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Anti-tumor activity of three novel derivatives of ginsenoside on colorectal cancer cells

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ABSTRACT

25-Hydroxyprotopanaxadiol (25-OH-PPD) is a natural compound isolated from *Panax ginseng*, and its anti-tumor activity has been studied in previous publication. In the current study, we investigated the anti-tumor activity of three novel derivatives synthesized from 25-OH-PPD, namely (20R)-12β-*O*-(L-chloracetyl)-dammarane-3β, 20, 25-triol (**xl**), (20R)-3β-*O*-(L-alanyl)-dammarane-12β, 20, 25-triol (**1c**), and (20R)-3β-*O*-(Boc-L-arginyl)-dammarane-12β, 20, 25-triol (**8b**). All three compounds significantly inhibited the growths of human colorectal cancer cells, while having lesser effect on the growth of normal primary muscle cells and spleno-lymphocytes. Further mechanistic study demonstrated that these compounds could induce apoptosis by activating the components of caspase-signaling pathways in HCT116 cells, but not in spleno-lymphocytes. Taken together, the results suggested that 25-OH-PPD derivatives exerted promising anti-tumor activity that is specific to human colorectal cancer cells, and may therefore represent a potential chemotherapeutic strategy for the treatment of colorectal cancer.

1. Introduction

Colorectal cancer remains the second leading cause of cancer death in the United States, although there has been an increase in patient survival as a result of early detection and treatment such as surgical operation, radiotherapy, and chemotherapy that uses agents such as 5-fluorouracil [1]. Given the high mortality rate and limited treatment options faced by patients, there is an urgent need to find new approaches for the treatment of this type of cancer.

Ginseng (*Panax ginseng*) is a highly valued herbal medicine, which has been used to treat a variety of ailments for thousands of years in China and other Asian countries. Although many classes of compounds can be extracted from the ginseng plant and the related *Panax notoginseng*, it is the ginsenosides (saponins) that possess the most activity. Currently, more than 60 ginsenosides have been isolated from *P. ginseng* and related plants. A number of studies have shown that the angiosuppressive effect of ginsenosides (including Rg3) correlates with their anti-tumor efficacy [2,3]. Another ginsenoside, Rb2, has been shown to inhibit angiogenesis and metastasis of melanoma in mice [4]. One clinical study specifically demonstrated that human subjects taking ginseng can decrease the risk of developing cancers of the lungs, oral cavity, and liver [5].

25-OH-PPD (20(R)-dammarane-3β, 12β, 20, 25-tetrol) is isolated from *P. ginseng* and its activity against breast and pancreatic cancers have been established in previous publications [6–8]. Three derivatives have been synthesized from 25-OH-PPD, namely (20R)-12β-*O*-(L-chloracetyl)-dammarane-3β, 20, 25-triol (**xl**), (20R)-3β-*O*-(L-alanyl)-dammarane-12β, 20, 25-triol (**1c**), (20R)-3β-*O*-(Boc-L-arginyl)-dammarane-12β, 20, 25-triol (**8b**). Their chemical structures are shown in Fig. 1. The aim of the present study was to determine whether these derivates of 25-OH-PPD could be developed into promising therapeutic agents for colorectal cancers. We examined the *in vitro* anti-cancer activity of these compounds and undertook a preliminary investigation into their mechanisms of action. The work described in this study constitutes part of our continuing effort in the search for safe and effective natural products that can be used as anti-cancer agents [9,10].

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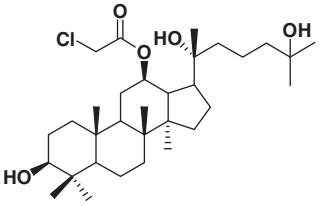
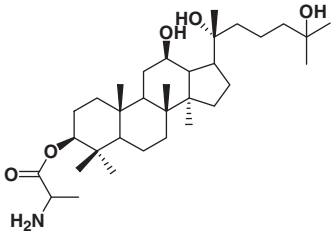
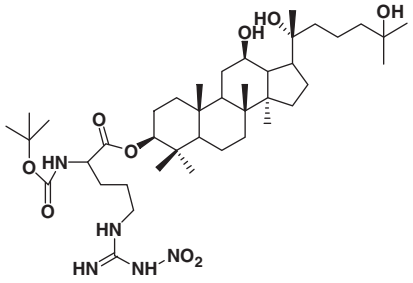


(20R)-12β-O-(L-chloracetyl)-dammarane-3β, 20, 25-triol(**xl**)
Chemical Formula: $C_{32}H_{55}ClO_5$
Exact Mass: 554.3738



(20R)-3β-O-(L-alanyl)-dammarane-12β, 20, 25-triol(**1c**)
Chemical Formula: $C_{33}H_{59}NO_5$
Exact Mass: 549.4393



(20R)-3β-O-(Boc-L-arginyl)-dammarane-12β, 20, 25-triol(**8b**)
Chemical Formula: $C_{41}H_{73}N_5O_9$
Exact Mass: 779.5408

Fig. 1. Chemical structures of 25-OH-PPD derivatives.

2. Materials and methods

2.1. Chemical and reagents

xl, **1c** and **8b** were synthesized as previously described [11,12]. FBS (Fetal Bovine Serum), RPMI 1640 medium and DMEM medium were bought from Thermo Fisher Scientific Co., Ltd. MTT reagent was acquired from Sigma Aldrich (St. Louis, MO, USA).

2.2. Cell culture

Human colorectal adenocarcinoma epithelial cell lines HCT116, HT29, LoVo and SW480 were obtained from ATCC. All these cells were cultured in DMEM containing 10% FBS.

Primary muscle cells were isolated from mouse thigh muscle under aseptic condition. In brief, muscle tissue was washed three times with PBS and then cut into pieces before immersing in DMEM medium. The sample was transferred to a centrifuge tube and centrifuged at 800*g* for 5 min. After discarding the supernatant, the precipitate was digested with trypsin and then transferred into a flask containing fresh DMEM medium plus 20% FBS. All media were supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin. Cells were incubated at 37 °C in a 5% CO_2 incubator.

Spleno-lymphocytes were isolated from six-weeks-old KM mouse (Animal Institute of Liaoning University of Traditional Chinese Medicine, Shenyang, China). In brief, spleen tissues were digested with collagenase, washed with PBS, and then centrifuged at 800*g* for 5 min. The supernatant was completely aspirated and the cell pellet was gently resuspended in 1 ml lysis buffer at room temperature. The cells were then washed several times with PBS to remove the red blood cells. Finally, the cells were gently resuspended in RPMI-1640 culture medium containing 20% FBS, 1 mM glutamine, 0.05 mM 2-mercaptoethanol, 100 μg/ml streptomycin, and 100 μg/ml penicillin for use in subsequent experiments [13].

2.3. Cell viability assay

Cell growth inhibition caused by **xl**, **1c** and **8b** was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay. HCT116, HT29, LoVo, SW480, primary muscle cells and spleno-lymphocytes were cultured in 96-well plates and under the conditions described above. Control cells were treated with 0.1% DMSO. Test cells were treated with different concentrations of either **xl**, **1c** or **8b** for 48 h. After that, the cells were incubated with 10 μl MTT (5 mg/mL) for 4 h, and the plates were then read by a plate reader (Bio-Rad, USA) at 495 nm to determine the percentage of surviving cells. Three replicates were performed for each treatment.

2.4. Determination of morphological changes of cells

HCT116 cells were seeded at a concentration of 1×10^5 cells/well in a six-well plate and grown for 16 h. The cells were then treated with **xl**, **1c** or **8b** for 24 h. Positive and negative control cells were treated with 30 μM Mitomycin C and 0.1% DMSO, respectively. Morphological changes of the cells were observed with an inverted fluorescence microscope [14].

A similar experiment was also carried out in which the cells were fixed with 70% ethanol for one hour at 4 °C after they had been treated with **xl**, **1c**, **8b**, Mitomycin C or 0.1% DMSO. After fixation, the cells were treated with 5 μM Propidium Iodide (PI) and 5 μM nucleic acid stain (NAS) at 37 °C and in the dark for 10 min [15], and then examined under a fluorescence microscope.

2.5. Detection of DNA fragmentation

HCT116 cells were collected after they were treated with **xl**, **1c**, **8b**, 0.1% DMSO or Mitomycin C for 24 h. Genomic DNA was extracted from the cells using the Animal Cell Genomic DNA Isolation Kit (DingGuo Biotech Ltd., Beijing). The extracted DNA was