SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 13: 4371-4377, 2017

Anti-proliferative and anti-metastasis effects of ten oligostilbenes from the seeds of *Paeonia suffruticosa* on human cancer cells

YING GAO[1] and CHUNNIAN HE[2]

[1]Tennessee Center for Botanical Medicine Research and the Department of Biology, Middle Tennessee State University, Murfreesboro, TN 37132, USA; [2]Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing 100193, P.R. China

Received June 12, 2016; Accepted November 21, 2016

DOI: 10.3892/ol.2017.5982

Abstract. *Paeonia suffruticosa* from the section *Moutan* of the genus *Paeonia* is an important Chinese medicinal herb. In our previous study, 10 oligostilbenes from the seeds of *P. suffruticosa*, including *trans*-resveratrol and its dimers and trimers, were isolated and identified. In the present study, the anti-proliferative effects of these 10 oligostilbenes were systemically evaluated in a panel of human lung, breast and bone cancer cell lines, and their apoptotic effects were analyzed using a high-content multiplex apoptosis assay and a fluorescent caspase-3/7 assay. Furthermore, their anti-metastasis effects were examined in an invasive breast cancer cell line. Among the ten compounds, two resveratrol dimers, *trans*- and *cis*-gnetin H, showed the most potent anti-proliferative and anti-metastasis effects. All *trans*-oligostilbenes were more effective than their *cis*-forms, and trimers of resveratrol were more effective than dimers and the resveratrol isomer. The structure-activity relationships revealed that the polymerization degree, the double bond in the stilbene skeleton, and the steric arrangement and conformation of oligostilbenes obviously affected their antitumor potential. The results from this study provide valuable information for future semi-synthesis of resveratrol derivatives to develop novel cancer chemopreventive agents.

Introduction

Cancer is one of the leading causes of mortality in humans worldwide. In 2016, there will be an estimated 1,685,210 new cancer cases diagnosed and 595,690 cancer-associated mortalities in the USA (1). Tumor metastasis, which is defined as the ability of cancer cells to spread to distant organs or tissues in a patient, is responsible for ~90% of cancer-related mortalities (2). Therefore, in addition to limiting the growth of existing tumors, blocking their metastasis is critical to improve the survival of cancer patients. Targeting cancer cell motility and invasion are the main strategies in the attempt to prevent metastasis (3).

Paeonia suffruticosa from the section *Moutan* of the genus *Paeonia* is an important Chinese medicinal herb. Previous investigations of this medicinal plant focused on paeonol, paeoniflorin and their analogs as the major bioactive constituents in the root bark (also called Cortex Moutan) (4,5). Nevertheless, researchers have also been interested in other parts of this medicinal plant, such as the flowers, fruits and seeds (6-8). In particular, recent studies demonstrated that the seeds of this herb are rich and unique sources of oligostilbenes (9,10).

Oligostilbenes are oligomers of the natural molecule resveratrol and have been reported to exhibit a broad variety of biological activities, including antioxidant, antitumor, anti-inflammatory and antimalarial activities (11-13). As one of the most promising naturally derived cancer chemopreventive agents, the *in vitro* and *in vivo* antitumor activity of resveratrol has been extensively characterized (13,14). It is of particular interest to determine whether naturally occurring oligostilbenes have comparable antitumor activities to resveratrol. Satyajit *et al* (15) first identified three oligostilbenes, suffruticosol A-C, from the seeds of *P. suffruticosa* in 1999. In 2002, Kim *et al* (6) isolated six oligostilbenes, *trans*-resveratrol, *trans*-ε-viniferin, *cis*-ε-viniferin, *trans*-gnetin H, suffruticosol A and suffruticosol B, from *Paeonia lactiflora* and evaluated their cytotoxicity against five cancer cell lines, HepG2 (human liver cancer cell line), MCF-7 (human breast cancer cell line), HeLa (human cervix cancer cell line), C6 (rat brain cancer cell line) and HT-29 (human colon cancer cell line). In 2003, Kang *et al* (7) isolated viniferin, *trans*-gnetin H and suffruticosol B from *P. lactiflora* and evaluated their effects on the proliferation and apoptosis of HL-60 cells. In our previous studies, we conducted several phytochemical analyses of the seedcases of *P. suffruticosa* and found that oligostilbenes were the major active ingredients, accounting for ~20% of the content (up to 200 mg/g) (9,10,16,17). In addition to their high abundance, some oligostilbenes identified in the seeds of *Paeonia*, such as suffruticosol A-C, have never been

Correspondence to: Dr Ying Gao, Tennessee Center for Botanical Medicine Research and the Department of Biology, Middle Tennessee State University, 1301 East Main Street, Murfreesboro, TN 37132, USA
E-mail: ying.gao@mtsu.edu

Key words: *Paeonia suffruticosa*, oligostilbene, resveratrol, anti-proliferation, anti-metastasis, apoptosis

reported in other plants. Therefore, a better understanding of the biological and pharmacological activities of these oligostilbenes is of great interest in the field of naturally derived cancer chemopreventive agents.

Previously, we identified ten oligostilbenes, *trans*-resveratrol, *cis*-ε-viniferin, *trans*-ε-viniferin, suffruticosol A, suffruticosol B, suffruticosol C, *cis*-suffruticosol D, *trans*-suffruticosol D, *cis*-gnetin H and *trans*-gnetin H, from the seedcases of *P. suffruticosa* (Fig. 1) (16). Recently, we investigated the biological effects of *cis*- and *trans*-gnetin H and *cis*- and *trans*-suffruticosol D, and showed that they suppressed the proliferation of cancer cells (18,19). In the present study, the antitumor activity of this unique and comprehensive collection of oligostilbenes was systematically evaluated, and their structure-activity relationships were determined based on their anti-proliferative and anti-metastasis effects.

Materials and methods

Plant material. The seeds of *P. suffruticosa* (1.2 kg) were collected in Tongling, Anhui province, China, and identified in 2012. The sample was authenticated by Professor Peigen Xiao and Dr Chunnian He from the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences. A voucher specimen (2012001) was deposited in the Seed Resource Bank of the Institute of Medicinal Plant Development and Peking Union Medical College, Beijing, China.

Simultaneous purification of ten oligostilbenes. The ten oligostilbenes, *trans*-resveratrol, *cis*-ε-viniferin, *trans*-ε-viniferin, suffruticosol A, suffruticosol B, suffruticosol C, *cis*-suffruticosol D, *trans*-suffruticosol D, *cis*-gnetin H and *trans*-gnetin H, were simultaneously purified from the dried seeds of *P. suffruticosa* as described previously (16). Their structures were characterized by ultraviolet (UV), infrared (IR), mass and nuclear magnetic resonance (NMR) spectroscopy, and the purities of all compounds were determined to be >95% (16). The compounds were resuspended in dimethyl sulfoxide (DMSO) (Sigma-Aldrich; Merck Millipore, Darmstadt, Germany) to yield a concentration of 10 mM, and stored at 4˚C.

Cell culture. Six human cancer cell lines were used in this study, including lung carcinoma (A549), breast carcinoma (BT20, MCF-7 and MDA-MB-231), osteosarcoma (U2OS) and cervix adenocarcinoma (HeLa). All cell lines were purchased from American Type Culture Collection (Manassas, VA, USA), except for the HPL1A cell line, which was obtained from Nagoya University, Japan. A549, BT20 and HeLa cells were grown in RPMI-1640 medium (Sigma-Aldrich; Merck Millipore). MCF-7 and MDA-MB-231 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich; Merck Millipore) supplemented with 2 mM L-glutamine (Sigma-Aldrich; Merck Millipore). U2OS cells were grown in McCoy's 5A medium (ATCC). HPL1A cells were grown in DMEM/F-12 medium (Sigma-Aldrich; Merck Millipore). All mediums were supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Inc., Waltham, MA, USA), 1% penicillin and streptomycin, and incubated in a humidified atmosphere containing 5% CO_2 at 37˚C.

Anti-proliferation assay and half maximal inhibitory concentration (IC_{50}) determination. Anti-proliferation activity was determined using a fluorescent staining assay. Cells were seeded into a 96-well tissue culture plate at a density of 4,000 cells/well and treated with the one of the ten compounds at final concentrations of 100, 50, 25, 12.5, 6.25 or 3.13 μM for 48 h. Cells treated with vehicle (1% DMSO) only was also included as an experimental control. Subsequently, 10% AlamarBlue dye (Invitrogen; Thermo Fisher Scientific, Inc.) was added to the medium and the cells were incubated at 37˚C in the CO_2 incubator for 1 h. The fluorescence intensity was read in a SpectraMax M5 microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) at excitation/emission (Ex/Em) wavelengths of 550/590 nm. The results are expressed as a percentage relative to the untreated control, and the IC_{50} values were calculated using non-linear regression analysis with GraphPad Prism software 6.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Multiplex apoptosis assay by high-content screening. MDB-MA-231 cells were seeded into a 96-well plate at a density of 8,000 cells/well, and treated with the test compound at 50 μM for 24 h. Apoptosis was assessed using a live-cell assay by fluorescein isothiocyanate (FITC)-Annexin V and propidium iodide (PI) staining, as described previously (20,21). Briefly, 10 μl of 10X binding buffer containing 1 μl Hoechst 33342, 5 μl FITC-Annexin V and 5 μl mg/ml PI (BD Biosciences, Franklin Lakes, NJ, USA) was added to cell culture medium following treatment with test compounds. Staurosporine (Sigma-Aldrich; Merck Millipore) at a concentration of 1 μM served as a positive control and cells treated with vehicle only served as a negative control. Removal of cell culture medium from wells was avoided because necrotic and poorly attached cells would be detached and removed during this process. Immediately following incubation at room temperature in the dark for 10 min, cells were imaged using an ArrayScan VTI High-Content Screening (HCS) reader (Thermo Fisher Scientific, Inc.) and the fluorescence intensity of each channel (Hoechst 33342, FITC-Annexin V and PI) was analyzed using HCS Studio software 6.5.0.

Caspase-3/7 assay. Caspase-3/7 activity was determined using the SensoLyte Homogeneous AMC Caspase-3/7 Assay kit (AnaSpec, Fremont, CA, USA), according to the manufacturer's instructions. Briefly, MDA-MB-231 cells (8,000 cells/well) were seeded into a 96-well plate and incubated at 37˚C overnight. The cells were treated with test compounds at 50 μM at 37˚C for 24 h. Staurosporine at a concentration of 1 μM served as a positive control and cells treated with vehicle only served as a negative control. After 24 h, 50 μl caspase-3/7 substrate solution was added to each well, and the plate was incubated in the dark at room temperature for 1 h. Fluorescence intensity was measured using a SpectraMax M5 microplate reader at Ex/Em wavelengths of 350/440 nm. Caspase-3/7 activities were calculated by subtracting the fluorescence levels of wells containing medium only.

In vitro cell migration assay. Prior to performing the *in vitro* migration and invasion assay, the cytotoxicity of the test compound was determined after a 16-h treatment. Only the concentration that had <20% toxicity on MDA-MB-231 cells

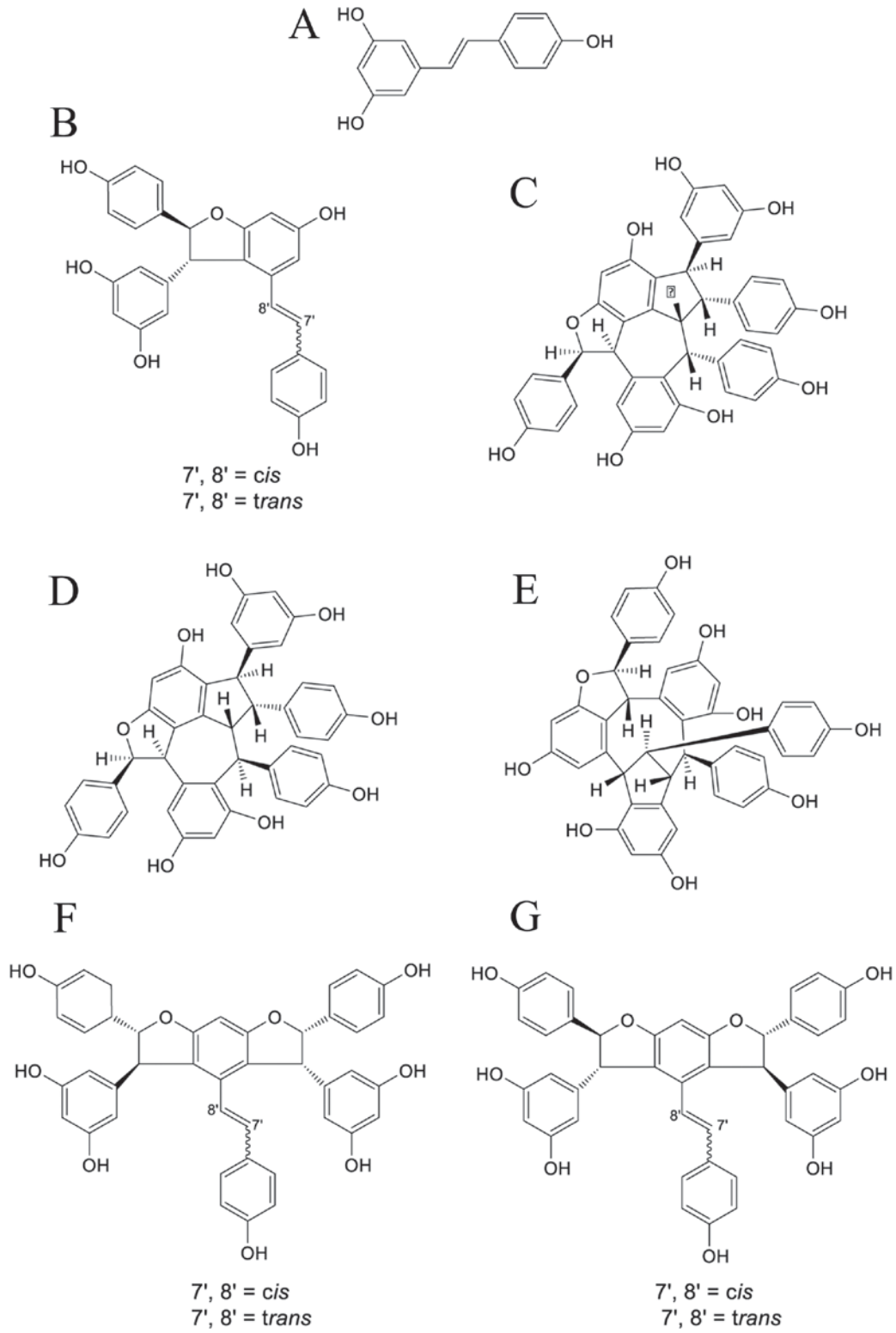


Figure 1. Chemical structures of ten oligostilbenes from *Paeonia suffruticosa* seeds. (A) resveratrol (E) -form, (B) *cis*- and *trans*-ε-viniferin, (C) suffruticosol A, (D) suffruticosol B, (E) suffruticosol C, (F) *cis*- and *trans*-suffruticosol D, and (G) *cis*- and *trans*-gnetin H.

was used for the migration and invasion assay. The *in vitro* migration assay was performed as described previously (22), with slight modifications. Millicell® Cell Culture Inserts (8-μm pore size; EMD Millipore, Billerica, MA, USA) were placed into a 24-well cell culture plate. MDA-MB-231 cells ($2x10^5$ cells/ml) in 500 μl serum-free medium were seeded into the upper chamber. The lower chamber was filled with 500 μl culture medium supplemented with 10% FBS. Each compound was added to the medium in both the upper and lower chambers to the desired concentration (10 μM). Doxycycline (Sigma-Aldrich; Merck Millipore) at a concentration of 100 μM served as a positive control and cells treated with vehicle only served as a negative control. The cells were incubated for 16 h to allow the cells to migrate through the filter

Table I. IC_{50} values (μM) of ten oligostilbenes against six different types of human cancer cell lines (A549, BT20, MCF-7, MDA-MB-231, U2OS and HeLa).

		IC_{50} (μM)					
Name	DP	A549	BT20	MCF7	MDA-MB-231	U2OS	HeLa
Resveratrol *(E)*-form	1	53.6[a]	91.3[a]	>100[a]	>100	>100[a]	17.1
cis-ε-viniferin	2	15.5	19.2	68.0	72.6	75.5	10.5
trans-ε-viniferin	2	6.0	13.1	53.5	59.0	59.7	8.7
Suffruticosol A	3	3.4	5.1	27.7	34.6	31.3	4.3
Suffruticosol B	3	4.8	10.9	17.8	16.7	12.2	3.3
Suffruticosol C	3	9.2	18.4	8.7	9.9	10.7	2.4
cis-suffruticosol D	3	17.1[b]	13.4[b]	46.8[b]	>100	24.6[b]	5.5
trans-suffruticosol D	3	11.9[b]	9.9[b]	15.8[b]	38.9	11.3[b]	2.3
cis-gnetin H	3	2.8[a]	2.4[a]	10.1[a]	9.7	15.9[a]	1.3
trans-gnetin H	3	2.6[a]	2.4[a]	7.7[a]	7.1	4.5[a]	0.9

DP, degree of polymerization; IC_{50}, half maximal inhibitory concentration. [a]Indicates values obtained in (18), and [b]indicates values obtained in (19).

pores. Subsequently, cells on the upper surface of the filter membrane were removed using a sterile cotton swab. Cells that had migrated to the lower surface were stained with 10% AlamarBlue dye at 37˚C for 1 h and read in a SpectraMax M5 microplate reader at Ex/Em wavelengths of 550/590 nm.

In vitro Matrigel invasion assay. The *in vitro* invasion assay followed the same procedure as the *in vitro* migration assay except that the filter inserts were pre-coated with Matrigel matrix. Briefly, 100 μl Matrigel matrix coating (Corning Incorporated, Corning, NY, USA) at 5 mg/ml was added to each insert and incubated at 37˚C for 2 h to form a continuous thin layer.

Statistical analysis. All experiments were performed in triplicate, and each experiment was repeated 3 times. Data were presented as the mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software, Inc.). Data were analyzed using one-way analysis of variance tests, where $P<0.05$ was considered to indicate a statistically significant difference.

Results

Ten oligostilbenes exhibit anti-proliferation activity in human breast, lung and bone cancer cells. The anti-proliferative activity of ten oligostilbenes was initially evaluated in six different human cancer cell lines (A549, MCF-7, BT20, MDA-MB-231, U2OS and HeLa). All oligostilbenes showed mild-to-potent *in vitro* cytotoxicity against these human cancer cells, and the oligomers of resveratrol showed superior antitumor activities compared with the resveratrol monomer (Table I). Concentration-dependent anti-proliferation effects were observed for all oligostilbenes in most of the tested cancer cell lines after 48 h of treatment. *Cis*- and *trans*-gnetin H showed the most potent anti-proliferation activities, with IC_{50} values ranging from 0.9 to 10.0 μM against the six cancer cell lines, representing a >20-fold increase in potency compared with resveratrol (data not shown). Generally, *trans*-ε-viniferin, *trans*-suffruticosol D and *trans*-gnetin H were more potent than their respective *cis*-forms, *cis*-ε-viniferin, *cis*-suffruticosol D and *cis*-gnetin H. Additionally, in most of the cancer cell lines, with the exception of A549, the trimers of resveratrol, suffruticosol A-C, *cis*- and *trans*-suffruticosol D and *cis*- and *trans*-gnetin H were more potent than the dimers of resveratrol *cis*- and *trans*-ε-viniferin.

It is notable that all the tested oligostilbenes inhibited the proliferation of three representative subtypes of breast carcinoma cells, including Basal A phenotype BT20 cells [estrogen receptor $(ER)^-$ progesterone $(PR)^-$ human epidermal growth factor receptor 2 $(HER2)^-$], Luminal A phenotype MCF-7 cells ($ER^+PR^+HER2^-$) and Basal B phenotype MDA-MB-231 cells ($ER^-PR^-HER2^-$). Generally they showed higher potency against BT20 cells than MCF-7 and MDA-MB-231 cells. Since Basal B cells are much more invasive than Basal A and luminal cells (23), Basal B phenotype MDA-MB-231 cells were selected for use in the following experiments to evaluate the anti-metastasis activity of the compounds.

Ten oligostilbenes induce apoptosis in human breast cancer cells. In an examination of the cancerous cells using an inverted microscope, it was observed that A549, BT20, MCF-7, MDA-MB-231, U2OS and HeLa cells treated with oligostilbenes at various concentrations (3.13-100 μM) for 24 and 48 h showed the characteristic morphological changes of apoptosis, including cell shrinkage and apoptotic bodies (data not shown). Therefore, a multiplex apoptosis assay of MDA-MB-231 cells was conducted using HCS to confirm the occurrence of apoptosis. Hoechst 33342 was used to stain the nucleus, and FITC-Annexin V and PI were used as indicators of apoptosis. To compare the apoptotic effect, the same dose of 50 μM was used for all oligostilbenes and the cells were treated for 24 h. As shown in Fig. 2A, cells treated with vehicle

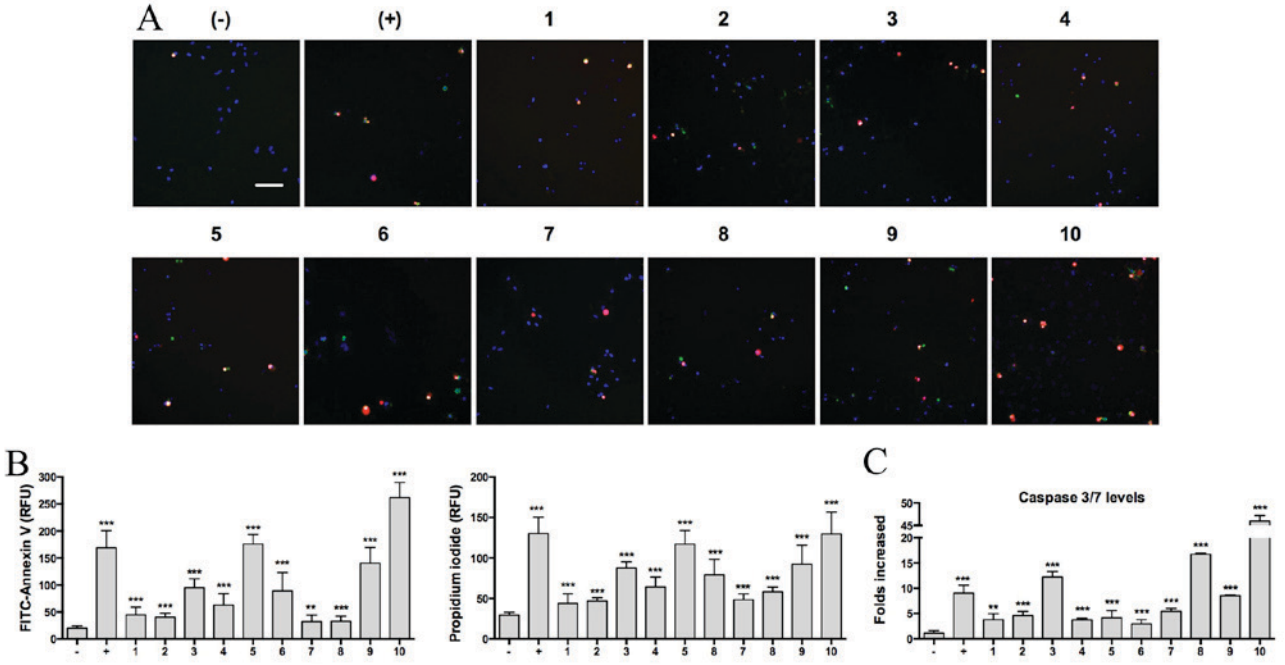


Figure 2. Effects of ten oligostilbenes on MDA-MB-231 cell apoptosis and caspase-3/7 activity. MDA-MB-231 cells were treated with 50 μM of the test compounds for 24 h. Staurosporine (1 μM) served as a positive control and cells treated with vehicle only served as a negative control. Cells were assessed for apoptosis using multiplex fluorescence staining and a HCS reader. (A) Representative HCS images indicative of Hoechst 33342, FITC-Annexin V and PI staining (scale bar=100 μm). (B) Quantitative fluorescent intensity of FITC-Annexin V and PI staining. (C) Caspase-3/7 activity measurements. Cells were assessed for caspase-3/7 activity using the SensoLyte® Homogeneous AMC Caspase-3/7 assay kit. Data are presented as the mean ± standard deviation (n=6). **P<0.01 and ***P<0.001 vs. the negative control. HCS, high-content screening; 1, resveratrol *(E)*-form; 2, *cis*-ε-viniferin; 3, *trans*-ε-viniferin; 4, suffruticosol A; 5, suffruticosol B; 6, suffruticosol C; 7, *cis*-suffruticosol D; 8, *trans*-suffruticosol D; 9, *cis*-gnetin H; 10, *trans*-gnetin H; FITC, fluorescein isothiocyanate; PI, propidium iodide; RFU, relative fluorescence units.

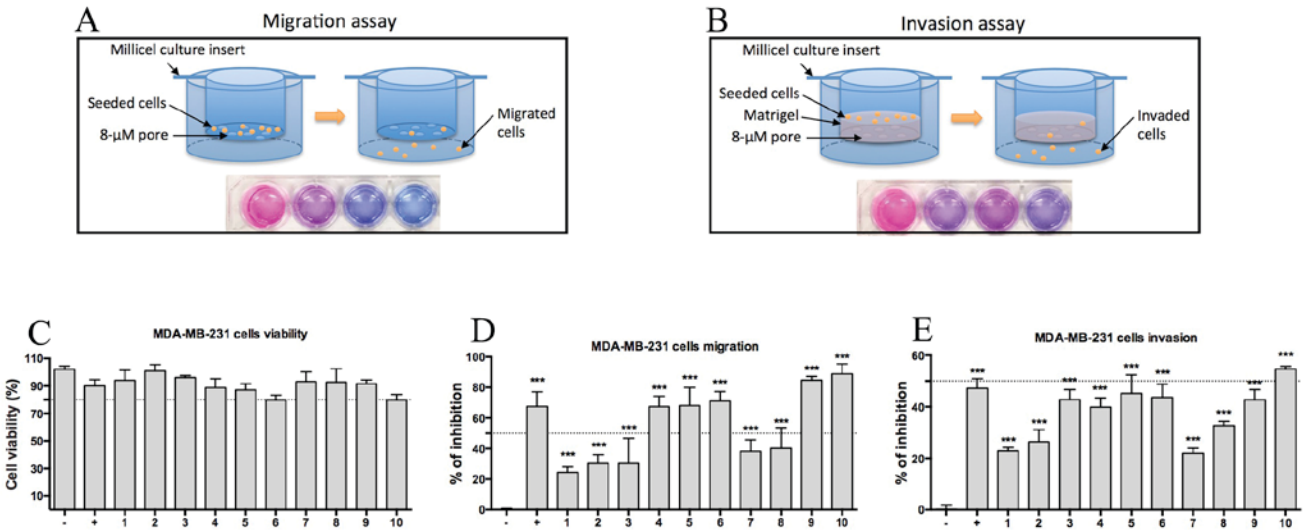


Figure 3. Effects of ten oligostilbenes on MDA-MB-231 cell migration and invasion. MDA-MB-231 cells were treated with 10 μM of the test compounds for 16 h. Doxycycline (100 μM) served as a positive control and cells treated with vehicle only served as a negative control. Migration and invasion were assessed using Millicell Culture Inserts with or without Matrigel matrix, and the number of cells that had migrated or invaded through the inserts was assessed using AlamarBlue staining. (A) Illustration of cell migration assay and representative images of inhibitory effects on cell migration (from left to right: negative control, positive control, *cis*-gnetin H, *trans*-gnetin H). (B) Illustration of cell invasion assay and representative images of inhibitory effects on cell invasion (from left to right: negative control, positive control, *cis*-gnetin H, *trans*-gnetin H). (C) Cell viability after treatment with the test compounds at 10 μM. (D) Inhibition of cell migration ability after treatment with the test compounds. (E) Inhibition of cell invasion ability after treatment with the test compounds. Data are presented as the mean ± standard deviation (n=6). ***P<0.001 vs. the negative control. 1, resveratrol (*E*)-form; 2, *cis*-ε-viniferin; 3, *trans*-ε-viniferin; 4, suffruticosol A; 5, suffruticosol B; 6, suffruticosol C; 7, *cis*-suffruticosol D; 8, *trans*-suffruticosol D; 9, *cis*-gnetin H; 10, *trans*-gnetin H; FITC, fluorescein Isothiocyanate.

only exhibited blue fluorescence due to Hoechst staining, cells undergoing early apoptosis exhibited green fluorescence due to Hoechst and FITC-Annexin V staining, and cells undergoing late apoptosis exhibited green and red fluorescence

due to FITC-Annexin V and PI staining, respectively. In cells treated with 50 μM oligostilbenes, both FITC-Annexin V and PI staining exhibited a significant increase compared with the negative control (P<0.01 or P<0.001; Fig. 2B). These results suggest that the oligostilbenes inhibit cancer cell growth by inducing apoptosis in the cancer cells.

To further examine the activities of caspase-3/7, which are the major effectors activated by the formation of the apoptosome in apoptotic cells (24), a fluorescent assay of MDA-MB-231 cells was performed. Subsequent to a 24-h treatment with oligostilbenes, caspase-3/7 levels were significantly increased in cancer cells. All oligostilbenes at 50 μM concentration had induced caspase-3/7 activity levels by at least 3-folds (P<0.001; Fig. 2C). Among the oligostilbenes, *trans*-gnetin H showed the most significant effect. The caspase-3/7 levels in *trans*-gnetin H-treated cells were 46-fold higher compared with the negative control. This result was consistent with the multiplex apoptosis assay where *trans*-gnetin H also showed the highest Annexin V and PI staining.

Ten oligostilbenes exhibit anti-migration and anti-invasion activities in human breast cancer cells at a low-toxicity dosage. Subsequently, the effects of oligostilbenes at a low-toxicity dosage on the migration and invasion of MDA-MB-231 breast cancer cells were investigated. Since all oligostilbenes displayed <20% cytotoxicity on the MDA-MB-231 cells at the dose of 10 μM following 18 h of treatment (Fig. 3A), we selected this concentration for the subsequent migration and invasion assays. All oligostilbenes inhibited the migration and invasion of MDA-MB-231 cells *in vitro*. In the migration assay, all oligostilbenes significantly affected the number of cells that migrated through the pores of the filter insert; the inhibition rate ranged from 24.4 to 88.9% (P<0.001; Fig. 3B). In the invasion assay, only those cells that passed through the Corning Matrigel Matrix layer and the filter membrane were detected, and the inhibition rate by oligostilbenes ranged from 22.0 to 54.6% (P<0.001; Fig. 3C).

Discussion

As one of the most promising naturally derived chemopreventive agents, the *in vitro* and *in vivo* antitumor activity of resveratrol has been extensively characterized (13,14,25,26). It is of particular interest to determine whether naturally occurring oligostilbenes or their derivatives have comparable antitumor activities to resveratrol. The present study systematically evaluated the antitumor activity of ten oligostilbenes that were simultaneously isolated from the seeds of *P. suffruticosa*, and demonstrated that the dimers and trimers of resveratrol had superior antitumor activities compared with resveratrol.

Cancer development involves the regulation of cell growth and metastasis (27). All test oligostilbenes showed mild-to-potent *in vitro* cytotoxicity against a panel of human cancer cells, and *cis*/*trans*-gnetin H showed the most potent activity among the test compounds; they were >20-fold more effective than the resveratrol monomer. The results of the present study suggested that. The present study also determined the activity levels of caspase-3/7, which are the major effectors activated by the formation of the apoptosome in apoptotic cells (24). In agreement with the results of the apoptosis assay, we found that caspase-3/7 activity levels were significantly increased in cancer cells treated with oligostilbenes.

Cancer metastasis is a complex process that involves sequential steps of invasion, migration, circulation, infiltration and colonization at a distant site (28,29). The present study focused on the effects of oligostilbenes on the migration and invasion of MDA-MB-231 breast cancer cells in order to determine their effects on the metastasis of tumor cells. At a lower dose that did not affect the cancer cell growth, all oligostilbenes inhibited the migration and invasion capability of MDA-MB-231 cells *in vitro*.

An analysis of the structure-antitumor activity relationships revealed three interesting findings. First, the degree of polymerization was closely associated with the antitumor activity of the oligostilbenes. Oligomers with more repeating resveratrol units were more active than smaller oligomers, as evidenced by the order of their potency: Trimers (*cis*- and *trans*-gnetin H, suffruticosol A-C, *cis*- and *trans*-suffruticosol D), followed by dimers (*cis*- and *trans*-ε-viniferin) and, lastly, the resveratrol monomer. This observation is consistent with previous findings of the structure-antioxidant activity of resveratrol oligomers (30,31). Secondly, the double bond in the stilbenic skeleton and its *trans*-isomerism were important to the antitumor activity of oligostilbenes. Resveratrol and its oligomers are known to be highly photosensitive compounds that are prone to UV-induced isomerization. Approximately 80% of *trans*-resveratrol was converted to *cis*-resveratrol upon UV light exposure for 1 h (32), and ~86% of *trans*-gnetin H was converted to *cis*-gnetin H upon UV light exposure for 6 h (18). It is well known that *cis*- and *trans*-isomers of naturally occurring compounds can differ in their bioactivities, and that *trans*-isomers are believed to be the more abundant and active form (33-35). In the present study, the three *cis*-isomers of oligostilbenes, in which the double bond is reduced, were significantly less effective than their *trans*-isomers. Thirdly, the steric arrangement and conformation of oligostilbenes also affected their antitumor activity. *cis*- and *trans*-suffruticosol D and *cis*- and *trans*-gnetin H are both trimers of resveratrol and both possess seven hydroxyl groups, differing only in their three-dimensional structures (16). Generally *cis*- and *trans*-gnetin H was 2-10 times more potent than *cis*- and *trans*-suffruticosol D, indicating that three-dimensional structures have a significant effect on cytotoxicity. The most likely reason was that the *trans* orientation of H-7"/H-8" in *cis*- and *trans*-gnetin H may lessen the steric hindrance between rings C1 and C2, and therefore enhance the bioactivity of *cis*- and *trans*-gnetin H.

Although numerous studies have demonstrated the potential of resveratrol as a cancer chemopreventive agent in the last two decades (13,14,26,36), the poor bioavailability of resveratrol due to its rapid metabolism and secretion from the body compromises its biological and pharmacological benefits (37). Hence, significant attention has been given to the derivatives of resveratrol to overcome these drawbacks. For example, a recent study on the pharmacokinetics of gnetin C, a resveratrol dimer, showed increased bioavailability when orally consumed compared with resveratrol (38). Since the oligostilbenes in the present study showed improved potency in cancer chemoprevention compared with resveratrol, further investigation of their bioavailability is warranted.

In conclusion, the present study assessed a group of ten naturally occurring oligostilbenes for their anti-proliferation and

anti-metastasis properties. The results provided valuable insight into the structure-activity relationship of oligostilbenes for the future development of novel cancer chemopreventive drugs.

Acknowledgements

The authors would like to thank the Tennessee Center of Botanical Medicinal Research (TCBMR) for providing the funding for this study.

References

1. American Cancer Society: Cancer Facts & Figures 2016. : American Cancer Society Inc., Atlanta, GA, 2016.
2. Spano D, Heck C, De Antonellis P, Christofori G and Zollo M: Molecular networks that regulate cancer metastasis. Semin Cancer Biol 22: 234-249, 2012.
3. Wells A, Grahovac J, Wheeler S, Ma B and Lauffenburger D: Targeting tumor cell motility as a strategy against invasion and metastasis. Trends Pharmacol Sci 34: 283-289, 2013.
4. Lau CH, Chan CM, Chan YW, Lau KM, Lau TW, Lam FC, Law WT, Che CT, Leung PC, Fung KP, *et al*: Pharmacological investigations of the anti-diabetic effect of Cortex Moutan and its active component paeonol. Phytomedicine 14: 778-784, 2007.
5. Wu M and Gu Z: Screening of bioactive compounds from moutan cortex and their anti-inflammatory activities in rat synoviocytes. Evid Based Complement Alternat Med 6: 57-63, 2009.
6. Kim HJ, Chang EJ, Bae SJ, Shim SM, Park HD, Rhee CH, Park JH and Choi SW: Cytotoxic and antimutagenic stilbenes from seeds of *Paeonia lactiflora*. Arch Pharm Res 25: 293-299, 2002.
7. Kang JH, Park YH, Choi SW, Yang EK and Lee WJ: Resveratrol derivatives potently induce apoptosis in human promyelocytic leukemia cells. Exp Mol Med 35: 467-474, 2003.
8. Tanaka T, Fukumori M, Ochi T and Kouno I: Paeonianins A-E, new dimeric and monomeric ellagitannins from the fruits of *Paeonia lactiflora*. J Nat Prod 66: 759-763, 2003.
9. He CN, Peng Y, Zhang YC, Xu LJ, Gu J and Xiao PG: Phytochemical and biological studies of paeoniaceae. Chem Biodivers 7: 805-838, 2010.
10. He CN, Peng Y, Wu QL, Xiao W, Peng B, Wang Z and Xiao PG: Simultaneous determination of ten stilbenes in the seeds of *Paeonia* species using HPLC-DAD. J Liq Chromatogr Relat Technol 36: 1708-1724, 2013.
11. He S, Lu Y, Jiang L, Wu B, Zhang F and Pan Y: Preparative isolation and purification of antioxidative stilbene oligomers from Vitis chunganeniss using high-speed counter-current chromatography in stepwise elution mode. J Sep Sci 32: 2339-2345, 2009.
12. Jung M, Park WH, Jung JC, Lim E, Lee Y, Oh S and Moon HI: Synthesis, structural characterization and biological evaluation of novel stilbene derivatives as potential antimalarial agents. Chem Biol Drug Des 73: 346-354, 2009.
13. Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S and Takada Y: Role of resveratrol in prevention and therapy of cancer: Preclinical and clinical studies. Anticancer Res 24: 2783-2840, 2004.
14. Cai H, Scott E, Kholghi A, Andreadi C, Rufini A, Karmokar A, Britton RG, Horner-Glister E, Greaves P, Jawad D, *et al*: Cancer chemoprevention: Evidence of a nonlinear dose response for the protective effects of resveratrol in humans and mice. Sci Transl Med 7: 298ra117, 2015.
15. Satyajit DS, Pensri W and Laurence D: Identification and ecdysteroid antagonist activity of three resveratrol trimers (Suffruticosols A, B and C) from *Paeonia suffruticosa*. Tetrahedron 55: 513-524, 1999.
16. He CN, Peng Y, Xu LJ, Liu ZA, Gu J, Zhong AG and Xiao PG: Three new oligostilbenes from the seeds of *Paeonia suffruticosa*. Chem Pharm Bull (Tokyo) 58: 843-847, 2010.
17. He C, Xiao W, Li M, Peng Y, Xu L, Gu J and Xiao P: Chemical Constituents from Seeds of *Paeonia suffruticosa*. Zhongguo Zhong Yao Za Zhi 35: 1428-1431, 2010 (In Chinese).
18. Gao Y, He C, Ran R, Zhang D, Li D, Xiao PG and Altman E: The resveratrol oligomers, cis-and trans-gnetin H, from *Paeonia suffruticosa* seeds inhibit the growth of several human cancer cell lines. J Ethnopharmacol 169: 24-33, 2015.
19. Almosnid NM, Gao Y, He CN, Park HS and Altman E: *In vitro* antitumor effects of two novel oligostilbenes, cis- and trans-suffruticosol D, isolated from *Paeonia suffruticosa* seeds. Int J Oncol 48: 646-656, 2016.
20. Arbab IA, Looi CY, Abdul AB, Cheah FK, Wong WF, Sukari MA, Abdullah R, Mohan S, Syam S, Arya A, *et al*: Dentatin induces apoptosis in prostate cancer cells via Bcl-2, Bcl-xL, Survivin downregulation, caspase-9, -3/7 activation, and NF-κB inhibition. Evid Based Complement Alternat Med 2012: 856029, 2012.
21. Martin HL, Adams M, Higgins J, Bond J, Morrison EE, Bell SM, Warriner S, Nelson A and Tomlinson DC: High-content, high-throughput screening for the identification of cytotoxic compounds based on cell morphology and cell proliferation markers. PLoS One 9: e88338, 2014.
22. Al-Nasiry S, Geusens N, Hanssens M, Luyten C and Pijnenborg R: The use of Alamar Blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells. Hum reprod 22: 1304-1309, 2007.
23. Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, Fevr T, Clark L, Bayani N, Coppe JP, Tong F, *et al*: A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. Cancer Cell 10: 515-527, 2006.
24. Li-Weber M: Targeting apoptosis pathways in cancer by Chinese medicine. Cancer Lett 332: 304-312, 2013.
25. Joe AK, Liu H, Suzui M, Vural ME, Xiao D and Weinstein IB: Resveratrol induces growth inhibition, S-phase arrest, apoptosis, and changes in biomarker expression in several human cancer cell lines. Clin Cancer Res 8: 893-903, 2002.
26. Bishayee A: Cancer prevention and treatment with resveratrol: From rodent studies to clinical trials. Cancer Prev Res 2: 409-418, 2009.
27. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. Cell 144: 646-674, 2011.
28. Nguyen DX, Bos PD and Massagué J: Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer 9: 274-284, 2009.
29. Weng CJ and Yen GC: Chemopreventive effects of dietary phytochemicals against cancer invasion and metastasis: Phenolic acids, monophenol, polyphenol, and their derivatives. Cancer Treat Rev 38: 76-87, 2012.
30. Mikulski D and Molski M: Quantitative structure-antioxidant activity relationship of trans-resveratrol oligomers, trans-4, 4'-dihydroxystilbene dimer, trans-resveratrol-3-O-glucuronide, glucosides: Trans-piceid, cis-piceid, trans-astringin and trans -resveratrol-4'-O-β-D-glucopyranoside. Eur J Med Chem 45: 2366-2380, 2010.
31. Lim KG, Gray AI, Anthony NG, Mackay SP, Pyne S and Pyne NJ: Resveratrol and its oligomers: Modulation of sphingolipid metabolism and signaling in disease. Arch Toxicol 88: 2213-2232, 2014.
32. Vian MA, Tomao V, Gallet S, Coulomb PO and Lacombe JM: Simple and rapid method for cis-and trans-resveratrol and piceid isomers determination in wine by high-performance liquid chromatography using Chromolith columns. J Chromatogr A 1085: 224-229, 2005.
33. Pettit GR, Melody N, Thornhill A, Knight JC, Groy TL and Herald CL: Antineoplastic Agents. 579. Synthesis and cancer cell growth evaluation of E-stilstatin 3: A resveratrol structural modification. J Nat Prod 72: 1637-1642, 2009.
34. Zhao DB, Rong CY, Jenkins S, Yin DL and Liu SB: Origin of the cis-effect: A density functional theory study of doubly substituted ethylenes. Acta Phys Chim Sin 29: 43-54, 2013.
35. Anisimova NY, Kiselevsky MV, Sosnov AV, Sadovnikov SV, Stankov IN and Gakh AA: Trans-, cis- and dihydro-resveratrol: A comparative study. Chem Cent J 5: 88, 2011.
36. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, *et al*: Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275: 218-220, 1997.
37. Neves AR, Lucio M, Lima JL and Reis S: Resveratrol in medicinal chemistry: A critical review of its pharmacokinetics, drug-delivery, and membrane interactions. Curr Med Chem 19: 1663-1681, 2012.
38. Tani H, Hikami S, Iizuna S, Yoshimatsu M, Asama T, Ota H, Kimura Y, Tatefuji T, Hashimoto K and Higaki K: Pharmacokinetics and safety of resveratrol derivatives in humans after oral administration of melinjo (Gnetum gnemon L.) seed extract powder. J Agric Food Chem 62: 1999-2007, 2014.