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

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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*-viniferin, *cis*-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...
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 seed cases 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 Chunnan 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 suspended 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 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 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 was used in the 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 CO2 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 IC50 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.

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 CO2 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 IC50 values were calculated using non-linear regression analysis with GraphPad Prism software 6.0 (GraphPad Software, Inc., La Jolla, CA, USA).
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.

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.
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*-viniferin, *trans*-suffruticosol D and *trans*-gnetin H were more potent than their respective *cis*-forms, *cis*-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*-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

**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).**

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

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*-viniferin, *trans*-suffruticosol D and *trans*-gnetin H were more potent than their respective *cis*-forms, *cis*-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*-viniferin.

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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, sulfurticosol A-C, cis- and trans-suffruticosol D), followed by dimers (cis- and trans-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-77/H-88 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 for Botanical Medicinal Research (TCBMR) for providing the funding for this study.

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