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MAO inhibitory activity modulation: 3-Phenylcoumarins versus 3-benzoylcoumarins

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ABSTRACT

With the aim of finding the structural features for the human MAO inhibitory activity and selectivity, in the present communication we report the synthesis, pharmacological evaluation and a comparative study of a new series of 3-phenylcoumarins (compounds **1**–**4**) and 3-benzoylcoumarins (compounds **5**–**8**). A bromo atom and a methoxy/hydroxy substituent were introduced in these scaffolds, at six and eight positions of the coumarin moiety, respectively. The synthesized compounds **1–8** were evaluated as MAO-A and B inhibitors using R-(−)-deprenyl and iproniazide as reference compounds. The presence or absence of a carbonyl group between the coumarin and the phenyl substituent in 3 position remarks, respectively, the MAO-A or MAO-B inhibitory activity. Some of the new compounds showed MAO-B inhibitory activities in the low nanomolar range. Compound **2** (IC_{50} = 1.35 nM) showed higher inhibitory activity than the R-(−)-deprenyl (IC_{50} = 19.60 nM) and higher MAO-B selectivity, with more than 74,074-fold inhibition level, respecting to the MAO-A isoform.

Coumarins, chalcone and their natural and/or synthetic derivatives are biologically interesting compounds because of their structural diversity. Due to this variability, these heterocyclic compounds occupy an important role not only in the Organic Chemistry but also in the Medicinal Chemistry realm.[1–6] They are described as anticancer, anti-inflammatory, antimicrobial, and antioxidant agents.[7–16] A number of studies pay special attention to coumarin derivatives as monoamine oxidase (MAO)[17–23] inhibitors. Recently, chalcone structure has also been identified as a valid scaffold for monoamine oxidase inhibitors (MAOI).[24] Therefore, recent findings reveal that MAO-A and MAO-B affinity and selectivity can be efficiently modulated by appropriate substitutions in different positions of the coumarin and chalcone moiety (Fig. 1).[19,25–27]

MAO is a FAD-containing enzyme (flavoenzyme) bound to the outer mitochondrial membrane in neuronal, glial and many other cells.[28,29] Two isoforms namely as MAO-A and MAO-B have been identified based on their amino acid sequences, three-dimensional structure, substrate preference and inhibitor selectivity.[30,31] These isoenzymes are responsible for the oxidative deamination of neurotransmitters and dietary amines. Therefore, they are responsible for the regulation of intracellular levels of biogenic amines in the brain and the peripheral tissues.[32,33] MAO-B preferentially deaminates phenylethylamine and benzylamine, while MAO-A has a higher affinity for noradrenaline and serotonin.[34,35] Despite of these differences, dopamine and tyramine are common substrates for both isoforms. These properties determine the pharmacological interest of MAOIs. Selective and irreversible MAO-B inhibitors, such as selegiline (R-(−)-deprenyl) and rasagiline are useful compounds for the treatment of Parkinson[36,37] and Alzheimer's diseases.[38,39] Selective MAO-A inhibitors, such as clorgyline (irreversible) and moclobemide (reversible), are useful for the treatment of neurological disorders, such as depression and anxiety.[40,41] All these findings have led us to an intensive search for novel, selective and efficient MAO inhibitors.

With the aim of finding novel and selective MAO-B inhibitors, we have previously synthesized 3-arylcoumarin derivatives in which both the coumarin nucleus and a 3-phenyl ring were differently substituted. The experimental data show that those compounds are potent and selective MAO-B inhibitors.[20–23] In particular, the 6,8-disubstituted coumarins proved to be very interesting derivatives.[22] Based on the previous 3-phenylcoumarins experimental results, in this paper we describe a new project with a comparative study between 3-phenylcoumarins (compounds **1**–**4**) and 3-benzoylcoumarins (compounds **5**–**8**), which are

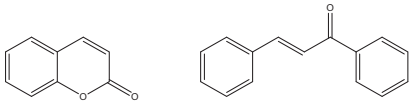

Figure 1. Chemical structures of coumarin and *trans*-chalcone.

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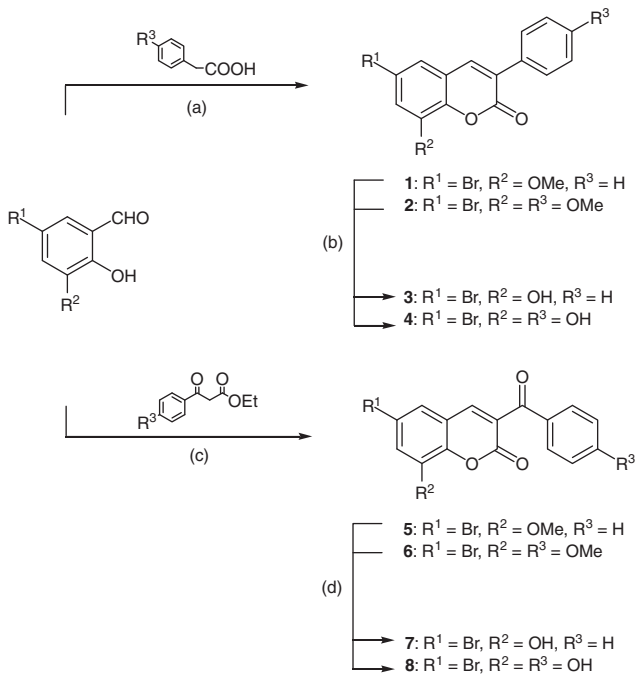

Scheme 1. Reagents and conditions: (a) DCC, DMSO, 110 °C, 24 h; (b) HI, AcOH, Ac_2O, 0 °C to reflux, 4 h; (c) ethanol, piperidine, reflux, 2–5 h; (d) BBr_3, DCM, 80 °C, 48 h.

interesting semi-rigid chalcones with the α,β-unsaturated double bond included in the coumarin skeleton (Scheme 1).

The 6-bromo-8-methoxycoumarins[42] were efficiently synthesized by Perkin[43–45] (**1** and **2**) and Knoevenagel[46,47] (**5** and **6**) reactions. The hydroxy derivatives (**3**, **4**, **7** and **8**)[42] were obtained by two different hydrolysis reactions,[48–50] according to the synthetic protocol outlined in Scheme 1. Treatment of the corresponding salicylaldehyde and the conveniently substituted phenylacetic acid with *N,N'*-dicyclohexylcarbodiimide (DCC) as dehydrating agent, in dimethyl sulfoxide (DMSO) at 110 °C during 24 h, afforded the 3-phenylcoumarins **1** and **2**. The consequent hydrolysis of **1** and **2** in acetic acid and acetic anhydride, with hydriodic acid 57%, for 4 h, yielded the hydroxy derivatives **3** and **4**.[42] The synthesis of the 3-benzoylcoumarins **5** and **6** was performed via condensation of the conveniently substituted salicylaldehyde with the corresponding β-ketoester, in ethanol at reflux temperature for 5 or 2 h respectively, using piperidine as basic catalyst. The resulting methoxy derivatives were treated with boron tribromide at 80 °C for 48 h, to give the corresponding hydroxy derivatives **7** and **8**. Starting from the same salicylaldehyde, we can afford two series, differing just in the presence (compounds **5–8**) or absence (compounds **1–4**) of a carbonyl group between the phenyl substituent at the 3 position and the coumarin scaffold.

The inhibitory MAO activity of compounds **1–8** was evaluated *in vitro* by the measurement of the enzymatic activity of human recombinant MAO isoforms expressed in BTI insect cells infected with baculovirus.[51] Subsequently, the IC_{50} values and MAO-B selectivity indexes [IC_{50} (MAO-A)]/[IC_{50} (MAO-B)] for inhibitory effects of both new types of compounds and reference inhibitors were calculated (Table 1).[51–53]

In the present communication, the effect of the presence or the absence of a carbonyl group between the coumarin and the 3-phenyl ring is studied. Substituents and their positions in the 3-phenylcoumarin nucleus have been selected based on previous results which showed very high MAOI activity for some derivatives. It was shown that introduction of both bromo and methoxy substituents at 6 and 8 positions respectively (compounds **1** and **2**) enhances the MAO-B inhibitory properties (potency and selectivity) of the described 3-phenylcoumarins, comparing to some of the other previously described compounds.[20–22] In addition, the introduction of a substituent in the *para* position of the 3-phenyl ring might help to improve the activity. Consequently, when this substituent is a methoxy group (compound **2**), the MAO-B inhibitory activity and selectivity improve in a great extent respecting to the other derivatives. However, replacement of the methoxy groups for hydroxyl groups at the indicated positions decreases the activity (compounds **3** and **4**) validating the previous information we already had[21] and it can even decrease selectivity. On the other hand, when we analyze the second series where a 3-benzoyl group has replaced the 3-phenyl substituent, the introduced carbonyl group decreases the MAOI activity against B isoform. The 6-bromo-8-hydroxy derivatives **7** and **8** are more active than the corresponding 6-bromo-8-methoxy ones (compounds **5** and **6**), being the structure activity relationship just the opposite of the 3-phenylcoumarins. Compounds **5** and **6** do not present any MAO inhibitory activity at the higher tested concentration (100 μM). However, compounds **7** and **8** increase the affinity for the MAO-A receptor, losing the MAO-B selectivity of the first series. A small change in the structure causes a big change in the affinity of the molecules for the receptor. These preliminary results allow us to understand slightly better interactions between molecule and receptor and the molecular fragments that are essential to maintain and improve the MAO activity and selectivity.

As conclusion, it was verified an important lost of the MAO-B inhibitory activity and selectivity when the 3-phenyl skeleton is substituted for the 3-benzoyl one. However, in some of the 3-benzoyl derivatives it was shown not only inactivity against MAO-B

Table 1
MAO-A and MAO-B inhibitory activity results for the synthesized compounds **1–8** and reference inhibitors

Compounds	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Selectivity Index
1	*	$83.48x10^{-3} \pm 5.60x10^{-3}$	>1,197[b]
2	*	$1.35x10^{-3} \pm 0.09x10^{-3}$	>74,074[b]
3	*	30.91 ± 2.09	>3.2[b]
4	20.74 ± 1.40	16.87 ± 1.14	1.2
5	*	*	–
6	*	*	–
7	46.81 ± 3.18[a]	73.92 ± 4.99	0.63
8	19.17 ± 1.29	**	0.19[c]
R-(−)-Deprenyl	67.25 ± 1.02[a]	$19.60x10^{-3} \pm 0.86x10^{-3}$	3,431
Iproniazide	6.56 ± 0.76	7.54 ± 0.36	0.87

* Inactive at 100 μM (highest concentration tested). At higher concentrations compound precipitate.
** 100 μM inhibits enzymatic activity around (by approximately) 45–50%. At higher concentrations compound precipitate.
[a] $P < 0.05$ versus the corresponding IC_{50} values obtained against MAO-B, as determined by ANOVA/Dunnett's.
[b] Values obtained under the assumption that the corresponding IC_{50} against MAO-A is the highest concentration tested (100 μM).
[c] Value obtained under the assumption that the corresponding IC_{50} against MAO-B is the highest tested concentration (100 μM).

isoenzyme, but showed MAO-A inhibitory activity and selectivity. Therefore, selectivity seems to depend on the nature of the coumarins' substituent. In the present study it was shown that 6-bromo-8-methoxy-3-phenylcoumarins are an interesting scaffold for MAO-B inhibitory studies, whereas the 6-bromo-8-hydroxy-3-benzoylcoumarins are an interesting moiety for MAO-A inhibitory ones. Compound **2**, with a *p*-methoxy substituent in the 3-phenyl ring was the most potent and selective molecule of the first series against MAO-B isoenzyme, with an IC_{50} in the low nanomolar range (IC_{50} = 1.35 nM). This compound is fifteen times more active and several times more selective than the R-(−)-deprenyl (IC_{50} = 19.6 nM, reference MAO-B inhibitor). The MAO selectivity is an important factor to discriminate the different potential therapeutic applications of these molecules. These findings encourage us to continue the efforts towards the optimization of the pharmacological profile of these structural types as important scaffolds in the neurodegenerative diseases realm.

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42. *General procedure for the preparation of 3-phenylcoumarins (**1** and **2**):* a solution of the conveniently substituted salicylaldehyde (0.56 mmol) and the correspondent phenylacetic acid (0.70 mmol) in DMSO and DCC (0.87 mmol) was heated in an oil-bath at 110 °C for 24 h. Triturate ice (20 mL) and acetic acid (3.0 mL) were added to the reaction mixture. After keeping it at room temperature for 2 h, the mixture was extracted with ether (3 x 25 mL). The organic layer was extracted with sodium bicarbonate solution (50 mL, 5%) and then with water (20 mL). The solvent was evaporated under vacuum and the dry residue was purified by FC (hexane/ethyl acetate 9:1) to give the desired compound.
*6-Bromo-8-methoxy-3-phenylcoumarin (**1**)*: it was obtained with a yield of 50%. Mp 153–154 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 3.97 (s, 3H, $-OCH_3$), 7.16 (d, 1H, H-7, J = 2.0), 7.26 (d, 1H, H-5, J = 2.0), 7.43–7.48 (m, 3H, H-3', H-4', H-5'), 7.68–7.73 (m, 3H, H-2', H-6', H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 57.2, 117.0, 117.3, 121.9, 122.1, 129.2, 129.8, 130.3, 134.8, 139.1, 142.8, 148.3, 160.0. MS m/z (%): 332 (99), 331 (30), 330 (M^+, 100), 304 (40), 302 (40), 261 (25), 259 (26), 194 (16), 165 (12), 153 (14), 152 (88), 151 (23), 102 (19), 76 (34). Anal. Calcd for $C_{16}H_{11}BrO_3$: C, 58.03; H, 3.35. Found: C, 58.01; H, 3.30.
*6-Bromo-8-methoxy-3-(4'-methoxyphenyl)coumarin (**2**)*: it was obtained with a yield of 53%. Mp 184–185 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 3.85 (s, 3H, $-OCH_3$), 3.96 (s, 3H, $-OCH_3$), 6.93–6.96 (m, 2H, H-3', H-5'), 7.12 (d, 1H, H-7, J = 1.8), 7.23 (d, 1H, H-5, J = 2.0), 7.63–7.67 (m, 2H, H-2', H-6'), 7.69 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 55.7, 56.8, 114.2, 116.2, 116.8, 121.5, 126.8, 129.4, 130.2, 130.8, 137.3, 142.2, 147.8, 159.8, 160.6. MS m/z (%): 363 (19), 362 (M^+, 100), 361 (19), 360 (59), 334 (24), 332 (23), 319 (33), 317 (34), 291 (11), 289 (11), 182 (18), 167 (17), 139 (21), 91 (11). Anal. Calcd for $C_{17}H_{13}BrO_4$: C, 56.53; H 3.63. Found: C, 56.55; H, 3.68.
*General procedure for the preparation of hydroxylated 3-phenylcoumarins (**3** and **4**).* a solution of the corresponding methoxy-3-phenylcoumarin (0.50 mmol) in acetic acid (5.0 mL) and acetic anhydride (5.0 mL), at 0 °C, was prepared. Hydriodic acid 57% (10.0 mL) was added dropwise. The mixture was stirred under reflux temperature for 3 h. The solvent was evaporated under vacuum and the dry residue was purified by recrystallization with CH_3CN.
*6-Bromo-8-hydroxy-3-phenylcoumarin (**3**)*: it was obtained with a yield of 61%. Mp 160–161 °C. ^{1}H NMR (DMSO-d_6) δ (ppm), J (Hz): 7.19 (d, 1H, H-7, J = 2.0), 7.43–7.49 (m, 4H, H-5, H-3', H-4', H-5'), 7.70 (dd, 2H, H-2', H-6', J = 7.6, J = 1.6), 8.13 (s, 1H, H-4), 10.79 (s, 1H, -OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 115.5, 119.9, 120.4, 121.8, 127.9, 128.2, 128.5, 128.8, 134.4, 139.7, 141.1, 145.6, 159.1. MS m/z (%): 319 (16), 318 (99), 317 (17), 316 (M^+, 100), 291 (12), 290 (78), 289 (13), 288 (81), 153 (22), 152 (51), 151 (12), 76 (25). Anal. Calcd for $C_{15}H_9BrO_3$: C, 56.81; H, 2.86. Found: C, 56.78; H, 2.82.
*6-Bromo-8-hydroxy-3-(4'-hydroxyphenyl)coumarin (**4**)*: It was obtained with a yield of 53%. Mp 249–250 °C. ^{1}H NMR (DMSO-d_6) δ (ppm), J (Hz): 6.83 (d, 2H, H-3', H-5', J = 8.8), 7.14 (t, 1H, H-7, J = 1.5), 7.36 (d, 1H, H-5, J = 2.0), 7.56 (d, 2H, H-2', H-6', J = 8.5), 8.00 (s, 1H, H-4). ^{13}C NMR (DMSO-d_6) δ (ppm): 115.6, 116.0, 119.9, 120.6, 122.5, 125.4, 128.2, 130.4, 138.0, 141.2, 146.0, 158.6, 159.8. MS m/z (%): 335 (35), 334 (99), 333 (45), 332 (M^+, 100), 307 (31), 306 (99), 305 (35), 304 (99), 225 (26), 197 (29), 169 (35), 168 (46), 153 (24), 141 (21), 140 (16), 139 (51), 118 (17), 115 (28), 98 (13), 89 (14), 84 (18), 84 (46), 75 (11), 63 (13). Anal. Calcd for $C_{15}H_9BrO_4$: C, 54.08; H, 2.72. Found: C, 54.00; H 2.69.
*General procedure for the preparation of 3-benzoylcoumarins (**5** and **6**).* To a solution of the appropriate β-ketoester and the corresponding salicylaldehyde in ethanol was added piperidine in catalytic amount. The reaction mixture was refluxed for 2–5 h and after completion, the reaction was cooled and the precipitated was filtered and washed with cold ethanol and ether to afford the desired compound. Compounds were further recrystallized in methanol/CH_2Cl_2.
*6-Bromo-8-methoxy-3-benzoylcoumarin (**5**)*: it was obtained with a yield of 49%. Mp 207–208 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 3.82 (s, 3H, $-OCH_3$), 7.09 (s, 1H, H-7), 7.14 (s, 1H, H-5), 7.24–7.51 (m, 3H, H-3', H-4', H-5'), 7.69 (d, 2H, H-2', H-6', J = 7.3), 7.78 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 57.2, 116.6, 118.6, 120.5, 123.0, 128.0, 129.2, 130.0, 134.5, 136.3, 143.3, 144.6, 147.8, 157.8, 191.9. MS m/z (%): 360 (18), 359 (M^+, 58), 358 (18), 357 (58), 105 (100), 77 (70). Anal. Calcd for $C_{17}H_{11}BrO_4$: C, 56.85; H, 3.09; Found: C, 56.79; H, 3.06.

6-Bromo-8-methoxy-3-(4́-methoxybenzoyl)coumarin (**6**): It was obtained with a yield of 90%. Mp 224–225 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 3.71 (s, 3H, $-OCH_3$), 3.82 (s, 3H, $-OCH_3$), 6.77 (d, 2H, H-3', H-5', J = 8.9), 7.14–7.16 (m, 2H, H-5, H-7), 7.69 (d, 2H, H-2', H-6', J = 8.9), 7.72 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 55.5, 56.1, 92.8, 95.2, 103.8, 113.7, 121.2, 129.7, 132.1, 141.5, 157.9, 158.3, 159.2, 163.8, 165.8, 190.6. MS m/z (%): 389 (87), 388 (17), 387 (M^+, 90), 135 (100), 77 (56). Anal. Calcd for $C_{18}H_{13}BrO_5$: C, 55.55; H, 3.37. Found: C, 55.53; H, 3.34.

*General procedure for the preparation of hydroxylated 3-benzoylcoumarins (**7** and **8**).* To the corresponding methoxy-3-benzoylcoumarin in DCM (1 mmol), BBr_3 in DCM (20 mmol) was added in a Schlenk tube. Tube was sealed, and the reaction mixture was heated at 80 °C for 48 h. The resulting crude was treated with MeOH and rotated to dryness. The obtained precipitated was recrystallized in MeOH to afford the desired hydroxy derivative.

*6-Bromo-8-hydroxy-3-benzoylcoumarin (**7**)*: it was obtained with a yield of 98%. Mp 240–242 °C. ^{1}H NMR (DMSO-d_6) δ (ppm), J (Hz): 7.29 (d, J = 2.2 Hz, 1H, H-7), 7.61–7.39 (m, 3H, H-3', H-4', H-5'), 7.76–7.67 (m, 1H, H-5), 7.93 (d, 2H, H-2', H6', J = 8.0), 8.30 (s, 1H, H-4), 10.92 (s, 1H, -OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 39.3, 39.5, 39.7, 39.9, 40.1, 40.4, 40.6, 116.2, 120.9, 121.8, 122.0, 127.8, 129.2, 130.0, 134.5, 136.3, 142.7, 144.8, 146.3, 157.9, 191.9. MS m/z (%): 345 (46), 343 (M^+, 45), 105 (100), 77 (58). Anal. Calcd for $C_{16}H_9BrO_4$: C, 55.68; H, 2.63. Found: C, 55.63; H, 2.59.

*6-Bromo-8-hydroxy-3-(4́-hydroxybenzoyl)coumarin (**8**):* it was obtained with a yield of 40%. Mp 293–295 °C. ^{1}H NMR (DMSO-d_6) δ (ppm), J (Hz): 6.86 (d, 2H, H-3', H-5', J = 8.7), 7.27 (d, 1H, H-7, J = 2.2), 7.46 (d, 1H, H-5 J = 2.2), 7.82 (d, 2H, H-2', H-6' J = 8.7), 8.18 (s, 1H, H-4), 10.62 (s, 1H, -OH), 10.89 (s, 1H, -OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 115.9, 116.2, 121.0, 121.5, 121.6, 127.6, 128.5, 133.0, 142.7, 143.5, 146.3, 157.9, 163.6, 190.0. MS m/z (%): 362 (29), 360 (M^+, 30), 121 (100), 93 (16), 65 (15). Anal. Calcd for $C_{16}H_9BrO_5$: C, 53.21; H, 2.51. Found: C, 52.97; H, 4.41.

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51. Determination of human monoamine oxidase (hMAO) isoform activity. The effects of the tested compounds on hMAO isoform enzymatic activity were evaluated by a fluorimetric method. Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing the tested drugs in several concentrations and adequate amounts of recombinant hMAO-A or hMAO-B required and adjusted to obtain in our experimental conditions the same reaction velocity [165 pmol of *p*-tyramine/min (hMAO-A: 1.1 mg protein; specific activity: 150 nmol of *p*-tyramine oxidized to *p*-hydroxyphenylacetaldehyde/min/mg protein; hMAO-B: 7.5 mg protein; specific activity: 22 nmol of *p*-tyramine transformed/min/mg protein)] were placed in the dark fluorimeter chamber and incubated for 15 min at 37 °C. The reaction was started by adding (final concentrations) 200 μM AmplexÒ 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 (FLX800TM, Bio-TekÒ Instruments, Inc., Winooski, VT, USA) 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 tested drugs with appropriate dilutions of the vehicles. In addition, the possible capacity of the above tested drugs for modifying the fluorescence generated in the reaction mixture due to non-enzymatic inhibition (e.g., for directly reacting with AmplexÒ Red reagent) was determined by adding these drugs to solutions containing only the AmplexÒ 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.
52. All IC_{50} values shown in the table are expressed as means ± SEM from five experiments.
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