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Synthesis of a novel ester of hydroxytyrosol and lipoic acid exhibiting an antiproliferative effect on human colon cancer HT-29 cells

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Abstract. A novel hydroxytyrosol-lipoic acid derivative has been synthesized. Key steps are an esterification reaction between tyrosol and α-lipoic acid derivatives and a regioselective aromatic hydroxylation of the monohydroxylated ester performed by 2-iodoxybenzoic acid (IBX) followed by an in situ reduction with sodium dithionite ($Na_2S_2O_4$). The novel ester exhibited an antiproliferative effect on the human colorectal adenocarcinoma HT-29 cell line significantly more potent than its parent compounds.

Introduction. α-Lipoic acid **1** (1,2-dithiolane-3-pentanoic acid or 6,8-thioctic acid) is a sulphur-containing cofactor present in wheat germ, beer yeast and red meat [1]. Hydroxytyrosol **2** [2-(3,4-dihydroxyphenyl)ethanol] is the most important component of the phenolic compounds found in virgin and extra olive oil [2]. Both molecules are components of the human diet and show several interesting pharmacological properties such as antioxidant, anti-inflammatory, anticancer activity [3].

On the basis of the data concerning the biological properties of hydroxytyrosol and α-lipoic acid, we projected the synthesis of the novel compound **3** obtained combining the two natural molecules (Figure 1). The novel ester was evaluated for its effect on the proliferation of the human colorectal adenocarcinoma HT-29 cell line, related to the corresponding parent natural compounds.

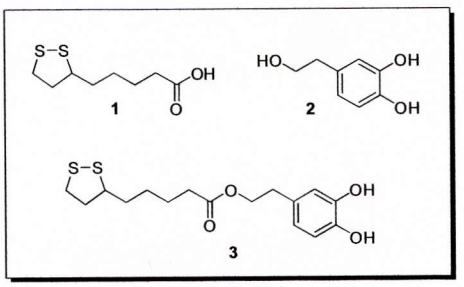

Figure 1

Material and Methods. Reagents and solvents were purchased from Sigma Aldrich. IBX was prepared in our laboratory as described [4]. HPLC analyses were performed on a Varian Prostar 325 apparatus equipped with an UV-Vis detector selected on λ=280 nm. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ (99.8% in deuterium) and in CD_3OD (99.8% in deuterium) using a Bruker 200 MHz spectrometer. Silica gel 60 F254 plates and silica gel 60 were furnished by Merck. The human colorectal adenocarcinoma HT-29 cell line was obtained from American Type Culture Collection (ATCC, Rockville, MD). Proliferation data were assessed using a BrdU–ELISA kit (Roche Diagnostics). Cell cycle was analyzed by flow cytometry. Results from proliferation and cell cycle experiments were evaluated by one-way ANOVA test.

Results and Discussion. Different synthetic approaches were followed [5]. The best results in term of yield of ester were obtained performing an esterification reaction between tyrosol selectively protected on the phenolic group and lipoic acid under Steglich conditions; then, a regioselective aromatic hydroxylation of the monohydroxylated ester with 2-iodoxybenzoic acid (IBX) and an *in situ* reduction with sodium dithionite ($Na_2S_2O_4$).
The novel ester was evaluated for its effect on the proliferation of the human colorectal adenocarcinoma HT-29 cell line related to hydroxytyrosol and α-lipoic acid (Figure 2). Experimental data demonstrated that it exerted an antiproliferative effect significantly more potent than the parent compounds.

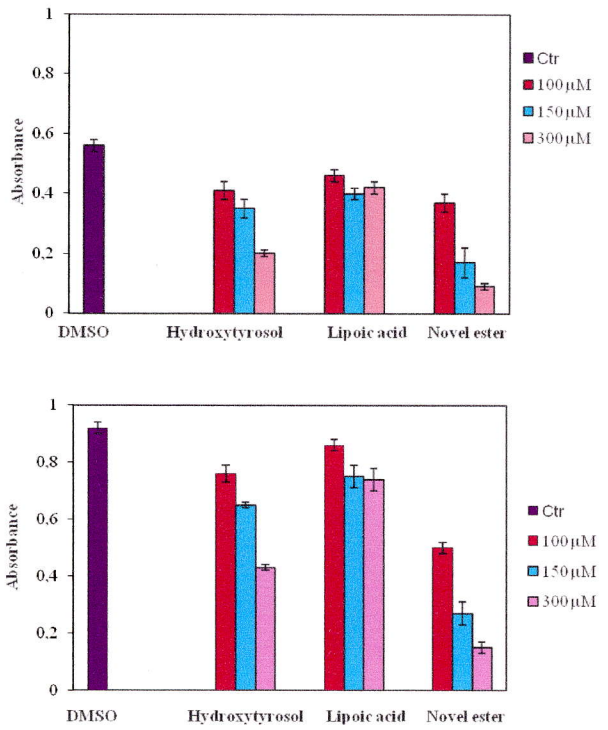

Figure 2. Inhibition of HT-29 cell proliferation after incubation for 24 h and for 48 h of the three compounds at different concentrations.

Mechanicistic studies of the cell cycle showed that the novel ester induced a block at the G2/M phase significantly stronger than hydroxytyrosol and α-lipoic acid, suggesting that the reduction in cancer cell growth is mediated by the induction of the G2 to M phase cell cycle arrest.

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