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Synthesis and anticancer activity of 7-hydroxycoumarinyl gallates

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ABSTRACT

Background: The search for anti-cancer agents includes naturally occurring substances and theirs modifications. Therefore we invented and designed compounds that represent fused derivatives of gallic acid with coumarins.
Methods: As a result, a series of 8 novel esters of gallic acid and 7-hydroxycoumarins were synthesized and evaluated for anticancer activity. The structures of the compounds were established by IR, ^{1}H, ^{13}C NMR and HR MS spectra. The esters were assayed for antiproliferative activity against human leukemia HL-60 and prostate cancer DU145 cell lines. The activity of novel esters was evaluated by cell viability assays as well as by analysis of cell cycle and cell death mechanism.
Results: The esters were found to be of similar or higher activity than gallic acid. No pronounced harmful effect was observed in non-cancer cells.
Conclusions: The novel compounds represent an excellent starting point for the further optimization and the design of therapeutically effective anti-cancerous drugs.

Introduction

Naturally occurring substances and their modifications are studied as potential therapeutics in many human diseases; especially in those, where standard therapy remains not effective or is highly toxic. Gallic acid (GA), found in a number of plants, and its semi-synthetic derivatives, alkyl esters, are widely known as chemopreventive and anticancer agents. As a component of green tea and grape seed extract, gallic acid has been shown to inhibit cell growth, induce cell cycle arrest and trigger apoptotic death in human prostate, breast, stomach, colon, lung and brain cancer cells [1,2]. The possible mechanisms of such complex effects induced by GA, postulated in the literature, include DNA-damage by reactive oxygen species (ROS) followed by up-regulation of p53 with subsequent G1/G0 cell cycle arrest and p53 triggered expression of pro-apoptotic proteins. All of these mechanisms can lead to intrinsic and extrinsic apoptosis induction and to cell death [3]. GA has been found to inhibit growth of cancer cells, with no harmful effects in non-tumor cells [4].

In animal studies, GA feeding inhibited the growth of DU145 and 22Rv1 PCa xenografts in nude mice. Immunohistochemical analysis revealed significant inhibition of tumor cell proliferation, induction of apoptosis, and reduction of microvessel density in tumor xenografts from gallic acid-fed mice, as compared to controls in both DU145 and 22Rv1 models [5].

The *in vivo* chemopreventive efficacy of GA against prostate cancer was established by evaluating its activity against prostate tumor growth and progression in transgenic adenocarcinoma of the mouse prostate (TRAMP) model [6]. It has been found that GA causes a significant imbalance of deoxynucleoside triphosphate pool sizes, indicating ribonucleotide reductase inhibition as one of the mechanisms of activity.

Moreover, GA induced dose-dependent apoptosis in HL-60 cells and attenuated progression from G0/G1 to the S phase of the cell cycle [7].

Another investigation showed that GA promotes the activity of macrophage phagocytosis in white blood cells from peripheral blood mononuclear cells and decreases macrophage phagocytosis in isolated peritoneal cells [8].

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The molecule of GA, allows obtaining a variety of ester derivatives with distinct pharmacological properties due to the presence of three phenolic and one carboxyl substituents. The alkyl esters, as well as 3,4,5-triacylated benzoic acid and its esters, have different physicochemical characteristics, especially the lipophilicity. Their pharmacokinetic and pharmacodynamic properties are also modified due to chemical changes in the molecule of GA. In many cases, alkyl esters demonstrated more favorable pharmacological properties.

Various alkyl gallates have been evaluated for their antiproliferative effects on human leukemia HL-60 cells. Dodecyl gallate showed the most potent activity. To clarify the molecular mechanism of the antiproliferative activity, the effects of this compound, on various factors have been investigated. Dodecyl gallate was found to induce apoptosis mediated by endoplasmic reticulum (ER)-stress-related caspase-12 [9]. In general, anticancer activity of gallates with eight or more carbon atoms was more pronounced than GA itself. The activity has been correlated to the amphipathic character of this alkyl ester. The hydrophobic moiety, probably contributes greatly to the activity, by increasing affinity for cell membranes and permeability [10].

Other naturally occurring substances, coumarins, have shown many biological activities, including anti-tumor activity. For example, 7-hydroxycoumarin inhibits the release of cyclin D1, which is overexpressed in many types of cancer [11].

These data inspired us with the idea to synthesize conjugates of GA with coumarins that would produce increased anti-tumor effect in comparison to individual substances.

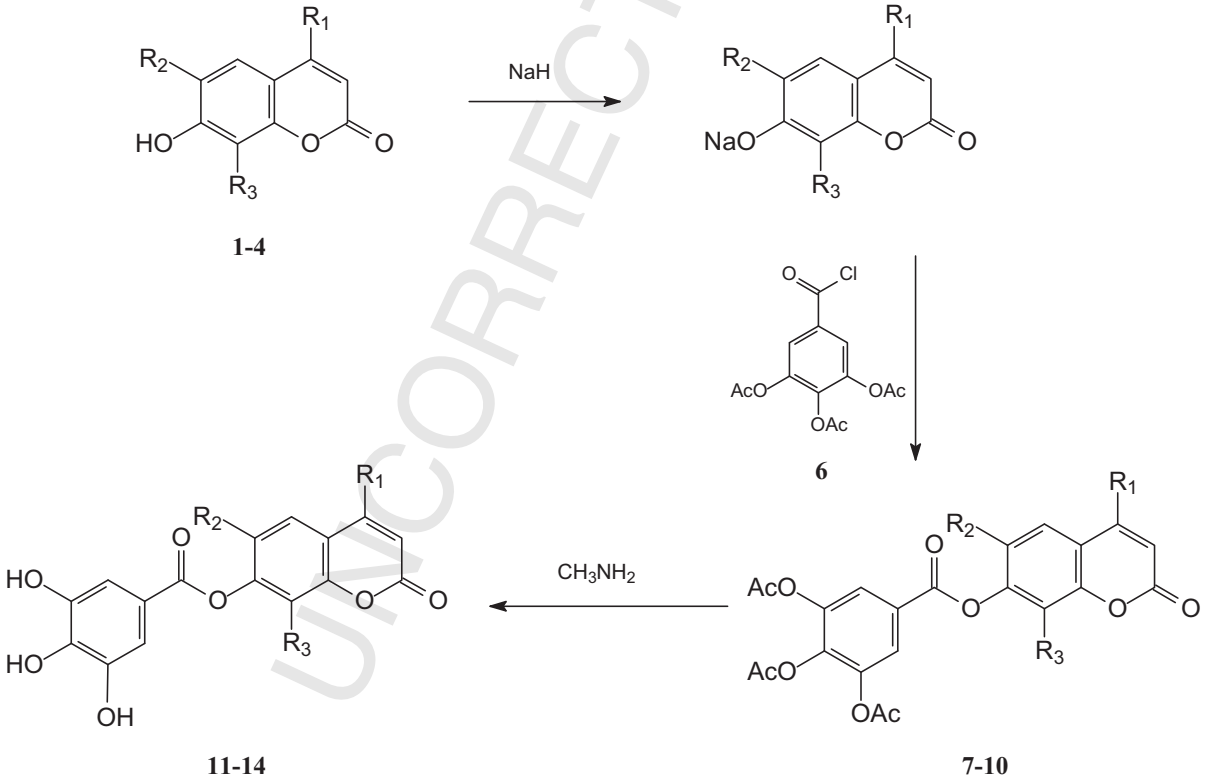
Tumor cells proliferate intensively and thus, are more sensitive to antitumor agents than non-proliferating somatic cells, however elimination of tumor cells requires often sub-toxic doses of cytostatics [12,13]. To overcome these difficulties, more selective agents are sought for. An ideal anti-tumor drug should affect tumor cells only, preserving healthy ones. Since GA derivatives and coumarins were shown to be only slightly toxic and to induce tumor cell cycle inhibition and apoptosis [10,14–17], in our study, novel coumarin esters of GA were synthesized in order to achieve cytotoxic activity exceeding that of parent compounds. Subsequently, the activity of novel esters in selected normal and tumor cell lines was determined and characterized.

Results and discussion

Chemistry

The objective of this approach was to synthesize the esters of GA and four derivatives of 7-hydroxycoumarin (7-hydroxycoumarin (**1**), 4-methyl-7-hydroxycoumarin (**2**), 6-acetyl-7-hydroxy-4-methylcoumarin (**3**) and 8-acetyl-7-hydroxy-4-methylcoumarin (**4**) (Scheme 1).

Firstly, the phenol groups of GA were protected by esterification with acetic anhydride (**5**). Then, compound **5** was converted to acid chloride **6** with use of thionyl chloride. The esterification was performed by initial forming of sodium salts 7-hydroxycoumarins **1**–**4**, followed by treatment with acid chloride **6**, to give novel protected coumarinyl gallates **7**–**10**. Due to the sensitivity of this ester bond to strong basic and acidic reaction conditions, the final cleavage of acetyl groups was a challenge. We considered possibility of deprotection with aqueous ammonia [18] and hydrazine hydrate [19] under conditions reported. Unfortunately,



1, 7, 11: R_1, R_2, R_3 = H, **2, 8, 12**: R_1 = CH_3, R_2, R_3 = H, **3, 9, 13**: R_1 = CH_3, R_2 = $COCH_3$, R_3 = H,
4, 10, 14: R_1 = CH_3, R_2 = H, R_3 = $COCH_3$,

Scheme 1. Synthesis of 7-hydroxycoumarinyl gallates.

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