use in a parallel test in vitro. Our strategy was to synthesize the racemic forms and successively resolve them by HPLC on polysaccharide-based chiral stationary phases (CPSs) on a semipreparative scale. Knowledge of the in vitro biological activity of homochiral forms could be useful for the in silico study of the interactions involving the receptor site.

Chemistry and HPLC Enantioseparation

All derivatives were obtained by direct reaction between 3-methylcyclohexanone or (*R*)-3-methylcyclohexanone and thiosemicarbazide in 2-propanol with catalytic amounts of acetic acid (Scheme 1). The desired thiosemicarbazones (3-MCET and (*R*)-3-MCET) were condensed with different and freshly synthesized α-halo-acetophenone to give the corresponding thiazoles (Hantzsch reaction) in high yields (84−99%) and enantiomeric excess (≥99%). All compounds were fully characterized by ^{1}H NMR and elemental analysis. The synthetic protocol used for the preparation of (*R*)-enantiomers of **1a**−**9a** starts from the commercially available (*R*)-3-methylcyclohexanone and does not involve the stereogenic center. Consequently, the absolute configuration of the thiazole derivatives in homochiral form was assigned by chemical correlation. The HPLC enantioseparation of the most active and selective compounds (**3a**, **5a**, **8a**, and **9a**) was carried out on the amylose-based Chiralpak AS-H CSP using binary mixtures *n*-hexane−ethanol as mobile phases. As shown in Figure 1, these conditions permitted a simultaneous diastereo- and enantioseparation and, scaled up at semipreparative level, isolation of milligram amounts of each enantiomer for single

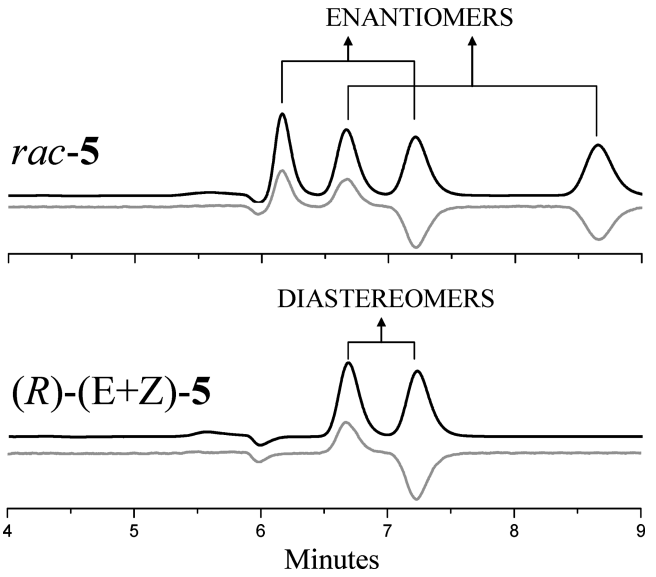


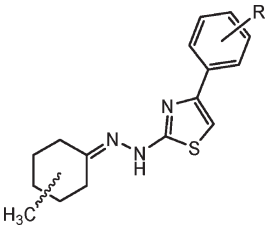
Figure 1. Chromatograms of rac-**5** and (*R*)-**5a** obtained by simultaneous UV (black) and CD (gray) detection at 310 nm: column, Chiralpak AS-H (250 mm × 4.6 mm i.d.); eluent, *n*-hexane−2-propanol 90/10 (v/v); flow-rate, 1 mL min^{-1}; temperature, 25 °C.

chromatographic run. The enantiomeric and diastereomeric relationships between the chromatographic peaks were established by spiking the racemic sample with the synthetic (*R*)-enantiomer and monitoring the CD signal during the HPLC stereoseparation as reported in our previous work.[19]

Biochemistry

The biological evaluation of the test drugs on hMAO activity was investigated by measuring their effects on the production of hydrogen peroxide (H_2O_2) from *p*-tyramine (a common substrate for hMAO-A and hMAO-B), using the Amplex Red MAO assay kit 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.[20] The production of H_2O_2 catalyzed by MAO isoforms can be detected using 10-acetyl-3,7-dihydroxyphenoxazine, a non-fluorescent and highly sensitive probe that reacts with H_2O_2 in the presence of horseradish peroxidase to produce a fluorescent product, resorufin. New compounds and reference inhibitors 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, hMAO-A displayed a Michaelis constant (K_m) equal to 457.17 ± 38.62 μM and a

Table 1. Structures and hMAO Inhibitory Activity of Derivatives and Reference Compounds[a]



3-methylcyclohexylidene					2-methylcyclohexylidene				
		IC$_{50}$ (μM)					IC$_{50}$ (μM)		
compd	R	hMAO-A	hMAO-B	ratio	compd	R	hMAO-A	hMAO-B	ratio
1a	H	37.84 ± 2.94[b]	2.23 ± 0.19	17	**1b**	H	41.23 ± 3.96[c]	0.71 ± 0.036	58
(*R*)-**1a**	H	38.61 ± 3.24[b]	0.81 ± 0.075	48					
2a	4-Cl	23.12 ± 1.85	21.90 ± 1.22	1.1	**2b**	4-Cl	35.22 ± 1.81[c]	13.12 ± 0.51	2.7
(*R*)-**2a**	4-Cl	22.76 ± 1.64[b]	9.55 ± 0.76	2.4					
3a	4-F	37.52 ± 3.21[b]	0.41 ± 0.023	92	**3b**	4-F	43.55 ± 3.61[c]	0.20 ± 0.008	214
(*R*)-**3a**	4-F	40.32 ± 3.31[b]	0.81 ± 0.068	50	(*R*)-**3b**	4-F	3.53 ± 0.12[c]	0.02 ± 0.001	160
(*R*S)-**3**	4-F	16.02 ± 0.78[b]	0.38 ± 0.021	42	(*S*)-**3b**	4-F	4.94 ± 0.09[c]	0.04 ± 0.002	105
4a	2,4-Cl	43.78 ± 4.17[b]	9.65 ± 0.0.78	4.5	**4b**	2,4-Cl	44.70 ± 5.23[c]	26.81 ± 2.74	1.7
(*R*)-**4a**	2,4-Cl	40.57 ± 3.45[b]	3.68 ± 0.35	11					
5a	2,4-F	42.66 ± 3.09[b]	0.056 ± 0.0015	762	**5b**	2,4-F	37.95 ± 3.41[c]	0.014 ± 0.0002	2,673
(*R*)-**5a**	2,4-F	38.84 ± 3.04[b]	0.018 ± 0.00068	2158	(*R*)-**5b**	2,4-F	6.83 ± 0.14[c]	0.03 ± 0.002	227
(*S*)-**5**	2,4-F	10.09 ± 0.65[b]	0.26 ± 0.017	39	(*S*)-**5b**	2,4-F	4.81 ± 0.03[c]	0.036 ± 0.003	133
6a	4-CH_3	[e]	2.86 ± 0.25	>35[g]	**6b**	4-CH_3	[e]	0.142 ± 0.008	>701[g]
(*R*)-**6a**	4-CH_3	[e]	0.15 ± 0.0088	>667[g]					
7a	4-OCH_3	5.77 ± 0.13[b]	4.51 ± 0.28	1.3	**7b**	4-OCH_3	2.76 ± 0.17[c]	2.37 ± 0.14	1.2
(*R*)-**7a**	4-OCH_3	4.26 ± 0.24[c]	3.12 ± 0.15	1.4					
8a	4-NO_2	[e]	0.41 ± 0.013	>244[g]	**8b**	4-NO_2	[e]	0.032 ± 0.002	>3,093[g]
(*R*)-**8a**	4-NO_2	[e]	0.086 ± 0.00041	>1163[g]	(*R*)-**8b**	4-NO_2	43.95 ± 1.08[c]	0.009 ± 0.0006	4,485
(*S*)-**8**	4-NO_2	[e]	0.21 ± 0.019	476[g]	(*S*)-**8b**	4-NO_2	42.31 ± 2.81[c]	0.016 ± 0.0008	2,552
9a	4-CN	20.57 ± 1.08[b]	0.12 ± 0.0023	171	**9b**	4-CN	31.03 ± 2.44[c]	0.026 ± 0.001	1,183
(*R*)-**9a**	4-CN	21.90 ± 0.86[b]	0.23 ± 0.0019	95	(*R*)-**9b**	4-CN	5.50 ± 0.26[c]	0.032 ± 0.002	169
(*S*)-**9**	4-CN	17.14 ± 0.97[b]	0.42 ± 0.029	41	(*S*)-**9b**	4-CN	7.22 ± 0.56[c]	0.063 ± 0.003	115
C		0.00446 ± 0.00032	61.35 ± 1.13	0.000073					
D		67.25 ± 1.02	0.020 ± 0.00086	3362					
I		6.56 ± 0.76	7.54 ± 0.36	0.87					
M		361.38 ± 19.37	[d]	<0.36[h]					
Is		[f]	18.75 ± 1.24	>5.3[g]					

[a] **C** = clorgyline. **D** = *R*-(−)-deprenyl. **I** = iproniazid. **M** = moclobemide. **Is** = Isatin. Ratio: hMAO-B selectivity index = IC$_{50}$(hMAO-A) /IC$_{50}$(hMAO-B). Each IC$_{50}$ value is the mean ± S.E.M. from five experiments (n = 5). [b] Level of statistical significance: $P < 0.01$ versus the corresponding IC$_{50}$ values obtained against hMAO-B, as determined by ANOVA/Dunnett's test. [c] Level of statistical significance: $P < 0.05$ versus the corresponding IC$_{50}$ values obtained against hMAO-B, as determined by ANOVA/Dunnett's test. [d] Inactive at 1 mM (highest concentration tested). [e] Inactive at 100 μM (highest concentration tested). At higher concentrations the compounds precipitate. [f] 100 μM inhibits the corresponding hMAO activity by approximately 40−45%. At higher concentration the compound precipitates. [g] Values obtained under the assumption that the corresponding IC$_{50}$ against hMAO-A is the highest concentration tested (100 μM). [h] Value obtained under the assumption that the corresponding IC$_{50}$ against hMAO-B is the highest concentration tested (1 mM).

maximum reaction velocity (V_{max}) in the control group of 185.67 ± 12.06 (nmol *p*-tyramine/min)/mg protein, whereas hMAO-B showed a K_m of 220.33 ± 32.80 μM and a V_{max} of 24.32 ± 1.97 (nmol *p*-tyramine/min)/mg protein (n = 5). Most tested drugs concentration-dependently inhibited this enzymatic control activity (Table 1).

Results and Discussion

Racemic 1-(4-arylthiazol-2-yl)-2-(3-methylcyclohexylidene)hydrazines (**1a**−**9a**) and their corresponding (*R*)-enantiomers were synthesized and evaluated for their ability to inhibit the A and B isoforms of hMAO. All compounds were selective hMAO-B inhibitors in the micromolar or submicromolar range, for which it was important to assay the pure enantiomers of the most active ones to evaluate the influence of stereochemistry on their inhibitory activity. With this in mind, we also performed a direct HPLC separation of the (*S*)-forms from the racemic mixtures.

In Table 1 we compared the biological results for the two series of derivatives obtained by shifting the chiral methyl group on the cycloaliphatic portion. As regards the racemic compounds (**1a**−**9a** and **1b**−**9b**), with the exception of derivatives **4a**, they all showed a better inhibitory activity in the 2-methylcyclohexylidene series, and the 3-methylcyclohexylidene series maintained the same trend and hMAO-B selectivity.

Structure−activity relationship within this small library revealed that racemic compounds with a 4-Cl, 4-CH_3, and 4-OCH_3 substituted phenyl group (**2a**, **6a**, and **7a**) or an unsubstituted (**1a**) one at the C4 position of thiazole ring displayed hMAO inhibitory activity in the micromolar range, with selectivity toward B isoform. The main difference in the hMAO inhibition of these derivatives originated from the replacement of the chlorine, methyl, and methoxy on the aromatic moiety by a fluorine atom (**3a** and **5a**), a nitro (**8a**), and a cyano (**9a**) group (inhibitory activity in the nanomolar range). The number and the position of fluorine

on the phenyl ring of the molecule seemed to be responsible for the best activity and selectivity toward hMAO-B, since **5a** (2,4-fluoro disubstituted derivative) had $IC_{50} = 0.056 \pm 0.0015$ μM and selectivity ratio was 762. Conversely, the introduction of two more sterically hindered chlorine atoms in ortho and para positions (**4a**) might reduce the compound binding in the active site cavity of the hMAO-B. A preliminary molecular modeling approach was carried out to justify the importance of this substituted aromatic ring in the interaction with both isoforms (see Supporting Information). Superimpositions of the best fully energy minimized hMAO-A and -B poses of the most selective derivative (**5a**) indicated that the aromatic and the thiazole moieties were located close to the FAD coenzyme, suggesting a functional role in catalysis as also demonstrated by the presence of a structural "aromatic cage" in hMAOs and in several flavin-dependent amine oxidizing enzymes described by others.[21] The sum of these interactions, in particular the hydrogen bond between Tyr326 and hydrazone NH, could also explain the better hMAO-B recognition of this scaffold.

The analysis of the influence of the stereochemistry on the biological behavior confirmed the results previously obtained.[16] Except for derivatives **3a** and **9a** (which, for both the racemic and the (*R*)-homochiral forms, displayed inhibitory activity in the same concentration range), the 3-methylcyclohexylidene series showed a stronger interaction with hMAO-B when assayed as pure enantiomer, the (*R*)-homochiral form being more active and selective than the corresponding (*S*)-enantiomers. This behavior could be also highlighted in the 2-methylcyclohexylidene series (compare **9a** to **9b** and (*R*)-**9a** to (*R*)-**9b**).

Moreover, as in the 2-methylcyclohexylidene series, we also decided to separate and evaluate the (*S*)-forms of the same derivatives (4-F, 2,4-F, 4-NO_2, and 4-CN) because in the 3-methylcyclohexylidene series they displayed high inhibitory activities as well. For **5a**, **8a**, and **9a**, it was possible to state that the (*S*)-homochiral form represented the distomer while the (*R*)-homochiral one was the eutomer. Instead the hMAO-B selectivity was only implemented for derivatives (*R*)-**8a** and (*R*)-**5a** with selectivity ratios of >1163 and 2158, respectively. In terms of anti-hMAO-A activity, the same substitutents (4-CH_3 and 4-NO_2) on the aromatic ring proved to be inactive in both series.

As a consequence, the shifting of the chiral methyl group on the cyclohexylidene moiety confirmed the choice of this pharmacophore and revealed that the CH_3 group on the asymmetric carbon of the six-membered ring may represent only the main (*R*)- and (*S*)-recognition difference allowing additional hydrophobic contacts (see Supporting Information). These findings increase our confidence in this model and stimulate us to continue investigations in designing more potent and selective analogues that may have interesting therapeutic potential as original chemical models (templates) for the design and subsequent development of new drugs useful for improving the pharmacological treatment of major depressive disorders and neurodegenerative diseases.

Experimental Section

Chemistry. Unless otherwise noted, starting materials and reagents were obtained from commercial suppliers and were used without further 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 Bruker spectrometer using $CDCl_3$ 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 indicated ≥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). The lipophilic parameter ClogP was calculated for each molecule by using ChemDraw Ultra 8.0. The synthesis of some compounds has been described in previously[22] and was performed with slight changes.

General Procedure for the Synthesis of Racemic Derivatives 1a−9a and (*R*)-Enantiomers. The appropriate carbonyl compound (50 mmol) was dissolved in 100 mL of 2-propanol and stirred with an equimolar quantity of thiosemicarbazide for 24 h at room temperature with catalytic amounts of acetic acid. The desired thiosemicarbazone precipitated from the mixture and was filtered, washed with suitable solvent, and dried under vacuum. Equimolar amounts of the prepared thiosemicarbazone (50 mmol) and freshly synthesized, according to classical methods, substituted α-halo-acetophenone (50 mmol), both stirred in 2-propanol, were reacted at room temperature for 2 h. The precipitate was filtered, washed with petroleum ether and diethyl ether, and dried under vacuum to give all compounds in high yields (84−99%). All the yields are on isolated basis.

Determination of hMAO Isoform Activity. Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing different concentrations of the test drugs (new compounds or reference inhibitors) 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) 165 pmol of *p*-tyramine/min (hMAO-A, 1.1 μg of 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) was 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 multimode microplate reader 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 (final saturating concentration). The production of H_2O_2 and consequently of resorufin was quantified at 37 °C in a multidetection microplate fluorescence reader (Fluostar Optima, BMG Labtech GmbH, Offenburg, Germany) based on the fluorescence generated (excitation, 545 nm; emission, 590 nm) over 15 min, 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 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. To determine the kinetic parameters of hMAO-A and hMAO-B (K_m and V_{max}), the corresponding enzymatic activity of both isoforms was evaluated (under the experimental conditions described above) in the presence of a number (a wide range) of *p*-tyramine concentrations. 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. In our experimental conditions, this background activity was practically negligible.

HPLC Stereoseparation. HPLC stereoseparations were performed using stainless-steel Chiralpak AS-H (250 mm × 4.6 mm i.d. and 250 mm × 10 mm i.d.) columns (Chiral Technologies Europe, Illkirch, France). HPLC-grade solvents were supplied

by Carlo Erba (Milan, Italy). The HPLC apparatus consisted of a Perkin-Elmer (Norwalk, CT) 200 lc pump equipped with a Rheodyne (Cotati, CA) injector, a 1000 μL sample loop, and a HPLC Dionex TCC-100 oven (Sunnyvale, CA). The detector for HPLC was a Jasco (Jasco, Tokyo, Japan) model CD 2095 Plus UV/CD. The signal was acquired and processed by Clarity software (DataApex, Prague, Czech Republic). The mobile phases were filtered and degassed by sonication just before use. After semipreparative separation, the collected fractions were analyzed by a chiral analytical column to determine their enantiomeric excess (ee).

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Supporting Information Available: Analytical data for new compounds, chromatography conditions, computational data, and details of pharmacological studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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