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New halogenated phenylcoumarins as tyrosinase inhibitors

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ABSTRACT

With the aim to find out structural features for the tyrosinase inhibitory activity, in the present communication we report the synthesis and pharmacological evaluation of a new series of phenylcoumarin derivatives with different number of hydroxyl or ether groups and bromo substituent in the scaffold. The synthesized compounds **5–12** were evaluated as mushroom tyrosinase inhibitors showing, two of them, lower IC_{50} than the umbelliferone. Compound **12** (IC_{50} = 215 μM) is the best tyrosinase inhibitor of this series.

Tyrosinase (EC 1.14.18.1) is a multifunctional dinuclear copper centre enzyme widely distributed in nature and mainly involved in the formation of pigments such as melanins and other polyphenolic compounds.[1] Tyrosinase oxidizes phenols and diphenols using a catalytic mechanism that depends on the presence of copper at its active site.[1] In fungi and vertebrates, tyrosinase catalyzes the two initial steps for the formation of the melanin pigments (melanogenesis), starting from the tyrosine.[2] This enzyme catalyses the conversion of tyrosine to DOPA and the oxidation of the resultant DOPA.[2] So, this enzyme is responsible for the pigmentation of the skin, eyes and hair.[3] In fact, tyrosinase inhibitors have been used as depigmenting agents for the treatment or prevention of hyperpigmentation disorders.[4] Also, tyrosinase is involved in the process to maintain the appearance, flavour, texture and nutritional value of many fresh-cut products.[5] The enzyme extracted from the mushroom *A. bisporus* has high homology with the mammalian one. So, it is suited as a model for melanogenesis and tyrosinase bio-pathways studies.

Coumarins are a large family of compounds, of natural and synthetic origin, which presents different pharmacological activities.[6] Structurally they are lactones of the cinnamic acid. Due to their structural variability, they occupy an important place in the realm of natural products and synthetic organic chemistry.[7] Recent studies pay special attention to their antioxidative, antiinflamatory, anticancer and enzymatic inhibition properties.[8–14]

Resveratrol –3,4′,5-trihydroxystilbene—is a natural polyphenolic compound present in grapes and red wine.[15] It is a phytoalexin produced by some species in response to an external or internal damage (such as a fungal infection).[15,16] In in vitro, ex vivo and in vivo experiments, resveratrol has shown important pharmacological activities including antiinflammatory, antioxidant, anticancer and cardioprotective properties, besides inhibitory activity towards several enzymes.[15–19] Therefore, this compound has been attracting a huge pharmacological interest since the last decade.

In recent studies, some coumarins proved to be mushroom tyrosinase inhibitors.[20,21] In these studies, esculetin and umbelliferone exhibited some of the strongest inhibitory activities of the tested series. Masamoto et al. investigated the structure–activity relationship of 18 coumarins for their inhibitory activity against mushroom tyrosinase. They found that esculetin exhibited the strongest inhibitory activity of those series.[20] Recently, and in contrast with the Masamoto's findings, Sollai et al. have shown that esculetin is considered to be a tyrosinase substrate rather than an inhibitor, whereas umbelliferone seems to be an inhibitor of the mentioned oxidase.[22] These recent findings revealed that tyrosinase affinity can be efficiently modulated by appropriate substitutions in the coumarin moiety. The introduction of hydroxyl groups in different positions is particularly suitable to these modifications.[20,21] Recently, it has been demonstrated that also resveratrol and other stilbenes[23] have inhibitory effects against mushroom tyrosinase activity.[24] Resveratrol showed stronger DOPA oxidase inhibitory activity than kojic acid (reference inhibitor).[25] In fact, until the last years, polyphenols are the largest scaffold in tyrosinase inhibition.[23,26] Their activity depends on

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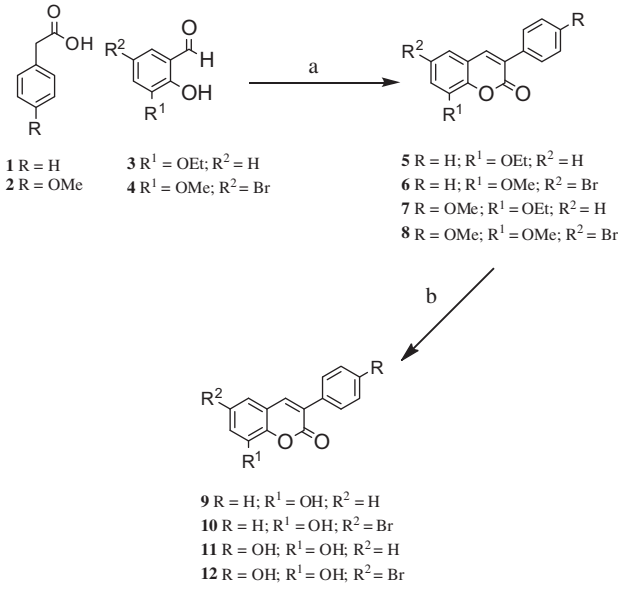
the presence and position of additional substituents whether a polyphenol may act as an inhibitor.[26] So, it was proved that both stilbene and coumarin derivatives show interesting inhibitory effects against tyrosinase.

Tyrosinase inhibitors could have broad applications. As the ideal drug candidate has not been attained, an intensive search for new and innovative tyrosinase inhibitors is still needed. This effort has considerably increased in recent years. In this context, and in an attempt to develop novel tyrosinase inhibitors, we had previously synthesized and described 3-arylcoumarin derivatives in which both the coumarin and the resveratrol templates were present.[21]

Resveratrol (Fig. 1, A) and umbelliferone (B) are very good tyrosinase inhibitors.[2,20] Umbelliferone-resveratrol hybrid (C) and 3-phenylumbelliferone (D) are less active than the mentioned compounds (IC_{50} 3.68 mM and >10, respectively). Although, the previously derivative described by us 6,8,3′,4′,5′-pentahydroxy proved to be the most active compound of the evaluated 3-arylcoumarin series, with an IC_{50} of 0.27 mM.[21] This molecule have two hydroxyl groups under the coumarin ring, being like the coumarin-resveratrol hybrid (E).

Based on these results,[21] in the present work we proposed to continue the 3-phenylcoumarin scaffold study, with different substitutions like methoxyl, ethoxyl, hydroxyl and/or bromo under the 6, 8 and 4′ positions (Scheme 1). We decided to explore the importance of the number and position of ether and hydroxyl groups located in the 3-phenyl ring or in the aromatic ring of the coumarin and, at the same time, the bromination of the coumarin moiety.

The coumarin derivatives **5–12**[27] were efficiently synthesized according to the synthetic protocol outlined in Scheme 1. The 3-phenylcoumarins **5–8** were prepared starting from the conveniently substituted phenylacetic acid **1** or **2** and the appropriate salicylaldehyde **3** or **4**, using dicyclohexylcarbodiimide (DCC) as dehydrating agent, by Perkin reaction,[28–31] in dimethylsulfoxide (DMSO). These reactions gave 55%, 59%, 61% and 60% yield, respectively. Hydrolysis of the methoxyl/ethoxyl groups, by treatment with hydriodic acid 57% in acetic acid/acetic anhydride (1:1),[32] gave the hydroxyl derivatives **9**, **10**, **11** and **12** in 64%, 60%, 61% and 53% yield, respectively. The obtained products were easy to purify by flash chromatography, using a mixture of hexane/ethyl acetate, in a proportion 9:1, as eluent.



Scheme 1. Reagents and conditions: (a) DCC, DMSO, 110 °C, 12 h; (b) HI/AcOH/Ac_2O, 0 °C–rt, 3 h.

The tyrosinase inhibitory activity of compounds **5–12** was evaluated in vitro by the measurement of the enzymatic activity of mushroom tyrosinase enzyme extracted from the mushroom specie *A. bisporus*.[33] Then, the IC_{50} values for inhibitory effects of the new compounds were calculated (Table 1).

In the present communication, the effect of the introduction of a halogen substituent into different hydroxy-3-phenylcoumarins was proposed. In fact, a higher tyrosinase inhibitory activity, regarding the non-halogenated compounds, was observed. The structural change obtained from compound **9** to compound **10** and from **11** to **12**, with the bromo atom in the coumarin nucleus, leads to a significant increase of the tyrosinase activity (from milimolar to micromolar range).

As it is shown in the Table 1, compound **12** is the most active compound of this series. This compound, with a bromo atom and two hydroxyl groups in the 3-phenylcoumarin moiety, has an IC_{50} in the micromolar range (IC_{50} = 215 μM). As expected, the more hydroxyl groups in the coumarin moiety, the better activity the compounds have. When compared with compound **10**, the introduction of one hydroxyl group more in compound **12** increases at least 1.5 times the tyrosinase inhibitory activity. Compound **12** shows, in fact, a lightly higher inhibitory activity than the 6,8-dihydroxy-3-(3′,4′,5′-trihydroxyphenyl)coumarin (IC_{50} = 270 μM), previously described by us.[21] The presence in 6 and 4′

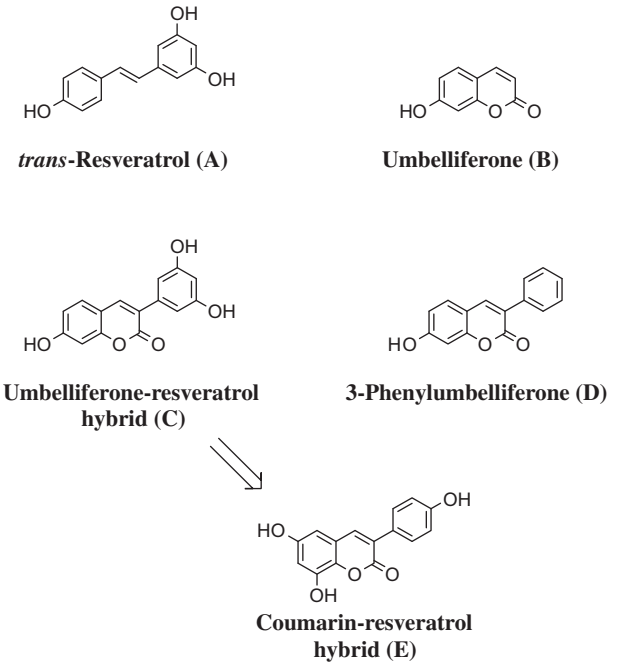


Figure 1. Tyrosinase inhibitors with coumarin/resveratrol derivative structures.

Table 1
Inhibitory effect of compounds **5–12** and umbelliferone on mushroom tyrosinase activity

Compounds	IC_{50} (mM) (L-DOPA 0.5 mM)
5	2.07 ± 0.07
6	>5.0
7	>5.0
8	1.36 ± 0.12
9	>5.0
10	0.302 ± 0.002
11	1.30 ± 0.08
12	0.215 ± 0.085
Umbelliferone	0.42[a]

[a] Obtained from data in Ref.[21]

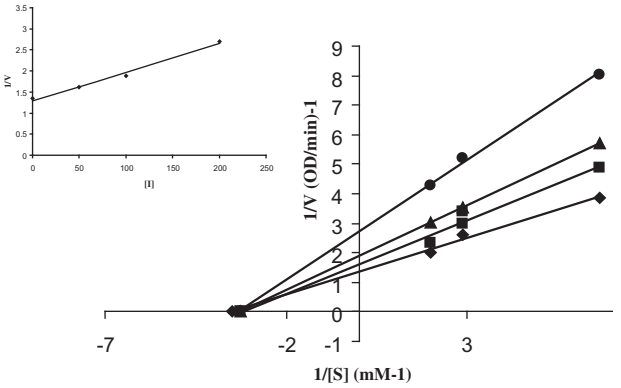


Figure 2. Lineweaver–Burk plots for inhibition of compound **12** on mushroom tyrosinase for catalysis of L-DOPA. Inhibitor concentrations were 0 (♦), 0.050 mM (■), 0.100 mM (▲), 0.200 mM (●). The inset represents the secondary plot of $1/V_{max}$ versus concentration of compound **12**, to determine the inhibition constant (K_i).

positions, at the same time, of a bromo and a hydroxyl substituent, respectively, contributed to an increase of the inhibitory activity. The experimental results show that the methoxyl and ethoxyl derivatives (compounds **5–8**) do not present any significant activity against the tested enzyme. These results suggest that the bromination of the hydroxycoumarins, in spite of the bromination of methoxycoumarins, could be an important step in the synthesis of novel tyrosinase inhibitors.

The inhibitory mechanism of the compound **12** was determined using a Lineweaver–Burk double reciprocal plot (Figure ure2). The data, displayed as a plot of 1/V versus 1/[S], gave three straight lines with different slopes and a horizontal line that intersected at the same point. With an increase in compound concentration, the V_{max} value decreased, whereas the K_m value was unchanged, suggesting that this compound is a non-competitive tyrosinase inhibitor. The inhibition constant of this compound (K_i = 0.189 mM) was determined by plotting the intercept values versus the concentration of the corresponding compound, as shown in Figure 2.

In conclusion, in the present study it was shown that the synthesized coumarin-resveratrol hybrid compounds have inhibitory activity against mushroom tyrosinase. Some of them present tyrosinase inhibitory activity in the micromolar range. The presence of a bromo atom in position 6 of the hydroxycoumarins improves the inhibitory activity respect to the other synthesized derivatives. So, the introduction of a bromo atom improves the pharmacological potential of these 3-phenylcoumarins, confirming that this lead could be effectively optimized in a candidate for the treatment of some hyperpigmentation skin diseases. These finds have encouraged us to continue the efforts towards the optimization of the pharmacological profile of these 3-phenylcoumarins.

Acknowledgement

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27. *8-Ethoxy-3-phenylcoumarin* (**5**). It was obtained with a yield of 55% mp 117–118 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 1.50 (t, 3H, –CH_3, J = 7.0), 4.21 (dd, 2H, –CH_2, J = 14.0 and J = 7.0), 7.09 (t, 2H, H-6, H-7, J = 6.9), 7.21 (t, 1H, H-5, J = 7.8), 7.42–7.48 (m, 3H, H-3′, H-4′, H-5′), 7.72 (dd, 2H, H-2′, H-6′, J = 7.7 and J = 1.4), 7.79 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 14.8, 65.0, 114.5, 119.3, 120.4, 124.3, 128.3, 128.4, 128.5, 128.8, 134.8, 140.1, 143.4, 146.3, 160.2. MS *m*/*z* (%): 267 (16), 266 (M^+, 83), 239 (16), 238 (20), 212 (14) 211 (20), 181 (15), 153 (25). Anal. Calcd for $C_{17}H_{14}O_3$: C, 76.68; H 5.30. Found: C, 76.66; H, 5.35.
6-Bromo-8-methoxy-3-phenylcoumarin (**6**). It was obtained with a yield of 59% 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), 7.42–7.47 (m, 3H, H-3′, H-4′, H-5′), 7.68–7.78 (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, 134.9, 139.1, 142.9, 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), 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.
8-Ethoxy-3-(4′-methoxyphenyl)coumarin (**7**). It was obtained with a yield of 61% mp 99–100 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 1.50 (t, 3H, –CH_3, J = 7.0), 3.84 (s, 3H, –OCH_3), 4.19 (dd, 2H, –CH_2, J = 14.0, J = 7.0), 6.84–7.26 (m, 5H, H-3′, H-5′, H-5, H-6, H-7), 7.69 (t, 2H, H-2′, H-6′, J = 7.7), 7.73 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 14.8, 55.4, 64.8, 113.8, 114.0, 119.1, 120.49, 124.2, 127.1, 127.8, 129.7, 129.8, 138.6, 138.7, 146.2, 160.0. MS *m*/*z* (%): 297 (35), 296 (M^+, 100), 268 (54), 240 (47) 225 (45), 197 (13), 152 (11), 139 (15). Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.91; H, 5.39.
6-Bromo-8-methoxy-3-(4′-methoxyphenyl)coumarin (**8**). It was obtained with a yield of 60% mp 183–184 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 3.85 (s, 3H, –OCH_3), 3.96 (s, 3H, –OCH_3), 6.94–6.98 (m, 2H, H-3′, H-5′), 7.12 (d, 1H, H-7, J = 1.8), 7.23 (dd, 1H, H-5, J = 2.1 and J = 0.9), 7.63–7.69 (m, 3H, H-4, H-2′, H-6′). ^{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), 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.
8-Hydroxy-3-phenylcoumarin (**9**). It was obtained with a yield of 64% mp 199–200 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 7.14–7.19 (m, 3H, H-5, H-6, H-7), 7.40–7.49 (m, 3H, H-3′, H-4′, H-5′), 7.70–7.78 (m, 2H, H-2′, H-6′), 8.19 (s, 1H, H-4), 10.25 (s, 1H, –OH). ^{13}C NMR ($CDCl_3$) δ (ppm): 118.0, 118.6, 120.4, 124.6, 126.7, 128.2, 128.5, 134.7, 141.0, 141.7, 144.3, 159.6. MS *m*/*z* (%): 239 (16), 238 (M^+, 100), 210 (80), 181 (13), 153 (22) 152 (20), 105 (9), 76 (15), 51 (6). Anal. Calcd for $C_{15}H_{10}O_3$: C, 75.62; H, 4.23. Found: C, 75.57; H, 4.28.
6-Bromo-8-hydroxy-3-phenylcoumarin (**10**). It was obtained with a yield of 60% mp 163–164 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 7.19 (d, 1H, H-7, J = 2.0), 7.42–7.51 (m, 4H, H-5, H-3′, H-4′, H-5′), 7.70 (dd, 2H, H-2′, H-6′, J = 7.6 and J = 1.6), 8.13 (s, 1H, H-4), 10.79 (s, 1H, –OH). ^{13}C NMR ($CDCl_3$) δ (ppm): 115.5, 119.9, 120.4, 121.8, 127.9, 128.3, 128.5, 128.8, 134.4, 139.7, 141.1, 145.6, 159.1. MS *m*/*z* (%):

318 (99), 317 (17), 316 (M^+, 100), 291 (12), 289 (13), 153 (22), 152 (51), 151 (12), 76 (25). Anal. Calcd for $C_{15}H_9BrO_3$: C, 56.81; H, 2.86. Found: C, 56.77; H, 2.81.

8-hydroxy-3-(4′-hydroxy)phenylcoumarin (**11**). It was obtained with a yield of 61% mp 237–238 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 6.81-6.85 (m, 2H, H-3′, H-5′), 7.03-7.12 (m, 3H, H-5, H-6, H-7), 7.55–7.59 (m, 2H, H-2′, H-6′), 8.05 (s, 1H, H-4), 9.80 (s, 1H, –OH), 10.15 (s, 1H, –OH). ^{13}C NMR ($CDCl_3$) δ (ppm): 115.3, 117.8, 118.6, 120.9, 124.7, 125.6, 126.8, 130.1, 139.2, 141.6, 144.5, 158.2, 160.1. MS *m*/*z* (%): 255 (18), 254 (M^+, 100), 227 (16), 226 (98), 197 (15), 169 (12), 115 (12). Anal. Calcd for $C_{15}H_{10}O_4$: C, 70.83; H, 3.87. Found: C, 70.80; H, 3.83.

6-Bromo-8-hydroxy-3-(4′-hydroxyphenyl)coumarin (**12**). It was obtained with a yield of 53% mp 249–250 °C. ^{1}H NMR ($CDCl_3$) 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 ($CDCl_3$) 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* (%): 334 (99), 333 (45), 332 (M^+, 100), 307 (31), 305 (35), 225 (26), 197 (29), 169 (35), 168 (46), 153 (24), 141 (21), 140 (16), 118 (17), 115 (28), 98 (13), 89 (14), 84 (18), 75 (11), 63 (13). Anal. Calcd for $C_{15}H_9BrO_4$: C, 54.08; H 2.72. Found: C 54.01, H 2.69.

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33. Pre-incubation with the enzyme: 1/15 M phosphoric acid buffer solution (pH 6.8, 1.8 mL), an aqueous solution of mushroom tyrosinase (1000 U/mL, Sigma Chemical Co., 0.1 mL) and DMSO (0.1 mL) with or without the sample. The mixture was incubated at 25 °C for 10 min. Then, a 1.05 mM of L-3,4-dihydroxyphenylalanine (DOPA) solution (1 mL) was added and the reaction was monitored at 475 nm, for 5 min. The percent of tyrosinase activity inhibition was calculated as: inhibition (%) = (A−B)/A × 100, where A represents the difference in the absorbance of control sample between 0.5 and 1.0 min, and B represents the difference in absorbance of the test sample between 0.5 and 1.0 min. The IC_{50} value, a concentration giving 50% inhibition of tyrosinase activity, was determinate by interpolation of dose–response curves. The mushroom tyrosinase activity was determinate by spectrophotometric assays (Varian Cary 50). Umbelliferone was used as a reference tyrosinase inhibitor.