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Short communication

Synthesis and in vitro antitumor evaluation of betulin acid ester derivatives as novel apoptosis inducers

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ABSTRACT

Nineteen betulin derivatives modified at the C-3 and C-28 positions were synthesized and assessed for antitumor activities against the MGC-803, PC3, Bcap-37, A375, and MCF-7 human cancer cell lines in vitro by MTT assay. Some derivatives (compounds **3a**–**3d** and **5**) displayed strong antitumor properties, with IC_{50} values between 4 and 18 μM. Compound **3c**, containing piperidine group at C-28 position, had IC_{50} values of 4.3, 4.5, 5.2, 7.5, and 5.2 μM on the five cancer cell lines, respectively. Subsequent fluorescence staining and flow cytometric analysis indicated that compound **3c** induced apoptosis in MGC-803 cell line, with an apoptosis ratio of 31.11% after 36 h of treatment at 10 μM **3c**.

1. Introduction

Betulin (BE; 1; lup-20(29)-ene-3β, 28-diol), is an abundant and naturally occurring pentacyclic triterpenoid [1,2]. This compound is a major effective component of many traditional Chinese medicines that is widely distributed in plants [3–5], and forms up to 30% of the dry weight of the extractive (e.g., the bark of birch trees) [6–8]. BE possesses various biological and pharmacological activities [9], such as anti-HIV [10], anti-inflammatory [11,12], antiretroviral [13], antibacterial properties [14], and anticancer properties [15,16]. Recently, some studies have shown that BE had marked antitumor activities against various types of cancer cell lines [17], such as colorectal (DLD-1), prostate (PC3), breast (MCF-7), and lung (A549) cancer cell lines, and could induce A549 cell-line apoptosis [18–21]. Furthermore, a recent report found BE to be more active against other melanoma lines (G361, SK-MEL-28), neuroblastoma cell lines (GOTO, NB-1), and leukemia lines (HL-60, U937, K562) [22]. However, compared to other pentacyclic triterpenoids, BE is inactive against melanoma cell line (MEL-2) [23]. Researchers had demonstrated that keeping a polar substituent at the C-3 position was essential for the pharmacological activities of pentacyclic triterpenes [24]. According to the structure–activity relationship, a hydrogen donor group at either C-3 or C-28 position can improve cell proliferation inhibition significantly [25]. In recent years, the drug, with introduced amino alkyl groups has attracted considerable attention. Recent studies have shown that the introduction of amino alky groups had unexpected improvements in the anti-HIV or antitumor activities of compounds [25]. In 2010, Kommera et al. found that the antitumor activities of BE derivatives with amino acyl groups introduced at C-28 position were significantly improved, and had the therapeutic potential in the treatment of gastric carcinoma [26]. Furthermore, previous work already indicated that the introduction of aromatic carboxyl groups to pentacyclic triterpenoids could result in good cytotoxicity [27,28].

Thus, in view of the previous rationale and in continuation of an ongoing program aiming at developing more potential anticancer

Abbreviations: ADM, adriamycin; AO/EB, acridine orange/ethidium bromide; BE, betulin; ^{13}C NMR, ^{13}C nuclear magnetic resonance; DMF, N, N-dimethylformamide; DMSO, dimethyl sulfoxide; HCPT, 10-hydroxyl camptothecine; ^{1}H NMR, proton nuclear magnetic resonance; IR, infra-red; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TUNEL, terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling.

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drugs, in the present study nineteen BE derivatives modified at C-3 and C-28 positions were designed and synthesized. The antitumor activities of these derivatives were screened in vitro by MTT assay using five human cancer cell lines MGC-803 (human gastric carcinoma cell line), PC3 (human prostate carcinoma cell line), Bcap-37 (human breast carcinoma cell line), A375 (human malignant melanoma cell line), and MCF-7 (human breast carcinoma cell line). Additionally, the antitumor mechanism of the BE derivatives was investigated through acridine orange/ethidium bromide staining (AO/EB), Hoechst 33258 staining, terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay, and flow cytometric analysis.

2. Results and discussion

2.1. Chemistry

The syntheses of BE derivatives are summarized in Scheme 1. The structure of BE was modified at C-3 and C-28 positions. Succinic anhydride and maleic anhydride were introduced at the C-28 position in dry dichloromethane (DCM) in the presence of pyridine to obtain compounds **2** and **4**. Compound **2** was treated with corresponding amines in dry DCM, in the presence of PyBOP to afford compounds **3a**–**3d**. The 28-OH and 3-OH of BE were acetylated with aromatic carboxyl group in the presence of DCC and DMAP in dry DCM at room temperature to gain the compounds **6a**–**6d**. Finally, only the 28-OH of BE was acetylated using EDCI instead of DCC to obtain the compounds **7a**–**7g** in high yields. All the compounds were fully characterized by various spectroscopic methods.

2.2. Evaluation of antitumor activities

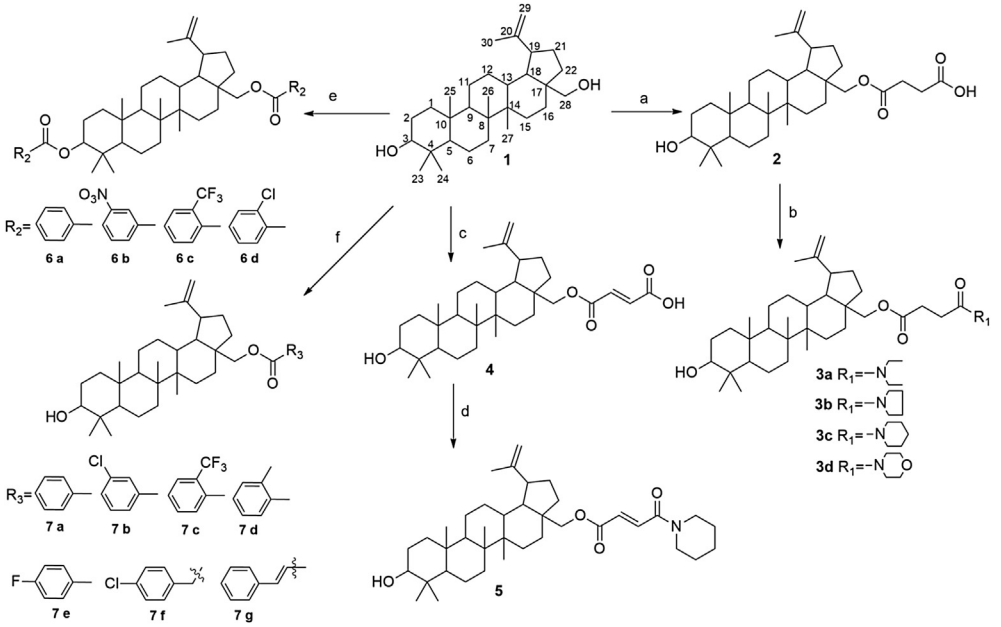
In vitro inhibitory activities of the synthesized BE derivatives were evaluated against MGC-803, PC3, Bcap-37, A375, and MCF-7 cell lines by MTT assay. Hydroxycamptothecine (HCPT) and Adriamycin (ADM) were used as positive controls. A culture medium containing 0.1% DMSO served as negative control. All BE derivatives including ADM and HCPT were dissolved in DMSO. Each experiment was repeated thrice. The IC_{50} values are summarized in Table 1.

Table 1 presents the modified compounds at the C-28 position with selected amino groups displaying potent antitumor activities against the five cancer cell lines. These compounds had IC_{50} values between 4 and 18 μM. Furthermore, the most active compound among the synthesized BE derivatives was compound **3c**. This compound had approximately four times more activity than that of BE, and the IC_{50} values were 4.3, 4.5, 5.2, 7.5, and 5.2 μM on the five cancer cell lines, respectively. Further experiments indicated that proliferation of the five cancer cells were significantly inhibited by compound **3c** in a concentration-dependent manner (Fig. 1).

Compounds **2** and **4**, with succinic anhydride and maleic anhydride introduced at the C-28 position, had more activity than that of the parent BE. This result may be attributed to the introduced carboxyl group, which was more hydrophilic than the hydroxyl group. This phenomena was similar to Drag–Zalesinska observations, wherein the most active compounds were those with the highest solubility in water [29].

Recently, some researchers studied the structure–activity relationships of other pentacyclic triterpenes including Betulinic acid, Ursolic acid, and Oleanolic acid. These pentacyclic triterpenes had similar structure–activity relationships despite the different structures.[30] Previous studies showed that the introduction of amino alkyl groups at the C-28 position further enhanced the antitumor activities. In this study, the compounds modified at the C-28 position with selected amino groups displayed potent antitumor activities (IC_{50} values, between 4 and 18 μM), identical with the previous reports [30].

Remarkably, when 28-OH was esterified with aromatic acid (**7a**–**7g**), the modification led to a decline in activity. In addition, when 28-OH and 3-OH were esterified at the same time (**6a**–**6d**), the activity was reduced or even disappeared. The low activity can be explained by the observation of Kommera et al. [26]: "One reason for low activity can be due to the straight and rigid phenyl groups and the possible interactions with the π-system of the phenyl ring lead to a much bulky molecule, thereby with a lower ability to penetrate the cell membrane."



Scheme 1. Reagents and conditions: (a) Succinic anhydride, DMAP, pyridine, DCM, r.t., 8 h; (b) R_1H, PyBOP, DCM, r.t., 12 h; (c) Maleic anhydride, DMAP, pyridine, DCM, r.t., 24 h; (d) Piperidine, PyBOP, DCM, r.t., 12 h; (e) R_2COOH, DCC, DMAP, DCM, r.t., 24 h; (f) R_3COOH, EDCI, DMAP, DCM, r.t., 24 h.