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Antimitotic activity and reversal of breast cancer resistance protein-mediated drug resistance by stilbenoids from *Bletilla striata*

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Abstract—Eight stilbenoids, 1-(*p*-hydroxybenzyl)-4,8-dimethoxyphenanthrene-2,7-diol (**1**), 2,7-dihydroxy-1,3-bis(*p*-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene (**2**), 4,7-dihydroxy-1-(*p*-hydroxybenzyl)-2-methoxy-9,10-dihydrophenanthrene (**3**), 3,3′-dihydroxy-2′,6′-bis(*p*-hydroxybenzyl)-5-methoxybibenzyl (**4**), 3′,5-dihydroxy-2-(*p*-hydroxybenzyl)-3-methoxybibenzyl (**5**), blestriarenes B (**6**) and C (**7**), and blestrianol A (**8**) have been isolated by the guidance of inhibitory effect of tubulin polymerization from the tubers of *Bletilla striata* (Orchidaceae). Among them, both of bisbenzyls **4** and **5** inhibited the polymerization of tubulin at IC_{50} 10 μM, respectively. Furthermore bisbenzyl **4** potentiated the cytotoxicity of SN-38 in BCRP-transduced K562 (K562/BCRP) cells.

Antimitotic agents that inhibit the microtubule formation and the mitotic arrest of eucaryotic cells, such as paclitaxel and vinblastine, are important components of current anticancer therapy.[1] Paclitaxel is potent inhibitor of cell proliferation and arrest cells in mitosis, but in contrast to vinblastine, promote the polymerization of purified tubulin, causing stabilization and bundling of microtubules.[2] The antimitotic agents have potential applications in drug development. Recently much effort has been directed to the isolation and synthesis of new antimitotic drugs that target the tubulin/microtubule system and display efficacy against drug-refractory carcinomas.[3]

Multidrug-resistance (MDR) is a phenomenon in which cancer cells display cross-resistance to structurally unrelated drugs. Breast cancer resistance protein (BCRP), also called ABCG2, mediates concurrent resistance to chemotherapeutic agents such as SN-38 (an active metabolite of CPT-11), mitoxantrone, and topotecan, presumably by pumping these compounds out of cell and thus decreasing their cytotoxic effects.[4]

During our search for bioactive compounds targeting the tubulin/microtubules from medicinal plants,[5] we found that the extract from the tubers of *Bletilla striata* remarkably inhibited the polymerization of tubulin. The tubers of *B. striata* (Orchidaceae) have been used as traditional medicine to treat pulmonary tuberculosis and as hemostatic agent.[6] Our efforts on identifying new agents that target tubulin resulted in the isolation of eight known stilbenoids: 1-(*p*-hydroxybenzyl)-4,8-dimethoxyphenanthrene-2,7-diol (**1**),[7] 2,7-dihydroxy-1,3-bis(*p*-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene (**2**),[8] 4,7-dihydroxy-1-(*p*-hydroxybenzyl)-2-methoxy-9,10-dihydrophenanthrene (**3**),[9] 3,3′-dihydroxy-2′,6′-bis(*p*-hydroxybenzyl)-5-methoxybibenzyl (**4**),[9] 3′,5-dihydroxy-2-(*p*-hydroxybenzyl)-3-methoxybibenzyl (**5**),[8] blestriarenes B (**6**)[10] and C (**7**),[10] and blestrianol A (**8**),[11] whose structures were established by spectroscopic data. This paper describes effects of these stilbenoids (**1**–**8**) on tubulin assembly as well as inhibitory effects of the stilbenoids on BCRP-mediated SN-38 resistance was also described.

The tubers of *B. striata* were extracted with MeOH, and the MeOH extract was in turn partitioned with hexane, EtOAc, $CHCl_3$, and *n*-BuOH. EtOAc-soluble materials inhibiting the polymerization of tubulin were subjected to a silica gel column ($CHCl_3$/MeOH, 1:0 → 0:1) followed by a C_{18} column (CH_3CN/0.1% TFA, 2:3) to afford stilbenoids **1**–**8**.

Keywords: Stilbenoid; Antimitotic activity; Reversal of breast cancer resistance protein-mediated drug resistance.
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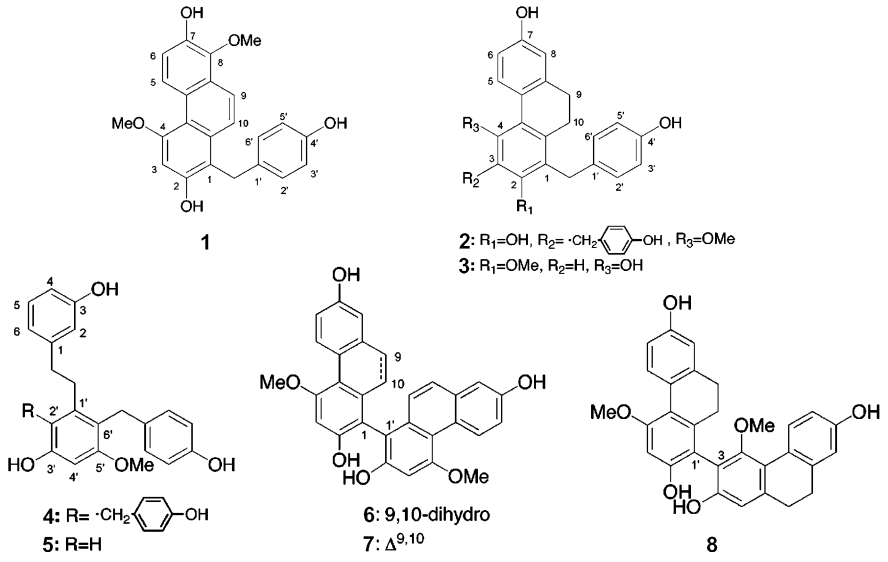


Generally antimitotic agents such as colchicines and vinblastine bind to either the colchicine binding site or the vinca alkaloid binding site. On the other hand, paclitaxel promotes the polymerization of tubulin by binding to and stabilizing the resulting microtubule polymer, which differs from those of colchicines, podophyllotoxin, and the vinca alkaloids.[1] Microtubules polymerized in the presence of paclitaxel are resistant to depolymerization by Ca^{2+} ions.

In this study, it was found that bisbenzyls **4** and **5** remarkably inhibited the polymerization of tubulin. Microtubule polymerization and depolymerization were monitored by the increase and the decrease in turbidity. Inhibitory effects of bisbenzyl **5** to tubulin polymerization are shown in Figure 1, in which tubulin polymerization was inhibited in a concentration-dependent manner. On the other hand, phenanthrene and dihydrophenanthrene with a benzyl moiety (**1** and **2**) and dimeric phenanthrenes (**6**–**8**) were found to be three times less potent (IC_{50}, 30 μM, respectively) than bisbenzyls **4** and **5**, indicating that the restricted biaryl ring system of phenanthrenes is unfavorable for tubulin binding. Substitution of the hydroxy group at C-4 is also critical as shown in Table 1. There was no difference for inhibitory effects of the polymerization of tubulin between phenanthrenes and dihydrophenanthrenes. The presence of *p*-hydroxy benzyl at C-2′ of bisbenzyl **4** had no influence in the polymerization of tubulin. In addition, inhibitory effects of the polymerization of tubulin by dimeric phenanthrenes was comparable with those by monomeric ones.

Antimitotic activity of stilbene related compounds have been well studied so far.[11] Especially combretastatin A-4 (CA-4) isolated from a South African willow tree *Combretum caffrum* is one of the most potent antimitotic agent and strongly inhibits the polymerization of tubulin by binding at the colchicine binding site (CLC site) (IC_{50}, 1.9 μM).[12] Common elements can be found in the structures of the active combretastatin congeners and of other well-known CLC site ligands such as colchicine,[13] steganacin,[14] and podophyllotoxin.[15] Common structural features among these compounds are the presence of two aromatic rings, which can be connected directly or through one or two atoms bridge spacer of single or double bond. Orientation of the two aromatic rings is required to be *cis*. In addition, the appropriate chiral torsion may be important in the conformation of the two aromatic rings. These structural features correspond

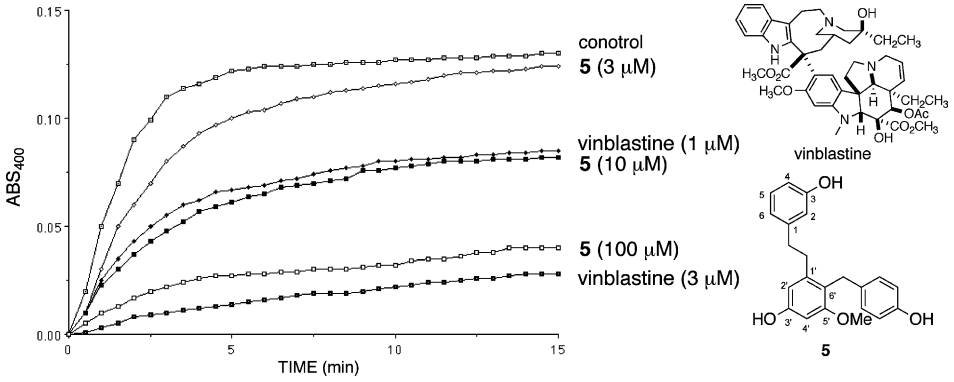


Figure 1. Inhibitory effects of bisbenzyl (**5**) and vinblastine to the polymerization of tubulin protein. Various concentrations of **5** were mixed with tubulin protein (1.5 mg/mL) at 0 °C and incubated at 37 °C. The absorbance at 400 nm was measured.

Table 1. Inhibitory effects of stilbenoids (**1**–**8**) and vinblastine to the polymerization of tubulin

Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Vinblastine
IC_{50} (μM)	30	30	300	10	10	30	30	30	1

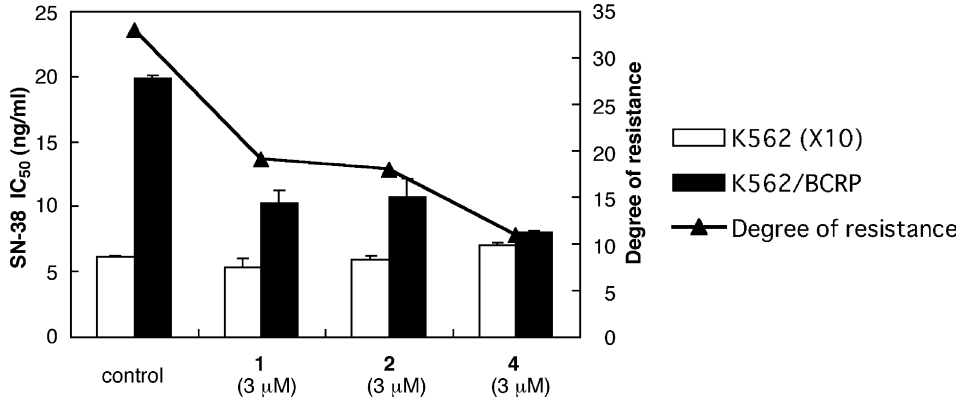
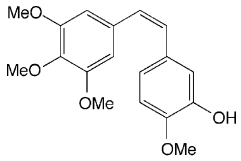


Figure 2. Inhibitory effects of stilbenoids (**1**), (**2**), and (**4**) on BCRP-mediated SN-38 resistance. K562 and K562/BCRP cells were cultured for five days in the absence or presence of 3 μM compound with increasing concentrations of SN-38. Cell numbers were determined using a cell counter, and then IC_{50} values were measured. Open bar represents nonresistance cells and black bar denotes resistance cells. The degree of resistance is the ratio of IC_{50} values of the cells to that of K562 cells under the indicated experimental conditions.

to the fact that, in this experiment, bisbenzyls with high flexibility such as **4** and **5**, which only showed high affinity to the tubulin protein.



combretastatin A-4

On the other hand, Estrone, 17β-estradiol, estrogen agonists, and estrogen antagonists reverse BCRP-mediated drug resistance. Recently, phytoestrogens with weak estrogenic activity such as flavonoids were also reported to potentiate the cytotoxicity of 7-ethyl-10-hydroxycamptothecin (SN-38) and mitoxantrone in BCRP-transduced K562 (K562/BCRP) cells.[4] In this study, we examined the potential reversal effects of stilbenoids from *B. striata*, since stilbenoids such as diethylstilbestrol and tamoxifen also interact with the same drug-binding site of BCRP.[4] As shown in Figure 2, 3 μM of **1**, **2**, and **4** strongly enhanced the cytotoxicity of SN-38 in K562/BCRP cells but not in K562 cells. Reversal indexes (ratios of IC_{50} measurements in the absence of reversing agents divided by levels in the presence of reversing agents) of 3 μM **1**, **2**, and **4** for SN-38 were 1.7, 1.8, and 3.0, respectively. These results suggested that stilbenoids sensitized K562/BCRP cells to SN-38 by inhibiting BCRP function.

In this work, we found that stilbenoids such as phenanthrene, dihydrophenanthrene, dimeric phenanthrene, and bisbenzyls from the tubers of *B. striata*, which have been used as traditional medicine, showed antimitotic activity and inhibited BCRP-mediated drug resistance.

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