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New halogenated 3-phenylcoumarins as potent and selective MAO-B inhibitors

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ABSTRACT

With the aim to find out the structural features for the MAO inhibitory activity and selectivity, in the present communication we report the synthesis and pharmacological evaluation of a new series of bromo-6-methyl-3-phenylcoumarin derivatives (with bromo atom in both different benzene rings of the skeleton) with and without different number of methoxy substituent at the 3-phenyl ring. The methoxy substituents were introduced, in this new scaffold, in the *meta* and/or *para* positions of the 3-phenyl ring. The synthesized compounds **3–7** were evaluated as MAO-A and B inhibitors using *R*-(−)-deprenyl (selegiline) and iproniazide as reference inhibitors, showing, most of them, MAO-B inhibitory activities in the low nanomolar range. Compounds **4** (IC_{50} = 11.05 nM), **5** (IC_{50} = 3.23 nM) and **6** (IC_{50} = 7.12 nM) show higher activity than selegiline (IC_{50} = 19.60 nM) and higher MAO-B selectivity, with more than 9050-fold, 30,960-fold and 14,045-fold inhibition levels, with respect to the MAO-A isoform.

Coumarins are a large family of compounds, of natural and synthetic origin, that presents different pharmacological activities.[1] Due to their structural variability, they occupy an important place in the realm of natural products and synthetic organic chemistry. Representatives of these groups of compounds are found to occur in the vegetable kingdom, either in the free or in the combined state.[2] Recent studies pay special attention to their antioxidative, anticancer, and enzymatic inhibition properties.[3–6] Some coumarins proved to be monoamine oxidase (MAO) inhibitors (MAOI).[7] Recent findings revealed that MAO-A and MAO-B affinity and selectivity can be efficiently modulated by appropriate substitutions in the coumarin moiety. The 3/4 and 6/7 positions are particularly suitable to these modifications.[8–14]

Resveratrol, 3,4′,5-trihydroxystilbene, is a natural polyphenolic compound present in grapes and red wine.[15] In in vitro, ex vivo, and in vivo experiments resveratrol has shown important biological activities including antiinflammatory, antioxidant, anticancer, and cardioprotective properties, besides inhibitory activity towards several enzymes.[15–20]

Therefore this compound has been attracting a huge interest since the last decade. Recently, it has been demonstrated that resveratrol also has MAO inhibitory activity.[16,21] To date, most pharmacological studies have considered the *trans* isomer to be more effective than the *cis*.[22]

MAOs are flavoenzymes (FAD-containing enzyme) bound to the outer mitochondrial membrane of neuronal, glial, and other cells.[23,24] These enzymes are responsible for the oxidative deamination of neurotransmitters and dietary amines.[25,26] Two isoforms, namely MAO-A and MAO-B, have been identified basis on their amino acid sequences, three-dimensional structure, substrate preference and inhibitor selectivity.[20,27] MAO-A has a higher affinity for serotonin and noradrenaline, while MAO-B preferentially deaminates phenylethylamine and benzylamine.[28] These properties determine the clinical interest of MAOIs. Selective MAO-A inhibitors, such as clorgyline (irreversible) and moclobemide (reversible), are used for the treatment of neurological disorders, like depression and anxiety,[29,30] whilst selective and irreversible MAO-B inhibitors, such as selegiline and rasagiline, are useful for the treatment of Parkinson's[31,32] and Alzheimer's diseases.[33,34] As the ideal drug candidate has not been attained, an intensive search for new and innovative MAOIs is still needed. This effort has considerably increased in recent years.[11]

In this context, and in an attempt to develop novel MAO-B selective inhibitors, we had previously synthesized 3-arylcoumarin derivatives in which both the coumarin and the resveratrol templates were present. These compounds proved to be potent and selective MAO-B inhibitors.[8,9] In this Letter, a subsequent project was developed based on a 6-methyl-3-phenylcoumarin scaffold, with a bromo pattern in both benzenic rings of the skeleton, and with several methoxyl substituents located in the 3-phenyl ring (Scheme 1).

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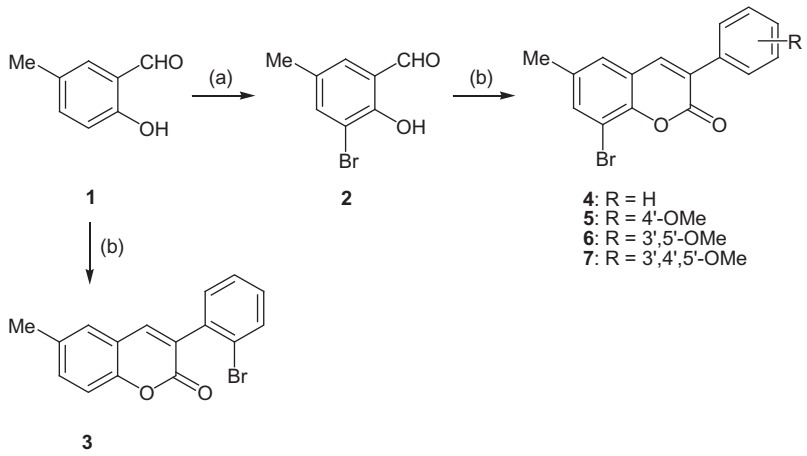

Scheme 1. Reagents and conditions: (a) NBS, AIBN, CCl_4, reflux, 18 h; (b) phenylacetic acids, DCC, DMSO, 110 °C, 24 h.

Table 1
MAO-A and MAO-B inhibitory activity results for compounds **3–7** and reference compounds

Compounds	MAO-A IC_{50} (nM)	MAO-B IC_{50} (nM)	Ratio
3	*	$4.3 \times 10^3 \pm 0.29 \times 10^3$	>23[b]
4	*	11.05 ± 0.81	>9050[b]
5	*	3.23 ± 0.49	>30,960[b]
6	*	7.12 ± 0.01	>14,045[b]
7	$31.20 \times 10^3 \pm 2.09 \times 10^3$	$4.89 \times 10^3 \pm 0.22 \times 10^3$	6.4
R-(−)-Deprenyl	$67.25 \times 10^3 \pm 1.02 \times 10^3$[a]	19.60 ± 0.86	3431
Iproniazide	$6.56 \times 10^3 \pm 0.76 \times 10^3$	$7.54 \times 10^3 \pm 0.36 \times 10^3$	0.87

* Inactive at 100 μM (highest concentration tested). At higher concentrations the compounds precipitate.
[a] P <0.01 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).

The coumarin derivatives **3–7** were efficiently synthesized according to the synthetic protocol outlined in Scheme 1. The experimental details are given in Ref. 35. The treatment of the precursor **1** with *N*-bromosuccinimide (NBS) in carbon tetrachloride (CCl_4) under reflux, using 2,2′-azo-bis-iso-butyronitrile (AIBN) as catalyst, afforded the bromo derivative **2**[36] in a yield of 44%. The preparation of the 8-bromo-6-methyl-3-phenylcoumarins (**4–7**) was performed via the classical Perkin reaction.[8,9,37–39] This reaction occurs by condensation of the 3-bromo-5-methylsalicylaldehyde (**2**) and the conveniently substituted phenylacetic acids, with *N*,*N*′-dicyclohexylcarbodiimide (DCC) as dehydrating agent, in dimethyl sulfoxide (DMSO), at 110 °C and during 24 h (Scheme 1).[8,9] The reaction is efficient and the compounds **4–7** were obtained with yields between 45% and 50%. Compound **3** was prepared in the same conditions described for compounds **4–7**, but starting from the 5-methylsalicylaldehyde **1** and the *o*-bromophenylacetic acid.

The inhibitory MAO activity of compounds **3–7** was evaluated in vitro by the measurement of the enzymatic activity of human recombinant MAO isoforms in BTI insect cells infected with baculovirus.[8,9,40] Then, the IC_{50} values and MAO-B selectivity ratios [IC_{50} (MAO-A)]/[IC_{50} (MAO-B)] for inhibitory effects of both new compounds and reference inhibitors were calculated (Table 1).[40–42]

In the present communication, the effect of the introduction of a halogen substituent into the 3-phenylcoumarin was studied. In fact a superior MAOI activity, regarding the non-halogenated compounds, was observed.[7,8] It was shown that the introduction of a bromo substituent enhance the MAO-B inhibitory properties (potency and selectivity) of the recently described 6-methyl-3-phenylcoumarin (IC_{50} = 284 nM).[7]

As it is shown in the Table 1, compound **3**, with the halogen atom in the 3-phenyl ring, has an IC_{50} in the micromolar range (IC_{50} = 4.3 μM). When compared with the 6-methyl-3-phenylcoumarin, compound **3** lost at least 15 times the MAO-B inhibitory activity. The structural change obtained with compound **4**, with the halogen atom in the coumarin nucleus, leads to a significant increase of the MAO-B activity (IC_{50} = 11.0 nM). In fact it was greater than the IC_{50} found for 6-methyl-3-phenylcoumarin and compound **3**. So, as consequence, the MAO-B selectivity had increased too (see Table 1). A change of the bromo atom position in the 3-phenylcoumarin nucleus, from 2′ to 8, was the other strategy performed to improve the activity (compounds **5–7**). Compound **5**, with a *p*-methoxy substituent in the 3-phenyl ring, was the most potent and selective molecule, against MAO-B isoenzyme, of this series, with an IC_{50} in the low nanomolar range (IC_{50} = 3.2 nM). This compound is six times more active and to a great extent more selective than the *R*-(−)-deprenyl (IC_{50} = 19.6 nM, reference MAO-B inhibitor). Compound **6**, with two methoxyl groups in the 3′ and 5′ positions, reveal also to be a potent MAOI-B. Its activity (IC_{50} = 7.1 nM) is still better than the non-phenylsubstituted compound **4**. Compound **7**, with three methoxyl groups, present a loss of activity (activity in the micromolar range) and selectivity in regard to the mono and dimethoxyl derivatives (compounds **5** and **6**, respectively). Compounds **3–6** do not exhibit MAO-A inhibitory activity for the highest tested concentration (100 μM). The MAO selectivity is an important factor to discriminate the potential therapeutic application of this kind of molecules. In summary one can say that the presence of a bromo atom in 8-position and a restricted number of methoxyl substituents (one or two) in the 3-phenyl ring seems to be important chemical features to modulate and improve the inhibitory enzymatic activity of the 6-methyl-3-phenylcoumarins.

In conclusion, in the present study it was shown that the synthesized resveratrol–coumarin hybrid compounds have high

selectivity for the MAO-B isoenzyme. Most of them present MAO-B inhibitory activity is in the low nanomolar range. The presence of a bromo atom in position 8 of the coumarin improves the activity respect to the presence of a bromo atom linked to the 3-phenyl ring. The introduction of one *para*-methoxy group in the 3-phenyl ring of the 8-bromo-6-methyl-3-phenylcoumarin improve to a great extent the MAO-B inhibitory activity respect to the other prepared derivatives. In fact, the introduction of a bromo atom improves the pharmacologic potential of the 6-methyl-3-phenylcoumarins confirming that this lead could be effectively optimized in a candidate for the treatment of neurodegenerative diseases. These finds have encouraged us to continue the efforts towards the optimization of the pharmacologic profile of 6-methyl-3-phenylcoumarin.

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References and notes

1. Borges, F.; Roleira, F.; Milhazes, N.; Santana, L.; Uriarte, E. *Curr. Med. Chem.* **2005**, *12*, 887.
2. Hoult, J. R. S.; Payá, M. *Gen. Pharmacol.* **1996**, *27*, 713.
3. Kontogiorgis, C.; Hadjipavlou-Litina, D. *J. Enzyme Inhib. Med. Chem.* **2003**, *18*, 63.
4. Kabeya, L.; Marchi, A.; Kanashiro, A.; Lopes, N.; Silva, C.; Pupo, M.; Lucisano-Valim, Y. *Bioorg. Med. Chem.* **2007**, *15*, 1516.
5. Carotti, A.; Altomare, C.; Catto, M.; Gnerre, C.; Summo, L.; De Marco, A.; Rose, S.; Jenner, P.; Testa, B. *Chem. Biodivers.* **2006**, *3*, 134.
6. Chilin, A.; Battistutta, R.; Bortolato, A.; Cozza, G.; Zanatta, S.; Poletto, G.; Mazzorana, M.; Zagotto, G.; Uriarte, E.; Guiotto, A.; Pinna, L.; Meggio, F.; Moro, S. *J. Med. Chem.* **2008**, *51*, 752.
7. Rendenbach-Müller, B.; Traut, M.; Weifenbach, H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1195.
8. Matos, M. J.; Viña, D.; Quezada, E.; Picciau, C.; Delogu, G.; Orallo, F.; Santana, L.; Uriarte, E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3268.
9. Matos, M. J.; Viña, D.; Picciau, C.; Orallo, F.; Santana, L.; Uriarte, E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5053.
10. Santana, L.; Uriarte, E.; González-Díaz, H.; Zagotto, G.; Soto-Otero, R.; Méndez-Álvarez, E. *J. Med. Chem.* **2006**, *49*, 1118.
11. Santana, L.; González-Díaz, H.; Quezada, E.; Uriarte, E.; Yáñez, M.; Viña, D.; Orallo, F. *J. Med. Chem.* **2008**, *51*, 6740.
12. Catto, M.; Nicolotti, O.; Leonetti, F.; Carotti, A.; Favia, A.; Soto-Otero, R.; Méndez-Álvarez, E.; Carotti, A. *J. Med. Chem.* **2006**, *49*, 4912.
13. Gnerre, C.; Catto, M.; Leonetti, F.; Weber, P.; Carrupt, P.; Altomare, C.; Carotti, A.; Testa, B. *J. Med. Chem.* **2000**, *43*, 4747.
14. Chimenti, F.; Secci, D.; Bolasco, A.; Chimenti, P.; Granese, A.; Befani, O.; Turini, P.; Alacaro, S.; Ortuso, F. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3697.
15. Frémont, L. *Life Sci.* **2000**, *66*, 663.
16. Vilar, S.; Quezada, E.; Santana, L.; Uriarte, E.; Yánez, M.; Fraiz, N.; Alcaide, C.; Cano, E.; Orallo, F. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 257.
17. Orallo, F. In *Resveratrol in Health and Disease*; Aggarwal, B. B., Shishodia, S., Eds.; CRC Press: USA, 2005; p 577.
18. Leiro, J.; Álvarez, E.; Arranz, J.; Laguna, R.; Uriarte, E.; Orallo, F. *J. Leukocyte Biol.* **2004**, *75*, 1156.
19. Orallo, F. *Curr. Med. Chem.* **2008**, *15*, 1887.
20. De Colibus, L.; Li, M.; Binda, C.; Lustig, A.; Edmondson, D. E.; Mattevi, A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12684.
21. Binda, C.; Li, M.; Hubalek, F.; Restelli, N.; Edmondson, D. E.; Mattevi, A. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9750.
22. Yánez, M.; Fraiz, N.; Cano, E.; Orallo, F. *Biochem. Biophys. Res. Commun.* **2006**, *344*, 688.
23. Binda, C.; Wang, J.; Pisani, L.; Caccia, C.; Carotti, A.; Salvati, P.; Edmondson, D. E.; Mattevi, A. *J. Med. Chem.* **2007**, *50*, 5848.
24. Novaroli, L.; Daina, A.; Favre, E.; Bravo, J.; Carotti, A.; Leonetti, F.; Catto, M.; Carrupt, P.; Reist, M. *J. Med. Chem.* **2006**, *49*, 6264.
25. Dostert, P.; Strolin Benedetti, M.; Jafre, M. In *Monoamine Oxidase: Basic and Clinical Frontiers*; Kamijo, K., Usdin, E., Nagausu, T., Eds.; Excerpta Medica: Amsterdam, 1982; p 197.
26. Singer, T. P., Muller, F., Eds.; CRC Press: London, 1991; p 437.
27. Binda, C.; Li, M.; Hubálek, F.; Restelli, N.; Edmondson, D. E.; Mattevi, A. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9750.
28. Yamada, M.; Yasuhara, H. *Neurotoxicology* **2004**, *25*, 11.
29. Rudorfer, M. V.; Potter, V. Z. *Drugs* **1989**, *37*, 713.
30. Palhagen, S.; Heinonen, E.; Hagglund, J.; Kaugesaar, T.; Maki-Ikola, O.; Palm, R. *Neurology* **2006**, *66*, 1200.
31. Guay, D. R. *Am. J. Geriatr. Pharmacother.* **2006**, *4*, 330.
32. Riederer, P.; Danielczyk, W.; Grunblatt, E. *Neurotoxicology* **2004**, *25*, 271.
33. Youdim, M. B. H.; Fridkin, M.; Zheng, H. *J. Neural Transm.* **2004**, *111*, 1455.
34. Cesura, A. M.; Pletscher, A. *Prog. Drug Res.* **1992**, *38*, 171.
35. *3-(2-Bromophenyl)-6-methylcoumarin* (**3**): A solution of 2-hydroxy-6-methylbenzaldehyde (**1**, 1.0 g, 7.34 mmol) and 2-bromophenylacetic acid (1.98 g, 9.18 mmol) in DMSO and DCC (2.36 g, 11.46 mmol) was heated in an oil-bath at 110 °C for 24 h. Triturate ice (100 mL) and acetic acid (10.0 mL) were added to the reaction mixture. After keeping it at room temperature for 2 h, the mixture was extracted with ether (3 × 25 mL). The organic layer was extracted with sodium bicarbonate solution (50 mL, 5%) and then water (20 mL). The solvent was evaporated under vacuum and the dry residue was purified by FC (hexane/ethyl acetate 9:1) to give **3** (1.36 g, 59%) as a white solid. Mp 141–142 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.45 (s, 3H, $-CH_3$), 7.28 (m, 1H, H-4′); 7.35 (m, 2H, H-7, H-8); 7.40 (m, 3H, H-5, H-5′, H-6′); 7.69 (d, 1H, H-3′); 7.71 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.8, 116.4, 118.7, 123.6, 127.4, 127.9, 128.6, 130.1, 131.3, 132.9, 133.0, 134.3, 135.9, 142.6, 152.0, 160.0. MS *m*/*z* (%): 316 (5), 315 (38), 314 (M^+, 100), 236 (32), 235 (80), 178 (22), 117 (7), 89 (7), 76 (9). Anal. Calcd for $C_{16}H_{11}BrO_2$: C, 60.98; H, 3.52. Found: C, 60.92; H, 3.47.
General procedure for the preparation of 8-bromo-6-methyl-3-phenylcoumarins (**4**–**7**): A solution of 3-bromo-2-hydroxy-6-methylbenzaldehyde (**2**, 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 × 25 mL). The organic layer was extracted with sodium bicarbonate solution (50 mL, 5%) and then water (20 mL). The solvent was evaporated under vacuum and the dry residue was purified by FC (hexane/ethyl acetate 9:1) to give a white solid.
8-Bromo-6-methyl-3-phenylcoumarin (**4**): It was obtained with a yield of 45%. Mp 158–159 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.41 (s, 3H, $-CH_3$), 7.28 (d, 1H, H-7, *J* = 2.3), 7.45 (m, 3H, H-3′, H-4′, H-5′), 7.58 (m, 1H, H-5), 7.70 (m, 3H, H-4, H-2′, H-6′). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.5, 109.4, 120.5, 127.1, 128.5, 129.0, 129.1, 134.3, 135.2, 135.6, 139.3, 148.3, 159.7. MS *m*/*z* (%): 316 (17), 315 (42), 314 (M^+, 100), 313 (99), 288 (36), 286 (15), 285 (36), 207 (30), 178 (26), 89 (10), 76 (10). Anal. Calcd for $C_{16}H_{11}BrO_2$: C, 60.98; H, 3.52. Found: C, 61.01; H, 3.56.
8-Bromo-6-methyl-3-(4′-methoxyphenyl)coumarin (**5**): It was obtained with a yield of 47%. Mp 144–145 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.40 (s, 3H, $-CH_3$), 3.87 (s, 3H, $-OCH_3$), 6.98 (d, 2H, H-3′, H-5′, *J* = 7.1), 7.26 (s, 1H, H-7), 7.56 (s, 1H, H-5), 7.67 (dd, 3H, H-4, H-2′, H-6′, *J* = 6.9 and *J* = 1.1). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.5, 55.4, 109.3, 114.0, 120.7, 126.7, 126.9, 128.5, 129.9, 135.2, 137.8, 148.1, 159.9, 160.3. MS *m*/*z* (%): 346 (99), 345 (15), 344 (M^+, 100), 303 (53), 301 (54), 275 (15), 207 (15), 166 (17), 165 (72), 138 (17), 89 (13), 76 (14), 58 (41). Anal. Calcd for $C_{17}H_{13}BrO_3$: C, 59.15; H, 3.80. Found: C, 59.17; H, 3.86.
8-Bromo-6-methyl-3-(3′,5′-dimethoxyphenyl) coumarin (**6**): It was obtained with a yield of 46%. Mp 165–166 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.40 (s, 3H, $-CH_3$), 3.83 (s, 6H, $(-OCH_3)_2$), 6.51 (t, 1H, H-4′ *J* = 2.3), 6.83 (d, 2H, H-2′, H-6′, *J* = 2.3), 7.26 (s, 1H, H-7), 7.57 (s, 1H, H-5), 7.70 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 21.0, 56.0, 101.6, 107.2, 109.9, 120.8, 127.6, 129.2, 135.7, 136.1, 136.6, 140.0, 148.7, 160.0, 161.2. MS *m*/*z* (%): 376 (99), 375 (26), 374 (M^+, 100), 238 (37), 209 (14), 181 (36), 165 (30), 153 (18), 152 (41), 151 (17), 126 (15), 76 (22). Anal. Calcd for $C_{18}H_{15}BrO_4$: C, 57.62; H, 4.03. Found: C, 57.63; H, 3.98.
8-Bromo-6-methyl-3-(3′,4′,5′-trimethoxyphenyl) coumarin (**7**): It was obtained with a yield of 50%. Mp 167–168 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.41 (s, 3H, $-CH_3$), 3.91 (d, 9H, $(-OCH_3)_3$, *J* = 5.8), 6.92 (s, 2H, H-2′, H-6′), 7.28 (d, 1H, H-7, *J* = 6.5), 7.59 (d, 1H, H-5, *J* = 1.3), 7.70 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.5, 56.1, 56.3, 60.9, 106.0, 106.2, 109.4, 120.4, 127.0, 128.7, 129.7, 135.3, 135.6, 138.9, 148.1, 153.1, 159.7. MS *m*/*z* (%): 407 (21), 406 (99), 405 (21), 404 (M^+, 100), 391 (56), 389 (56), 363 (19), 361 (19), 303 (21), 247 (13), 187 (15), 186 (15), 139 (19). Anal. Calcd for $C_{19}H_{17}BrO_5$: C, 56.31; H, 4.23. Found: C, 56.28; H, 4.19.
36. Li, W.; Huanwang, J.; Xiuli, B.; Tao, C.; Lili, J.; Yongmin, L. *Catal. Commun.* **2007**, *8*, 80.
37. Hans, N.; Singhi, M.; Sharma, V.; Grover, S. K. *Indian J. Chem., Sect B* **1996**, *35B*, 1159.
38. Mohanty, S.; Makrandi, J. K.; Grover, S. K. *Indian J. Chem., Sect B* **1989**, *28B*, 766.
39. Kamat, S. P.; ÐSouza, A. M.; Paknikar, S. K.; Beaucahmp, P. S. *J. Chem. Res., Synop.* **2002**, 242.
40. *Determination of human monoamine oxidase (hMAO) isoform activity*: The effects of the test 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 test drugs in 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 [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 placed in the dark fluorimeter chamber and incubated for 15 min at 37 °C. 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, 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 test drugs 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 non-enzymatic 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. 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 ($n = 20$).

41. All IC_{50} values shown in the table are expressed as means ± SEM from five experiments.
42. Yáñez, M.; Fraiz, N.; Cano, E.; Orallo, F. *Biochem. Biophys. Res. Commun.* **2006**, *344*, 688.