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A new series of 3-phenylcoumarins as potent and selective MAO-B inhibitors

Maria Joao Matos [a,b,*], Dolores Viña [a,c], Elias Quezada [a,b], Carmen Picciau [b], Giovanna Delogu [b], Francisco Orallo [c], Lourdes Santana [a], Eugenio Uriarte [a]

[a] Departamento de Química Orgánica, Facultad de Farmacia, 15782 Santiago de Compostela, Spain
[b] Dipartimento Farmaco Chimico Tecnologico, Facoltà di Farmacia, 09124 Cagliari, Italy
[c] Departamento de Farmacología, Facultad de Farmacia, 15782 Santiago de Compostela, Spain

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ABSTRACT

6-Methyl-3-phenylcoumarins **3–6** were designed, synthesized and evaluated as monoamine oxidase A and B (MAO-A and MAO-B) inhibitors. The synthesis of these new compounds (resveratrol–coumarin hybrids) was carried out with good yield by a Perkin reaction, from the 5-methylsalicylaldehyde and the corresponding phenylacetic acid. They show high selectivity to the MAO-B isoenzyme, with IC_{50} values in the nanomolar range. Compound **5** is the most active compound and is several times more potent and selective than the reference compound, *R*-(−)-deprenyl.

Coumarins (or benzopyrones) are a large family of compounds, of natural and synthetic origin, that show numerous biological activities.[1] Recent studies pay special attention to their antioxidative, anticarcinogenic and enzymatic inhibition properties.[2–6] In regard to the monoamine oxidase (MAO) inhibition, the recent findings revealed that MAO-A and MAO-B affinity and selectivity can be efficiently modulated by appropriate substitutions in the coumarin ring, in particular in the 3:4 and 6:7 positions.[7–11]

The resveratrol (3,4′,5-trihydroxystilbene) is a phytoalexin of natural origin present in spermatophytes species such as vines, in response to damage. Resveratrol was already studied as antioxidant, anti-inflammatory, cardioprotective (vasodilatory and platelet antiaggregatory activities), anticancer, enzymatic inhibitor, proving to be very efficient in a large group of in vitro, ex vivo and/or in vivo experiments.[12–15] The resveratrol's cis and trans isomers are inhibitors of the MAO activity. *cis*-Resveratrol is less effective than *trans*-resveratrol as inhibitor of MAO-A and MAO-B activities.[16]

Due to these coincident properties, it seems to be interesting to design and synthesize hybrids that incorporate the skeleton of those two kinds of molecules.[17,18] In the present compounds the resveratrol nucleus is blocked by the coumarin ring and can only assume the trans isomeric form. A series of these molecules, with different number and position of methoxy groups in the 3-phenyl ring (compounds **3–6**), was synthesized and evaluated as MAO inhibitors (iMAO). These modifications were studied to find out how these changes can contribute to the biological activity of these molecules, helping to understand a structure–activity relationship (SAR).

MAO is a FAD-containing enzyme with two known isoforms (MAO-A and MAO-B) and is present in the mitochondrial outer membrane of glial, neuronal and other cells.[19] MAO enzymes intervene in the monoamines degradation and carry out an important physiologic function in the adrenaline, noradrenaline and serotonin deamination (preferentially MAO-A) and in the β-phenylethylamine and benzylamine deamination (preferentially MAO-B).[20] This enzymatic function increases the synaptic concentration of these neurotransmitters and conditions to a great extent the neurone's excitement of those possessing receptors for these mediators.[21]

The iMAO are a class of compounds that act by blocking the MAO enzymatic action, being used by several years in the treatment of the depression and anxiety diseases (iMAO-A) or in Parkinson's disease (iMAO-B).[22] Nowadays is being studied also in the Alzheimer's disease.[23]

The active sites of these two isoenzymes (MAO-A and MAO-B) are not completely known and for this reason there is a lack of information in respect of how the inhibitors act selectively in one of the two of them.[10] Recent X-ray crystal structures of MAO-B[24,25] and MAO-A,[21,26] completed with irreversible and reversible inhibitors, can be used in the future as a tool to the structural basis to help understanding the selective enzyme–ligand recognition and drug development of iMAOs.[21]

* Corresponding author. Tel.: +34 981 563100.
E-mail address: mariacmatos@gmail.com (M.J. Matos).

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With the aim of finding out new structural features for the MAO inhibitory activity and selectivity, we decided in this work to explore the importance of the number and position of different methoxy groups under the benzenic ring in 3-position (compounds **4**[27,28]**–6**), to establish a relation between them and with the non substituted analogue (compound **3**[27–29]).

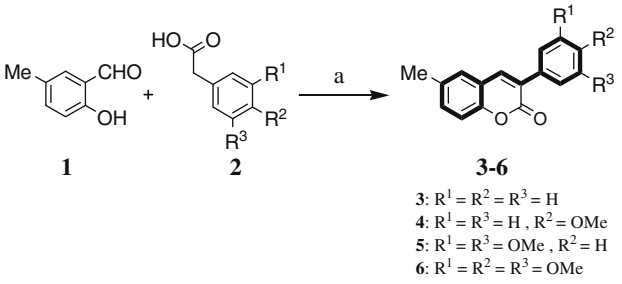
The preparation of these 6-methyl-3-phenylcoumarins was performed via the classical Perkin reaction.[29] This reaction was carried out by condensation of the 5-methylsalicylaldehyde **1** and the conveniently substituted phenylacetic acids **2**, with *N,N′*-dicyclohexylcarbodiimide (DCC) as dehydrating agent, under DMSO reflux, during 24 h (Scheme 1). The reaction to obtain **3–6** is very clean and the yields are between 60% and 70%.[30–33] The obtained products are easy to purify by flash chromatography, using a mixture of hexane/ethyl acetate in a proportion 9:1 as eluent.

The inhibitory MAO activity of compounds **3–6** was evaluated in vitro by the measurement of the enzymatic activity of human recombinant MAO isoforms in BTI insect cells infected with baculovirus.[8,34] 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).[35]

The prepared series of compounds proved to be selective as inhibitor of the MAO-B isoenzyme. Compound **3**, none substituted in the phenyl ring, is by itself very active and selective against MAO-B isoenzyme. Compound **4** (with a *p*-methoxy group) has a MAO-B IC_{50} similar to the *R*-(−)-deprenyl (reference MAO-B inhibitor) and is more selective than this one. The most potent molecule of this family is compound **5**, bearing two methoxy groups in 3′- and 5′-positions (IC_{50} = 8.98 ± 1.42 nM). This one is two times more active and several times more iMAO-B selective than the *R*-(−)-deprenyl. Compound **6**, with 3 methoxy groups, is more active than **3** (none substituted) but it loses activity in respect to the mono and dimethoxy derivatives (compounds **4** and **5**, respectively). None of the described compounds showed a MAO-A inhibitory activity for the highest concentration tested (100 μM). This iMAO-B selectivity is an important factor to discriminate the potential therapeutic application of this kind of molecules.

Comparing the iMAO-B activities of **3** and **4**, the introduction of a *p*-methoxy group increases the inhibitory activity. Substitution with two methoxy groups in the 3′- and 5′-positions on the phenyl ring, compound **5**, improves the iMAO-B activity. Increasing the number of methoxy substituent to three, compound **6**, decreases the enzymatic inhibitory activity. However, compound **6** is even better than none substituted coumarin **3**. The presence of methoxy substituent in the 3-phenyl ring seems to be important to modulate and improve the inhibitory enzymatic activity of the 6-methyl-3-phenylcoumarins.

These hybrid compounds with resveratrol–coumarin skeleton show high selectivity against MAO-B isoenzyme, being active in the nanomolar range. Introduction of methoxy groups in the phenyl ring improves the activity, giving more active and selective compounds than the reference ones. These modifications, which we studying more deeply, can improve the pharmacologic profile of the synthesized coumarins in the Parkinson's disease.



Scheme 1. Reagents and conditions: (a) DCC, DMSO, 110 °C, 24 h.

Table 1
MAO-A and MAO-B inhibitory activity results for compounds **3–6** and reference compounds

Compd	MAO-A IC_{50}	MAO-B IC_{50}	Ratio
3	*	283.75 ± 0.98 nM	>352[b]
4	*	13.05 ± 0.90 nM	>7663[b]
5	*	8.98 ± 1.42 nM	>11,136[b]
6	*	160.64 ± 1.01 nM	>623[b]
R-(−)-Deprenyl	67.25 ± 1.02 μM[a]	19.60 ± 0.86 nM	3431
Iproniazide	6.56 ± 0.76 μM	7.54 ± 0.36 μM	0.87

* Inactive at 100 μM (highest concentration tested). At higher concentrations 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).

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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. Biod.* **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. Santana, L.; Uriarte, E.; González-Díaz, H.; Zagotto, G.; Soto-Otero, R.; Méndez-Álvarez, E. *J. Med. Chem.* **2006**, *49*, 1118.
8. Santana, L.; González-díaz, H.; Quezada, E.; Uriarte, E.; Yáñez, M.; Viña, D.; Orallo, F. *J. Med. Chem.* **2008**, *51*, 6740.
9. Catto, M.; Nicolotti, O.; Leonetti, F.; Carotti, A.; Favia, A.; Soto-Otero, R.; Méndez-Álvarez, E.; Carotti, A. *J. Med. Chem.* **2006**, *49*, 4912.
10. Gnerre, C.; Catto, M.; Leonetti, F.; Weber, P.; Carrupt, P.; Altomare, C.; Carotti, A.; Testa, B. *J. Med. Chem.* **2000**, *43*, 4747.
11. 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.
12. Frémont, L. *Life Sci.* **2000**, *66*, 663.
13. Orallo, F. In *Resveratrol in Health and Disease*; Aggarwal, B. B., Shishodia, S., Eds.; CRC Press: USA, 2005; p 577.
14. Leiro, J.; Álvarez, E.; Arranz, J.; Laguna, R.; Uriarte, E.; Orallo, F. *J. Leukocyte Biol.* **2004**, *75*, 1156.
15. Orallo, F. *Curr. Med. Chem.* **2008**, *15*, 1887.
16. Yánez, M.; Fraiz, N.; Cano, E.; Orallo, F. *Biochem. Biophys. Res. Commun.* **2006**, *344*, 688.
17. 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.
18. Vilar, S.; Quezada, E.; Alcaide, C.; Orallo, F.; Santana, L.; Uriarte, E. *Qsar Comb. Sci.* **2007**, *26*, 317.
19. De Colibus, L.; Li, M.; Binda, C.; Lustig, A.; Edmondson, D. E.; Mattevi, A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12684.
20. Grimsby, J.; Lan, N. C.; Neve, R.; Chen, K.; Shih, J. C. *J. Neurochem.* **1990**, *55*, 1166.
21. Edmondson, D. E.; Mattevi, A.; Binda, C.; Li, M.; Hubalek, F. *Curr. Med. Chem.* **2004**, *11*, 1983.
22. Harfenist, H.; Heuseur, D. J.; Joyner, C. T.; Batchelor, J. F.; White, H. L. *J. Med. Chem.* **1996**, *39*, 1857.
23. Wouters, J. *Curr. Med. Chem.* **1998**, *5*, 137.
24. Binda, C.; Newton-Vinson, P.; Hubalek, F.; Edmondson, D. E.; Mattevi, A. *Nat. Struct. Biol.* **2002**, *9*, 22.
25. Binda, C.; Li, M.; Hubalek, F.; Restelli, N.; Edmondson, D. E.; Mattevi, A. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9750.

26. Ma, J.; Yoshimura, M.; Yamashita, E.; Nakagawa, A.; Ito, A.; Tsukihara, T. *J. Mol. Biol.* **2004**, *338*, 103.
27. Hans, N.; Singhi, M.; Sharma, V.; Grover, S. K. *Indian J. Chem., Sect. B* **1996**, *35*, 1159.
28. Mohanty, S.; Makrandi, J. K.; Grover, S. K. *Indian J. Chem., Sect. B* **1989**, *28*, 766.
29. Kamat, S. P.; ÓSouza, A. M.; Paknikar, S. K.; Beaucahmp, P. S. *J. Chem. Res. (S)* **2002**, 242.
30. 6-Methyl-3-phenylcumarin **(3)**. It was obtained with a yield of 68%. Mp 148–149 °C (biblio. 145–147 °C, 149–150 °C). ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.42 (s, 3H, $-CH_3$), 7.27 (m, 1H, H-7), 7.34 (m, 2H, H-4′ and H-8), 7.43 (m, 3H, H-2′,H-4′ and H-5), 7.70 (dd, 2H, H-1′ and H-5′, *J* = 7.7 and 1.8), 7.77 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 21.29, 116.67, 119.57, 128.17, 128.70, 128.95, 129.02, 129.26, 132.95, 134.65, 135.35, 140.39, 152.16, 161.31. DEPT ($CDCl_3$) δ (ppm): 21.29, 100.60, 116.67, 128.17, 128.95, 129.02, 129.26, 132.95, 140.39. MS *m/z* (%): 236 (M^+, 100), 208 (50), 178 (8), 165 (8) 76 (6), 51 (69). Anal. Calcd for $C_{16}H_{12}O_2$: C, 81.34; H, 5.12; O, 13.54. Found: C, 81.44; H, 4.84.
31. 3-(4′-Methoxy)phenyl-6-methylcumarin **(4)**. It was obtained with a yield of 61%. Mp 144–145ºC (biblio. 143 °C). ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.40 (s, 3H, $-CH_3$), 3.84 (s, 3H, $-OCH_3$), 6.96 (dd, 2H, H-3′ and H-5′, *J* = 6.8 and 2.1), 7.26 (m, 3H, H-4, H-7 and H-8), 7.66 (m, 3H, H-3, H-2′ and H-6′). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.75, 55.32, 113.85, 116.04, 119.54, 127.19, 127.46, 127.66, 129.77, 132.02, 134.03, 138.47, 151.41, 160.05, 160.96. DEPT ($CDCl_3$) δ (ppm): 20.76, 55.32, 113.85, 116.04, 127.46, 129.78, 132.01, 138.48. MS *m/z* (%): 266 (M^+, 100), 223 (60), 195 (24), 165 (16), 152 (13), 115 (5), 89 (5), 63 (7), 50 (6). Anal. Calcd for $C_{17}H_{14}O_3$: C, 76.68; H, 5.30; O, 18.02. Found: C, 76.88; H, 5.32.
32. 3-(3′,5′-Dimethoxy)phenyl-6-methylcumarin **(5)**. It was obtained with a yield of 60%. Mp 110–111ºC. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.40 (s, 3H, $-CH_3$), 3.82 (s, 6H, $-(OCH_3)_2$), 6.50 (t, 1H, H-4′, *J* = 2.1), 6.83 (d, 2H, H-2′ and H-6′, *J* = 2.1), 7.22–7,33 (m, 3H, H-5, H-7 and H-8), 7.74 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.72, 55.41, 100.89, 106.72, 116.06, 119.22, 127.69, 127.93, 132.49, 134.10, 136.68, 140.02, 151.60, 160.48, 160.61. DEPT ($CDCl_3$) δ (ppm): 20.72, 55.40, 100.89, 106,71, 116.06, 127.69, 132.49, 140.02. MS *m/z* (%): 296 (M^+, 100), 295 (17), 267 (16), 210 (8), 181 (22), 152 (13), 139 (6), 105 (8), 76 (9). Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44; O, 21.60. Found: C, 73.23; H, 4.90.
33. 6-Methyl-3-(3′,4′,5′-trimethoxy)phenylcumarin **(6)**. It was obtained with a yield of 70%. Mp 165–166 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.43 (s, 3H, $-CH_3$), 3.90 (s, 3H, $-OCH_3$), 3,92 (s, 6H, $-(OCH_3)_2$), 6.93 (d, 2H, H-2′and H-6′, *J* = 2.1), 7.24–7.35 (m, 3H, H-5, H-7 and H-8), 7.76 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.70, 56.19, 60.72, 105.94, 116.03, 119.24, 127.48, 127.58, 130.04, 130.44, 132.38, 134.12, 139.48, 151.45, 153.02, 160.66. DEPT ($CDCl_3$) δ (ppm): 20.76, 56.23, 60.77, 105.97, 116.08, 127.63, 132.43, 139.54. MS *m/z* (%): 326 (M^+, 100), 311 (89), 283 (43), 253 (12), 225 (30), 197 (10), 169 (10), 148 (5), 115 (8). Anal. Calcd for $C_{19}H_{18}O_5$: C, 69.93; H, 5.56; O, 24.51. Found: C, 70.14; H, 5.58.
34. *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 following the experimental protocol previously described by us. 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).
35. All IC_{50} values shown in the table are expressed as means ± SEM from five experiments.