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Synthesis of new ferrocenyl dehydrozingerone derivatives and their effects on viability of PC12 cells

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ABSTRACT

A series of novel compounds deriving from the conjugation of ferrocene with curcumin-related bioactive molecules as dehydrozingerone, zingerone and their biphenyl dimers was prepared by Claisen–Schmidt condensation of the suitable aromatic aldehydes and acetylferrocene in different conditions according to the starting material. The obtained compounds were fully characterized by NMR spectroscopy and cyclic voltammetry and reversible electrochemical behavior was recorded for monomer derivatives. The cell viability of PC12 cells after exposure to the organometallic compounds was also evaluated and a reduced toxicity with respect to the ferrocene was detected. In comparison with biphenyl **4**, a compound that manifested antiproliferative and apoptotic activities and was quite toxic on PC12 cells, the exposure to the ferrocenyl analogue **14** resulted in roughly fourfold increase in the cell viability. Ferrocenyl chalcones **14** and **16**–**18** significantly increased the oxidative stress generated by hydrogen peroxide, a molecule generally accumulated in cancer cells and, recently, studied as prodrug.

1. Introduction

Many efforts have been devoted to the development of new agents that, by targeting simultaneously multiple etiologies of a disease, may be more beneficial than selective agents for a single receptor site [1,2].

Curcumin **1** is a well-established active compound in dealing with different biochemical pathways leading to cancer. It is the major metabolite extracted from the rhizome of *Curcuma longa*, a plant routinely used in the preparation of curry spice as component of Asian traditional medicine (Fig. 1) [3,4].

A wide spectrum of biological properties (*e.g.* anti-inflammatory, anticancer, neuroprotective) attributed to curcumin has been related with its excellent free radical scavenging and antioxidant activities due to the presence of two guaiacyl moieties that proved to be very effective in stabilizing phenoxy radicals [5,6]. Unfortunately, curcumin has low solubility in aqueous and physiological solutions wherein it undergoes rapid degradation into ferulic acid, vanillin and dehydrozingerone **2** (Fig. 1) [7,8]. Like curcumin, dehydrozingerone **2** is known for its interesting anti-inflammatory and anticancer activities [9].

In a previous study we prepared compound **4**, the dimer of *O*Me-dehydrozingerone **3**, as the first curcumin-related biphenyl. We found that compound **4** was more active in inhibiting malignant melanoma and neuroblastoma cells growth when compared to curcumin itself (Fig. 1) [10]. Normal fibroblasts proliferation was not affected by this treatment therefore compound **4** could represent a good candidate in developing new therapies against neural crest-derived tumors.

Recently, we found that biphenyl **4** and biphenyl **5**, the latter being the biphenyl analogue of dehydrozingerone **2**, are able to partially inhibit the aggregation process of α-synuclein, suggesting the potential role of a hydroxylated biphenyl scaffold in the design of α-synuclein aggregation inhibitors in neurodegenerative pathologies [11]. Protective effects against oxidative stress induced in PC12 cells, a neuronal cell model, was also investigated for biphenyls **4** and **5** and their derivatives.

Compared to phenols, hydroxylated biphenyls display higher antioxidant activity in virtue of the presence of two hydroxyl groups at the *ortho–ortho′* positions generally providing reduction in toxicity compared with the corresponding phenolic monomer [12,13].

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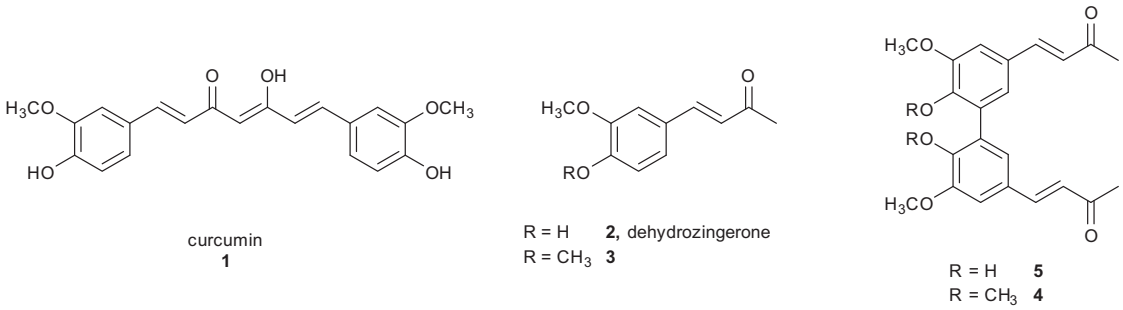

Fig. 1. Natural and natural-like phenols and biphenols.

Ferrocene is a neutral, chemically stable, relatively non toxic molecule whose good reversible redox proprieties seem to be strongly associated with the biological activity. As an example, the activity of tamoxifen, the most common drug used to treat patients diagnosed with breast cancer, was enhanced when a unit of ferrocene is covalently bonded to the molecule of hydroxytamoxifen, the active metabolite and it was thought that the extended π-system in hydroxytamoxifen-ferrocene conjugate plays an important role in the mode of action of the drug [14,15].

The organometallic approach has been successfully applied to other bioactive compounds like flavones [16], amino acids [17,18], chalcones [19,20], quinolinones [21], ellagitannins [22], cyclodextrin [23] and curcumin [24,25]. Although several ferrocenyl-curcumin derivatives were prepared by different groups, all of them contain a β-diketoeptadiene or pentenedienone chain in their structure [24,25].

Ferrocene derivatives of dehydrozingerone, zingerone and the corresponding C_2-symmetric dimers have not been synthetized so far and here we report their preparation and characterization. The electrochemical properties and cytotoxic activity in PC12 cells of the obtained compounds was also evaluated in comparison with data of the corresponding compounds lacking in ferrocene unit.

2. Experimental section

2.1. Instrumentation

^{1}H and ^{13}C NMR spectra were registered in $CDCl_3$ at 400.13 and 100.69 MHz respectively on a Bruker Avance™ 400 instrument. 2D-NMR experiments were performed using standard Bruker microprograms. All NMR spectra were recorded using $CDCl_3$ unless otherwise specified. Chemical shifts (δ) are given as ppm relative to the residual solvent peak and coupling constants (J) are in Hz. In the NMR assignments, Cp and Cp′ refer to substituted and unsubstituted cyclopentadienyl rings, respectively. UV spectra were recorded with a spectrometer Perkin-Elmer Lambda 35 in dichloromethane at concentration of 0.74×10^{-5} M. Elemental analyses were obtained from the Department of Pharmaceutical Sciences, University of Catania. Melting points are uncorrected. Cyclic voltammetries were performed with an eDAQ QuadStat, an e-Corder 410 and the Echem software (eDAQ Europe, Poland).

2.2. Materials

Sodium hydroxide microprills were purchased from Riedel-de-Haën (Germany). Column chromatography was performed on Si 60 (230–400 mesh) silica gel using the specified eluants. Ferrocene, veratraldehyde, hydrogen peroxide were purchased from Sigma–Aldrich (Milan, Italy), vanillin from Alfa Aesar GmbH & Co KG (Karlsruhe, Germany) and zingerone from Chemos GmbH (Regenstanf, Germany). All solvents of purity >98% (GC) were used as received.

2.3. General procedure for the O-benzylation of vanillin and divanillin

To a solution of the suitable aldehyde (1 mmol) in DMF (5 mL), K_2CO_3 (2 eqv.) and 4-methylbenzylbromide (1.1 eqv.) were added and the suspension stirred at room temperature until complete conversion of the substrate was detected by TLC analysis (12–20 h). The solvent was then evaporated *in vacuo* and the residue dissolved in AcOEt (15 mL) and treated with satd. NH_4Cl solution (3×10 mL). The organic phase, extracted, then was washed with brine, dried over Na_2SO_4 and taken to dryness to give a residue that was purified by chromatographic column (*n*-hexane:AcOEt 9:1) to give pure *O*-(4-methyl)benzyl derivatives.

2.3.1. 3-Methoxy-4-(4-methyl)benzyloxy-benzaldehyde (**9**)

95% yield, white solid, mp = 69–71 °C; ^{1}H NMR: δ 2.36 (s, 3H, Me), 3.94 (s, 3H, OMe), 5.21 (s, 2H, CH_2), 6.99 (d, 1H, J = 8.0, H-5), 7.20 (d, 2H, J = 8.0, ArH), 7.33 (d, 2H, J = 8.0, ArH), 7.39 (dd, 1H, J = 1.6 and 8.0, H-6), 7.43 (d, 1H, J = 1.6, H-3), 9.84 (s, 1H, CHO); ^{13}C NMR: δ 21.1 (Me), 55.9 (OMe), 70.7 (CH_2), 109.2 (ArH), 112.3 (ArH), 126.5 (ArH), 127.2 (2× ArH), 129.3 (2× ArH), 130.1 (Ar), 132.8 (Ar), 137.9 (Ar), 150.0 (Ar), 153.6 (Ar), 190.8 (CHO). *Anal.* Calc. for $C_{16}H_{16}O_3$: C, 74.97; H, 6.30. Found: C, 75.05; H, 6.28%.

2.3.2. 2,2′-Di(4-methyl)benzyloxy-3,3′-dimethoxy-5,5′-diformyl-1,1′-biphenyl (**13**)

93% yield, white solid, mp = 128–129 °C; ^{1}H NMR: δ 2.27 (s, 6H, Me), 4.00 (s, 6H, OMe), 4.89 (s, 4H, CH_2), 6.88 (d, 4H, J = 8.0, ArH), 6.96 (d, 4H, J = 8.0, ArH), 7.14 (d, 2H, J = 1.6, H-6 and H-6′), 7.49 (d, 2H, J = 1.6, H-4 and H-4′), 9.77 (s, 2H, CHO); ^{13}C NMR: δ 21.0 (Me), 56.0 (OMe), 74.5 (CH_2), 109.6 (Ar-H), 128.1 (ArH), 128.2 (2× ArH), 128.7 (2× ArH), 131.7 (Ar), 132.4 (Ar), 133.7 (Ar), 137.6 (Ar), 150.0 (Ar), 153.4 (Ar), 191.0 (CHO). *Anal.* Calc. for $C_{32}H_{30}O_6$: C, 75.26; H, 5.93. Found: C, 75.32; H, 5.90%.

2.4. Solvent-free procedure for the synthesis of ferrocenyl chalcones (Method A)

A 10-mL sealed vial charged with the suitable *O*-protected aldehyde (0.5 mmol) and acetylferrocene (0.5 mmol) was placed in a bath oil at 100 °C and solid NaOH (1.0 mmol) was added. The mixture was stirred vigorously and left to react until the TLC analysis showed complete disappearance of substrates (1–3 h). After addition of CH_2Cl_2 (10 mL) the mixture was partitioned with satd. NH_4Cl solution (3×5 mL) and the organic layer, extracted, was washed with brine and dried over Na_2SO_4. The organic solvent was then removed *in vacuo* and the residue recrystallized from *n*-hexane/CH_2Cl_2 to give pure chalcones.