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Remarkable antioxidant properties of a series of hydroxy-3-arylcoumarins ☆

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ABSTRACT

In the present work we synthesized a series of hydroxy-3-arylcoumarins (compounds **1**–**9**), some of them previously described as MAO-B selective inhibitors, with the aim of evaluating their antioxidant properties. Theoretical evaluation of ADME properties of all the derivatives was also carried out. From the ORAC-FL, ESR and CV data it was concluded that these derivatives are very good antioxidants, with a very interesting hydroxyl, DPPH and superoxide radicals scavenging profiles. In particular compound **9** is the most active and effective antioxidant of the series (ORAC-FL = 13.5, capacity of scavenging hydroxyl radicals = 100%, capacity of scavenging DPPH radicals = 65.9% and capacity of scavenging superoxide radicals = 71.5%). Kinetics profile for protection fluorescein probe against peroxyl radicals by addition of antioxidant molecule **9** was also performed. Therefore, it can operate as a potential candidate for preventing or minimizing the free radicals overproduction in oxidative-stress related diseases.

1. Introduction

Phenolic compounds are bioactive substances widely distributed in the vegetable kingdom. Generally, this group of compounds has one or more aromatic rings in their structure and one or more hydroxyl groups. They have been described to act as natural antioxidants and their presence contributes to prevent or minimize several types of oxidative processes.[1] Due to their antioxidant activity their ingestion is correlated with interesting benefits to health. Therefore, the research and characterization of new bioactive phenolic substances from diet has been intensified in the last years, either for the development of nutraceutics or medicines.[1] Due to their antioxidant properties they can protect cells from the oxidative damage of the reactive oxygen species (ROS). In fact, the overproduction of free radicals have been related to cellular membrane and DNA damage, and indirectly with aging and oxidative-stress related diseases like cancer, cardiovascular and neurodegenerative pathologies.[2] Therefore, antioxidants are very important for protecting the organisms from oxidative disorders, in which ROS are also involved.[3,4] Antioxidants are capable of decrease or prevent oxidation processes through different mechanisms, such as scavenging free radicals, inhibition of pro-oxidant enzymes or chelation of transition metal ions.[5]

An increasing number of reports suggested the involvement of oxidative stress in neurodegenerative diseases (ND), where the increased formation of ROS can contribute to neuronal damage and cell death.[3,4]

Suggestion has been made that the etiology of Parkinson's (PD) and Alzheimer's (AD) diseases may be closely linked to biochemical changes resultant from this oxidative stress.[6–8] Dopamine (DA) auto-oxidation naturally produces oxidative species and may contribute to ND such as PD and ischemia/reperfusion-induced damage. Monoamine oxidase (MAO) enzyme (particularly MAO-B) is responsible for metabolizing DA and plays an important role in oxidative stress through altering the redox state of neuronal and glial cells, leading to neuronal death.[9] Consequences are an over-production of MAO and non-MAO initiated hydrogen peroxide (H_2O_2) by proliferated reactive microglia and inability of neurons to dispose of H_2O_2 and other recative species like peroxyl radicals.[10] H_2O_2 produces highly toxic ROS, namely hydroxyl

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radical, by Fenton reaction that is catalyzed by iron and neuromelanin.[11] Concerning the mechanism of the clinical efficacy of MAO-B inhibitors in PD, the inhibition of DA degradation (a symptomatic effect) and also the prevention of the formation of neurotoxic DA degradation products, that is, ROS and DA derived aldehydes have been speculated.[12] The neuroprotective effect of rasagiline, a well-known MAO-B inhibitor, might be explained through multiple mechanisms, possibly due to reduction of DA catabolism with a subsequent increased activity on dopaminergic D_2 receptors and suppressing the action of ROS as well.[13] So, the possible mechanism of neuroprotection of MAO-B inhibitors may be related not only to MAO-B inhibition but also to induction and activation of multiple factors related with oxidative stress and apoptosis.[14]

Coumarins are a family of compounds widely distributed in the nature.[15] Due to their structural features, and biological properties, namely anticancer, anti-inflammatory, antioxidant, antithrombotic, vasorelaxant, antiviral and enzymatic inhibition agents, they have been ascribed as important building blocks in Organic Chemistry and Medicinal Chemistry.[16–23]

Recently, it was shown by our group that 3-substituted aryl coumarins are potent and selective MAO-B inhibitors.[24–30] In addition, it has been found that hydroxycoumarins are antioxidants scavenging ROS and/or chelating transition metals, exhibiting tissue-protective properties.[6,31–33] The complementarity of these activities for 3-arylcoumarins was not previously studied and described. The versatility of the used reactions allowed obtaining a family of compounds with hydroxyl and/or methyl substituents in different positions of the molecule. The election of these derivatives has considered the previously MAO-B inhibitory pharmacological evaluation and the low cost of the commercial reagents to begin with. Also, the influence of the substituents in the desired activity was taken into account.

2. Materials and methods

2.1. Chemistry

Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using TMS as internal standard (chemical shifts in δ values, J in Hz). Mass spectra were obtained using a Hewlett–Packard 5988A spectrometer. Elemental analyses were performed using a Perkin–Elmer 240B microanalyser and were within ±0.4% of calculated values in all cases. Silica gel (Merck 60, 230-00 mesh) was used for flash chromatography (FC). Analytical thin layer chromatography (TLC) was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm).

2.1.1. General procedure for the preparation of methoxy-3-arylcoumarins

To a solution of the conveniently substituted *ortho*-hydroxybenzaldehyde (7.34 mmol) and the corresponding phenylacetic acid (9.18 mmol) in dimethyl sulfoxide (15 mL), *N*,*N*′-dicyclohexylcarbodiimide (11.46 mmol) was added. The mixture was heated at 110 °C for 24 h. Then, ice (100 mL) and acetic acid (10 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 layers were combined and washed with sodium bicarbonate solution (50 mL, 5%) and water (20 mL). Subsequently, the solvent was evaporated under vacuum and the dry residue was purified by flash chromatography (hexane/ethyl acetate 9:1), to give the desired methoxy-3-arylcoumarins.[23,28]

2.1.2. General procedure for the preparation of hydroxy-3-arylcoumarins

To a solution of a methoxy-3-arylcoumarin (0.50 mmol) in acetic acid (5 mL) and acetic anhydride (5 mL), at 0 °C, hydriodic acid 57% (10 mL) was added dropwise. The mixture was stirred under reflux, for 3 h. The solvent was evaporated under vacuum and the dry residue was purified by crystallization (CH_3CN).[23,28,34]

2.1.2.1. 3-(3′,4′-Dihydroxyphenyl)-6-methylcoumarin (4).

Yield: 92%; mp 199–200 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.43 (s, 3H, –CH_3), 6.43 (s, 1H, H-2′), 7.01 (d, J = 7.1 Hz, 1H, H-5′), 7.14 (d, J = 7.0 Hz, 1H, H-6′), 7.28–7.32 (m, 2H, H-7, H-8), 7.57 (d, J = 2.2 Hz, 1H, H-5), 7.83 (s, 1H, H-4), 10.40 (s, 2H, –OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.7, 100.1, 110.7, 111.6, 115.7, 119.4, 120.1, 127.3, 127.4, 132.1, 133.7, 138.8, 146.5, 146.8, 151.3, 161.6; EI MS *m*/*z*: 269 (13), 268 (M^+, 100), 241 (31), 240 (70), 239 (22), 165 (30), 125 (12), 111 (10); Anal. Calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.60; H, 4.49.

2.1.2.2. 3-(3′,4′-Dihydroxyphenyl)-8-methylcoumarin (5).

Yield: 85%; mp 205–206 °C; ^{1}H NMR (300 MHz, DMSO-$\boldsymbol{d_6}$): $\boldsymbol{\delta}$ 2.50 (s, 3H, –$\boldsymbol{CH_3}$), 6.80 (d, $\boldsymbol{J}$ = 8.3 Hz, 1H, H-5′), 7.05 (dd, $\boldsymbol{J}$ = 8.2, $\boldsymbol{J}$ = 2.2 Hz, 1H, H-6′), 7.22 (d, $\boldsymbol{J}$ = 2.2 Hz, 1H, H-2′), 7.25 (d, $\boldsymbol{J}$ = 7.6 Hz, 1H, H-6), 7.44 (dd, $\boldsymbol{J}$ = 7.4, $\boldsymbol{J}$ = 1.0 Hz, 1H, H-7), 7.57 (dd, $\boldsymbol{J}$ = 7.6, $\boldsymbol{J}$ = 1.1 Hz, 1H, H-5), 8.09 (s, 1H, H-4), 9.09 (s, 1H, –OH), 9.24 (s, 1H, –OH); ^{13}C NMR (75 MHz, DMSO-$\boldsymbol{d_6}$): $\boldsymbol{\delta}$ 14.9, 115.4, 116.0, 119.5, 119.9, 124.1, 124.6, 125.7, 126.1, 126.5, 132.2, 138.7, 144.8, 146.2, 150.9, 159.9; EI MS ***m*/*z***: 270 (12), 269 (76), 268 (M^+, 82), 241 (47), 240 (100) 239 (57), 211 (23), 166 (19), 165 (58), 152 (18), 139 (14), 125 (30), 111 (28), 82 (19); Anal. Calcd for $\boldsymbol{C_{16}H_{12}O_4}$: C, 71.64; H, 4.51. Found: C, 71.63; H, 4.49.

2.1.2.3. 3-(3′,5′-Dihydroxyphenyl)-8-methylcoumarin (6).

Yield: 90%; mp 180–181 °C; ^{1}H NMR (300 MHz, DMSO-$\boldsymbol{d_6}$): $\boldsymbol{\delta}$ 2.49 (s, 3H, –$\boldsymbol{CH_3}$), 6.70 (s, 3H, H-2′, H-4′, H-6′), 7.26 (t, $\boldsymbol{J}$ = 7.6 Hz, 1H, H-6), 7.48 (d, $\boldsymbol{J}$ = 7.8 Hz, 1H, H-7), 7.62 (d, $\boldsymbol{J}$ = 7.7 Hz, 1H, H-5), 8.11 (s, 1H, H-4), 10.27 (s, 2H, OH); ^{13}C NMR (75 MHz, DMSO-$\boldsymbol{d_6}$): $\boldsymbol{\delta}$ 15.3, 103.3, 107.2, 119.6, 124.5, 125.1, 126.8, 127.2, 133.1, 136.6, 140.9, 151.6, 158.5, 160.0; EI MS ***m*/*z***: 269 (21), 268 (M^+, 100), 241 (12), 240 (63), 239 (26); Anal. Calcd for $\boldsymbol{C_{16}H_{12}O_4}$: C, 71.64; H, 4.51. Found: C, 71.60; H, 4.50.

2.1.2.4. 3-(3′,4′,5′-Trihydroxyphenyl)-8-methylcoumarin (7).

Yield: 82%; mp 189–190 °C. ^{1}H NMR (300 MHz, DMSO-$\boldsymbol{d_6}$): $\boldsymbol{\delta}$ 2.49 (s, 3H, –$\boldsymbol{CH_3}$), 6.95 (s, 2H, H-2′, H-6′), 7.20 (t, $\boldsymbol{J}$ = 7.4 Hz, 2H, H-6, H-7), 7.38 (d, $\boldsymbol{J}$ = 7.4 Hz, 1H, H-5), 7.79 (s, 1H, H-4), 10.55 (s, 2H, –OH), 10.60 (s, 1H, –OH); ^{13}C NMR (75 MHz, DMSO-$\boldsymbol{d_6}$DMSO-$\boldsymbol{d_6}$): $\boldsymbol{\delta}$ 15.6, 106.1, 119.4, 124.2, 125.4, 125.7, 127.7, 130.4, 132.7, 135.6, 139.8, 146.7, 146.9, 160.6; EI MS ***m*/*z***: 285 (16), 284 (M^+, 100), 283 (84), 256 (32), 181 (10) 141 (10); Anal. Calcd for $\boldsymbol{C_{16}H_{12}O_5}$: C, 67.60; H, 4.25. Found: C, 67.61; H, 4.28.

2.2. Antioxidant assays

2.2.1. Oxygen radical antioxidant capacity-fluorescein (ORAC-FL)

The ORAC analyses were carried out on a Synergy HT multi detection microplate reader, from Bio-Tek Instruments, Inc. (Winooski, USA), using white polystyrene 96-well plates, purchased from Nunc (Denmark). Fluorescence was read from the top, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by Gen 5 software. The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4), and 200 μL final volume. FL (70 nM, final concentration) and hydroxy-3-arylcoumarin solutions in methanol with