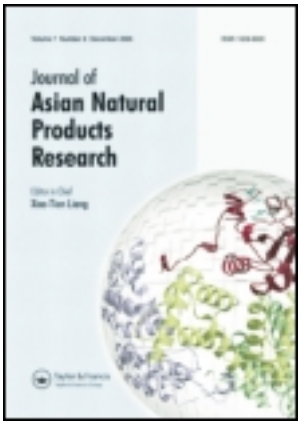

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Antitumor effect of resorcinol derivatives from the roots of *Ardisia brevicaulis* by inducing apoptosis

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Two new resorcinol derivatives 2-methoxy-4-hydroxy-6-(8*Z*-pentadecenyl)-benzene-1-*O*-acetate (**1**) and 2-methoxy-4-hydroxy-6-pentadecyl-benzene-1-*O*-acetate (**2**), together with four known compounds 2-methoxy-4-hydroxy-6-tridecyl-benzene-1-*O*-acetate (ardisiphenol D, **3**), 5-(8*Z*-pentadecenyl)resorcinol (**4**), 5-pentadecylresorcinol (**5**), 5-tridecylresorcinol (**6**), have been isolated from the roots of *Ardisia brevicaulis* in our previous work. In the present study, the inhibitory effect of **1**–**6** on the proliferation of human pancreatic PANC-1, human lung A549, human gastrointestinal carcinoma SGC 7901, human breast MCF-7, and human prostate PC-3 cancer cells was evaluated by the methyl thiazolyl tetrazolium method. Compounds **1**–**6** all showed inhibitory activities against the proliferation of PANC-1, A549, SGC7901, MCF-7, and PC-3 cancer cells. Compound **3**, the most active agent and the main constituent with the highest yield, induced apoptosis of PANC-1 cells (the most sensitive cell line among the cell lines screened) via the activation of caspase-3 and caspase-9, up-regulation of the ratio of bax/bcl-2 protein expression.

Keywords: *Ardisia brevicaulis*; resorcinol derivative; antitumor; apoptosis

1. Introduction

Ardisia species have been used as ornamental plants, food, and medicines. Recent studies demonstrated that species of *Ardisia* are a rich source of structurally diverse natural products [1]. Phytochemical studies of *Ardisia* species resulted in the isolation of compounds with various interesting biological activities, such as a cyclic depsipeptide with antiplatelet effects from leaves of *Ardisia crenata* [2], ardisiacrispins A and B with antitumor activity from *A. crenata* and *Ardisia crispa* [3], ardipusillosides I and II with immunostimulatory and anticancer properties from *Ardisia pusilla* [4], bergenin and norbergenin from *Ardisia japonica* with weak anti-HIV activity [5], alk(en)ylphenols with antioxidative and cytotoxic activities from the fruits of *Ardisia colorata* [6], and quinones from *A. crispa* showing antimetastatic and integrin receptor-binding antagonistic activities [7]. Although great efforts have been made to discover the bioactive constituents from *Ardisia* species, several species still remain unexplored due to taxonomic confusions and difficulties for obtaining the plant material. In this context, we have collected two species (*Ardisia gigantifolia* and *Ardisia brevicaulis* Diels), growing in China and poorly investigated. In a

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previous contribution, two new resorcinol derivatives 2-methoxy-4-hydroxy-6-(8*Z*-pentadecenyl)-benzene-1-*O*-acetate (**1**) and 2-methoxy-4-hydroxy-6-pentadecyl-benzene-1-*O*-acetate (**2**), together with four known compounds 2-methoxy-4-hydroxy-6-tridecyl-benzene-1-*O*-acetate (ardisiphenol D, **3**), 5-(8*Z*-pentadecenyl)-resorcinol (**4**), 5-pentadecylresorcinol (**5**), 5-tridecylresorcinol (**6**), have been isolated and identified from the roots of *A. brevicaulis*, a small shrub distributed in south and southwest of China (Figure 1). The roots of this plant have been used as 'Xuedang' for the treatment of menstrual disorders, injuries from falls, and rheumatic pain in traditional medicines [8]. This paper reports the inhibitory activities of resorcinol derivatives **1**–**6** on the proliferation of cancer cell lines and their molecular mechanisms.

2. Results

2.1 Inhibitory effects of compounds 1–6 on the proliferations of PANC-1, A549, SGC7901, MCF-7, and PC-3 tumor cells

The *in-vitro* cytotoxic activities on various human cancer cell lines of resorcinol derivatives **1**–**6** were evaluated by the methyl thiazolyl tetrazolium (MTT) assay. The concentrations that caused 50% inhibition on cancer cell growth are expressed as IC_{50} values that are reported in Table 1. The different cancer cell lines showed distinctive sensitivity toward resorcinol derivatives **1**–**6**. The proliferations of all five cancer cells were significantly inhibited by compound **3** in a dose-dependent manner (IC_{50} values lower than 20 μmol/L). Compared with cisplatin, which is widely used in anti-tumor chemotherapy in clinics, **3** exhibited an equivalent potency in the inhibitory effect on the proliferations of SGC7901, MCF-7, and PC-3 cells, and much potent inhibitory activity on the proliferations of PANC-1 and A549 cells.

2.2 Effect of resorcinol derivative 3 on morphological changes of PANC-1 cells

To determine whether the loss of cell viability is relative to apoptosis, are monitored the nuclear morphology of PANC-1 cells by Hoechst 33258 staining. As illustrated in Figure 2, the untreated cells were stained roundly and uniformly, but the cells treated with different concentrations of resorcinol derivative **3** obviously appeared as apoptotic features. These results indicated that **3** could induce the apoptosis of PANC-1 cells.

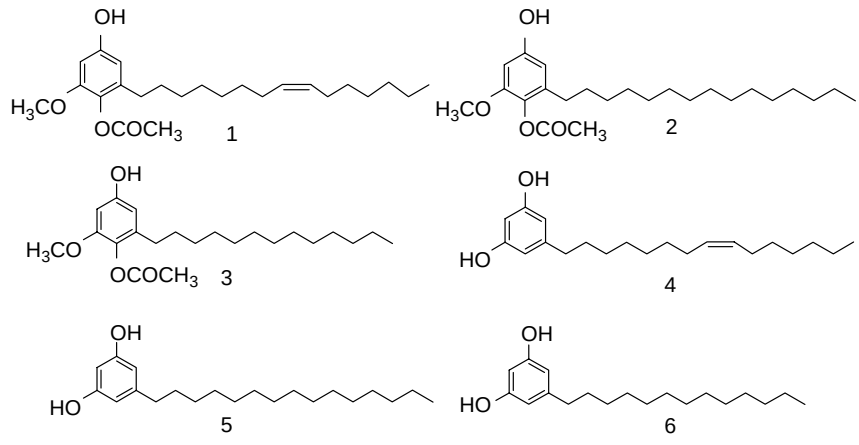

Figure 1. Chemical structures of resorcinol derivatives **1**–**6**.

Table 1. Cytotoxic activities of compounds **1**–**6** isolated from *A. brevicaulis*.

Comp.	IC_{50} (μmol/L)				
	PANC-1	A549	SGC7901	MCF-7	PC-3
1	27.9 ± 1.4	2.96 ± 0.18	75.4 ± 3.9	55.8 ± 2.8	>100
2	15.5 ± 0.7	3.19 ± 0.26	50.2 ± 2.2	43.1 ± 2.3	86.0 ± 4.1
3	0.27 ± 0.08	1.00 ± 0.05	11.9 ± 0.7	10.7 ± 0.9	7.81 ± 3.2
4	34.8 ± 2.2	16.1 ± 0.7	46.4 ± 2.1	39.4 ± 1.1	91.2 ± 4.6
5	40.7 ± 2.1	19.0 ± 0.9	40.2 ± 2.0	48.7 ± 2.4	28.1 ± 1.9
6	40.2 ± 1.5	12.5 ± 0.6	31.7 ± 1.6	67.8 ± 3.0	32.2 ± 2.3
cisplatin	18.2 ± 1.0	18.7 ± 0.9	11.6 ± 0.7	17.0 ± 1.1	12.6 ± 0.4

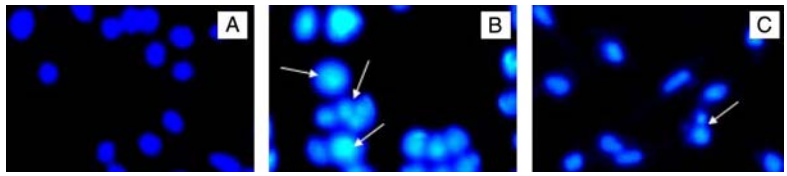

Figure 2. Effect of compound **3** on morphological changes of PANC-1 cells. A, untreated; B, 0.19 μmol/L; C, 1.56 μmol/L. Arrow, the apoptotic bodies of PANC-1 cells and nuclear cleavage.

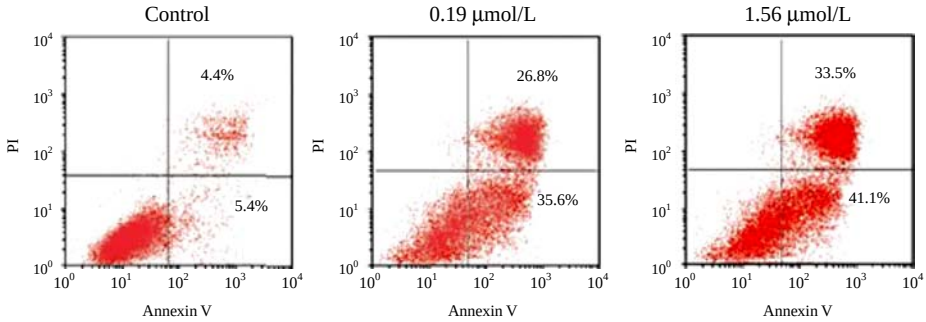

Figure 3. Effect of compound **3** on the induction of apoptosis in PANC-1 cells.

2.3 Resorcinol derivative 3 induced apoptosis of PANC-1 cells

As shown in Figure 3, lower left corner was signed as normal cells, lower right corner was signed as early phase apoptotic cells, and upper right corner was signed as necrotic cells. In the untreated group, the cell apoptotic rate was 5.4%. After treating with 0.19 μmol/L resorcinol derivative **3**, the cell apoptotic rate increased to 35.6%, indicating that resorcinol derivative **3** significantly induced apoptosis of PANC-1 cells. After treating with 1.56 μmol/L resorcinol derivative **3**, the cell apoptotic rate further increased to 41.1% (Table 2), which showed good dose dependency. All results are expressed as means ± SD ($n = 3$). Statistical comparison was conducted using Student's *t*-test after

Table 2. Effect of compound **3** on cell apoptosis by flow cytometry method.

Group	Dose (μmol/L)	Apoptotic cell (%)
Control	0	5.4 ± 0.1
3	0.19	35.6 ± 0.7**
	1.56	41.1 ± 1.2**

Note: **significant vs. control group $P < 0.01$.

ANOVA. The results are considered to be significant when $P < 0.05$.

2.4 *Resorcinol derivative 3 activates caspase-3 and caspase-9 enzymes in PANC-1 cells*

There are multiple ways for inducing apoptosis in cancer cells. However, caspase plays a principal role in the execution of apoptosis by a variety of stimuli. There are two well-established pathways of caspase activation for propagating death signals [9–10]. Nevertheless, both pathways result in the activation of the major downstream effector caspase-3 and caspase-9 that cleaves various cellular targets and leads to cell death [11]. On the basis of the above reasons, PANC-1 cells were treated with resorcinol derivative **3** and the activities of caspase-3 and caspase-9 enzymes were determined. The proteolytic activities of caspase-3 and caspase-9 were measured in terms of their ability to cleave Ac-DEVD-*p*NA, which is a specific substrate for caspase-3 and caspase-9 and can be measured at 405 nm. As shown in Figure 4, the levels of caspase-3 and caspase-9 enzymatic activity after being treated with compound **3** (0.19 and 0.39 μmol/L) at the indicated period of time (1, 2, 4, 8, and 24 h) were found to be significantly

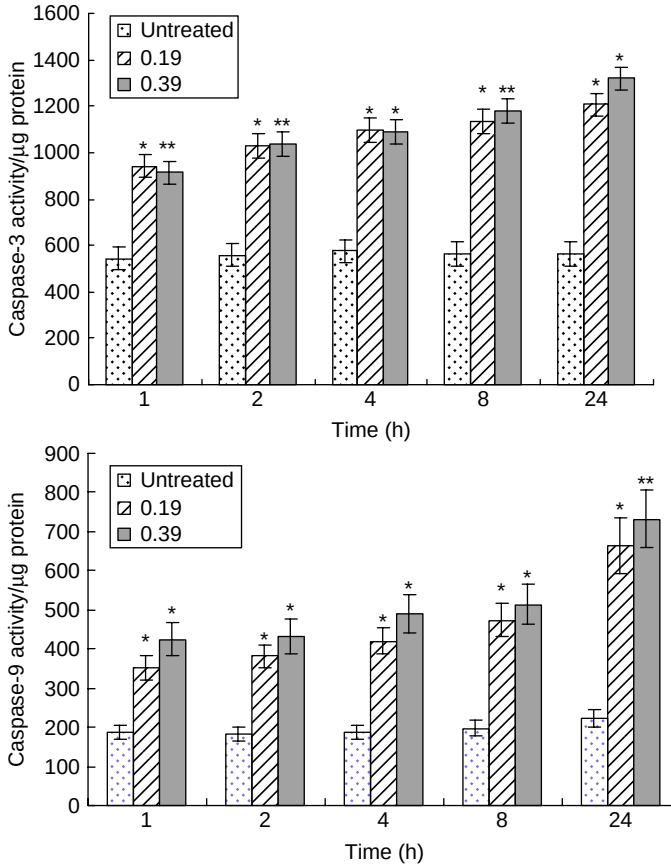

Figure 4. Effect of compound **3** on the enzyme activities of caspase-3 and caspase-9. The enzyme activities of caspase-3 and caspase-9 in PANC-1 cells were measured using a caspase enzyme activity assay kit. Values are expressed as mean ± SE for three independent experiments, in which each measurement was performed in triplicate. $*P < 0.05$, $**P < 0.01$ vs. untreated control group.

increased compared with those of control group (one unit in the figure means the amount of enzyme that will cleave 1.0 mmol/L of the colorimetric substrate Ac-DEVD-*p*NA per hour at 37°C under saturated substrate concentration).

2.5 Effect of resorcinol derivative 3 on the expression of bax, bcl-2, caspase-3, and caspase-9 proteins in PANC-1 cells

The most important protein among apoptotic regulatory proteins is the bcl-2 family that includes anti-apoptotic member bcl-2 and pro-apoptotic member bax [12]. One important underlying mechanism of the pro-apoptotic function of bax is to promote the release of pro-apoptotic factors such as cytochrome C from mitochondria [13–14]. It has been proposed that bax may stimulate the opening of the permeability transition pore (PTP) through interaction with the adenine nucleotide translocation. As a result of PTP opening, mitochondria would swell, leading to the rupture of the outer mitochondrial membrane and the release of cytochrome C [13]. Contradictorily, bcl-2 is a mitochondrial protein family, which inhibits apoptotic process and promotes cell survival by heterodimerization with pro-apoptotic bcl-2 family member such as bax [15–16]. To elucidate the mechanism of resorcinol derivative **3** inducing apoptosis, the levels of both bax and bcl-2 together with those of caspase-3 and caspase-9 were investigated. As shown in Figures 5–7, the up-regulation of bax/bcl-2 ratio was notably observed when treated with resorcinol derivative **3**. Furthermore, the protein level of procaspase-3 was significantly down-regulated when treated with resorcinol derivative **3**. The cleavage of caspase-3 and caspase-9

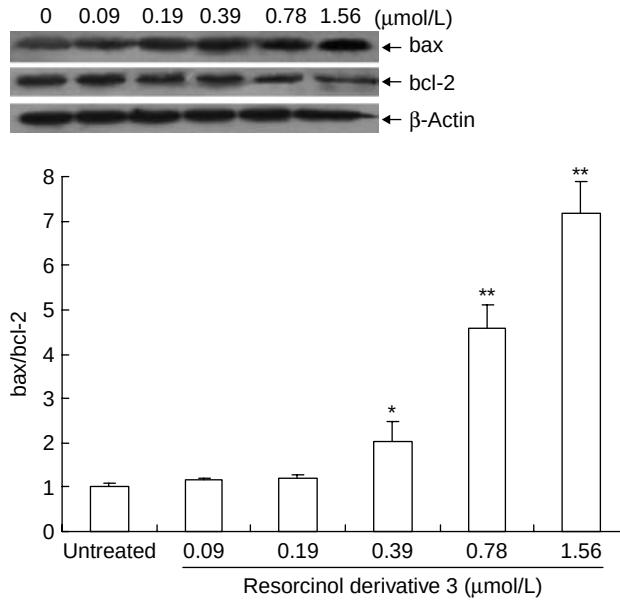

Figure 5. Effect of compound **3** on the expressions of bax and bcl-2 in PANC-1 cells. PANC-1 cells were treated with 0 (lane 1), 0.09 (lane 2), 0.19 (lane 3), 0.39 (lane 4), 0.78 (lane 5), and 1.56 μmol/L (lane 6) of compound **3** for 48 h. The expressions of bax and bcl-2 protein were assessed by the Western blot analysis. Detection of β-actin was carried out to confirm equal loading of proteins. Densitometric analysis of bax protein and bcl-2 protein expression represented the mean from three separate experiments. Data were normalized on the basis of β-actin levels. $*P < 0.05$, $**P < 0.01$ vs. untreated control group.

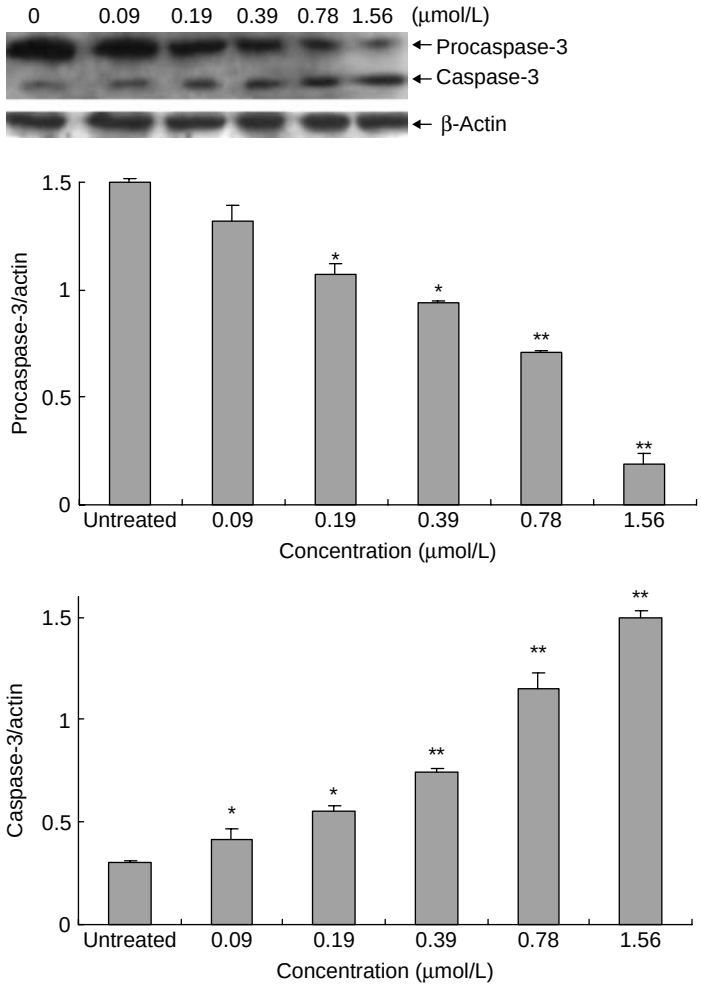

Figure 6. Effect of compound **3** on the expressions of caspase-3 of PANC-1 cells. PANC-1 cells were treated with 0 (lane 1), 0.09 (lane 2), 0.19 (lane 3), 0.39 (lane 4), 0.78 (lane 5), and 1.56 μmol/L (lane 6) of compound **3** for 48 h. The expressions of caspase-3 protein were assessed by the Western blot analysis. Detection of β-actin was carried out to confirm equal loading of proteins. Densitometric analysis of caspase-3 protein expression represented the mean from three separate experiments. Data were normalized on the basis of β-actin levels. $*P < 0.05$, $**P < 0.01$ vs. untreated control group.

proteins can be observed clearly. The above results suggested that **3** induced apoptosis of PANC-1 cells via the activation of caspase-3 and caspase-9, and the up-regulation of the ratio of bax/bcl-2 protein level.

3. Discussion

Although the cytotoxic effects of various constituents isolated from *Ardisia* species have been reported, the biological studies on the resorcinol derivatives remain rare. In the present study, the cytotoxic effects of two new resorcinol derivatives (**1** and **2**) and four known resorcinol derivatives (**3**–**6**) on the proliferation of human pancreatic PANC-1, human lung A549, human gastrointestinal carcinoma SGC 7901, human breast MCF-7, and human prostate PC-3 cancer cells have been studied. Compounds **1**–**6** all

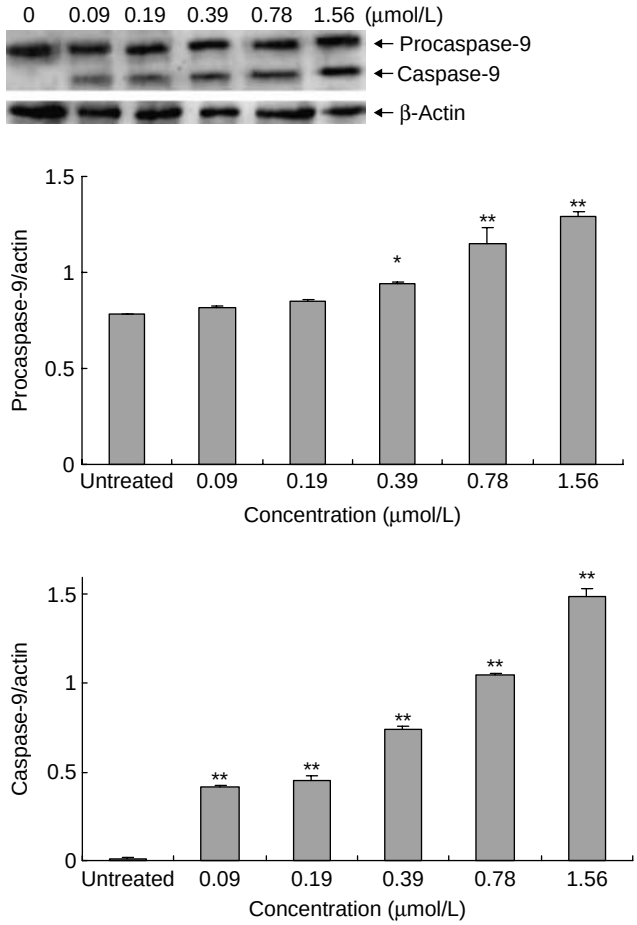

Figure 7. Effect of compound **3** on the expressions of caspase-9 of PANC-1 cells. PANC-1 cells were treated with 0 (lane 1), 0.09 (lane 2), 0.19 (lane 3), 0.39 (lane 4), 0.78 (lane 5), and 1.56 μmol/L (lane 6) of compound **3** for 48 h. The expressions of caspase-9 protein were assessed by the Western blot analysis. Detection of β-actin was carried out to confirm equal loading of proteins. Densitometric analysis of caspase-9 protein expression represented the mean from three separate experiments. Data were normalized on the basis of β-actin levels. $*P < 0.05$, $**P < 0.01$ vs. untreated control group.

showed inhibitory activities against the proliferation of PANC-1, A549, SGC7901, MCF-7, and PC-3 cancer cells. Compound **3**, the most active agent and the main constituent with the highest yield, induced apoptosis of PANC-1 cells (the most sensitive cell line among the cell lines screened) via activation of caspase-3 and caspase-9 enzymes, and up-regulation of the ratio of bax/bcl-2 protein expression. These results explained the molecular mechanism of the antitumor effect of resorcinol derivatives, which may suggest the potency of such resorcinol derivatives to be developed as antitumor agents. Further *in-vivo* studies of **3** in various animal models are in progress.

4. Experimental

4.1 General experimental procedures

RPMI 1640 and DMEM liquid mediums and fetal bovine serum were purchased from Invitrogen Co. (New York, USA).

Penicillin–streptomycin and trypsin solution were purchased from Yantai Science & Biotechnology Co. Ltd. (Yantai, China). MTT and Hoechst 33258 were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). The caspase-3 and caspase-9 enzyme activity detection kits and Annexin V-FITC/PI apoptosis detection kit were purchased from Beyotime Institute of Biotechnology (Haimen, China). All primary antibodies for the Western blot experiment (bax, bcl-2, caspase-3, caspase-9, and β-actin) were purchased from Santa Cruz Biotechnology, Inc. (CA, USA). Cisplatin was purchased from Shandong Lunan Pharmaceutical Factory (Linyi, China). Resorcinol derivatives **1–6** were isolated and identified as previously reported [17–18]. Compounds **1–6** were dissolved in cell culture level DMSO and stored at −20°C until use.

4.2 Cell culture

Human pancreatic PANC-1 and human prostate PC-3 cancer cells were cultured in DMEM; human lung A549, human gastrointestinal carcinoma SGC7901, and human breast MCF-7 cancer cells were cultured in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum, 2 mmol/L L-glutamine, 100 U/ml of penicillin, and 100 μg/ml of streptomycin in a humidified incubator with 5% CO_2 and 95% air at 37°C. The medium was routinely changed every two days. The cells were passed by trypsinization until they attained confluence.

4.3 MTT assay

PANC-1, A549, SGC7901, MCF-7, and PC-3 cancer cells in logarithmic growth phase were seeded in a 96-well plate at a density of 1×10^5 cells/ml and cultured for 24 h. Resorcinol derivatives **1–6** were added at different concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.79, 0.39, 0.19, 0.1, and 0.05 μmol/L). Control group received an equal amount of DMSO, which resulted in a final concentration of 0.2% DMSO in the culture medium. The mitochondrial-dependent reduction of MTT to formazan was used to measure cell respiration as an indicator of cell viability. In brief, after 48 h incubation with or without compounds **1–6** (0.05 ~ 100 μmol/L), an MTT solution (final concentration was 200 μg/ml) was added and the cells were incubated for another 4 h at 37°C. After removing the supernatant, 200 μl of DMSO was added to dissolve the formazan. The absorbance of each group was measured using a microplate reader at wavelength of 570 nm, using 450 nm as reference wavelength. The control group consisting of untreated cells was considered as 100% of viable cells. The inhibitory rates were calculated by the following formula: inhibitory rate $= (1 - A_{samplae}/A_{control}) \times 100\%$. Results given in Table 1 are expressed as IC_{50} values.

4.4 Hoechst 33258 staining and morphological observation of apoptotic cells

PANC-1 cells in logarithmic growth phase were seeded in a 6-well plate at a density of 2×10^5 cells/ml for 24 h. After treating with the indicated concentrations of compound **3** (0.19 and 1.56 μmol/L) for 48 h, the cells were fixed with 2% formaldehyde in phosphate-buffered saline (PBS) for 10 min, and then washed with PBS for 2–3 times. The cells were then incubated with 20 μg/ml of Hoechst 33258 on ice for 20 min. After being washed with PBS, the cells were observed under a fluorescence microscope and the cell morphology was observed and photographed. The cells exhibiting condensed chromatin were scored as apoptotic cells.

4.5 Flow cytometry

PANC-1 cells in logarithmic growth phase were seeded in a 12-well plate at a density of 2×10^5 cells/ml for 24 h. After being treated

with the indicated concentrations of compound **3** (0.19 and 1.56 μmol/L) for 48 h, the cells were harvested by trypsinization and resuspended in PBS buffer. After being centrifuged at 1000*g* for 5 min, the cells were collected and 200 μl of an Annexin V-FITC binding solution was added and then incubated for 10 min. After being centrifuged at 1000*g* for 5 min, the cells were collected. Two hundred microliters of PI staining solution were added and the cells were observed by flow cytometry.

4.6 Measurement of enzymatic activities of caspase-3 and caspase-9

The enzymatic activities of caspase-3 and caspase-9 were measured using a caspase enzyme activity assay kit. PANC-1 cells in logarithmic growth phase were seeded in 60 mm Petri dishes at a density of 4×10^5 cells/ml (6 ml/each dish) for 24 h. After being treated with the indicated concentrations of compound **3** (0.19 and 0.39 μmol/L) for 1, 2, 4, 8, and 24 h, the cells were centrifuged at 600*g* for 5 min, the supernatant was removed, and the cells were washed with PBS and then lysed in a lysis buffer for 15 min on ice. The total protein concentration of the cell lysate was determined by the Bradford method. Twenty-five microliters of cell lysate were incubated with a caspase-3 or caspase-9 colorimetric substrate (Ac-DEVD-*p*NA) for 4 h at 37°C. Absorbance was measured by a universal microplate reader (at 405 nm) subsequently, and the activities of caspase-3 and caspase-9 were calculated according to the manufacturer's instructions.

4.7 Western blot analysis

PANC-1 cells in logarithmic growth phase were seeded in 60-mm Petri dishes at a density of 4×10^5 cells/ml (6 ml/each dish) for 24 h. After being treated with the indicated concentrations of compound **3** (0.09, 0.19, 0.39, 0.78, and 1.56 μmol/L) for 48 h, the cells were washed with cold PBS and then lysed immediately by sonication in PBS containing 1% phenylmethanesulfonyl fluoride. The lysate was centrifuged at 12,000 rpm for 5 min, and the supernatant was collected and the total protein concentration was assayed with a Bradford reagent. Equal amounts of protein (50 μg) were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then electrotransferred onto PVDF membranes. The membranes were blocked with 5% skim milk solution in Tris-bufferred saline-Tween 20 at room temperature for 2 h. After blocking, the membranes were incubated with an appropriate dilution of specific primary antibodies (bax, bcl-2, caspase-3, caspase-9, and β-actin) over night. The membranes were washed four times and then incubated with a 1:1000 dilution of horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. The membranes were washed and then the blots were developed using an enhanced chemiluminescence kit. Images were collected and the bands corresponding to bax, bcl-2, caspase-3, caspase-9, and β-actin protein were quantitated by densitometric analysis using DigDoc100 program (Alpha Ease FC software). Data of bax, bcl-2, caspase-3, and caspase-9 were normalized on the basis of β-actin level. Densitometric analysis of bax, bcl-2, caspase-3, and caspase-9 protein expression represents the mean from three separate experiments.

Acknowledgements

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References

[1] H. Kobayashi and E. De Mejía, *J. Ethnopharmacol.* **96**, 347 (2005).
[2] M. Fujioka, S. Koda, Y. Morimoto, and K. Biemann, *J. Org. Chem.* **53**, 820 (1988).

[3] C. Jansakul, H. Baumann, L. Kenne, and G. Samuelsson, *Planta Med.* **53**, 405 (1987).
[4] Q.H. Zhang, S.L. Huang, and X.J. Wang, *China J. Chin. Mater. Med.* **9**, 545 (1993).
[5] S. Piacente, C. Pizza, N. De Tommasi, and N. Mahmood, *J. Nat. Prod.* **59**, 565 (1996).
[6] Y.H. Kang, W.H. Kim, M.K. Park, and B.H. Han, *Int. J. Cancer* **93**, 736 (2001).
[7] M. Sumino, T. Sekine, N. Ruangrungsi, and F. Ikegami, *Chem. Pharm. Bull.* **49**, 1644 (2001).
[8] B. Dai, C.E. Qin, L.N. Zhou, S.F. Chen, and Z.D. Li, *Chin. Tradit. Herb. Drugs* **27**, 621 (1996).
[9] P.T. Daniel, *Leukemia* **12**, 2035 (2000).
[10] X.M. Sun, M. Macfarlane, J. Zhuang, B.B. Wolf, D.R. Green, and G.M. Cohen, *J. Biol. Chem.* **8**, 5053 (1999).
[11] C. Stroh and K. Schulze-Osthoff, *Cell Death Differ.* **12**, 997 (1998).
[12] S. Cory and J.M. Adams, *Nat. Rev. Cancer* **9**, 647 (2002).
[13] R. Eskes, S. Desagher, B. Antonsson, and J.C. Martinou, *Mol. Cell Biol.* **3**, 929 (2000).
[14] S. Desagher and J.C. Martinou, *Trends Cell Biol.* **9**, 369 (2000).
[15] M.O. Hengartner, *Nature* **6805**, 770 (2000).
[16] X. Yi, X.M. Yin, and Z. Dong, *J. Biol. Chem.* **19**, 16992 (2003).
[17] H.W. Liu, F. Zhao, R.Y. Yang, M.Y. Wang, M.Q. Zheng, Y.S. Zhao, X. Zhang, F. Qiu, and H.S. Wang, *Phytochemistry* **6**, 773 (2009).
[18] H.W. Liu, Y.S. Zhao, R.Y. Yang, M.Q. Zheng, M.Y. Wang, X. Zhang, F. Qiu, H.S. Wang, and F. Zhao, *Helv. Chim. Acta* **93**, 249 (2010).