Accepted Manuscript

Synthesis and *in vitro* and *in vivo* antitumour activity study of 11-hydroxyl esterified bergenin/cinnamic acid hybrids

Chengyuan Liang, Shaomeng Pei, Weihui Ju, Minyi Jia, Danni Tian, Yonghong Tang, Gennian Mao

PII: S0223-5234(17)30220-9

DOI: 10.1016/j.ejmech.2017.03.053

Reference: EJMECH 9313

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 3 January 2017

Revised Date: 23 March 2017

Accepted Date: 24 March 2017

Please cite this article as: C. Liang, S. Pei, W. Ju, M. Jia, D. Tian, Y. Tang, G. Mao, Synthesis and *in vitro* and *in vivo* antitumour activity study of 11-hydroxyl esterified bergenin/cinnamic acid hybrids, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.03.053.

Synthesis and *in vitro* and *in vivo* antitumour activity study of 11-hydroxyl esterified bergenin/cinnamic acid hybrids

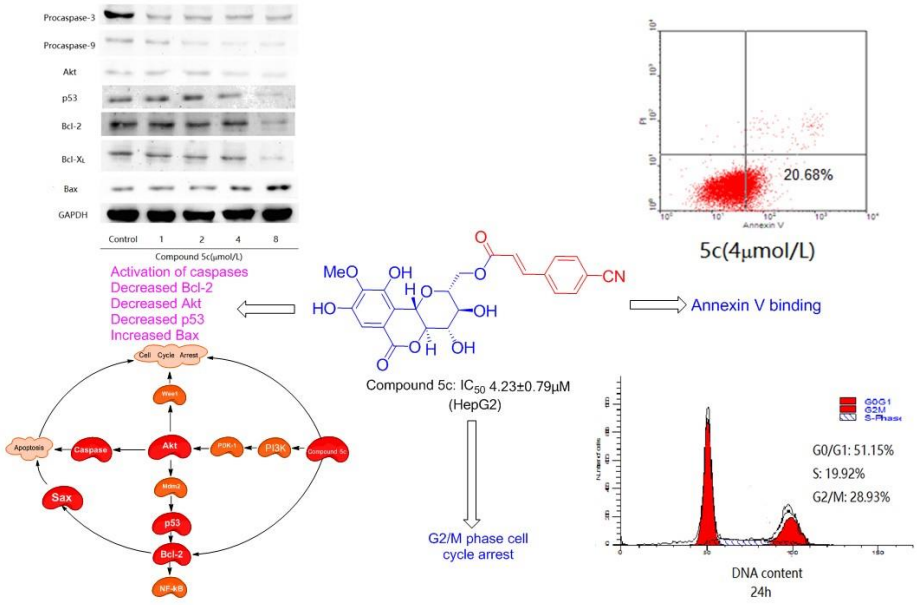
Chengyuan Liang[1,2,*] , Shaomeng Pei[1], Weihui Ju[1], Minyi Jia[1], Danni Tian[1], Yonghong Tang[2], Gennian Mao[1,*]

1. Faculty of Pharmacy, Shaanxi University of Science and Technology, Xi'an 710021, People's Republic of China;
2. Xi'an Taikomed Pharmaceutical Technology Co., LTD, Xi'an 710077, People's Republic of China;

*Correspondence: C.Y. Liang: E-mail: chengyuanliang@gmail.com Phone: +86-18292490697;

G..N. Mao: E-mail: maogn@sust.edu.cn

Novel bergenin/cinnamic acid hybrids were synthesized and evaluated for their antitumour activity both *in vitro* and *in vivo*. The most potent compound, **5c**, arrested HepG2 cells (IC_{50}=4.23±0.79μM) in the G2/M phase and induced mitochondria-mediated apoptosis, which could serve as a novel Akt/Bcl-2 inhibitor for further preclinical studies



Abstract

Fourteen bergenin/cinnamic acid hybrids were synthesized, characterized and evaluated for their antitumour activity both *in vitro* and *in vivo*. The most potent compound, **5c,** arrested HepG2 cells (IC_{50}=4.23±0.79μM) in the G2/M phase and induced cellular apoptosis. Moreover, compound **5c** was also found to suppress the tumour growth in Heps xenograft-bearing mice with low toxicity. In the mechanistic study, **5c** administration ignited a mitochondria-mediated apoptosis pathway of HepG2 cell death. Furthermore, **5c** activated Akt-dependent pathways and further decreased the expression of the Bcl-2 family of proteins. The downstream mitochondrial p53 translocation was also significantly activated, accompanied by an increase of the caspase-9, caspase-3 activation. These data imply that bergenin/cinnamic acid hybrids could serve as novel Akt/Bcl-2 inhibitors for further preclinical studies.

Keywords: bergenin, cinnamic acid, antitumour, apoptosis, Akt/Bcl-2

1 Introduction

Polyphenols of plant origin are important resources in natural medicament, which are often used for pharmacological applications [1-3]. Bergenin (Fig. 1), a dihydroisocoumarin derivative, is the major bioactive component of *Saxifragaceae* [4]. Bergenin is a C-glucoside of 4-O-methyl gallic acid isolated from several medicinal plants, such as *Bergenial crassifolia* [5], *Corylopsis spicata* [6], *Mallotus japonicus* [7], *Mallotus philippinensis* [8], *Caesalpinia digyna* [9], Peltophorum africanum [10], etc. Bergenin is found to possess versatile biological activities, including anticancer [11], antihepatotoxic [7], antioxidant [12], antiarrhythmic [12], anti-HIV [13], anti-inflammatory [14] and neuroprotective properties [15]. Because of the few side effects, low toxicity and lack of drug resistance of bergenin, structural modifications and biological activity research have been conducted by medicinal chemists [16-21]. Several studies have demonstrated that bergenin can induce cell cycle arrest and enhance apoptosis in human prostate cancer cell lines and murine breast cancer cell lines. Additionally, bergenin has an anti-angiogenesis effect, which could suppress vessel endothelial cell growth [22-24].

Fig. 1

Cinnamic acid (Fig. 2) and its derivatives, which consist of a phenyl ring substituted with an acrylic acid group, are widely present in propolis, coffee, fruits and wine [25]. Cinnamic acid exhibits extinguishing pharmacological properties, such as antioxidant [26] and antimicrobial [27].

Recent studies have also revealed that cinnamic acid possesses potential antitumour activity in melanoma, glioblastoma, gastric adenocarcinoma, prostate and lung carcinoma cells [28, 29] by inducing apoptosis and cytoskeleton damage. However, medicinal applications of cinnamic acid have been hindered by its poor solubility in water and low bioavailability caused by the propenoic chain.

Fig. 2

In contrast, highly water-soluble bergenin was endowed with two phenolic hydroxyl groups and three alcoholic hydroxyl groups (one of which is a primary hydroxyl group), which can be easily modified under molecular hybridization drug R&D practices. Molecular hybridization is a valuable structural modification concept, which involves the design of new hybrid compounds with ameliorated efficacy by the combination of pharmacophoric units of different bioactive moieties targeted at increasing the biological affinity and efficacy and reducing the undesirable defects compared to the parent compounds [30-33]. Herein, bergenin and cinnamic acid are both perceived as promising lead compounds for antitumour agents [34, 35]. The rational design is based on the molecular hybridization between bergenin and a variety of cinnamic acids aimed at discovering new hybrid compounds with improved aqueous solubility, bioavailability and anticancer activity. Additionally, with drug-like criteria in mind, the M.W. (reflection of lattice energy) and logP (key parameter to solubility and permeability) of bergenin/cinnamic acid hybrids do not violate Lipinski's Rules of Five.

Based on this hypothesis, a series of bergenin/cinnamic acid hybrids were synthesized and evaluated for antitumour activity against a panel of human tumour cell lines using 5-FU as a potent antitumour reference. The most promising molecule was selected for further *in vivo* antitumour activity and pharmacological mechanistic studies.

2 Chemistry

The synthetic route of 11-hydroxyl-modified bergenin derivatives (**5a-n**) is outlined in Scheme 1. Cinnamic acid (**1**) and bergenin (**3**) were bought from J&K Scientific and used as starting materials. Acid chlorides (**2a-n)** were synthesized through the reaction between cinnamic acid analogues (**1a-n)** and thionyl chloride with CH_2Cl_2 as a catalyst. For the synthesis of ester

analogues (**5a–n)** of bergenin at the C-11 position, the phenolic hydroxyl groups of bergenin were protected by converting bergenin into dibenzyl bergenin (**4**) with benzyl bromide at room temperature using DMF as a catalyst. Finally, compound **4** was treated with acid chlorides (**2a-n)** in the presence of dimethylaminopyridine (DMAP）in pyridine and CH_2Cl_2, followed by hydrogenation with Pd/C to obtain the bergenin ester derivatives (**5a–n),** as shown in Table 1.

More significantly, the esterification reaction occurred only at the primary alcohol group in all cases. Compound **5a** in this study was previously synthesized; however, there were no immediate reports of its antitumour potential. Thus, both compound **5a** and the novel compounds **5b-n** synthesized herein were evaluated for their antitumour activity. The structures of the resulting esters were characterized by ^{1}H and ^{13}C NMR.

Scheme 1

Table 1

3 Biological Activity

3.1 Cytotoxicity evaluation

The synthesized compounds, **5a-n**, were preliminarily evaluated *in vitro* for their cytotoxicity to a panel of five human cancer cell lines consisting of PC-3, SGC-7901, A549, MCF-7 and HepG2 by applying the standard MTT method with 5-FU as the positive control. The half-inhibitory concentration (IC_{50}) values are listed in Table 2. The results show that most of the evaluated compounds displayed potent antiproliferative activities against the tested cell lines. For PC-3 cells, compounds **5a**, **5b**, **5c**, **5d**, **5f** and **5j** displayed more potential cytotoxic activity with IC_{50} values ranging from 12.06 μM to 30.15 μM compared to 5-FU (IC_{50} = 38.61 μM). For A549 cells, compounds **5b**, **5c**, **5d** and **5f** exhibited significant cytotoxicities compared to that of 5-FU (IC_{50} = 10.65 μM) with IC_{50} values of 5.46 μM, 10.66 μM, 12.35 μM and 10.32 μM, respectively. For SGC-7901 cells, compounds **5a**, **5b**, **5c** and **5f** showed equal to or stronger cytotoxicity effects than that of 5-FU (IC_{50} =13.25 μM) with IC_{50} values of 9.67 μM, 7.53 μM, 8.32 μM, 11.38 μM, respectively. For MCF-7 cells, **5c** and **5d** displayed stronger cytotoxic activity with IC_{50} values of 7.34 μM and 6.25 μM, respectively, compared to that of 5-FU (IC_{50} = 7.67 μM). For HepG2 cells,

the cytotoxic activity of compounds **5b**, **5c**, **5d** and **5j** were more potent than that of 5-FU (IC_{50} = 17.44 μM) with IC_{50} values of 6.62 μM, 5.23 μM, 16.83 μM, 17.15 μM, respectively. Compound **5c** exhibited the strongest cytotoxicity activity against all five cancer cells compared to 5-FU with IC_{50} values ranging from 5.23 μM to 26.96 μM. Fig. 3 indicates that **5c** exhibits both dose- and time-dependent effects on HepG 2 proliferation. Thus, compound **5c** was regarded as the most promising compound and was selected for further investigation.

Table 2

Fig. 3

3.2 Structure-activity relationship (SAR) of the bergenin/cinnamic acid hybrids

A structure activity relationship (SAR) analysis for the test compounds **5a-n** showed that the LogP values of all the newly synthesized hybrids were generally between that of bergenin and the corresponding cinnamic acids (Bergenin's LogP: -1.4, Table 1), which indicates equilibrium between lipid and water solubilities. The substitution of cyan or methoxy groups (**5b-e**) led to a significant increase in activity, which is in accordance with the literature results [36-38] that revealed that the introduction of methoxy groups could apparently enhance the antitumour activity. The cytotoxic activities were found to be more potent when two methoxyl groups were located adjacent to one another (**5c** compared to **5b**, **5d** and **5e**). Inversely, the substitution of halogen (chloro- and fluoro-) and methyl groups (**5f-k**) led to a slight loss of activity. The replacement of substituents with electron-donating groups, such as methoxy (**5b-e**) and methyl (**5f**), would enhance the antiproliferative activity. However, the introduction of a nitro group (**5l-n**) could lead to a significant loss of activity. In addition, the cytotoxic activity of bergenin/cinnamic hybrids (**5a**) was visibly superior to that of bergenin, which may be related to the α,β-unsaturated ketone pharmacophore in the cinnamic acid moiety.

3.3 The effect of 5c on cell cycle

To further understand the mechanism of the antiproliferative effect of compound **5c**, we examined the effect of **5c** on cell cycle distribution by flow cytometry. As shown in Fig. 4, **5c** can induce a cell arrest at the G2/M phase. Cell populations in the control group were approximately 64.31% in the G0/G1 phase, 25.28% in the S phase, and 10.41% in the G2/M phase. After incubation with 2 μM/L 5c for 24 h, the cells in the S phase and the G2/M phase increased to

19.92% and 28.93%, respectively.

Fig. 4

3.4 The effect of compound 5c on apoptosis

To examine whether or not compound **5c** induces cell apoptosis, HepG 2 cells were treated with compound **5c** at concentrations of 0.1 μM, 2 μM and 4 μM for 24 h, stained with Annexin V-FITC and propidium iodide, and then analysed by flow cytometry. As shown in Fig. 5, after incubation for 24 h, the apoptotic cell rates at 1 μM, 2 μM and 4 μM were 5.58%, 10.68% and 20.68%, respectively, which were higher than that of the control group. The results of the cell apoptosis assay suggested that compound **5c** can induce apoptosis in a dose-dependent manner in HepG 2 cells.

Fig. 5

3.5 *In vivo* antitumour activity of compound 5c

Compound **5c** showed the highest cytotoxic activity *in vitro* among all of the bergenin/cinnamic acid ester derivatives. Consequently, **5c** was selected for *in vivo* antitumour evaluation on the tumour-bearing mice. Compound **5c** was injected intraperitoneally every day for 14 days at doses of 15 and 30 mg/kg. Saline of blank control and 5-FU of positive control were injected in the same manner. As indicated in Fig. 6A, the average body weights of the tumour-bearing mice treated with **5c** were higher than those of the control group at a dose of 15 mg/kg. In contrast, the average body weights of the tumour-bearing mice treated with 5-fluorouracil (5-FU, 20 mg/kg) significantly decreased compared to the control group, which indicate that compound **5c** is less toxic than 5-FU.

However, compound 5c significantly attenuated the tumour-bearing mice in both tumour weight (Fig. 6B) and tumour size (Fig. 6C) in a dose-dependent manner. Treatment with compound **5c** (30 mg/kg) caused a reduction in the tumour weight by 61.02%, whereas 5-FU (20 mg/kg) treatment resulted in a 52.18% tumour growth regression (Table 3), suggesting that compound **5c** can significantly inhibit tumour growth *in vivo*, which was consistent with its *in vitro* cytotoxicity. Furthermore, treatment with compound **5c** resulted in improved thymus and spleen indexes, which were superior to those treated with 5-FU (Fig. 6D, Fig. 6E). These results

proved that compound **5c** possesses stronger antitumour activity with fewer side effects than 5-FU and could be a potential antitumour compound.

Table 3

Fig. 6

3.6 Western blot assay

To clarify the mechanism of apoptosis caused by compound **5c**, activation of procaspase-3, procaspase-9, Akt, p53, Bcl-2, Bcl-XL and Bax were evaluated by western blot analysis. HepG2 cells were treated with different concentrations (1 μM, 2 μM, 4 μM, and 8 μM) of compound 5c, to which a vehicle control was added (0.5% DMSO). As shown in Fig. 7, upon treatment with compound **5c**, the down regulation of expression of apoptotic procaspase-3 and procaspase-3 is associated with decreased expression of Bcl-2, Bcl-XL protein. Moreover, decreased expression levels of Akt and p53 were also observed, suggesting that down-regulation of Akt protein expression would affect the expression of downstream mediator p53 protein. In addition, compound **5c** down-regulated the expression of Bcl-2, Bcl-XL and up-regulated the expression of proapoptotic proteins, Bax, in both a time- and dose-dependent manner in HepG2 cells. All of the results indicate that the mitochondria-dependent apoptosis pathway was fully activated. Compound 5c could activate Akt and regulate the expression of the Bcl-2 family of proteins as well as p53 and Bax, which play key roles in the regulation of apoptosis by controlling the mitochondrial membrane [39,40].

Fig. 7

4 Conclusion

A series of novel 11-hydroxyl-modified bergenin/cinnamic acid hybrids were synthesized and evaluated in an effort to seek safe and effective anti-tumour lead compounds. The present study demonstrated that most compounds significantly suppressed the viability of five human tumour cells (PC-3, SGC-7901, A549, MCF-7, and HepG2). After screening, compound **5c** was found to display the most potent antitumour activity both *in vitro* and *in vivo*, significantly attenuating tumour growth in a dose-dependent manner without decreasing tumour-bearing mice body weight. Further mechanistic studies showed that compound **5c** arrested the cell cycle at the G2/M phase and induced typical apoptotic features in a dose-dependent manner. Moreover, the western blot assay revealed that compound **5c** can activate Akt and regulate the expression of the

Bcl-2 family of proteins, promote Bax translocation to mitochondria, then trigger caspase-9 and caspase-3, which are essential prerequisites for induction of apoptosis. A preliminary structure-activity relationship (SAR) analysis suggested that the cyan or methoxy substitution of the cinnamic acid phenyl group was essential for antiproliferative activity. Further chemical modifications and preclinical studies, including PK (pharmacokinetic)/PD(pharmacodynamic) properties are currently underway in our laboratories.

5 Experimental protocols

5.1. Chemistry

Unless noted, all solvents and reagents in this study were commercially available. All the reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica GF 254 plates and visualized with UV lamp (254 nm and 365 nm). Melting points (mp) were determined on X-4 micro melting point apparatus (Beijing) and without correction. ^{1}H NMR and ^{13}C NMR spectra were measured in DMSO solution on a 400 Bruker NMR spectrometer at ambient temperature using tetramethylsilane (TMS) as the internal standard. Chemical shifts (δ values) and coupling constant (*J* values) were reported in parts per million (ppm) and hertz (Hz), respectively. Multiplicities were given as s (singlet), d (doublet), t (triplet), m (multiplet), dd (double doublet). Flash column chromatography was carried out on silica gel 200–300 mesh. Mass spectra experiments were performed on electrospray ionization (ESI) techniques. The purities of all compounds were over 96% identified by HPLC.

5.2. General procedure for the synthesis of 5a-n

Cinnamic acid derivatives **1a-n** (1 equiv.) was dissolved in thionyl chloride ($SOCl_2$, 5 equiv.), the reaction mixture was refluxed in the presence of sufficient amount of DMF as the catalyst for 3-5 h. The solvent was removed by reduced pressure distillation to obtain the product **2a-n** in the form of solid residue. Meanwhile, bergenin (1 equiv.) and K_2CO_3 (3 equiv.) were dissolved in dry DMF at 0 °C and benzyl bromide (3 equiv.) was added. After the mixture was stirred for 12 h at room temperature, the compound **4** was obtained through extraction with ethyl acetate, drying with anhydrous sodium sulfate and desolventizing by evaporated in vacuo. The compound **4** was directly added to an ice-cold stirred solution of above solid residue in dry pyridine (200mg/mL).

Then warm the mixture to room temperature and stir for 20 h with CH_2Cl_2 added and DMAP as a catalytic. After completion of the reaction (monitored by TLC: ethyl acetate /petroleum ether =2:1), the mixture was hydrogenated by Pd/C under H_2 atmosphere for 15 h. The mixture was filtered, evaporated under vacuum and purified using column chromatography to afford the compound **5a-n** (Scheme 1). Characteristic data for all bergenin ester derivatives are as follows:

5.2.1. Compound **5a**:11-O-cinnamaldehyde bergenin

White crystals. Yield: 48%, mp: 167-169°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.78 (1H, s), 8.46 (1H, s), 7.64-7.73 (2H, m), 7.49-7.58 (2H, m), 7.46 (1H, d, *J*=11.6 Hz), 7.35 (1H, m), 6.98 (1H, s), 6.32 (1H, d, *J*=9.8 Hz), 5.57 (1H, d, *J*=10.3 Hz), 5.05 (1H, d, *J*=15.7 Hz), 4.12-4.24 (1H, m), 3.96-4.08 (2H, m), 3.72-3.84 (1H, m), 3.58-3.61 (1H, m), 3.45 (3H, s), 3.40 (2H, s), 3.27 (1H, dd, *J*=11.2, 11.2 Hz); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 167.6, 167.4, 150.7,148.0, 147.3, 140.5, 134.2, 130.2, 128.9, 128.8, 128.2, 119.2, 118.0, 109.4, 81.9, 79.7, 73.9, 72.1, 70.6, 61.2, 59.8; HRMS (ESI) calcd. for $C_{23}H_{22}O_{10}$ $[M + H]^+$, 458.1213; found, 458.1219.

5.2.2. Compound **5b**: 11-O-(E)-3-(3,4,5-trimethoxyphenyl)acrylaldehyde bergenin

White crystals. Yield: 26%, mp: 199-201°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.76 (1H, s), 8.42 (1H, s), 7.50 (1H, d, *J*=13.4 Hz), 6.95 (1H, s), 6.80 (2H, s), 6.32 (1H, d, *J*=11.8 Hz), 5.47 (1H, d, *J*=11.8), 4.52-4.61 (1H, m), 4.26-4.35 (2H, m), 4.25 (1H, m), 3.84 (12H, s), 3.88-3.87 (1H, m), 3.56 (2H, s), 3.40 (1H, dd, *J*=10.5, 10.5 Hz); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 167.5, 167.3, 153.5, 149.2, 146.7, 145.9, 145.2, 138.6, 126.5, 125.9, 118.8, 117.2, 109.2, 104.0, 81.4, 79.7, 75.3, 74.6, 63.4, 61.1, 60.0, 56.2; HRMS (ESI) calcd. for $C_{26}H_{28}O_{13}$ $[M + H]^+$, 548.1530; found, 548.1534.

5.2.3. Compound **5c**: 11-O-(E)-4-(3-oxoprop-1-en-1-yl)benzonitrile bergenin

White crystals. Yield: 37%. mp: 194-196°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.82 (1H, s), 8.46 (1H, s), 7.88 (2H, d, *J*=10.6 Hz), 7.58 (1H, d, *J*=9.6 Hz), 7.48 (2H, d, *J*=12.9 Hz), 6.98 (1H, s), 6.32 (1H, d, *J*=12.0 Hz), 5.48 (1H, d, *J*=10.5), 4.53-4.62 (1H, m), 4.26-4.35 (2H, m), 4.27 (1H, m), 3.84 (3H, s), 3.93-4.01 (1H, m), 3.58 (2H, s), 3.42 (1H, dd, *J*=10.8, 10.8 Hz); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 166.6, 166.4, 149.2, 146.7, 145.9, 145.2, 139.7, 132.3, 128.9, 125.9, 118.9, 117.8, 116.4, 109.2, 81.4, 79.6, 75.2, 74.6, 72.0, 63.4, 61.0; HRMS (ESI) calcd. for

$C_{24}H_{21}NO_{10}$ $[M + H]^+$, 483.4242; found, 483.4246.

5.2.4. Compound **5d**: 11-O-(E)-3-(3,5-dimethoxyphenyl)acrylaldehyde bergenin

White crystals. Yield: 37%, mp: 172-174°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.74 (1H, s), 8.26 (1H, s), 7.48 (1H, d, *J*=12.8 Hz), 6.99 (1H, s), 6.74 (2H, s), 6.32 (1H, d, *J*=10.6 Hz), 6.27 (1H, s), 5.49 (1H, d, *J*=11.2 Hz), 4.54-4.63 (1H, m), 4.26-4.32 (2H, m), 4.24 (1H, m), 3.92-4.01 (1H, m), 3.84 (9H, s), 3.58 (2H, s), 3.40 (1H, dd, *J*=9.8, 9.8 Hz); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 166,7 166.5, 161.7, 149.2, 146.7, 145.9, 145.2, 134.2, 126.0, 117.9, 116.4, 109.2, 105.6, 99.8, 81.5, 79.7, 75.3, 74.6, 72.1, 63.4, 61.1, 56.0; HRMS (ESI) calcd. for $C_{25}H_{26}O_{12}$ $[M + H]^+$, 518.4667; found, 518.4670.

5.2.5. Compound **5e**: 11-O-(E)-3-(4-methoxyphenyl)acrylaldehyde bergenin

White crystals. Yield: 31%, mp: 175-177°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.86 (1H, s), 8.54 (1H, s), 7.64 (2H, d, *J*=8.8 Hz), 7.49 (1H, d, *J*=12.8 Hz), 7.02 (1H, s), 6.96 (2H, d, *J*=8.5 Hz), 6.32 (1H, d, *J*=11.0 Hz), 5.48 (1H, d, *J*=11.8), 4.52-4.61 (1H, m), 4.27-4.36 (2H, m), 4.25 (1H, m), 3.84 (6H, s), 3.91-4.00 (1H, m), 3.56 (2H, s), 3.40 (1H, dd, *J*=10.9, 10.9 Hz); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 166.6, 166.4, 159.9, 149.2, 146.7, 145.9, 145.2, 130.4, 127.6, 125.9, 117.9, 116.4, 114.4, 109.2, 81.4, 79.6, 75.3, 74.6, 72.0, 63.4, 61.0, 55.9; HRMS (ESI) calcd. for $C_{24}H_{24}O_{11}$ $[M + H]^+$, 488.4408; found, 488.4412.

5.2.6. Compound **5f**: 11-O-(E)-3-(p-tolyl)acrylaldehyde bergenin

White crystals. Yield: 22%, mp: 220-222°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.80 (1H, s), 8.48 (1H, s), 7.60 (2H, d, *J*=7.9 Hz), 7.49 (1H, d, *J*=12.5 Hz), 7.20 (2H, d, *J*=8.2 Hz), 7.00 (1H, s), 6.32 (1H, d, *J*=11.5 Hz), 5.48 (1H, d, *J*=10.8), 4.52-4.60 (1H, m), 4.28-4.37 (2H, m), 4.26 (1H, m), 3.84 (3H, s), 3.91-4.00 (1H, m), 3.58 (2H, s), 3.42 (1H, dd, *J*=12.1, 11.1 Hz), 2.36 (3H, s); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 166.6, 166.4, 149.2, 146.7, 145.9, 145.2, 137.8, 132.4, 129.1, 128.7, 125.9, 117.9, 116.4, 109.2, 81.4, 79.6, 75.3, 74.6, 72.0, 63.4, 61.0, 21.5; HRMS (ESI) calcd. for $C_{24}H_{24}O_{10}$ $[M + H]^+$, 472.4414; found, 472.4416.

5.2.7. Compound **5g**: 11-O-(E)-3-(3,4-dimethylphenyl)acrylaldehyde bergenin

White crystals. Yield: 28%, mp: 211-213°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.77 (1H, s), 8.12 (1H, s), 7.48 (1H, d, *J*=13.7 Hz), 7.42 (1H, d, *J*=7.8 Hz), 7.18 (1H, d, 8.6 Hz), 7.00 (1H, s),

6.98 (1H, s), 6.33(1H, d, *J*=12.4 Hz), 5.48 (1H, d, *J*=11.8 Hz), 4.54 (1H, m), 4.28 (1H, m), 4.16-4.25 (2H, m),3.84 (3H, m), 3.58 (2H, s), 3.44-3.51 (1H, m), 3.40 (1H, dd, *J*=11.2, 11.2 Hz), 2.36 (6H, s); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 166.8, 166.5, 149.2, 146.7, 145.9, 145.3, 136.9, 136.0, 132.3, 132.2, 131.5, 125.9, 125.7, 117.9, 116.4, 109.1, 81.6, 79.8, 75.3, 74.6, 72.1, 63.4, 61.0, 19.4, 18.9; HRMS (ESI) calcd. for $C_{25}H_{26}O_{10}$ $[M + H]^+$, 486.4679; found, 486.4684.

5.2.8. Compound **5h**: 11-O-(E)-3-(3,4-dichlorophenyl)acrylaldehyde bergenin

White crystals. Yield: 43%, mp: 187-189°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.76 (1H, s), 8.23 (1H, s), 7.58 (1H, d, *J*=8.5 Hz), 7.48 (1H, d, *J*=12.6 Hz), 7.40 (1H, d, 9.5 Hz), 7.26 (1H, s), 6.98 (1H, s), 6.33(1H, d, *J*=12.9 Hz), 5.49 (1H, d, *J*=10.5 Hz), 4.51-4.60 (1H, m), 4.28-4.35 (2H, m), 4.27 (1H, m), 3.85 (3H, m), 3.65-3.74 (1H, m), 3.58 (2H, s), 3.41 (1H, dd, *J*=10.3, 10.3 Hz); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 166.8, 166.5, 149.2, 146.7, 145.9, 145.3, 134.9, 133.5, 132.8, 130.3, 127.9, 127.3, 125.9, 117.9, 116.4, 109.1, 81.6, 79.8, 75.3, 74.6, 72.1, 63.4, 60.9; HRMS (ESI) calcd. for $C_{23}H_{20}Cl_2O_{10}$ $[M + H]^+$, 527.3049; found, 527.3054.

5.2.9. Compound **5i**: 11-O-(E)-3-(4-chlorophenyl)acrylaldehyde bergenin

White crystals. Yield: 25%, mp: 179-181°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.82 (1H, s), 8.57 (1H, s), 7.70 (2H, d, *J*=7.4 Hz), 7.48 (1H, d, *J*=11.5 Hz), 7.45 (2H, d, *J*=8.5 Hz), 6.98 (1H, s), 6.32 (1H, d, *J*=12.5 Hz), 5.48 (1H, d, *J*=11.3), 4.52-4.60 (1H, m), 4.28-4.37 (2H, m), 4.26 (1H, m), 3.84 (3H, s), 3.91-4.00 (1H, m), 3.58 (2H, s), 3.42 (1H, dd, *J*=11.7, 11.7 Hz); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 166.6, 166.4, 149.2, 146.7, 145.9, 145.2, 133.8, 133.4, 129.1, 128.7, 125.9, 117.9, 116.4, 109.2, 81.4, 79.6, 75.3, 74.6, 72.0, 63.4, 61.0; HRMS (ESI) calcd. for $C_{23}H_{21}ClO_{10}$ $[M + H]^+$, 492.8598; found, 492.8602.

5.2.10. Compound **5j**: 11-O-(E)-3-(4-fluorophenyl)acrylaldehyde bergenin

White crystals. Yield: 29%, mp:163-165°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.87 (1H, s), 8.50 (1H, s), 7.74 (2H, d, *J*=8.4 Hz), 7.48 (1H, d, *J*=12.6 Hz), 7.21 (2H, d, *J*=8.9 Hz), 6.98 (1H, s), 6.32 (1H, d, *J*=12.8 Hz), 5.48 (1H, d, *J*=10.8), 4.53-4.62 (1H, m), 4.26-4.37 (2H, m), 4.27 (1H, m), 3.84 (3H, s), 3.93-4.01 (1H, m), 3.58 (2H, s), 3.44 (1H, dd, *J*=12.6, 12.6 Hz); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 166.6, 166.4, 162.2, 149.2, 146.7, 145.9, 145.2, 130.9, 130.6, 125.9, 117.9, 115.5, 116.4, 109.2, 81.4, 75.3, 74.6, 72.0, 63.4, 61.0; HRMS (ESI) calcd. for $C_{23}H_{21}FO_{10}$ $[M + H]^+$,

476.4052; found, 476.4056.

5.2.11. Compound **5k**: 11-O-(E)-3-(3,5-difluorophenyl)acrylaldehyde bergenin

White crystals. Yield: 35%, mp: 156-158°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.83 (1H, s), 8.52 (1H, s), 7.48 (1H, d, *J*=13.1 Hz), 6.99 (1H, s), 6.74 (2H, s), 6.58 (1H, s), 6.32 (1H, d, *J*=11.6 Hz), 5.49 (1H, d, *J*=9.5 Hz), 4.54-4.62 (1H, m), 4.26-4.32 (2H, m), 4.24 (1H, m), 3.94-4.02 (1H, m), 3.84 (3H, s), 3.58 (2H, s), 3.46 (1H, dd, *J*=9.8, 9.8 Hz); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 166,7 166.5, 161.7, 160.9, 149.2, 146.7, 145.9, 145.2, 138.6, 126.0, 117.9, 116.4, 109.6, 104.0, 81.5, 79.7, 75.3, 74.6, 72.1, 63.4, 61.0; HRMS (ESI) calcd. for $C_{23}H_{20}F_2O_{10}$ $[M + H]^+$, 494.1025; found, 494.1029.

5.2.12. Compound **5l:** 11-O-(E)-3-(3,4-dimethoxyphenyl)acrylaldehyde bergenin

White crystals. Yield: 29%, mp: 188-190°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.80 (1H, s), 8.38 (1H, s), 7.48 (1H, d, *J*=12.6 Hz), 7.23 (1H, s), 7.19 (1H, d, *J*=7.7 Hz), 7.00 (1H, s), 6.98(1H, d, 9.8 Hz), 6.33(1H, d, *J*=11.8 Hz), 5.48 (1H, d, *J*=10.8 Hz), 4.54 (1H, m), 4.28 (1H, m), 4.16-4.25 (2H, m),3.84 (9H, m), 3.69-3.76 (1H, m), 3.58 (2H, s), 3.40 (1H, dd, *J*=10.4, 10.4 Hz); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 166.8, 166.5, 150.0,149.2, 146.7, 145.9, 145.3, 127.5, 126.0, 122.7, 117.9, 116.4, 112.0, 111.7, 109.2, 81.6, 79.8, 75.3, 74.6, 72.1, 63.4, 61.0, 56.3; HRMS (ESI) calcd. for $C_{25}H_{26}O_{12}$ $[M + H]^+$, 518.4667; found, 518.4669.

5.2.13. Compound **5m**: 11-O-(E)-3-(4-nitrophenyl)acrylaldehyde bergenin

White crystals. Yield: 46%, mp: 167-169°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.78 (1H, s), 8.54 (1H, s), 8.23 (2H, d, *J*=12.1 Hz), 8.05 (2H, d, *J*=10.7 Hz), 7.64 (1H, d, *J*=11.4 Hz), 6.99 (1H, s), 6.62 (1H, d, *J*=12.6 Hz), 5.49 (1H, d, *J*=9.6), 4.52-4.60 (1H, m), 4.28-4.36 (2H, m), 4.26 (1H, m), 3.85 (3H, s), 3.96-4.05 (1H, m), 3.60 (2H, s), 3.45 (1H, dd, *J*=13.2, 13.2 Hz); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 166.6, 166.4, 149.2, 147.3, 146.7, 145.9, 145.2, 141.5, 129.2, 125.9, 124.0, 117.8, 116.4, 109.2, 81.4, 79.6, 75.2, 74.6, 72.0, 63.4, 60.9; HRMS (ESI) calcd. for $C_{23}H_{21}NO_{12}$ $[M + H]^+$, 503.4123; found, 503.4126.

5.2.14. Compound **5n**: 11-O-(E)-3-(3,5-dinitrophenyl)acrylaldehyde bergenin

White crystals. Yield: 22%, mp: 202-204°C. ^{1}H-NMR(400MHz, DMSO-d_6) δ: 9.85 (1H, s), 8.72 (2H, s), 8.66 (1H, s), 8.27 (1H, s), 7.60 (1H, d, *J*=12.8 Hz), 6.98 (1H, s), 6.58 (1H, d, *J*=13.6

Hz), 5.48 (1H, d, *J*=8.7 Hz), 4.54-4.62 (1H, m), 4.28-4.35 (2H, m), 4.25 (1H, m), 3.94-4.02 (1H, m), 3.85 (3H, s), 3.59 (2H, s), 3.41 (1H, dd, *J*=10.4, 10.4 Hz); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 166,7 166.5, 149.2, 148.8, 146.7, 145.9, 145.2, 137.2, 128.9, 126.0, 117.9, 117.4, 116.4, 109.2, 81.5, 79.7, 75.3, 74.6, 72.0, 63.4, 60.8; HRMS (ESI) calcd. for $C_{23}H_{20}N_2O_{14}$ $[M + H]^+$, 548.4099; found, 548.4103.

5.3. Cytotoxicity assay

5.3.1. Cell lines and culture

Five human cancer cell lines, including PC-3 (human prostate cancer), SGC-7901 (human gastric cancer), A549 (human non-small cell lung cancer), MCF-7 (human breast cancer), and HepG2 (human hepatoma cell) were obtained from Cancer Cell Repository (Shanghai cell bank). Cell lines were maintained in DMEM supplemented with 10% (v/v) heat inactivated foetal bovine serum (FBS), 100 IU/mL penicillin, and 100 mg/mL streptomycin. All cancer cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C.

5.3.2. *In vitro* anti-proliferative activity assays

All of the synthesized compounds (**5a-n)** were assessed for their cytotoxic activity via the MTT assay using the PC-3, SGC-7901, A549, MCF-7 and HepG2 cancer cell lines. 5000 cells per well were planted into 96-well microtiter plates in 180 μL growth medium (5% FBS in DMEM) and incubated for 24 h. Then, the cells were exposed to each compound in DMSO at four different concentrations (0.1, 1, 10, and 100 μmol/L) for 72 h. The mixture was incubated at 37°C under a humidified 5% CO_2 atmosphere. After incubation, 20 μL of MTT (5 mg/mL in PBS) was added to each well and the cells were further incubated for 4 h at 37°C. The supernatant of each well was removed and the formazan contained in the cells was dissolved in 150 μL of DMSO. The plate was shaken to ensure the crystals were dissolved completely. Then, the absorbance (OD values) was recorded by spectrophotometric quantification at 570 nm as a marker for cell viability. The IC_{50} (50% cell viability inhibition) values were calculated by means of PRISM 6, Graph Pad software. The independent experiment was repeated at least three times.

5.4 Flow cytometry cell cycle analysis

HepG2 cells were seeded 3×10^5 per well in a 6-well plate and incubated for 24 h at 37°C. Then, the media was replaced and the cells were treated with compound **5c** at different concentrations for 24 h. The cells were harvested and washed two times with cold PBS, and fixed with cold 70% alcohol at -20°C for at least 24 h. After centrifugation of the suspensions at 1500 rpm for 5 min, the cell pellets were treated with RNase A (50 mg/ml in PBS) and stained with propidium iodide (50 mg/mL) at 37°C for 30 min. Flow cytometry analysis was performed by a flow cytometer (Becton, Dickinson and Company).

5.5. Apoptosis analysis

HepG2 cells were planted at 1.5×10^5 per well in a 6-well plate and incubated at 37°C. After 24 h, the media was replaced and the cells were exposed to different concentrations of compound 5c for 48 h. The cells were harvested and washed two times with cold PBS. The percentages of apoptotic cells were evaluated by staining with Annexin V-FITC/PI Apoptosis Detection Kit (APOAF; Sigma, St. Louis, MO, USA) according to the manufacturer's instructions. The cells were analysed with a flow cytometer (Becton, Dickinson and Company).

5.6. *In vivo* antitumour activity assay with compound 5c

Female Swiss mice with body weights of 20-22 g, obtained from the Experimental Animal Center of Xi'an Jiaotong University Health Science Center, were maintained in a conventional animal colony and freely available for food and water. HepG2 cells (3×10^6) were injected subcutaneously into the right flank to generate the xenograft-bearing mice model. After the tumour cells were inoculated for 7-21 days, 24 tumour-bearing mice were randomly divided into four groups, with 6 mice in each group: saline control group, **5c** 15 mg/kg group, **5c** 30 mg/kg group and 5-FU (positive control) 20 mg/kg group. All groups were given a daily intraperitoneal injection. The body weights were recorded every day and the tumour wet weights were measured on the last day of the experiment (all mice were killed on Day 14). In addition, the toxicity of **5c** on the thymus and spleen were also observed. The thymus and spleen indexes were calculated by using the following formulas: thymus index = (thymus weight/final body weight); spleen index = (spleen weight/final body weight).

5.7. Western blot assay

HepG2 cells were seeded into 12-well plates at 5×10^5 cells per well with different concentrations of compound **5c** and incubated at 37°C for 24 h in the presence of 10% FBS. The controls were treated with vehicle (0.5% DMSO). The cells were lysed into ice-cold cell lysis buffer for 30 min and centrifuged at 13000 g for 15 min at 4°C. The protein concentrations of the supernatant were determined by a BCA protein Assay Kit. Equal amounts of protein lysates were separated on 10% SDS-PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis) and transferred onto PVDF membranes. The membranes were blocked with 5% non-fat milk in TBST (0.1% Tween 20 in TBS) buffer for 2 h, incubated with primary antibodies at 1:2000 dilutions at 4°C, and washed by TBST four times. Then, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies at 1:2000 dilutions at room temperature for 2 h and washed four times with TBST. The western blotting was detected by enhanced chemiluminescence reagent (Beyotime Institute of Biotechnology, China). GAPDH was used as a loading control.

5.8. Statistical analysis

The experimental results are expressed as the mean ± standard deviation (SD) and mean ± standard error of the mean (S.E.M). The statistical differences were analysed according to analysis of variance (ANOVA) test, one-way and two-way. P values less than 0.05 ($P<0.05$) were considered statistically significant and represented by: *$P<0.05$, **$P<0.01$. All statistics were calculated using the statistical progam PRISM 6, Graph Pad software.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (Grant No.81602967) and the China Postdoctoral Science Foundation (Grant No.2016M592898XB), the Shaanxi Postdoctoral Science Foundation (2015), the Basic Research Plan of the Education Department of Shaanxi Province National (Grant No.15JK1076), the College Students' Innovative Entrepreneurial Training Program (Grant No.201510708172, 201610708019). We appreciate the support from Center for Instrumental Analysis, China Pharmaceutical University, for their contribution to the structural confirmation.

References

[1] Asensi M, Ortega A, Mena S, Feddi F, Estrela JM. Natural polyphenols in cancer therapy[J]. Critical Reviews in Clinical Laboratory Sciences. 2011,48(48):197-216.

[2] Ebrahimi A, Schluesener H. Natural polyphenols against neurodegenerative disorders: potentials and pitfalls[J]. Ageing Research Reviews. 2012,11(2):329-45.

[3] Lecour S, Lamont KT. Natural polyphenols and cardioprotection[J]. Mini Reviews in Medicinal Chemistry. 2011,11(14):1191-9(9).

[4] Tanaka N, Kraus FJ, Fujioka T, Fujii H, Mihashi K, Ishimaru K. Bergenin and fragrance molecules in Saxifraga stolonifera root cultures[J]. Current Topics in Phytochemistry, Volume 5: 85-89. 2002.

[5] Chen XM, Yoshida T, Hatano T, Fukushima M, Okuda T. Galloylarbutin and other polyphenols from Bergenia purpurascens[J]. Phytochemistry. 1987,26(2):515–7.

[6] Patel DK, Patel K, Kumar R, Gadewar M, Tahilyani V. Pharmacological and analytical aspects of bergenin: a concise report[J]. Asian Pacific Journal of Tropical Disease. 2012,2(2):163-7.

[7] Lim HK, Kim HS, Choi HS, Oh S, Choi J. Hepatoprotective effects of bergenin, a major constituent of Mallotus japonicus, on carbon tetrachloride-intoxicated rats[J]. Journal of Ethnopharmacology. 2000,72(3):469–74.

[8] Haribabu K, Ajitha M, Ramesh B, Babu K, Rao J. Quantification of bergenin from Mallotus philippinensis by HPTLC-MS and study on different extraction methods[J]. JPC - Journal of Planar Chromatography - Modern TLC. 2012,25:445-9.

[9] Kumar R, Patel DK, Prasad SK, Laloo D, Krishnamurthy S, Hemalatha S. Type 2 antidiabetic activity of bergenin from the roots of Caesalpinia digyna Rottler[J]. Fitoterapia. 2012,83(2):395–401.

[10] Mebe PP, Makuhunga P. 11-(E)-p-Coumaric acid ester of bergenin from Peltophorum africanum[J]. Phytochemistry. 1992,31(9):3286-7.

[11] Chen Z, Liu YM, Yang S, Song BA, Xu GF, Bhadury PS, et al. Studies on the chemical constituents and anticancer activity of Saxifraga stolonifera (L) Meeb[J]. Bioorganic & Medicinal Chemistry. 2008,16(3):1337-44.

[12] Arfan M, Amin H, Khan I, Shah MR, Shah H, Khan AZ, et al. Molecular simulations of bergenin as a new urease inhibitor[J]. Medicinal Chemistry Research. 2012,21(9):2454-7.

[13] Piacente S, ., Pizza C, ., Tommasi N, De, Mahmood N, . Constituents of Ardisia japonica and their *in vitro* anti-HIV activity[J]. Journal of Natural Products. 1996,59(6):565-9.

[14] Gao XJ, Guo MY, Zhang ZC, Wang TC, Cao YG, Zhang NS. Bergenin Plays an Anti-Inflammatory Role via the Modulation of MAPK and NF-κB Signaling Pathways in a Mouse Model of LPS-Induced Mastitis[J]. Inflammation. 2014:1-9.

[15] Takahashi H, Kosaka M, Watanabe Y, Nakade K, Fukuyama Y. Synthesis and neuroprotective activity of bergenin derivatives with antioxidant activity[J]. Bioorganic & Medicinal Chemistry. 2003,11(8):1781-8.

[16] D., H., T., Y., J., C., et al. A carbon-carbon-coupled dimeric bergenin derivative biotransformed by Pleurotus ostreatus[J]. Bioorganic & Medicinal Chemistry Letters. 2005,15(18):4073–5.

[17] De-Biao, ZHANG, Dong-Ping, Ming, Wen-Yuan, FENG, et al. Synthesis and cytotoxic activity of 3, 4, 11-trihydroxyl modified derivatives of bergenin[J]. Chinese Journal of Natural Medicines. 2014,12(12):929-36.

[18] Kumar TV, Tiwari AK, Robinson A, Babu KS, Kumar RSC, Kumar DA, et al. Synthesis and antiglycation potentials of bergenin derivatives[J]. Bioorganic & Medicinal Chemistry Letters. 2011,21(16):4928-31.

[19] Nighat N, Surrinder K, Mushtaq Ahmad Q, Muzaffar Hussain N, Mohammed Iqbal Z. Evaluation of antioxidant and antimicrobial activities of Bergenin and its derivatives obtained by chemoenzymatic synthesis[J]. European Journal of Medicinal Chemistry. 2011,46(6):2415-20.

[20] Shah MR, Arfan M, Amin H, Hussain Z, Qadir MI, Choudhary MI, et al. Synthesis of new bergenin derivatives as potent inhibitors of inflammatory mediators NO and TNF-α[J]. Bioorganic & Medicinal Chemistry Letters. 2012,22(8):2744–7.

[21] Zamarrud, Ali I, Hussain H, Ahmad VU, Qaiser M, Amyn A, et al. Two new antioxidant bergenin derivatives from the stem of Rivea hypocrateriformis[J]. Fitoterapia. 2011,82(4):722–5.

[22] Manh Heun K, Sung Yi H, Myeong Hwan O, Hyuk KH, So Ra K, Won LM. Anti-oxidative and anti-proliferative activity on human prostate cancer cells lines of the phenolic compounds from Corylopsis coreana Uyeki[J]. Molecules. 2013,18(5):4876-86.

[23] Megumi S, Toshikazu S, Nijsiri R, Kazuei I, Fumio I. Ardisiphenols and other antioxidant principles from the fruits of Ardisia colorata[J]. Chemical & Pharmaceutical Bulletin. 2002,50(11):1484-7.

[24] Newell AMB, Yousef GG, Lila MA, Ramírez-Mares MV, Mejia EGD. Comparative *in vitro* bioactivities of tea extracts from six species of Ardisia and their effect on growth inhibition of HepG2 cells[J]. Journal of Ethnopharmacology. 2010,130(3):536–44.

[25] Hoskins JA. The occurrence, metabolism and toxicity of cinnamic acid and related compounds[J]. Journal of Applied Toxicology. 1984,4(6):283-92.

[26] Gülçin İ. Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid)[J]. Toxicology. 2006,217(s 2–3):213-20.

[27] Sova M. Antioxidant and antimicrobial activities of cinnamic acid derivatives[J]. Mini Reviews in Medicinal Chemistry. 2012,12(8):749-67(19).

[28] De P, ., Baltas M, ., Bedos-Belval F, . Cinnamic acid derivatives as anticancer agents-a review[J]. Current Medicinal Chemistry. 2011,18(11):1672-703(32).

[29] Qi-Fu L, Song-Lin S, Qing-Rong L, Jian T, Jianye S, Ying L. Anticancer effects of ginsenoside Rg1, cinnamic acid, and tanshinone IIA in osteosarcoma MG-63 cells: Nuclear matrix downregulation and cytoplasmic trafficking of nucleophosmin[J]. International Journal of Biochemistry & Cell Biology. 2008,40(9):1918–29.

[30] Bosquesi PL, Trf M, Vizioli EO, dos Santos JL, Chung MC. Anti-Inflammatory Drug Design Using a Molecular Hybridization Approach[J]. Pharmaceuticals. 2011,4(12):1450-74.

[31] Cloete TT, De KC, Smith PJ, N'Da DD. Synthesis, *in vitro* antiplasmodial activity and cytotoxicity of a series of artemisinin–triazine hybrids and hybrid-dimers[J]. European Journal of Medicinal Chemistry. 2014,76(7):470–81.

[32] Meunier B. Hybrid molecules with a dual mode of action: dream or reality?[J]. Accounts of Chemical Research. 2008,41(1):69-77.

[33] Singh K, Kaur H, Chibale K, Balzarini J. Synthesis of 4-aminoquinoline-pyrimidine hybrids as potent antimalarials and their mode of action studies[J]. European Journal of Medicinal Chemistry. 2013,66C(15):314-23.

[34] Ting Z, Qian S, Bastow KF, Kuo-Hsiung L. Antitumor agents 286. Design, synthesis, and structure-activity relationships of 3'R,4'R-disubstituted-2',2'-dimethyldihydropyrano[2,3-f]chromone (DSP) analogues as potent chemosensitizers to overcome multidrug resistance[J]. Journal of Medicinal Chemistry. 2010,53(24):8700-8.

[35] Wang-Fun F, Xiao-Ling S, Christoph G, Michael W, Guang-Ying C, Guo-Yuan Z, et al. Methoxylation of 3',4'-aromatic side chains improves P-glycoprotein inhibitory and multidrug resistance reversal activities of 7,8-pyranocoumarin against cancer cells[J]. Bioorganic & Medicinal Chemistry. 2008,16(7):3694–703.

[36] Xu CC, Deng T, Fan ML, Lv WB, Liu JH, Yu BY. Synthesis and *in vitro* antitumor evaluation of dihydroartemisinin-cinnamic acid ester derivatives[J]. European Journal of Medicinal Chemistry. 2015,107.

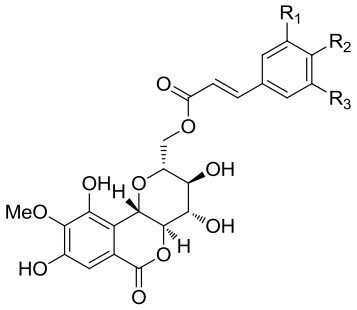
[37] Yan J, Guo Y, Wang Y, Fei M, Ling H, Li X. Design, synthesis, and biological evaluation of benzoselenazole-stilbene hybrids as multi-target-directed anti-cancer agents[J]. European Journal of Medicinal Chemistry. 2015,95:220-9.

[38] Yang X, Wei W, Tan J, Song D, Ming L, Dan L, et al. Synthesis of a series of novel dihydroartemisinin derivatives containing a substituted chalcone with greater cytotoxic effects in leukemia cells. Bioorg Med Chem Lett[J]. Bioorganic & Medicinal Chemistry Letters. 2009,19(15):4385-8.

[39] Cory S, Huang DC, Adams JM. The Bcl-2 family: roles in cell survival and oncogenesis[J]. Oncogene. 2003,22(53):8590-607.

[40] Nazir N, Koul S, Qurishi M A, et al. Evaluation of antioxidant and antimicrobial activities of Bergenin and its derivatives obtained by chemoenzymatic synthesis.[J]. European Journal of Medicinal Chemistry, 2011, 46(6):2415-20.

Table 1. Structure of 11-hydroxyl-modified bergenin/cinnamic acid hybrids



5a-n

Compound	Substituents			Yield	MP (°C)	LogP[a]	LogP
	R_1	R_2	R_3	（%）		(Compound)	(Cinnamic acid)
5a	H	H	H	48	167-169	1.07	1.93
5b	OMe	OMe	OMe	26	199-201	0.69	1.55
5c	H	CN	H	37	194-196	1.11	1.97
5d	OMe	H	OMe	37	172-174	0.81	1.68
5e	H	OMe	H	31	175-177	0.94	1.81
5f	H	Me	H	22	220-222	1.55	2.42
5g	Me	Me	H	28	211-213	2.04	2.91
5h	Cl	Cl	H	43	187-189	2.18	3.05
5i	H	Cl	H	25	179-181	1.62	2.49
5j	H	F	H	29	163-165	1.22	2.09
5k	F	H	F	35	156-158	1.38	2.25
5l	OMe	OMe	H	29	188-190	0.81	1.68
5m	H	NO_2	H	46	167-169	1.21	1.92
5n	NO_2	H	NO_2	22	202-204	1.47	1.88

[a]Theoretical values of logP were calculated using commercially available ACD LAB/logP release 10, product version 10.08. Bergenin's LogP: -1.4

Table 2. *In vitro* antiproliferative activity (IC_{50} in μM) of the synthesized compounds **5a-n** against PC-3, SGC-7901, A549, MCF-7 and HepG2 cell lines.

Compound	IC_{50} (μM)[a]				
	PC-3	A549	SGC-7901	MCF-7	HepG2
bergenin	>100	77.28±3.47	23.56±1.97	>100	34.56±3.12
5a	13.46±0.33	56.89±2.65	9.67±1.68	>100	18.46±1.55
5b	12.06±0.24	5.46±0.26	7.53±0.16	11.69±1.01	6.62±0.53
5c	**26.96±0.15**	**10.66±0.10**	**8.32±1.62**	**7.34±0.88**	**5.23±0.79**
5d	26.32±1.76	12.35±0.95	27.82±2.18	6.25±1.74	16.83±1.90
5e	>100	26.38±1.07	15.86±1.25	18.65±3.23	29.71±3.67
5f	30.15±3.12	10.32±1.86	11.38±1.46	20.22±1.49	>100
5g	>100	>100	>100	59.54±2.68	>100
5h	>100	63.24±3.36	>100	>100	>100
5i	86.52±2.21	>100	>100	>100	>100

5j	22.87±1.98	>100	>100	35.75±3.18	17.15±1.55
5k	46.24±1.25	28.36±2.21	>100	72.13±2.88	>100
5l	>100	>100	58.94±2.50	>100	86.13±1.71
5m	>100	>100	>100	>100	>100
5n	>100	>100	>100	>100	>100
5-FU[b]	38.61±2.16	10.65±0.43	13.25±0.32	7.67±0.18	17.44±0.85

All data are presented as the mean±SD (n = 3).

[a]Concentration that promotes 50% of cell growth inhibition after 48 h of treatment. IC_{50} was determined by the MTT assay. The independent experiment was run at least three times.

[b]Used as a positive control.

[c]When a 50% inhibition could not be reached at the highest compound concentration, >100 μM was given.

Table 3. Inhibitory effect of compound **5c** on HepS tumour xenograft.

Compounds	Rate of inhibition (%)
5c (15 mg/kg)	45.36
5c (30 mg/kg)	61.02
5-FU (20 mg/kg)	52.18

Figure Legends

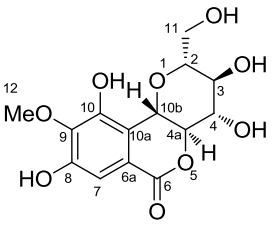


Fig. 1 Structure of bergenin

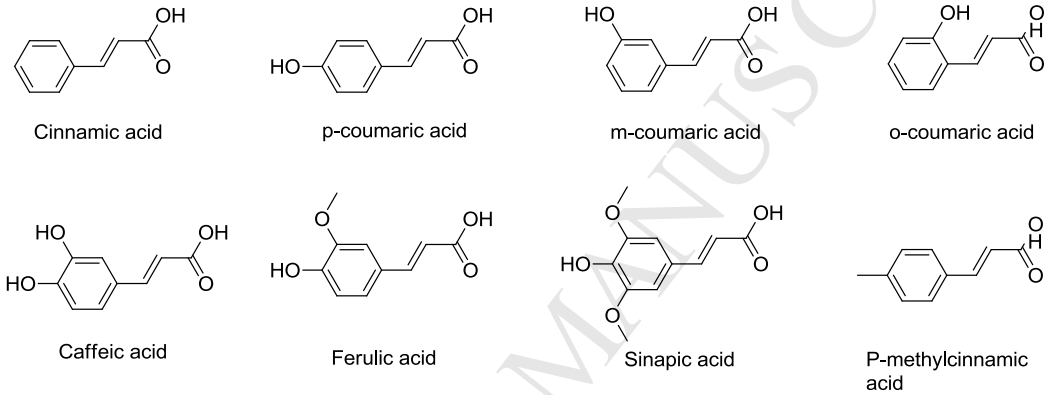


Fig. 2 Structures of natural and synthetic cinnamic acids

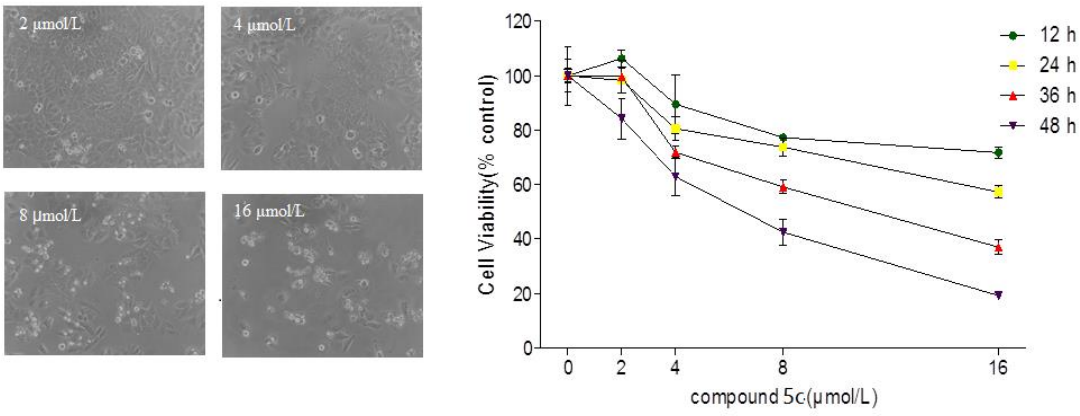


Fig.3 Dose- and time-dependent effect of compound **5c** on HepG 2 proliferation

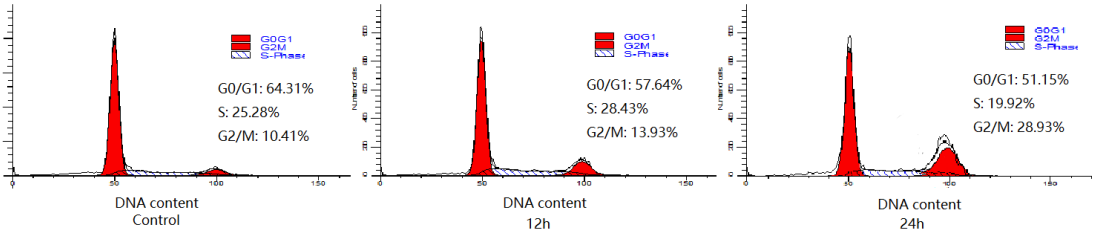


Fig.4 Compound **5c** (2μmol/L) induced G2/M arrest in HepG 2 cells for 24 h, harvested and stained with propidium iodide (PI) for 20 min. Cells were then subjected to flow cytometric analysis for cell distributions at each phase of cell cycle.

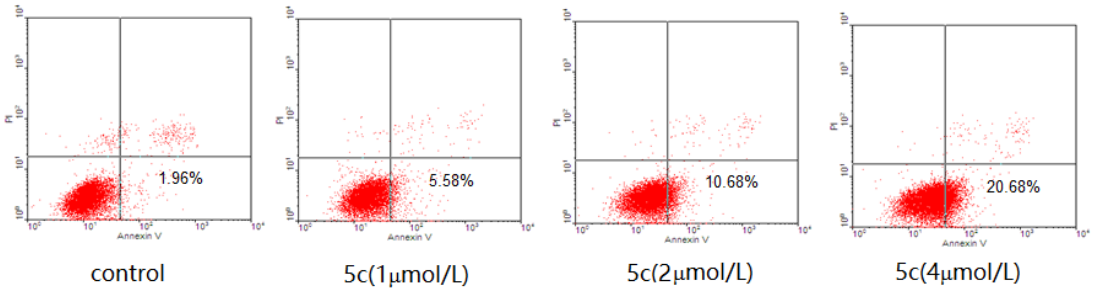


Fig.5 Compound **5c** induced apoptosis in HepG2 cells was assessed by flow cytometric analysis after staining with Annexin V-PI.

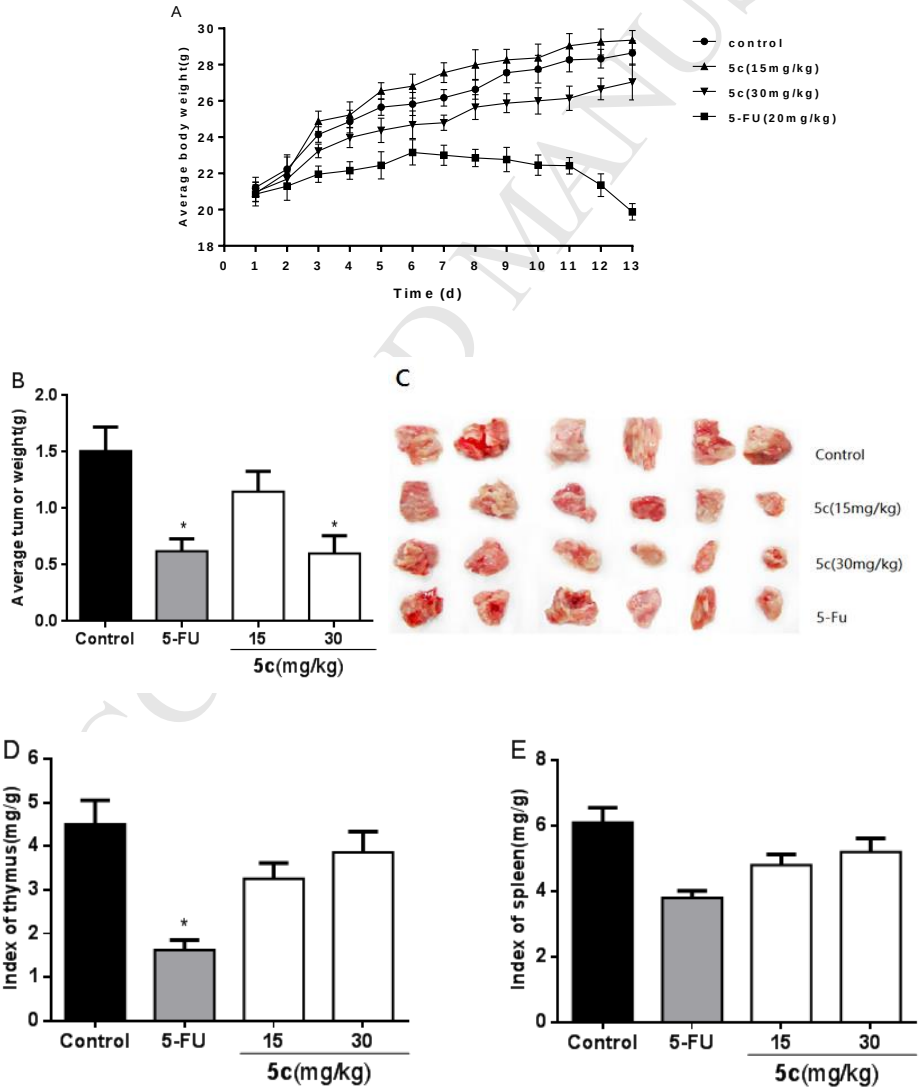


Fig.6 *In vivo* antitumor efficacy assay of compound **5c**. Swiss HepS xenografts-bearing mice were

given a daily intraperitoneal injection for 14 days with normal saline, **5c** (15, 30 mg/kg), 5-FU (20mg/kg). The body weight changes as a function of time (A); the tumor weight (B); the tumor mode (C); effects of **5c** against thymus (D) and spleen index (E). Data represent mean ± S.E.M (n = 6) from three independent experiments, *P < 0.05, vs control.

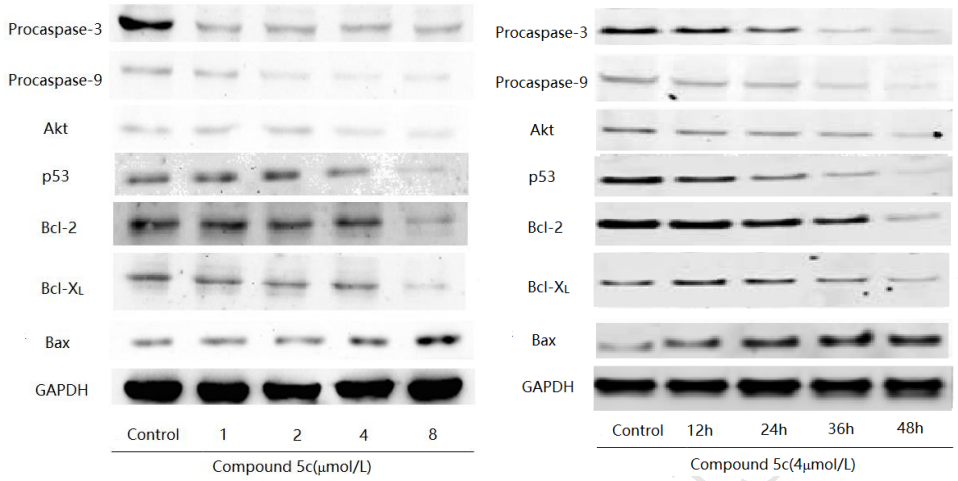
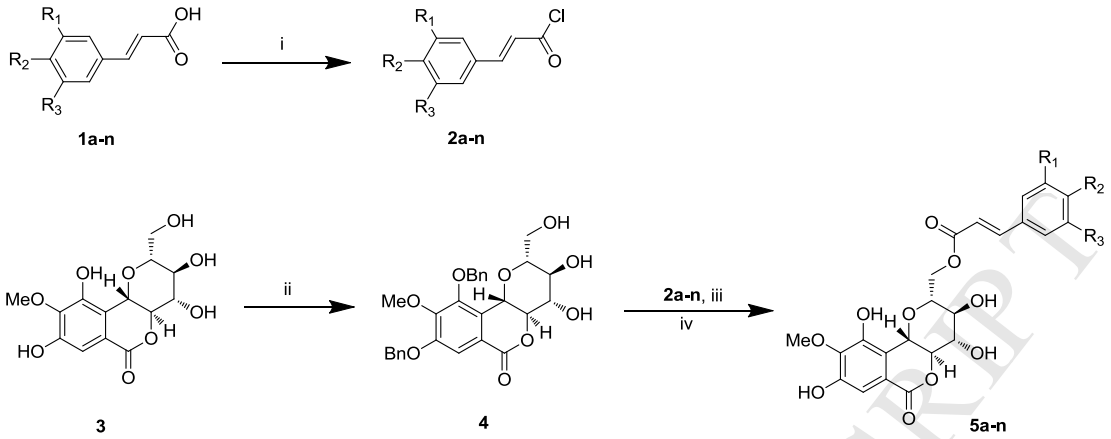


Fig. 7 Western blot analysis of Akt, p53, Bcl-2, Bcl-X_L and Bax in HepG2 cells treated with different concentrations (for 24 h) and different time (for 4 μmol/L) of compound **5c**. GAPDH was used as a loading control.

Scheme 1 The synthesis of **5a-n**.



Reaction conditions and reagents: (i) thionyl chloride, CH_2Cl_2; (ii) K_2CO_3, BnBr, DMF/acetone; (iii) pyridine, DMAP; (iv) H_2/Pd/C, MeOH/CH_2Cl_2, rt.

Research Highlights

1. Novel bergenin/cinnamic acid hybrids with better aqueous and lipid solubility balance.

2. Superior *in vitro* and moderate *in vivo* antitumor activity as well as low toxicity.

3. **5c** arrested HepG2 cells in G2/M phase and induced mitochondria-mediated apoptosis.

4. **5c** inhibited the expression of Akt and Bcl-2 family proteins in a dose- and time- dependent manner.

5. **5c** could be novel Akt/Bcl- 2 signaling pathway inhibitor for further preclinical studies.