Ginkgolide B derivative synthesis and their effects on the viability of SKOV3 cells

Zi-Li Feng

Shaanxi University of Technology

Zhi-Bin Zhu

Shaanxi University of Technology

Wang Chen (✉ chenwang@snut.edu.cn)

Shaanxi University of Technology <https://orcid.org/0000-0003-0565-9157>

Jia-Yuan Xu

Shaanxi University of Technology

Yu Bai

Shaanxi University of Technology

Dai-Hua Hu

Shaanxi University of Technology

Zheng-Dong Zhao

Shaanxi Cuicheng Biomedical Tehnology CO., Ltd.

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Abstract

The natural product GB was used as a raw material and modified by esterification on C10-OH or C1-OH to obtain 11 derivatives (**1** to **11**), which were structurally characterized with nuclear magnetic resonance spectroscopy. An MTT assay-based *in vitro* tumor proliferation inhibitory activity test showed that compounds **2**, **3**, **6**, **7**, **10**, and **11** exhibited strong inhibitory activity against the human ovarian cancer cells SKOV3, with IC₅₀ values of 16.05 µmol/L, 15.65 µmol/L, 32.00 µmol/L, 63.30 µmol/L, 23.20 µmol/L, and 31.10 µmol/L, respectively. Annexin V/PI double staining assay showed that compound **2** induced apoptosis in SKOV3 cells to a slightly greater extent than GB and compounds **5**, and **9**, with an apoptosis rate of 31.68%.

Introduction

Ginkgolide B (GB) is a diterpenoid isolated from Ginkgo biloba, which is associated with various pharmacological activities, such as anti-platelet aggregation, anti-inflammatory, antioxidant, and free radical scavenging [1–5]. In recent years, GB has also been reported to have anti-tumor effects [6]; it inhibits the growth and proliferation of various tumor cells, such as ovarian, breast, lung, colorectal, bladder, and prostate cancers, with a rich set of anti-tumor targets and pathways [7–15].

The PAF/PAFR signaling axis has emerged as an important determinant of the aggressive phenotype in several malignancies, including ovarian cancer [16–17]. GB, as a potent PAFR antagonist, may be a key regulator in treating tumor cells. Jiang Wei et al. [7–8] conducted *in vivo* and *in vitro* experiments and found that GB could inhibit the proliferation of ovarian cancer cells and growth of nude mice tumors with a tumor suppression rate of 48%, and up to 78.2% in combination with cisplatin (CDDP), which could be used as an adjuvant drug for the treatment of ovarian cancer. The mechanism may be that GB inhibits the expression of platelet-activating factor (PAF), platelet-activating factor receptor (PAFR), and the tyrosine kinase Src and the p38 mitogen-activated protein kinase (p38MAPK), preventing p38MAPK from activating downstream transcription factors [18], including the cyclic adenosine monophosphate (cAMP) response element binding protein (CREB), which is closely related to cell invasion. This results in the inability of CREB to bind to the promoter in the upstream of the matrix metalloproteinase 2/9 (MMP2/MMP9) gene and the inability of MMP2 mRNA/MMP9 mRNA to be expressed, which inhibits the migration of ovarian cancer cells. In addition, ginkgolide B upregulated 25 proteins with antitumor effects (e.g., p53) and downregulated 22 proteins associated with tumor migration (e.g., β-catenin).

Yu Y et al. [19] demonstrated that the PAFR upregulation in CDDP-treated ovarian cancer cells provided additional support for the function of the PAF/PAFR signaling axis as a resistance mechanism, with the possible mechanism being that cisplatin (CDDP) acts on the ovarian cancer cells and causes nuclear factor kappa-B (NF-κB) and hypoxia-inducible factor (HIF-1α) to accumulate in the nucleus, leading to the upregulation of the platelet-activating factor receptor PAFR. In addition, GB inhibited the PAFR activity, which may block the downstream signaling pathways of phosphoinositide 3-kinase (PI3K) and the

extracellular regulated protein kinase (ERK), thereby enhancing the sensitivity of ovarian cancer cells to CDDP, intensifying drug efficacy and significantly reducing tumor growth.

Ginkgolide B has a unique rigid caged dodeca-carbon skeleton structure, and it was found that the synthesis of GB ester derivatives by converting the hydroxyl groups at the C-1 and (or) C-10 positions of GB into aromatic-containing groups such as ester groups significantly enhanced the anti-PAF activity [20–21]. To explore the effect of GB parent structure and the introduction of small molecule side chains of benzoic acid series on the hydroxyl groups at C-1 and (or) C-10 positions on pharmacological activity, this study constructed GB ester derivatives by the esterification reaction of GB as the parent nucleus with compounds with aromatic groups such as p-chlorobenzoic acid, p-fluorobenzoic acid, p-nitrobenzoic acid, p-methoxybenzoic acid, 3-methoxybenzoic acid, and 3'5'-dinitrobenzoic acid by reference to the design of nicotinic acid esters, cinnamic acid esters, and benzoic esters [22–30]. The GB derivatives were structurally characterized by nuclear magnetic resonance spectroscopy (NMR), tested for *in vitro* tumor proliferation inhibitory activity by thiazolyl blue (MTT), and detected for ovarian cancer SKOV3 cell apoptosis by the Annexin V/PI double staining assay.

Materials And Methods

Chemistry

All reagents and solvents used in this study were purchased from Tianjin Fuyu Fine Chemical Co., Ltd. (P.R. China), Shanghai Macklin Biochemical Technology Co., Ltd. (P.R. China), and Energy Chemical (P.R. China), and used as received without further purification unless otherwise noted. In addition, some biological reagents were purchased from Sigma or GIBCO, USA.

TLC was carried out on Silica Gel GF254 plates (Qingdao Haiyang Chemical Co., Ltd) and spots were visualized by iodine vapors or by irradiation with UV light (254 nm). All water employed was ultrapure (> 18.2 MΩcm⁻¹ at 25°C, Milli-Q, Millipore, Billerica, MA). The NMR spectra were measured by Bruker Avance 600 NMR analyzer (Bruker, Switzerland) in the indicated solvents. Chemical shifts are expressed in ppm (δ units) relative to TMS signal as internal reference, and the coupling constant values (J) are shown in Hertz. Signal multiplicities are reported by the following abbreviations: s (singlet), d (doublet), t (triplet), dd (double doublet), q (quartet), m (multiplet), and brs (broad signal). HB DAC-50 was used for preparative liquid chromatograph (Jiangsu Hanbon Science & Technology Co., Ltd.). Agilent 1260 high performance liquid chromatograph (Agilent Technologies Co. Ltd, USA), SCIENTZ-10N vacuum freeze dryer (Ningbo Xinzhi Biotechnology Co. Ltd.), YG-875B ultra clean bench (Suzhou Medical Equipment Factory), inverted phase contrast microscope (Zeiss, Germany), enzyme marker (Biotek, USA), CO₂ cell incubator (Thermo Fisher Scientific), and GB (98.24% purity, lab-made) were used.

Tumor cell growth inhibitory assay

The SKOV3 cells used in the following cell experiments were obtained from the Shanghai EK-Bioscience Co., Ltd. (P.R. China). SKOV3 cells were maintained in DMEM containing 10% fetal bovine serum (FBS).

All cells were grown at 37°C in a humidified atmosphere of 5% CO₂. All the reagents used in this study, unless otherwise indicated, were purchased from Sigma (USA). The compounds with inhibitory activity against human ovarian cancer cells SKOV3 were screened by the MTT assay [31]. Briefly, SKOV3 cells were cultured at a density of 80 % to 90 % and inoculated in 96-well plates at 2×10⁴/well. The attached cells were placed overnight and treated with increasing concentrations of drug for 24 h. Blank controls (no drug treatment), and experiment groups (containing 5 µmol/L, 10 µmol/L, 20 µmol/L, 40 µmol/L, 60 µmol/L, 80 µmol/L, and 100 µmol/L) of ginkgolide B derivatives compounds **1** to **11** were used to treat ovarian cancer cells, respectively. After 24 h of cell culture, 20 µL of MTT was added to the cell medium of each well, and the plates were mixed by gently tapping. After continuing incubation for 4 h, the culture supernatant in the wells was aspirated and discarded, 150 µL of DMSO solution was added to each well and shaken for 10 min on a shaker. Cell growth was examined by detecting the absorbance value (A) of each well at a wavelength of 550 nm by an enzyme marker to preliminarily investigate the optimal concentration of compounds **1~11** on the viability of SKOV3 cells. Three replicate wells were set up for each concentration. The inhibition rate was calculated according to the following formula, and IC₅₀ was calculated using the software Prism 5 (GraphPad Software, Inc.).

$$\text{Inhibition rate} = [1 - (OD \text{ of treatment} - OD \text{ of blank}) / (OD \text{ of control} - OD \text{ of blank})] \times 100\%$$

$$\lg IC_{50} = X_m - I[P - (3 - P_m - P_n)/4]$$

X_m – lg Maximum dose

I – lg (maximum dose/adjacent dose)

P – (sum of positive rate)

P_m – (Maximum positive rate)

P_n – (minimum positive rate)

Annexin V/PI double staining assay to observe SKOV3 cell apoptosis

Annexin V-FITC Apoptosis Detection Kit was purchased from Sigma (USA) and used according to the manufacturer's instructions. Human ovarian cancer cells SKOV3 at the logarithmic growth stage were inoculated in 6-well plates at 37°C, 5% CO₂, and cultured overnight. Drug-treated groups were treated with compound **1**, **3**, **6**, and **10** at the optimal mass concentration for 48 h. The negative control group was treated with McCoy's 5A complete medium only. After digestion of cells with EDTA-free trypsin, cells were collected by centrifugation at 4 °C, 7000 rpm/min for 5 min. Cells were resuspended in precooled sterilized PBS at 4°C and 7000 rpm/min for 5 min, and the supernatant was discarded. The 1 × binding buffer (195 µL) was added. After suspending the cells, 5 µL of Annexin V-FITC was added and mixed gently. Ten µL of propidium iodide staining solution was added, mixed gently, and incubated at room temperature for 15 min protected from light. Stained samples were analyzed immediately by a FACScan

flow cytometer (Beckman coulter EPICS xL). The fractions of cell population in different quadrants were analyzed using quadrant statistics. Cells in the lower right quadrant represented apoptosis while cells in the upper right quadrant represented necrosis or post apoptotic necrosis.

General procedure for the synthesis of GB derivatives 1-11

The synthetic route was shown in Fig. 1. A 100.0 mL round bottom flask was added with 212.2 mg of GB (0.5 mmol), 122.2 mg of 4-dimethylaminopyridine (DMAP) (1.0 mmol), 191.7 mg of 1-ethyl-carbodiimide hydrochloride (EDC-HCl) (1.0 mmol) and 6 benzoic acids (*p*-chlorobenzoic acid, *p*-fluorobenzoic acid, *p*-nitrobenzoic acid, *p*-methoxybenzoic acid, 3-methoxybenzoic acid, 3,5-dinitrobenzoic acid) with 1.0 mmol, respectively. Anhydrous dichloromethane 50.0 mL was added and stirred under room temperature. The reaction was detected by TLC after 6 h at 1 h intervals and fumigated with sulfuric acid-ethanol solution or acetic acid-iodine. The reaction was considered complete when the spots of GB disappeared. After recovering the dichloromethane solution under reduced pressure, the residue was dissolved with 30.0 mL of ethyl acetate and underwent reverse extraction three times with water, with 15.0 mL/time. The ethyl acetate layer was dried by anhydrous sodium sulfate and concentrated under reduced pressure to dryness. The product of each synthesis was dissolved in acetonitrile to make a sample solution of 30.0 mg/mL. After passing through a 0.45 μ m filter membrane, the product was purified by high pressure preparative chromatography eluting with acetonitrile/water to produce the corresponding derivatives.

1-(4-chlorobenzoyl) GB (1)

White powder, yield 12.12%, $\lambda_{\max}$ = 254 nm. ^1H NMR (600 MHz, DMSO- d_6) δ 8.04 (m, 2H), 7.65 (dd, J = 11.2, 4.4 Hz, 2H), 6.71 (d, J = 4.9 Hz, 1H), 6.67 (s, 1H), 6.16 (d, J = 93.6 Hz, 1H), 5.69 (dd, J = 61.2, 3.8 Hz, 1H), 5.64 (d, J = 3.6 Hz, 1H), 4.96 (m, 1H), 4.93 (d, J = 5.6 Hz, 1H), 2.97 (m, 1H), 2.22 (dd, J = 13.6, 4.5 Hz, 1H), 2.00 (ddd, J = 36.1, 28.3, 24.2 Hz, 1H), 1.79 (ddd, J = 31.8, 14.3, 4.4 Hz, 1H), 1.13 (dd, J = 28.9, 5.1 Hz, 3H), 1.01 (d, J = 6.7 Hz, 9H); ^{13}C NMR (151 MHz, DMSO- d_6) δ 176.45, 173.78, 170.41, 163.44, 139.07, 131.89, 129.51, 128.51, 109.59, 99.31, 91.71, 83.85, 79.84, 75.40, 71.04, 68.72, 68.30, 48.88, 41.70, 40.54, 37.07, 32.45, 29.35, 8.62. ESI-MS: m/z 563.3 $[\text{M}+\text{H}]^+$.

10-(4-chlorobenzoyl) GB (2)

White powder, 15.48% yield, $\lambda_{\max}$ = 254 nm. ^1H NMR (600 MHz, DMSO- d_6) δ 8.06 (m, 2H), 7.54 (m, 2H), 6.82 (s, 1H), 6.48 (s, 1H), 6.25 (s, 1H), 6.22 (s, 1H), 5.46 (s, 1H), 4.62 (t, J = 21.1 Hz, 1H), 4.09 (d, J = 5.3 Hz, 1H), 2.86 (q, J = 7.0 Hz, 1H), 2.14 (m, 1H), 1.75 (d, J = 12.6 Hz, 2H), 1.07 (d, J = 7.2 Hz, 3H), 0.90 (s, 9H, t-Bu); ^{13}C NMR (151 MHz, DMSO- d_6) δ 176.72, 170.41, 168.96, 164.18, 139.94, 132.47, 129.55, 127.05, 110.46, 100.16, 94.36, 83.33, 78.66, 74.38, 72.38, 70.72, 67.45, 48.89, 41.88, 40.53, 37.22, 32.21, 30.57, 28.89, 8.51. ESI-MS: m/z 563.3 $[\text{M}+\text{H}]^+$.

1-(4-fluorobenzoyl) GB (3)

White powder, yield 15.13%, $\lambda_{\max}$ = 210 nm, ^1H NMR (600 MHz, DMSO- d_6) δ 8.00 (m, 2H), 7.32 (m, 2H), 6.64 (t, J = 4.3 Hz, 1H), 6.61 (s, 1H), 6.01 (s, 1H), 5.68 (d, J = 4.1 Hz, 1H), 5.46 (dd, J = 14.8, 5.1 Hz, 1H), 4.91 (d, J = 6.4 Hz, 1H), 4.84 (m, 1H), 2.91 (m, 1H), 2.14 (m, 1H), 1.98 (m, 1H), 1.71 (m, 1H), 1.07 (m, 3H), 0.92 (m, 9H); ^{13}C NMR (151 MHz, DMSO- d_6) δ 176.46, 173.78, 170.44, 166.64, 165.18, 163.31, 133.00, 126.30, 116.54, 109.58, 99.36, 91.78, 83.85, 79.86, 75.31, 70.10, 69.14, 68.31, 48.88, 41.69, 40.48, 37.07, 32.45, 29.35, 8.64. ESI-MS: m/z 547.2 $[\text{M}+\text{H}]^+$.

10-(4-fluorobenzoyl) GB (4)

White powder, 36.47% yield, $\lambda_{\max}$ = 210 nm, ^1H NMR (600 MHz, DMSO- d_6) δ 8.21 (m, 2H), 7.32 (t, J = 8.8 Hz, 2H), 6.82 (d, J = 4.9 Hz, 1H), 6.47 (s, 1H), 6.25 (s, 1H), 6.22 (s, 1H), 5.46 (d, J = 2.9 Hz, 1H), 4.64 (d, J = 6.4 Hz, 1H), 4.09 (dd, J = 6.3, 5.1 Hz) 6.3, 5.1 Hz, 1H), 2.87 (q, J = 7.1 Hz, 1H), 2.14 (m, 1H), 1.77 (m, 2H), 1.07 (m, 3H), 0.90 (s, 9H, t-Bu); ^{13}C NMR (151 MHz, DMSO- d_6) δ 176.72, 170.42, 169.03, 165.41, 164.00, 133.74, 133.68, 124.83, 116.62, 110.84, 100.16, 94.39, 83.35, 78.66, 74.40, 72.39, 70.59, 67.47, 48.89, 41.88, 40.48, 37.23, 32.21, 28.89, 8.51. ESI-MS: m/z 547.2 $[\text{M}+\text{H}]^+$.

1-(4-methoxybenzoyl) GB (5)

White powder, yield 8.40%, molecular formula: $\text{C}_{28}\text{H}_{30}\text{O}_{12}$, $\lambda_{\max}$ = 254 nm, ^1H NMR (600 MHz, DMSO- d_6) δ 7.93 (dd, J = 12.1, 8.8 Hz, 2H), 7.08 (dd, J = 8.8, 3.8 Hz, 2H), 6.66 (m, 1H), 6.07 (s, 1H), 5.93 (m, 1H), 5.74 (m, 1H), 5.52 (dd, J = 14.8, 4.9 Hz, 1H), 4.90 (m, 1H), 3.85 (d, J = 5.6 Hz, 3H), 2.92 (m, 1H), 2.21 (dd, J = 12.2, 8.1 Hz, 1H), 2.05 (m, 1H), 1.92 (td, J = 13.6, 3.7 Hz, 1H), 1.79 (m, 1H), 1.27 (m, 2H), 1.16 (d, J = 7.2 Hz, 1H), 0.98 (s, 9H, t-Bu); ^{13}C NMR (151 MHz, DMSO- d_6) δ 176.82, 169.76, 164.33, 163.83, 131.98, 121.01, 114.54, 110.03, 108.64, 104.27, 98.48, 85.44, 83.90, 80.24, 73.23, 70.20, 68.88, 68.44, 56.03, 49.92, 48.38, 42.03, 36.72, 31.48, 29.45, 8.72. ESI-MS: m/z 559.3 $[\text{M}+\text{H}]^+$.

10-(4-methoxybenzoyl) GB (6)

White powder, yield 55.57%, $\lambda_{\max}$ = 254 nm, ^1H NMR (600 MHz, DMSO- d_6) δ 8.15 (dd, J = 16.9, 8.8 Hz, 2H), 7.08 (m, 2H), 6.80 (t, J = 47.4 Hz, 1H), 6.53 (s, 1H), 6.23 (m, 1H), 5.52 (m, 1H), 4.65 (dd, J = 50.4, 15.3 Hz, 1H), 4.16 (dd, J = 41.0, 35.2 Hz, 1H), 3.79 (m, 3H), 2.93 (m, 1H), 2.17 (m, 1H), 1.80 (m, 1H), 1.13 (m, 4H), 1.03 (dd, J = 16.2, 9.1 Hz, 1H), 0.96 (s, 9H, t-Bu); ^{13}C NMR (151 MHz, DMSO- d_6) δ 176.74, 169.88, 169.25, 164.54, 132.92, 131.08, 120.43, 114.64, 110.35, 100.27, 94.51, 83.44, 78.66, 74.46, 72.48, 70.16, 67.52, 56.04, 48.91, 41.85, 40.48, 37.17, 32.19, 30.61, 28.90, 8.60. ESI-MS: m/z 559.3 $[\text{M}+\text{H}]^+$.

1-(4-Nitrobenzoyl) GB (7)

White powder, 38.62% yield, $\lambda_{\max}$ = 254 nm, ^1H NMR (600 MHz, DMSO- d_6) δ 8.38 (m, 2H), 8.24 (m, 2H), 6.72 (m, 1H), 6.09 (d, J = 11.5 Hz, 1H), 5.78 (t, J = 11.0 Hz, 1H), 5.53 (m, 1H), 5.05 (t, J = 11.1 Hz, 1H), 4.93 (m, 1H), 2.98 (m, 1H), 2.24 (m, 1H), 2.03 (m, 1H), 1.78 (m, 1H), 1.28 (m, 1H), 1.14 (m, 3H), 1.01 (s, 9H, t-Bu);

^{13}C NMR (151 MHz, DMSO- d_6) δ 176.44, 173.81, 170.33, 162.89, 150.94, 134.95, 131.44, 124.39, 109.66, 99.24, 91.52, 83.85, 79.83, 75.87, 70.99, 69.17, 68.30, 48.87, 41.73, 40.55, 37.12, 32.49, 29.34, 14.07, 8.56. ESI-MS: m/z 574.4 $[\text{M}+\text{H}]^+$.

10-(4-Nitrobenzoyl) GB (8)

White powder, 26.1% yield, $\lambda_{\text{max}} = 254$ nm, ^1H NMR (600 MHz, DMSO- d_6) δ 8.38 (m, 2H), 8.23 (dd, $J = 18.7, 8.7$ Hz, 2H), 6.77 (d, $J = 4.7$ Hz, 1H), 6.07 (m, 1H), 5.74 (t, $J = 30.6$ Hz, 1H), 5.55 (m, 1H), 5.05 (t, $J = 11.1$ Hz, 1H), 4.91 (dd, $J = 34.0, 4.7$ Hz, 1H), 2.92 (m, 1H), 2.24 (m, 1H), 2.05 (m, 1H), 1.79 (m, 1H), 1.28 (ddd, $J = 60.3, 40.8, 20.6$ Hz, 1H), 1.18 (s, 3H), 1.01 (s, 9H, t-Bu); ^{13}C NMR (151 MHz, DMSO- d_6) δ 176.44, 173.81, 170.33, 162.89, 150.94, 134.96, 131.53, 124.43, 110.88, 99.25, 91.52, 83.85, 80.54, 75.87, 70.99, 68.74, 68.30, 48.88, 41.72, 40.50, 37.12, 32.49, 30.79, 29.34, 8.56. ESI-MS: m/z 574.4 $[\text{M}+\text{H}]^+$.

1-(5-methoxybenzoyl) GB (9)

White powder, yield 8.72%, $\lambda_{\text{max}} = 254$ nm, ^1H NMR (600 MHz, DMSO- d_6) δ 7.78 (ddd, $J = 13.3, 4.6, 3.6$ Hz, 2H), 7.47 (m, 2H), 6.82 (t, $J = 41.7$ Hz, 1H), 6.52 (d, $J = 45.7$ Hz, 1H), 6.31 (s, 1H), 6.29 (s, 1H), 5.51 (m, 1H), 4.64 (m, 1H), 4.42 (m, 1H), 4.17 (dt, $J = 22.0, 11.0$ Hz, 1H), 3.80 (m, 1H), 2.88 (m, 1H), 2.21 (m, 1H), 1.85 (m, 1H), 1.14 (d, $J = 7.3$ Hz, 1H), 1.03 (m, 4H), 0.96 (s, 9H, t-Bu); ^{13}C NMR (151 MHz, DMSO- d_6) δ 177.64, 169.76, 169.06, 165.10, 159.72, 130.23, 129.47, 122.12, 114.87, 110.40, 100.19, 94.27, 83.60, 79.06, 74.39, 72.59, 70.54, 67.89, 55.85, 48.89, 42.27, 33.73, 31.75, 30.61, 29.12, 8.43. ESI-MS: m/z 559.3 $[\text{M}+\text{H}]^+$.

10-(5-methoxybenzoyl) GB (10)

White powder, yield 38.90%, $\lambda_{\text{max}} = 254$ nm, ^1H NMR (600 MHz, DMSO- d_6) δ 7.56 (m, 1H), 7.49 (m, 2H), 7.27 (m, 1H), 6.69 (dt, $J = 35.6, 19.0$ Hz, 1H), 6.06 (m, 1H), 5.76 (m, 1H), 5.56 (ddd, $J = 31.9, 19.4, 5.0$ Hz, 1H), 5.02 (dd, $J = 36.5, 17.5$ Hz, 1H), 4.89 (dd, $J = 33.1, 5.1$ Hz, 1H), 4.08 (s, 1H), 3.87 (m, 3H), 2.96 (m, 1H), 2.25 (m, 1H), 2.01 (m, 1H), 1.81 (m, 1H), 1.23 (m, 1H), 1.18 (m, 2H), 0.99 (s, 9H, t-Bu); ^{13}C NMR (151 MHz, DMSO- d_6) δ 176.48, 173.38, 170.44, 164.25, 159.74, 131.14, 122.09, 119.67, 114.76, 109.59, 99.26, 91.79, 83.46, 79.49, 76.46, 73.32, 71.06, 68.50, 55.83, 49.40, 40.89, 36.97, 32.49, 29.34, 13.16, 8.60. ESI-MS: m/z 559.3 $[\text{M}+\text{H}]^+$.

1-(3-5-Dinitrobenzoyl) GB (11)

White powder, 61.25% yield, $\lambda_{\text{max}} = 254$ nm, ^1H NMR (600 MHz, DMSO- d_6) δ 9.06 (m, 1H), 8.98 (dd, $J = 15.0, 2.1$ Hz, 2H), 6.82 (m, 2H), 6.12 (s, 1H), 5.86 (d, $J = 4.1$ Hz, 1H), 5.53 (dd, $J = 30.6, 5.3$ Hz, 1H), 5.21 (d, $J = 6.9$ Hz, 1H), 4.93 (m, 1H), 2.98 (m, 1H), 2.24 (dt, $J = 41.8, 21.0$ Hz, 1H), 1.97 (m, 1H), 1.73 (m, 1H), 1.13 (m, 3H), 0.99 (m, 9H); ^{13}C NMR (151 MHz, DMSO- d_6) δ 176.43, 173.80, 170.22, 161.54, 148.97, 132.60,

130.40, 129.27, 123.39, 109.78, 99.04, 91.18, 83.58, 79.67, 76.52, 70.86, 69.22, 68.25, 48.86, 41.76, 40.53, 36.94, 30.88, 29.30, 8.37. ESI-MS: m/z 619.4 $[M+H]^+$.

Results And Discussion

According to the reaction described in Scheme 1, eleven novel GB derivatives **1–11** were synthesized by esterification using GB as a raw material. Two products can be simultaneously obtained in one pot reaction. Due to the higher reaction activity of C-10 hydroxyl in GB, the yields of esterification at the C-10 position were higher than at the C-1 position. Conformational analysis showed that compounds **1, 3, 5, 7, 9** and **11** were hydroxyesterification products at the C-1 position of GB, and compounds **2, 4, 6, 8**, and **10** were hydroxyesterification products at the C-10 position of GB. A method for the rapid separation and purification of GB derivatives by preparative chromatography was established. The structure of all the final compounds **1–11** was confirmed by ^1H NMR and ^{13}C NMR. For the NMR spectra of compounds **1–11**, a singlet at δ 1.0-0.9 ppm that corresponded to tert butyl functionality, signals at δ 9.1-6.0 ppm revealed the presence of the benzene ring.

On the basis of previous studies, the final products **1–11** and GB were evaluated for their *in vitro* antiproliferative activity against ovarian cancer SKOV3 cell lines by MTT assay. The proliferation inhibitory activity of these compounds was measured at different concentrations. The IC_{50} values for each sample were obtained by plotting the inhibition rate against the drug concentration. The results are presented in Table 1. As we defined that compounds showing less than 50% inhibitory rate at 100 μM were inactive. Compounds **3, 4, 7, 8** and **11** hardly showed any activity, the IC_{50} values against the SKOV3 cell lines are all above 100 μM . The rest of the compounds showed moderate to good activity on SKOV3 cell lines with IC_{50} values 15.65–63.30 μM . Obviously, as compared with compound **1** and **2**, the esterification position of C-1 or C-10 has little effect on the activity of the compounds. However, the inhibitory activities were increased by introduce of the electron-donating group into the benzyl group.

Table 1
In vitro antiproliferative activity of GB derivatives. IC_{50} values are shown in μM against SKOV3 cell line, respectively.

Compounds	IC_{50}	Compounds	IC_{50}
GB	45.4 ± 1.24	6	63.30 ± 2.04
1	16.05 ± 0.86	7	> 100
2	15.65 ± 0.78	8	> 100
3	> 100	9	23.20 ± 0.97
4	> 100	10	31.10 ± 1.05
5	32.00 ± 1.12	11	> 100

The apoptosis of ovarian cancer SKOV3 cells was induced by 100 $\mu\text{mol/L}$ of GB, compounds **2**, **5**, and **9**. The apoptosis rates were found to be 7.98%, 31.68%, 8.82%, and 9.46%, respectively. Preliminary *in vitro* anti-tumor cell proliferation activity test and the Annexin V/PI double staining assay showed that compound **2** had the highest inhibition rate against ovarian cancer SKOV3 cells and could inhibit the proliferation of tumor cells and induce apoptosis. Ginkgolide B derivatives hydroxyesterified at the C-10 position showed significantly higher apoptosis rates than the hydroxyesterified product at the C-1 position in ovarian cancer cells SKOV3.

Conclusion

In this study, 11 novel GB derivatives **1–11** were synthesized and evaluated for their *in vitro* antiproliferative activities against human ovarian cancer SKOV3 cell lines. The majority of the compounds showed moderate to good activity on SKOV3 cell lines. Among these derivatives, compound **2** was found to exhibit better cytotoxic activity on against SKOV3 cells with IC_{50} values 15.65 μM . Annexin V/PI double staining assay showed that compound **2** induced apoptosis in SKOV3 cells to a slightly greater extent than GB and compounds **5**, and **9**, with an apoptosis rate of 31.68%. The above findings will be of great significance for the development of GB derivatives as potential antitumor agents.

Declarations

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

Figure 1

Synthesis route of Ginkgolide B derivatives

Figure 2

Annexin V/PI double staining assay to detect the effect of Ginkgolide B and its derivatives on the apoptosis of human SKOV3 cells.