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Synthesis and evaluation of 6-methyl-3-phenylcoumarins as potent and selective MAO-B inhibitors

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ABSTRACT

A series of 6-methyl-3-phenylcoumarins **3-6** were synthesized and evaluated as monoamine oxidase A and B (MAO-A and MAO-B) inhibitors. A comparative study between the three possible mono methoxy 3-phenyl derivatives and the *p*-hydroxy analogue is reported. The synthesis of these new resveratrol-coumarin hybrids was carried out by a Perkin reaction between the 5-methylsalicylaldehyde and the corresponding phenylacetic acids. The *p*-methoxy substituted compound **3** was hydrolyzed to **6** by a traditional reaction with hydriodic acid. The prepared compounds show high selectivity to the MAO-B isoenzyme, some of them with IC_{50} values in the low nanomolar range. Compound **4**, with the methoxy group in meta position, is the most active of this series, with an IC_{50} against MAO-B of 0.80 nM, and is several times more potent and MAO-B selective than the *R*-(−)-deprenyl (reference compound).

Coumarins are present in remarkable amounts in the nature. They have attracted considerable interest due to their numerous biological activities depending on their substitution pattern.[1] These compounds have been shown to possess antioxidative and anticarcinogenic properties and to inhibit several enzymes.[2–6] Some coumarin derivatives of natural and synthetic origin have been characterized as monoamine oxidase inhibitors (MAOIs).[7–12]

Monoamine oxidase (MAO) is an FAD-containing enzyme bound to the mitochondrial outer membrane of neuronal, glial, and other cells.[10,13] This enzyme regulates levels of biogenic amines (including neurotransmitters) in the brain and the peripheral tissues by catalyzing their deamination.[11] MAO exists as two distinct enzymatic isoforms, MAO-A and MAO-B, based on their substrate and inhibitor specificities.[14,15]

MAO-A preferentially deaminates serotonin, adrenaline and noradrenaline. That isoenzyme is irreversibly inhibited by low concentrations of clorgyline. MAO-B preferentially deaminates β-phenylethylamine and benzylamine and is irreversibly inhibited by *R*-(−)-deprenyl.[16] The MAOIs have been used for several years in the treatment of depression and anxiety diseases (MAO-A inhibitors) and in Parkinson's disease (MAO-B inhibitors).[17]

Resveratrol, structurally 3,4′,5-trihydroxystilbene, is a natural phenolic component of *Vitis vinifera* L. and other spermatophyte species, produced in response to an exterior or interior damage.[18] Resveratrol shows a large number of pharmacological activities, including antiinflammatory, antioxidant, anticancer, and cardioprotective properties and enzyme inhibition.[19–23] *cis* and *trans*-resveratrol proved to be MAO activity inhibitors, the trans isomer being more effective than the cis.[24]

Because of their similar characteristics, it was interesting to design and synthesize hybrids that incorporate the nucleus of the coumarins and resveratrol molecules.[19,25] In previous work, our research group had reported a comparative study of the importance to the MAOI activity of the different number of methoxy groups on the phenyl ring in the 3 position of coumarin. This study contributed to establish a relationship between them and with the non-substituted analogue.[8] Based on this, and with the aim of helping to better understand a structure/activity relationship for the MAO inhibitory activity and selectivity, in this paper we report the synthesis and evaluation of a new series. Maintaining the 6-methyl-3-phenylcoumarin structure, the three possible different positions of one methoxy group in 3-phenyl ring were explored. We also explored the importance of the hydrolysis of this methoxy group.

The synthesis of the 6-methyl-3-phenylcoumarins was carried out via the classical Perkin reaction.[26] This reaction is performed by condensation of the 5-methylsalicylaldehyde **1** and the appropriately substituted phenylacetic acids **2**, with *N,N′*-dicyclohexylcarbodiimide (DCC) as dehydrating agent, in DMSO, at 110 °C, for

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24 h (Scheme 1). Compounds **3**,[8] **4**[27] and **5**[28] were obtained in yields of 61%, 53%, and 59%, respectively. The reaction mixture was purified by flash chromatography, using hexane/ethyl acetate, in a proportion of 9:1, as eluent.

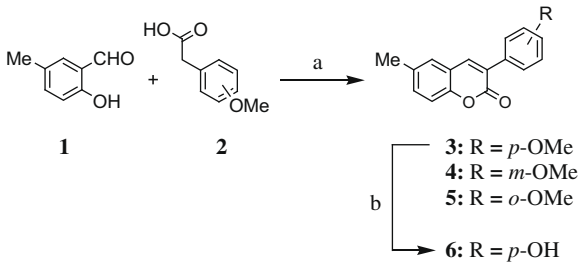
The *p*-methoxy derivative **3** was hydrolyzed with hydriodic acid, in the presence of acetic acid and acetic anhydride, at 110 °C, for 5 h (Scheme 1). The residue was purified by crystallization of acetonitrile, and the phenol derivative **6**[29] was obtained with a yield of 63%.

MAO inhibiting 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] Then, the IC_{50} values and MAO-B selectivity ratio [IC_{50} (MAO-A)]/[IC_{50} (MAO-B)] for inhibitory effects of both new compounds and reference inhibitors were calculated (Table 1).[30]

The resveratrol–coumarin hybrid compounds **3**, **4,** and **6** showed high selectivity for the MAO-B isoenzyme and inhibitory activity in the nano to picomolar range. Compound **4** was the most active compound of this series, making the *meta* methoxy position the most interesting position at which to improve the MAO-B-inhibiting activity. Compound **5** has no MAOI activity up to the highest tested concentration, proving that the methoxy group in the *ortho* position is not favorable to the measured enzymatic inhibition. Changes on the methoxy substituent position on the phenyl ring in coumarin's 3 position can modulate the pharmacologic potential of the synthesized coumarins.

Comparing compound **3** with its hydroxyl derivative **6**, it was shown that the hydrolysis of methoxy groups is not, in this case, a strategy to improve the MAOI activity. The IC_{50} of compound **6** for inhibition of MAO-B activity is approximately 10 times bigger than compound **3**. However, this value is also in the nanomolar range, and the molecule is also a potent MAOI, selective for the MAO-B isoenzyme.

In conclusion, the synthesized resveratrol–coumarin hybrid compounds show high selectivity for the MAO-B isoenzyme. Most of them present activity in the low nanomolar range. The introduction of one *meta* methoxy group in the 3-phenyl ring improves several times the MAO-B inhibitory activity in respect to *ortho* and *para* positions. Compound **4** is about 24 times more active that *R*-(−)-deprenyl, and several times more selective than this drug. The hydrolysis of methoxy groups is not a strategy to get better MAOI activity. These studied modifications can interestingly improve the pharmacologic potential of the 3-phenylcoumarins in the treatment of Parkinson's disease.



Scheme 1. Reagents and conditions: (a) DCC, DMSO, 110 °C, 24 h; (b) HI, AcOH, Ac_2O, 110 °C, 5 h.

Table 1
MAO-A and MAO-B inhibition by the prepared compounds **3–6** and for the reference compounds

Compounds	MAO-A IC_{50}	MAO-B IC_{50}	Ratio
3	*	13.05 ± 0.90 nM	>7663[b]
4	*	802.60 ± 53.75 pM	>124,595[b]
5	*	*	
6	*	155.59 ± 17.09 nM	>643[b]
R-(−)-Deprenyl	67.25 ± 1.02 μM[a]	19.60 ± 0.86 nM	3431
Iproniazid	6.56 ± 0.76 μM	7.54 ± 0.36 μM	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).

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27. *3-(3′-Methoxy)phenyl-6-methylcumarin* (**4**): It was obtained with a yield of 53%. Mp 84–85 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 2.44 (s, 3H, –CH_3), 3.88 (s, 1H, –OCH_3), 6.97 (m, 1H, H-4′), 7.26–7.42 (m, 6H, H-5, H-7, H-8, H-2′, H-5′ and H-6′) 7.78 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.77, 55.37, 114.15, 114.38, 116.10, 119.28, 120.86, 127.70, 129.43, 132.48, 134.12, 136.12, 139.91, 140.10, 151.60, 159.45 160.66. MS *m/z* (%): 267 (48), 266 (M^+, 100), 239 (16), 238 (70), 237 (20), 195 (48), 194 (16), 166 (10), 165 (29), 152 (23). Anal. Calcd for $C_{17}H_{14}O_3$: C, 76.68; H, 5.30. Found: C, 76.76; H, 5.21.
28. *3-(2′-Methoxy)phenyl-6-methylcumarin* (**5**): It was obtained with a yield of 59%. Mp 177–178 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 2.41 (s, 3H, –CH_3), 3.82 (s, 1H, –OCH_3), 7.02 (m, 2H, H-3′, H-4′), 7.24–7.41 (m, 5H, H-5, H-7, H-8, H-5′ and H-6′) 7.69 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.80, 55.81, 111.31, 116.21,

119.24, 120.57, 124.20, 126.36, 127.58, 130.14, 130.80, 132.21, 133.89, 141.84, 151.82, 157.22 160.55. MS *m/z* (%): 267 (22), 266 (M^+, 100), 265 (10), 249 (29), 237 (22), 235 (14), 223 (22), 220 (12), 195 (29), 173 (26), 165 (25), 152 (17), 145 (19), 118 (19). Anal. Calcd for $C_{17}H_{14}O_3$: C, 76.68; H, 5.30. Found: C, 76.76; H, 5.22.

29. *3-(4′-Hydroxy)phenyl-6-methylcumarin* (**6**): It was obtained with a yield of 63%. Mp 217–218 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.37 (s, 3H, $-CH_3$), 6.84 (d, 2H, H-3′ and H-5′, *J* = 8.8), 7.31 (d, 1H, H-8, *J* = 8.4), 7.40 (dd, 1H, H-7, *J* = 1.9 and 8.4), 7.56 (m, 3H, H-2′, H-6′ and H-5), 8.06 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.31, 115.04, 115.52, 119.45, 125.29, 126.66, 127.92, 129.83, 132.00, 133.67, 138.46, 150.79, 157.95, 160.04. MS *m/z* (%): 253 (13), 252 (M^+, 75), 224 (58), 223 (26), 165 (12) 152 (15), 143 (23), 99 (37), 98 (25), 83 (12), 70 (20), 56 (100), 55 (27). Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 75.99; H, 4.69.
30. All IC_{50} values shown in the table are expressed as means ± SEM from five experiments.