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Combined dual effect of modulation of human neutrophils' oxidative burst and inhibition of colon cancer cells proliferation by hydroxycinnamic acid derivatives

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ABSTRACT

Colon cancer is one of the most incident cancers in the Western World. While both genetic and epigenetic factors may contribute to the development of colon cancer, it is known that chronic inflammation associated to excessive production of reactive oxygen and nitrogen species by phagocytes may ultimately initiate the multistep process of colon cancer development. Phenolic compounds, which reveal antioxidant and antiproliferative activities in colon cancer cells, can be a good approach to surpass this problem. In this work, hydroxycinnamic amides and the respective acid precursors were tested in vitro for their capacity to modulate human neutrophils' oxidative burst and simultaneously to inhibit growth of colon cancer cells. A phenolic amide derivative, caffeic acid hexylamide (CAHA) (**4**) was found to be the most active compound in both assays, inhibiting human neutrophils' oxidative burst, restraining the inflammatory process, inhibiting growth of colon cancer cells and triggering mitochondrial dysfunction that leads cancer cells to apoptosis. Altogether, these achievements can contribute to the understanding of the relationship between antioxidant and anticancer activities and based on the structure–activity relationships (SAR) established can be the starting point to find more effective phenolic compounds as anticancer agents.

1. Introduction

Colon cancer is one of the most incident cancers in the Western World[1] and nowadays it is also spreading into Asian countries, probably due to the adoption of Western diet. In spite of relevant improvement in survival over the past decade, a significant number of patients relapse after surgical and conventional therapies and do not respond to metastatic cancer treatment.[2,3] On the other hand, it is well known that cytotoxic drugs used in chemotherapy protocols have several adverse effects namely the weakening of patients' immune system, also destroying peripheral blood mononuclear cells, which are critical components of the immune system to fight opportunistic infections as well as cancer cells. Taking this knowledge into account, the search for new therapeutic options for the chemoprevention and/or the treatment of colon cancer is a matter of interest.

In addition, it has been assumed that excessive production of reactive oxygen (ROS) and nitrogen (RNS) species by phagocytes, namely neutrophils, may lead to chronic inflammation and ultimately initiate the multistage process of colon cancer development.[4,5] Further, it has been described that ROS are involved, not only in the initiation of the carcinogenesis process, but also in cancer promotion and progression.[6,7] In particular, the inflammatory bowel disease, namely the ulcerative colitis, has been linked to an increased risk of colorectal cancer being the oxidative reactions an important part of the inflammatory response.[8,9]

A large amount of scientific evidence reported in the literature suggests that phenolic compounds present in diet or consumed

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alone can act as chemopreventive and/or chemotherapeutic agents.[10] Among these, hydroxycinnamic acid derivatives constitute a major group of antioxidant compounds with inhibitory activity in proliferation of several cancer cell lines. Particularly, caffeic acid (CA) showed a protective effect on paclitaxel induced antiproliferation and apoptosis of lung cancer cells[11] however it also presented antiproliferative effects against colon,[12,13] fibrosarcoma,[14] breast,[15,16] cervical,[16] liver,[15,17] and leukemia cancer cells.[18] Ferulic acid (FA) is described as an antiproliferative agent against breast and liver cancer cells[15] and revealed to delay the cell cycle progression, specifically in the S and G2/M phases of colon cancer cells.[19] 3,4,5-Trihydroxycinnamic acid (OHCA) showed antiproliferative activities in cervical, colon, prostate and oral cavity cancer cell lines.[20] Recently, new lipophilic caffeic and ferulic acid derivatives were synthesized and their cytotoxicity was compared with that of the parent compounds showing increased cytotoxicity towards breast cancer cell lines. These results indicated that the new compounds inhibited cell proliferation and induced cell cycle alterations and cell death in the referred cancer cells.[21]

Based on the above-mentioned considerations, we aimed to find new and more effective agents suitable for chemoprevention and/or chemotherapeutic purposes against colon cancer which will be able to simultaneously modulate human neutrophils' oxidative burst, restraining the inflammatory process, and to inhibit growth of colon cancer cells. For this purpose, *n*-hexylamide derivatives of caffeic, ferulic and 3,4,5-tryhydroxycinnamic acids with superior lipophilicity and consequently with improved ability to cross cell membranes were synthesized (Scheme 1). Subsequently, they were screened along with their parent acids, to test their anti-inflammatory activity against human neutrophils' oxidative burst as well as in terms of cytotoxicity, on two colon adenocarcinoma cell lines with different genetic background and origin localization, WiDr (rectosigmoid) and C2BBe1 (descending colon). Finally, some structure–activity considerations were inferred.

2. Materials and methods

2.1. Chemistry

Reactions were controlled by thin layer chromatography (TLC) using silica gel 60 F254 plates. Column chromatography was performed using silica gel 60 (0.063–0.200 mm). Melting points (MPs) were determined on a Reichert Thermopan hot block apparatus and were not corrected. IR spectra were recorded on a Jasco 420 FT/IR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 600 MHz and 150 MHz, respectively on a Varian Unity 600. Chemical shifts were recorded in δ values in parts per million (ppm) downfield from tetramethylsilane as an internal standard. All *J* values are given in Hz. Caffeic (**1**) and ferulic (**2**) acids were purchased from Sigma–Aldrich (Schnelldorf, Germany) and 3,4,5-trihydroxycinnamic acid (**3**) to Apin Chemicals Limited (Abingdon, Oxon, United Kingdom). Reagents and solvents were used as obtained from suppliers without further purification.

2.2. General procedure to obtain the cinnamic acid hexylamides 4, 5 and 6

To synthesize the amides CAHA (**4**), FAHA (**5**) and OHCAHA (**6**), cinnamic acids CA (**1**), FA (**2**) and OHCA (**3**), respectively (Scheme 1) were dissolved in dimethylformamide (DMF) and triethylamine (TEA). The solution was then cooled in an ice-water bath and hexylamine was added, followed by a solution of (benzotriazol-1-yloxy) tris(dimethylamino)phosphonium hexafluorophosphate (BOP) in dichloromethane. The mixture was stirred at 0 °C for 30 min and then at room temperature for specific periods of time. Dichloromethane was removed under reduced pressure and the remaining solution was diluted with water (100 mL). The mixture was then extracted with ethyl acetate (2 × 100 mL). The extracts were washed with 1 N HCl (2 × 100 mL), water (2 × 100 mL), $NaHCO_3$ 5% (3 × 100 mL) and finally with water (2 × 100 mL), dried over anhydrous $MgSO_4$, filtered and concentrated. The obtained residues were purified by column chromatography yielding the corresponding hexylamides (**4**, **5** and **6**).

2.2.1. *N*-Hexyl-3-(3,4-dihydroxyphenyl)-2-propenamide (4)

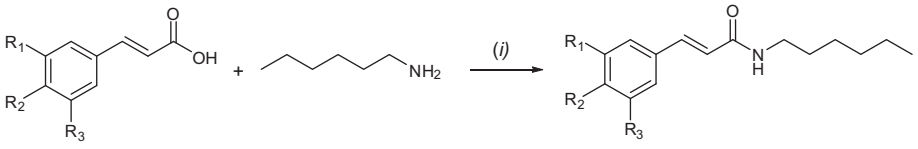
As described before.[22]

2.2.2. *N*-Hexyl-3-(4-hydroxy-3-methoxyphenyl)-2-propenamide (5)

As described before.[22]

2.2.3. *N*-Hexyl-3-(3,4,5-trihydroxyphenyl)-2-propenamide (6)

Compound **3** (250.0 mg, 1.27 mmol); DMF (2.9 mL); TEA (0.18 mL); hexylamine (0.17 mL, 1.27 mmol); BOP (561.7 mg, 1.27 mmol); CH_2Cl_2 (3 mL). The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 5 h 30 min. The residue obtained after work-up was purified by silica gel column chromatography (hexane/ethyl acetate) giving the pure compound **6** in 39% yield. $Mp_{(hexane/ethyl\ acetate)}$ 93–97 °C. IR (NaCl plates, $CHCl_3$) ν_{max} cm^{-1}: 3267 (N—H), 1642 (C=O), 1315 (C—O phenolic alcohol). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.85 (3H, m, $-CH_3$), 1.28 (6H, m, $-CH_2(3'–5')$), 1.41 (2H, m, $-CH_2(2')$), 3.12 (2H, dd, J = 12.9, J = 6.9, $-CH_2(1')$), 6.26 (1H, d, J = 15.6, –CH (α)), 6.47 (2H, s, –CH(2 and 6)), 7.11 (1H, d, J = 15.6, –CH(β)),



(**1**) $R_1 = R_2 = OH$; $R_3 = H$

(**2**) $R_1 = OCH_3$; $R_2 = OH$; $R_3 = H$

(**3**) $R_1 = R_2 = R_3 = OH$

(**4**) $R_1 = R_2 = OH$; $R_3 = H$

(**5**) $R_1 = OCH_3$; $R_2 = OH$; $R_3 = H$

(**6**) $R_1 = R_2 = R_3 = OH$

(**1**) caffeic acid (CA)
(**2**) ferulic acid (FA)
(**3**) 3,4,5-trihydroxycinnamic acid (OHCA)
(**4**) caffeic acid hexylamide (CAHA)
(**5**) ferulic acid hexylamide (FAHA)
(**6**) 3,4,5-trihydroxycinnamic acid hexylamide (OHCAHA)

Scheme 1. Synthesis and structures of hydroxycinnamic acid *n*-hexylamide derivatives. Reagents and conditions: (i) dimethylformamide (DMF), triethylamine (TEA), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), dichloromethane, rt.

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