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## Cytotoxic Constituents of *Soymida febrifuga* from Myanmar

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The 70% ethanol extract of *Soymida febrifuga* was found to kill PANC-1 human pancreatic cancer cells preferentially under nutrition-deprived conditions at a concentration of 10  $\mu\text{g/mL}$ . Phytochemical investigation led to the isolation of 27 compounds including four new compounds [(3*R*)-6,4'-dihydroxy-8-methoxyhomoisoflavan (**1**), (2*R*)-7,4'-dihydroxy-5-methoxy-8-methylflavan (**2**), 7-hydroxy-6-methoxy-3-(4'-hydroxybenzyl)coumarin (**3**), and 6-hydroxy-7-methoxy-3-(4'-hydroxybenzyl)coumarin (**4**)]. 2',4'-Dihydroxychalcone (**8**) displayed the most potent preferential cytotoxicity ( $\text{PC}_{50}$  19.0  $\mu\text{M}$ ) against PANC-1 cells. In addition, the cytotoxic activity against colon 26-L5 carcinoma (colon 26-L5), B16-BL6 melanoma (B16-BL6), lung A549 adenocarcinoma (A549), cervix HeLa adenocarcinoma (HeLa), and HT-1080 fibrosarcoma (HT-1080) cell lines and their structure–activity relationship are discussed. The cytotoxic activity of 4'-hydroxy-3,5-dimethoxystilbene (**6**) against colon 26-L5 ( $\text{IC}_{50}$  2.96  $\mu\text{M}$ ) was found to be stronger than the positive control, doxorubicin, at  $\text{IC}_{50}$  3.12  $\mu\text{M}$ .

Cancer is the leading cause of death worldwide. A new American Cancer Society report estimates 7.6 million cancer deaths (about 20 000 deaths a day) worldwide in 2007, which is expected to increase to an incidence of 15.5 million with 11.5 million deaths by 2030.<sup>1</sup> Consequently, the battle against cancer has not been successful yet. Among all the forms of cancers, pancreatic cancer has the lowest 5-year survival rate.<sup>2</sup> The outcome for patients with metastatic disease is particularly dismal, with an estimated survival time of only 2 to 4 months in the absence of treatment.<sup>3</sup> It is largely resistant to almost all known chemotherapeutic agents such as 5-fluorouracil, Taxol, doxorubicin, cisplatin, and camptothecin.<sup>4</sup> Therefore, discovery of effective chemotherapeutic agents are urgently needed. Pancreatic cancer cells have remarkable tolerance against extreme nutrition starvation,<sup>5</sup> enabling them to survive under hypovascular conditions. Discovery of drugs that retard cancer cells' tolerance to nutrition starvation may serve as a novel biochemical approach to cancer therapy.<sup>5–7</sup> Working on this hypothesis, we carried out antiausterity strategy based screening of medicinal plant extracts using the PANC-1 human pancreatic cancer cell line.<sup>6–12</sup> Recently, we observed that *Soymida febrifuga* collected from Myanmar caused 100% cell death of PANC-1 cells in nutrient-deprived medium (NDM) at a concentration of 10  $\mu\text{g/mL}$ . Therefore, it was subjected to phytochemical analysis, which led to the isolation of 27 compounds including four new compounds (**1–4**). In this paper, we report the structure elucidation of these new compounds together with in vitro preferential cytotoxicity of the isolates against the PANC-1 cancer cell line in NDM. In addition, the cytotoxic activity of the isolated compounds against the panel of five additional cancer cell lines and their structure–activity relationship are discussed.

### Results and Discussion

**Isolation and Identification.** The bark of *S. febrifuga* was extracted with EtOH by sonication. The EtOH extract was separated into  $\text{CH}_2\text{Cl}_2$ -soluble and -insoluble fractions by dissolution in  $\text{CH}_2\text{Cl}_2$ . The  $\text{CH}_2\text{Cl}_2$ -soluble fraction was subjected to a series of chromatographic separations, which furnished four new compounds [(3*R*)-6,4'-dihydroxy-8-methoxyhomoisoflavan (**1**), (2*R*)-7,4'-dihydroxy-5-methoxy-8-methylflavan (**2**), 7-hydroxy-6-methoxy-3-(4'-hydroxybenzyl)coumarin (**3**), and 6-hydroxy-7-methoxy-3-(4'-hydroxybenzyl)coumarin (**4**)], together with 23 known compounds.

The known compounds were identified by spectroscopic analysis as 4'-hydroxy-7-methoxyflavan (**5**),<sup>13</sup> 4'-hydroxy-3,5-dimethoxystilbene (**6**),<sup>14</sup> 5,7-dihydroxyflavanone (**7**),<sup>15</sup> 2',4'-dihydroxychalcone (**8**),<sup>15</sup> 5,7-dihydroxy-4'-methoxyhomoisoflavanone (**9**),<sup>16</sup> 4'-hydroxy-2,4-dimethoxydihydrochalcone (**10**),<sup>17</sup> 6,4'-dihydroxy-7-methoxyhomoisoflavan (**11**),<sup>18</sup> 7,4'-dihydroxy-8-methylflavan (**12**),<sup>19</sup> 7,4'-dihydroxyhomoisoflavan (**13**),<sup>20</sup> 7,4'-dihydroxyflavan (**14**),<sup>21</sup> *p*-hydroxybenzoic acid (**15**),<sup>22</sup> 5,7,4'-trihydroxy-6-methoxyhomoisoflavanone (**16**),<sup>16</sup> 3,4'-dihydroxy-5-methoxystilbene (**17**),<sup>23</sup> 5,7,4'-trihydroxyhomoisoflavanone (**18**),<sup>16</sup> 4-hydroxy-2,6,4'-trimethoxydihydrochalcone (**19**),<sup>24</sup> 7,4'-dihydroxyhomoisoflavanone (**20**),<sup>20</sup> 2,2',4'-trihydroxychalcone (**21**),<sup>25</sup> 2,4'-dihydroxy-4-methoxydihydrochalcone (**22**),<sup>13</sup> 2,4,4'-trihydroxydihydrochalcone (**23**),<sup>26</sup> 4,4'-dihydroxy-2,6-dimethoxydihydrochalcone (**24**),<sup>27</sup> guaiacylglycerol (**25**), 2,4'-dihydroxy-2'-methoxychalcone (**26**),<sup>28</sup> and 2,4'-dihydroxy-4-methoxychalcone (**27**).<sup>13</sup>

Compound **1** was obtained as an orange, amorphous powder with  $[\alpha]_{\text{D}}^{25} +41.5$  ( $c$  0.33, MeOH). Its molecular formula was determined as  $\text{C}_{17}\text{H}_{19}\text{O}_4$  by HRFABMS. The IR spectrum of **1** indicated the presence of a hydroxy ( $3520\text{ cm}^{-1}$ ) and an aromatic ring ( $1600$ ,  $1445\text{ cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum showed signals due to two aromatic protons ( $\delta_{\text{H}}$  6.47, H-5;  $\delta_{\text{H}}$  6.43, H-7), a 1,4-disubstituted benzene ring ( $\delta_{\text{H}}$  7.05, d,  $J = 8.6$ ;  $\delta_{\text{H}}$  6.77, d,  $J = 8.6$ ), an oxymethylene ( $\delta_{\text{H}}$  4.11, 3.74), an *O*-methyl group ( $\delta_{\text{H}}$  3.79), two methylene protons ( $\delta_{\text{H}}$  2.68, 2.41;  $\delta_{\text{H}}$  2.63, 2.51), and a methine proton ( $\delta_{\text{H}}$  2.23) (Table 1). The  $^{13}\text{C}$  NMR spectrum displayed 17 signals including 12 aromatic carbons, an oxygenated methylene, and an *O*-methyl carbon (Table 1). These data closely resembled those of 6,4'-dihydroxy-7-methoxyhomoisoflavan (**11**),<sup>18</sup> isolated from the same extract, suggesting compound **1** to be a homoisoflavan. However, **1** and **11** slightly differed in the chemical shifts of A-ring protons. The HMBC correlations (Figure 1a) observed between H-5 and C-4 ( $\delta_{\text{C}}$  30.7), C-6 ( $\delta_{\text{C}}$  144.7), and C-7 ( $\delta_{\text{C}}$  103.1) and between H-7 and C-5 ( $\delta_{\text{C}}$  112.0) and C-8 ( $\delta_{\text{C}}$  141.0) indicated that ring A in **1** had substituents at C-6 and C-8, instead of C-6 and C-7 in **11**. The location of the *O*-methyl group was determined to be at C-8 on the basis of the HMBC correlation between the *O*-methyl protons ( $\delta_{\text{H}}$  3.79) and C-8 ( $\delta_{\text{C}}$  141.0). Finally, the absolute configuration of **1** was determined from CD analysis. Compound **1** showed a positive Cotton effect ( $[\theta]_{282} +1080$ ) in the  $^1\text{L}_b$  transition

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**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data of Compounds **1** and **2** in  $\text{CDCl}_3$  ( $J$  values in parentheses)

position	<b>1</b>		<b>2</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
2	4.11 ddd (10.7, 3.2, 1.4) 3.74 dd (10.7, 8.3)	69.8	4.85 dd (10.0, 2.2)	78.5
3	2.23 m	34.3	2.11 m; 1.85 m	20.5
4	2.68 dd (16.4, 5.9) 2.41 dd (16.4, 9.0)	30.7	2.66 ddd (16.4, 6.1, 3.2) 2.58 ddd (16.4, 11.0, 6.3)	30.8
4a		115.3		103.3
5	6.47 s	112.0		157.1
6		144.7	6.03 s	92.0
7	6.43 s	103.1		155.0
8		141.0		105.0
8a		148.7		155.5
9	2.63 dd (14.2, 7.1) 2.51 dd (14.2, 8.1)	37.1	1.96 s	8.0
1'		131.5		134.7
2',6'	7.05 d (8.6)	130.1	7.23 d (8.8)	128.2
3',5'	6.77 d (8.6)	115.3	6.77 d (8.8)	116.0
4'		154.1		157.9
5-OCH <sub>3</sub>			3.72 s	55.7
8-OCH <sub>3</sub>	3.79 s	56.6		

region and a negative Cotton effect ( $[\theta]_{217} -2072$ ) near the  $^1\text{L}_a$  transition region, suggesting the absolute configuration at C-3 as *R*.<sup>29</sup> Therefore, the structure of **1** was assigned as (3*R*)-6,4'-dihydroxy-8-methoxyhomoisoflavan.

Compound **2** was obtained as a red, amorphous solid with  $[\alpha]_{\text{D}}^{25} +52.7$  (*c* 0.19, MeOH). Its molecular formula was deduced by HRFABMS to be  $\text{C}_{17}\text{H}_{19}\text{O}_4$ . The IR spectrum of **2** showed absorption bands of the hydroxy group ( $3270\text{ cm}^{-1}$ ) and the aromatic ring ( $1595\text{ cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum exhibited an aromatic singlet ( $\delta_{\text{H}}$  6.03, s), a 1,4-disubstituted benzene ring ( $\delta_{\text{H}}$  7.23, d,  $J = 8.8\text{ Hz}$ ;  $\delta_{\text{H}}$  6.77, d,  $J = 8.8\text{ Hz}$ ), an oxymethine ( $\delta_{\text{H}}$  4.85), an *O*-methyl group ( $\delta_{\text{H}}$  3.72), two methylene protons ( $\delta_{\text{H}}$  2.66, 2.58;  $\delta_{\text{H}}$  2.11, 1.85), and a methyl group ( $\delta_{\text{H}}$  1.96) (Table 1). The  $^{13}\text{C}$  NMR spectrum displayed 17 signals including 12 aromatic carbons, an oxygenated methylene carbon, a methoxy carbon, and a methyl carbon (Table 1). These data were similar to those of 6,4'-dihydroxy-7-methoxy-8-methylflavan, previously reported from the resin of *Dracaena cochinchinensis*,<sup>18</sup> but the spectra slightly differed in the chemical shift of H-6. In the HMBC experiment of **2**, correlations were observed between H-6 ( $\delta_{\text{H}}$  6.03) and C-5 ( $\delta_{\text{C}}$  157.1), C-7 ( $\delta_{\text{C}}$  155.0), and C-8 ( $\delta_{\text{C}}$  105.0) and between the methyl protons ( $\delta_{\text{H}}$  1.96) and C-7 ( $\delta_{\text{C}}$  155.0), C-8 ( $\delta_{\text{C}}$  105.0), and C-8a ( $\delta_{\text{C}}$  155.5) (Figure 1b). This suggested the location of the methyl group at C-8. Further, the HMBC correlation between the *O*-methyl protons ( $\delta_{\text{H}}$  3.72) and C-5 ( $\delta_{\text{C}}$  157.1) indicated its location at C-5. Finally, the positive  $[\alpha]_{\text{D}}$  value indicated the absolute configuration at C-2 as *R*.<sup>20</sup> Therefore, the structure of **2** was assigned as (2*R*)-7,4'-dihydroxy-5-methoxy-8-methylflavan.

Compound **3** was isolated as a yellow, amorphous solid, and its molecular formula was found to be  $\text{C}_{17}\text{H}_{15}\text{O}_5$  by HRFABMS. The  $^1\text{H}$  NMR spectrum showed signals due to an olefinic proton ( $\delta_{\text{H}}$  7.44, s), two aromatic singlets ( $\delta_{\text{H}}$  6.99;  $\delta_{\text{H}}$  6.74), a 1,4-disubstituted benzene ring ( $\delta_{\text{H}}$  7.09, d,  $J = 8.5$ ;  $\delta_{\text{H}}$  6.73, d,  $J = 8.5$ ), an *O*-methyl group ( $\delta_{\text{H}}$  3.85), and a methylene group ( $\delta_{\text{H}}$  3.69, s, 2H). The  $^{13}\text{C}$  NMR spectrum indicated the presence of 17 carbon signals, which were classified as a lactone carbonyl carbon ( $\delta_{\text{C}}$  164.5), 12 aromatic carbons, two olefinic carbons, an *O*-methyl carbon, and a methylene carbon.

In the HMBC spectrum, the correlations between the olefinic proton ( $\delta_{\text{H}}$  7.44, H-4) and C-5 ( $\delta_{\text{C}}$  109.6), C-8a ( $\delta_{\text{C}}$  150.3), and the lactone carbonyl carbon ( $\delta_{\text{C}}$  164.5, C-2), between H-5 ( $\delta_{\text{H}}$  6.99) and C-4 ( $\delta_{\text{C}}$  141.7), C-6 ( $\delta_{\text{C}}$  147.1), C-7 ( $\delta_{\text{C}}$  152.0), and C-8a, and between H-8 ( $\delta_{\text{H}}$  6.74) and C-4a ( $\delta_{\text{C}}$  113.0), C-6, C-7, and C-8a indicated the presence of a coumarin skeleton with two oxygen substituents on ring A. Similarly, the correlations between the 1,4-disubstituted benzene ring protons ( $\delta_{\text{H}}$  7.09, H-2',6') and the

oxygenated aromatic carbon ( $\delta_{\text{C}}$  157.2, C-4') and the methylene carbon ( $\delta_{\text{C}}$  36.4, C-9) indicated the presence of a 4-hydroxybenzyl group. Moreover, HMBC of the methylene protons ( $\delta_{\text{H}}$  3.69, H<sub>2</sub>-9) with C-2, C-3, and C-4 suggested the location of the 4-hydroxybenzyl group at C-3. Finally, the location of the *O*-methyl group was assigned to be at C-6 on the basis of the NOE enhancement of H-5 upon irradiation of 6-OCH<sub>3</sub> ( $\delta_{\text{H}}$  3.85). Therefore, the structure of **3** was assigned as 7-hydroxy-6-methoxy-3-(4'-hydroxybenzyl)-coumarin.

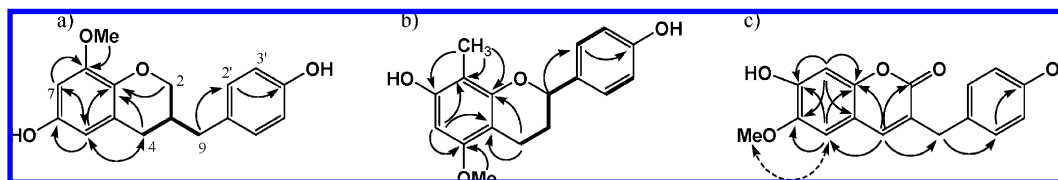
Compound **4** was isolated as yellow, amorphous solid. The IR and UV data of **4** showed similar patterns to those of **3**. HRFABMS of **4** also exhibited the same molecular formula,  $\text{C}_{17}\text{H}_{15}\text{O}_5$ , as **3**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra closely resembled those of **3** except for the slight difference in the chemical shifts for an *O*-methyl group ( $\delta_{\text{H}}$  3.93), H-5 ( $\delta_{\text{H}}$  6.93), and H-8 ( $\delta_{\text{H}}$  6.87). Therefore, the structure of **4** was assumed to be 6-hydroxy-7-methoxy-3-(4'-hydroxybenzyl)coumarin, a regioisomer of **3**.

**Preferential Cytotoxic Activities against the PANC-1 Human Pancreatic Cancer Cell Line.** The isolated compounds comprised mainly flavonoid derivatives that include three homoisoflavans (**1**, **11**, **13**), four homoisoflavanones (**9**, **16**, **18**, **20**), five flavanones (**2**, **5**, **7**, **12**, **14**), two 3-benzylcoumarins (**3**, **4**), three chalcones (**8**, **21**, **26**), five dihydrochalcones (**10**, **19**, **22**, **23**, **24**), two stilbenes (**6**, **17**), and two miscellaneous compounds (**15**, **16**). Compound **4** was the minor compound (0.4 mg). All the isolated compounds except for **4** were tested for their preferential cytotoxic activity against the PANC-1 cell line in NDM. The tested compounds exhibited different cytotoxic potencies (Tables 3–10). 2',4'-Dihydroxychalcone (**8**) displayed the most potent preferential cytotoxicity ( $\text{PC}_{50}$  19.0  $\mu\text{M}$ ). Contrary to this, 2,2',4'-trihydroxychalcone (**21**) and 2,4'-dihydroxy-2'-methoxychalcone (**26**) were inactive. This indicated the unique requirement of the substituents in chalcones for the activity. Similarly, 4'-hydroxy-3,5-dimethoxystilbene (**6**) showed stronger activity ( $\text{PC}_{50}$  44.4  $\mu\text{M}$ ) than 3,4'-dihydroxy-5-methoxystilbene (**17**) ( $\text{PC}_{50}$  71.4  $\mu\text{M}$ ). The other isolated compounds either displayed mild activity or were inactive (Tables 3–10). The conventional anticancer drug paclitaxel was also inactive ( $\text{PC}_{50} >100\text{ }\mu\text{M}$ ). Arctigenin, an antiausterity-based anticancer agent, was used as a positive control and displayed total preferential cytotoxic activity at 1  $\mu\text{M}$ .

**Cytotoxic Activity against Other Cancer Cell Lines and Their Structure–Activity Relationships.** The *in vitro* cytotoxic activities of compounds **1**–**27**, except **4**, were further tested against the panel of five cancer cell lines, viz., two murine cancer cell lines [colon 26-L5 carcinoma (colon 26-L5),<sup>30</sup> B16-BL6 melanoma (B16-BL6)<sup>31</sup>] and three human cancer cell lines [lung A549 adenocarcinoma (A549),<sup>32</sup> cervix HeLa adenocarcinoma (HeLa),<sup>33</sup> HT-1080 fibrosarcoma (HT-1080)<sup>34</sup>]. The conventional anticancer drugs in clinical use, 5-fluorouracil and doxorubicin, were used as positive controls. The results are listed in Tables 3–10. 4'-Hydroxy-3,5-dimethoxystilbene (**6**) showed the most potent activities against HeLa ( $\text{IC}_{50}$  9.43  $\mu\text{M}$ ), colon 26-L5 ( $\text{IC}_{50}$  2.96  $\mu\text{M}$ ), and B16-BL6 ( $\text{IC}_{50}$  15.9  $\mu\text{M}$ ) cell lines. In particular, the observed  $\text{IC}_{50}$  value of **6** against colon 26-L5 was comparable to that of the positive control doxorubicin ( $\text{IC}_{50}$  3.12  $\mu\text{M}$ ). Interestingly, **6** also showed mild preferential cytotoxicity against PANC-1 cells.

**Homoisoflavans and Homoisoflavanones.** Homoisoflavans and homoisoflavanones were found to show selective cytotoxicity to colon 26-L5 cells. In general, the homoisoflavans showed stronger cytotoxic activity than homoisoflavanones against all the tested cell lines (Table 3, Table 4). The presence of the *O*-methyl group at C-7 rather than C-8 in homoisoflavans seems to favor activity (**11**  $\gg$  **1**).

**Flavans and Flavanones.** The flavans (**2**, **12**, **14**), with the exception of **5**, displayed cytotoxicity against all the tested cell lines. On the other hand, flavanone **7** was only mildly active toward colon



**Figure 1.** COSY (bold lines) and HMBC (arrows;  $^1\text{H}$ – $^{13}\text{C}$ ) correlations and difference NOE (dashed arrow) in **1** (a), **2** (b), and **3** (c).

**Table 2.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data of Compounds **3** and **4** in Methanol- $d_4$  ( $J$  values in parentheses)

position	<b>3</b>		<b>4</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
2		164.5		164.5
3		126.4		127.4
4	7.44 s	141.7	7.42 s	141.4
4a		113.0		114.1
5	6.99 s	109.6	6.93 s	112.6
6		147.1		147.5
7		152.0		52.6
8	6.74 s	103.6	6.87 s	100.4
8a		150.3		149.2
9	3.69 s	36.4	3.70 s	36.5
1'		130.6		130.5
2', 6'	7.09 d (8.5)	131.2	7.10 d (8.5)	131.2
3', 5'	6.73 d (8.5)	116.4	6.73 d (8.5)	116.4
4'		157.2		157.2
6-OCH <sub>3</sub>	3.85 s	56.8		
7-OCH <sub>3</sub>			3.93 s	56.8

26-L5 and HT-1080 cells (Table 5). This suggests that the absence of a ketone carbonyl group enhances the cytotoxic activity.

**Chalcones and Dihydrochalcones.** Chalcones showed cytotoxic activities against all the tested cell lines with the exception of the B16-BL6 cell line (Table 7). Chalcones without a B-ring substituent were found to be preferable for the activity against all the cell lines (**8** > **21**, **8** > **26**) except for B16-BL6 cells. Interestingly, the activity of **8** was comparable to that of the positive control doxorubicin against colon 26-L5 cells. Among dihydrochalcones, only **24** displayed cytotoxic activity against all the tested cell lines. The activity of **22** was found to be the strongest in A549 ( $\text{IC}_{50}$  12.0  $\mu\text{M}$ ). Dihydrochalcones without any substituents (**22**) were inactive in all the tested cell lines.

**Stilbenes.** The stilbenes displayed cytotoxic activity against all the tested cell lines. Stilbenes with two instead of one *O*-methyl group in ring B were found to be more active against all the tested cancer cell lines (**6** > **17**). The activity of **6** was very strong against HeLa, colon 26-L5, and B16-BL6 cell lines.

**Miscellaneous Compounds.** *p*-Hydroxybenzoic acid (**15**) showed mild activity against HeLa, colon 26-L5, and B16-BL6 cell lines, while the new coumarin **3** displayed mild cytotoxicity against all tested cell lines.

In conclusion, *S. febrifuga* collected from Myanmar showed preferential cytotoxicity against PANC-1 human pancreatic cancer cells in NDM. Phytochemical investigation of this plant extract led to the isolation of 27 compounds including four new compounds (**1**–**4**). 2',4'-Dihydroxychalcone (**8**) showed the most potent preferential cytotoxicity against PANC-1 cells in NDM with a  $\text{PC}_{50}$  value of 19.0  $\mu\text{M}$ . Furthermore, the isolated compounds were tested against the panel of five additional cancer cell lines, and their structure–activity relationships were determined. 4'-Hydroxy-3,5-dimethoxystilbene (**6**) showed the most potent activity against the colon 26-L5 ( $\text{IC}_{50}$  2.96  $\mu\text{M}$ ) cell line, which is stronger than the positive control doxorubicin ( $\text{IC}_{50}$  3.12  $\mu\text{M}$ ).

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a JASCO DIP-140 digital polarimeter. CD measurements were carried out on a JASCO J-805 spectropolarimeter. IR spectra were

measured with a Shimadzu IR-408 spectrophotometer in  $\text{CHCl}_3$  solutions. NMR spectra were recorded on a JEOL JNM-LA400 spectrometer with TMS as an internal standard, and chemical shifts are expressed in  $\delta$  values. HRFABMS measurements were carried out on a JEOL JMS-700T spectrometer, and glycerol was used as matrix. MPLC was performed with a Büchi MPLC double gradient pump system. Column chromatography was performed with normal-phase silica gel (silica gel 60N, spherical, neutral, 40–50  $\mu\text{m}$ , Kanto Chemical Co., Inc.). Analytical and preparative TLC were carried out on precoated silica gel 60F<sub>254</sub> and RP-18F<sub>254</sub> plates (Merck, 0.25 or 0.50 mm thickness).

**Plant Material.** The bark of *S. febrifuga* was collected at Ywarrngon Village, Shan State, Myanmar, in November 2004. The plant was identified by Associate Professor T. M. Ohn (Department of Botany, University of Yangon, Myanmar). A voucher specimen (TMPW 26500) was deposited at the Museum for Materia Medica, Institute of Natural Medicine, University of Toyama, Japan.

**Extraction and Isolation.** The bark of *S. febrifuga* (100 g) was extracted with 70% EtOH under sonication (1 L, 90 min  $\times$  3) at room temperature, and the solvent was evaporated under reduced pressure to give a 70% EtOH extract (13.0 g). The 70% EtOH extract was again sonicated with  $\text{CH}_2\text{Cl}_2$  (100 mL  $\times$  2) to give  $\text{CH}_2\text{Cl}_2$ -soluble (3.20 g) and  $\text{CH}_2\text{Cl}_2$ -insoluble (8.45 g) fractions.

The  $\text{CH}_2\text{Cl}_2$ -soluble fraction was chromatographed on silica gel with an EtOAc–*n*-hexane gradient system to give 12 fractions (fr.1: 5% EtOAc–*n*-hexane eluate, 227 mg; fr.2: 10% EtOAc, 174 mg; fr.3: 20% EtOAc, 92.4 mg; fr.4: 30% EtOAc, 59.6 mg; fr.5: 30% EtOAc, 8.5 mg; fr.6: 30% EtOAc, 1080 mg; fr.7: 50% EtOAc, 191 mg; fr.8: 50% EtOAc, 73.2 mg; fr.9: 50% EtOAc, 303 mg; fr.10: 75% EtOAc, 454 mg; fr.11: 75% EtOAc, 482 mg; and fr.12: 30% MeOH– $\text{CH}_2\text{Cl}_2$  eluate, 201 mg). Fractions 3 and 9 afforded **5** (92.4 mg) and **22** (303 mg), respectively.

Fraction 4 was subjected to normal-phase preparative TLC (pTLC) with 30% EtOAc–*n*-hexane to afford two subfractions (fr.4-1, 17.3 mg; fr.4-2, 21.3 mg). Subfraction 4-1 was purified with normal-phase pTLC with  $\text{CH}_2\text{Cl}_2$  and then by reversed-phase pTLC with  $\text{CH}_3\text{CN}$ – $\text{H}_2\text{O}$  (3:2) to give **5** (3.2 mg), **6** (1.5 mg), **7** (3.2 mg), and **8** (3.4 mg). Subfraction 4-2 was further purified by normal-phase pTLC with 10% MeOH– $\text{CH}_2\text{Cl}_2$  to give **6** (8.6 mg).

Fraction 5 was purified by normal-phase pTLC with 30% EtOAc–hexane to give **9** (5.7 mg).

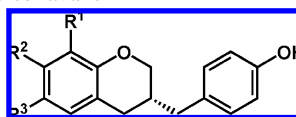
Fraction 6 was subjected to normal-phase pTLC with 3% MeOH– $\text{CHCl}_3$  to give **10** (58.7 mg), **12** (20.1 mg), **13** (8.8 mg), **14** (7.6 mg), and a mixture (33.6 mg). Purification of this mixture by normal-phase pTLC with 3% MeOH–benzene gave **1** (12.1 mg) and **11** (2.5 mg).

Fraction 7 was subjected to reversed-phase pTLC with  $\text{CH}_3\text{CN}$ –MeOH– $\text{H}_2\text{O}$  (1:1:1) to give **15** (2.6 mg), **19** (52.7 mg), and a mixture (89.7 mg). This mixture was separated by normal-phase pTLC with 5% MeOH– $\text{CHCl}_3$  to afford four subfractions (fr.7-1, 21.7 mg; fr.7-2, 9.0 mg; fr.7-3, 13.1 mg; fr.7-4, 13.2 mg). Fraction 7-1 afforded **1** (21.7 mg). Subfraction 7-2 was further purified by reversed-phase pTLC with  $\text{CH}_3\text{CN}$ –MeOH– $\text{H}_2\text{O}$  (2:2:3) to give **2** (1.5 mg) and **16** (2.9 mg). Subfraction 7-3 was subjected to normal-phase pTLC with 30% EtOAc–hexane, followed by reversed-phase pTLC with acetone–MeOH– $\text{H}_2\text{O}$  (3:3:4), to give **13** (5.0 mg) and **17** (1.4 mg). Subfraction 7-4 was purified by normal-phase pTLC with 30% EtOAc–*n*-hexane to give **14** (7.4 mg) and **18** (3.0 mg).

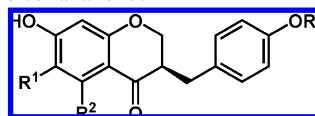
Fraction 8 was subjected to reversed-phase pTLC with  $\text{CH}_3\text{CN}$ –MeOH– $\text{H}_2\text{O}$  (1:1:1) to afford two subfractions (fr.8-1, 21.2 mg; fr.8-2, 13.1 mg). Subfraction 8-1 was separated by normal-phase pTLC with 3% MeOH– $\text{CH}_2\text{Cl}_2$  to give **20** (11.2 mg). Subfraction 8-2 was purified by normal-phase pTLC with 3% MeOH– $\text{CH}_2\text{Cl}_2$  to give **21** (3.8 mg).

Fraction 10 was rechromatographed on silica gel with MPLC (flow rate: 2.5 mL/min) by using a MeOH– $\text{CH}_2\text{Cl}_2$  gradient system to afford five

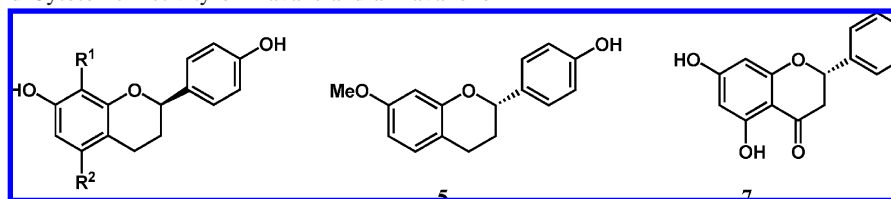


**Table 3.** Structures and Cytotoxic Activity of Homoisoflavans

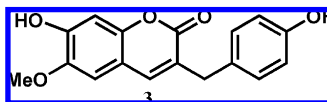
compound	PC <sub>50</sub> [μM]			IC <sub>50</sub> [μM]					
	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	PANC-1	HT-1080	A549	HeLa	colon 26-L5	B16-BL6
<b>1</b>	OMe	H	OH	>100	70.2	>100	65.6	49.5	>100
<b>11</b>	H	OMe	OH	63.0	17.5	19.1	37.8	22.2	75.4
<b>13</b>	H	OH	H	79.0	79.6	>100	>100	44.5	>100
doxorubicin					0.41	0.84	0.62	3.12	3.07
5-fluorouracil					8.58	5.91	0.44	0.41	7.39

**Table 4.** Structures and Cytotoxic Activity of Homoisoflavones

compound	PC <sub>50</sub> [μM]			IC <sub>50</sub> [μM]					
	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	PANC-1	HT-1080	A549	HeLa	colon 26-L5	B16-BL6
<b>9</b>	H	OH	Me	>100	>100	>100	>100	30.6	64.3
<b>16</b>	OMe	OH	H	>100	96.2	>100	>100	89.5	>100
<b>18</b>	H	OH	H	>100	>100	>100	>100	57.8	>100
<b>20</b>	H	H	H	>100	>100	>100	>100	69.3	99.6
doxorubicin					0.41	0.84	0.62	3.12	3.07
5-fluorouracil					8.58	5.91	0.44	0.41	7.39

**Table 5.** Structures and Cytotoxic Activity of Flavans and a Flavanone

compound			PC <sub>50</sub> [ $\mu$ M]	IC <sub>50</sub> [ $\mu$ M]				
R <sup>1</sup>	R <sup>2</sup>	PANC-1	HT-1080	A549	HeLa	colon 26-L5	B16-BL6	
<b>2</b>	Me	OMe	70.1	37.0	35.5	74.0	59.1	89.1
<b>5</b>			74.0	>100	>100	>100	52.8	40.8
<b>7</b>			72.9	72.8	>100	>100	71.0	>100
<b>12</b>	Me	H	85.8	24.1	39.7	89.2	40.2	98.9
<b>14</b>	H	H	>100	50.1	94.0	93.4	43.9	>100
doxorubicin				0.41	0.84	0.62	3.12	3.07
5-fluorouracil				8.58	5.91	0.44	0.41	7.39

**Table 6.** Structures and Cytotoxic Activity of Coumarins

compound	PC <sub>50</sub> [μM]	IC <sub>50</sub> [μM]				
	PANC-1	HT-1080	A549	HeLa	colon 26-L5	B16-BL6
<b>3</b>	86.7	40.3	88.1	69.5	61.8	>100
doxorubicin		0.41	0.84	0.62	3.12	3.07
5-fluorouracil		8.58	5.91	0.44	0.41	7.39

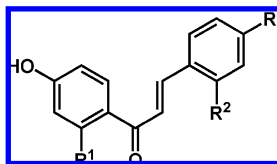
subfractions (fr.10-1, 51.0 mg; fr.10-2, 65.2 mg; fr.10-3, 61.4 mg; fr.10-4, 66.1 mg; fr.10-5, 63.7 mg). Subfraction 10-1 was subjected to normal-phase pTLC with 40%-EtOAc-*n*-hexane to give four subfractions (fr.10-1-1, 2.3 mg; fr.10-1-2, 12.9 mg; fr.10-1-3, 5.9 mg; fr.10-1-4, 13.2 mg). Subfraction 10-1-1 was identified as **24** (2.3 mg). Subfraction 10-1-2 was further purified by normal-phase pTLC with 3% MeOH-CH<sub>2</sub>Cl<sub>2</sub> to give **3** (3.2 mg), **4** (0.4 mg), and **25** (2.2 mg). Subfraction 10-1-2 was subjected to reversed-phase pTLC with CH<sub>3</sub>CN-MeOH-H<sub>2</sub>O (3:3:4) to afford **24** (1.5 mg), **26** (6.1 mg), and **27** (0.8 mg).

**(3R)-6,4'-Dihydroxy-8-methoxyhomoisoflavan (1)**: orange, amorphous powder; [α]<sub>D</sub><sup>25</sup> +41.5 (*c* 0.33, MeOH); CD (*c* 5.59 × 10<sup>-4</sup> M,

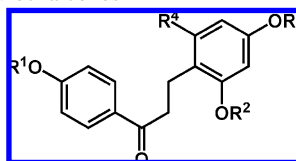
EtOH) [θ]<sub>282</sub> +1080, [θ]<sub>217</sub> -2072; IR ν<sub>max</sub> (CHCl<sub>3</sub>) 3520, 3280, 1600, 1510, 1445 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HRFABMS *m/z* 287.1270 [M + H]<sup>+</sup> (calcd for C<sub>17</sub>H<sub>19</sub>O<sub>4</sub>, 287.1283).

**(2R)-7,4'-Dihydroxy-5-methoxy-8-methylflavan (2)**: red, amorphous solid; [α]<sub>D</sub><sup>25</sup> +52.7 (*c* 0.19, MeOH); IR ν<sub>max</sub> (CHCl<sub>3</sub>) 3270, 1595, 1110, 830 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HRFABMS *m/z* 287.1258 [M + H]<sup>+</sup> (calcd for C<sub>17</sub>H<sub>19</sub>O<sub>4</sub>, 287.1283).

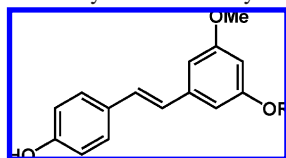
**7-Hydroxy-8-methoxy-3-(4'-hydroxybenzyl)coumarin (3)**: yellow, amorphous solid; UV λ<sub>max</sub> nm (log ε) 345 (3.16), 210 (3.53); IR ν<sub>max</sub> (CHCl<sub>3</sub>) 1695, 1580 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 2; HRFABMS *m/z* 299.0948 [M + H]<sup>+</sup> (calcd for C<sub>17</sub>H<sub>15</sub>O<sub>5</sub>, 299.0920).

**Table 7.** Structures and Cytotoxic Activity of Chalcones

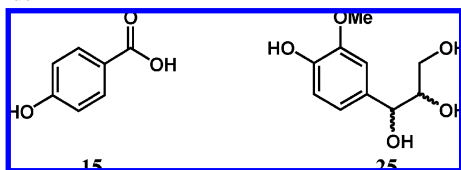
compound	PC <sub>50</sub> [ $\mu$ M]			IC <sub>50</sub> [ $\mu$ M]					
	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	PANC-1	HT-1080	A549	HeLa	colon 26-L5	B16-BL6
<b>8</b>	OH	H	H	19.0	17.0	23.2	24.4	4.71	>100
<b>21</b>	OH	OH	H	>100	40.4	89.9	89.1	28.9	>100
<b>26</b>	OMe	H	OH	>100	26.7	66.5	33.3	14.1	49.9
doxorubicin					0.41	0.84	0.62	3.12	3.07
5-fluorouracil					8.58	5.91	0.44	0.41	7.39

**Table 8.** Structures and Cytotoxic Activity of Dihydrochalcones

compound	PC <sub>50</sub> [ $\mu$ M]				IC <sub>50</sub> [ $\mu$ M]					
	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	PANC-1	HT-1080	A549	HeLa	colon 26-L5	B16-BL6
<b>10</b>	H	Me	Me	H	83.2	>100	89.5	>100	32.2	55.5
<b>19</b>	Me	Me	H	OMe	85.7	>100	>100	>100	68.1	70.1
<b>22</b>	H	H	Me	H	>100	>100	12.0	46.1	81.4	57.8
<b>23</b>	H	H	H	H	>100	>100	>100	>100	>100	>100
<b>24</b>	H	Me	H	OMe	69.7	67.2	42.9	49.0	40.2	84.4
doxorubicin						0.41	0.84	0.62	3.12	3.07
5-fluorouracil						8.58	5.91	0.44	0.41	7.39

**Table 9.** Structures and Cytotoxic Activity of Stilbenes

compound	R	PC <sub>50</sub> [ $\mu$ M]		IC <sub>50</sub> [ $\mu$ M]					
		PANC-1	HT-1080	A549	HeLa	colon 26-L5	B16-BL6		
<b>6</b>	Me	44.4	93.4	71.7	9.43	2.96	15.9		
<b>17</b>	H	71.4	89.1	91.8	48.4	35.8	>100		
doxorubicin			0.41	0.84	0.62	3.12	3.07		
5-fluorouracil			8.58	5.91	0.44	0.41	7.39		

**Table 10.** Structures and Cytotoxic Activity of Other Types of Compounds

compound	PC <sub>50</sub> [ $\mu$ M]		IC <sub>50</sub> [ $\mu$ M]					
	PANC-1	HT-1080	A549	HeLa	colon 26-L5	B16-BL6		
<b>15</b>	>100	>100	>100	36.2	33.3	63.3		
<b>25</b>	>100	>100	>100	>100	96.9	>100		
doxorubicin		0.41	0.84	0.62	3.12	3.07		
5-fluorouracil		8.58	5.91	0.44	0.41	7.39		

**8-Hydroxy-7-methoxy-3-(4'-hydroxybenzyl)coumarin (4):** yellow, amorphous solid; UV  $\lambda_{\max}$  nm (log  $\epsilon$ ) 345 (2.91), 210 (3.67); IR  $\nu_{\max}$  (CHCl<sub>3</sub>) 1700, 1600, 1580 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 2; HRFABMS  $m/z$  299.0961 [M + H]<sup>+</sup> (calcd for C<sub>17</sub>H<sub>15</sub>O<sub>5</sub>, 299.0920).

**Agents.** 5-Fluorouracil was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan), and doxorubicin hydrochloride was from Kyowa

Hakko Co. Ltd. (Tokyo, Japan).  $\alpha$ -Modified minimum essential medium (MEM $\alpha$ ) and RPMI 1640 medium were obtained from Wako Pure Chemicals Ind., Ltd. (Osaka, Japan). Dulbecco's modified Eagle's medium (DMEM) and Dulbecco's phosphate-buffered saline (PBS) were purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Sodium bicarbonate was purchased from Nacalai Tesque Inc. (Kyoto, Japan), and fetal bovine serum (FBS) was from Gibco BRL Products (Gaithersburg, MD). Antibiotic antimycotic solution and 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT) were from Sigma-Aldrich Inc. (St. Louis, MO). WST-8 cell counting kit was from Dojindo (Kumamoto, Japan). Cell culture flasks and 96-well plates were from Corning Inc. (Corning, NY).

**Cancer Cell Lines.** The PANC-1 human pancreatic cancer cell line was kindly provided by Dr. Hiroyasu Esumi (National Cancer Center Research Institute East). Highly liver metastatic murine colon 26-L5 carcinoma cell line was obtained from Dr. I. Saiki (Institute of Natural Medicine, University of Toyama) and highly liver metastatic murine B16-BL6 melanoma cell line was obtained from Dr. I. J. Fidler (M. D. Anderson Cancer Center, Houston, TX) and maintained in our laboratory. Highly metastatic human HT-1080 fibrosarcoma cell line (ATCC#CCL-121) was obtained from the American Type Culture Collection (Rockville, MD). Human lung A549 adenocarcinoma (RCB0098) and human cervix HeLa adenocarcinoma (RCB0007) cell lines were purchased from Riken Cell Bank (Tsukuba, Japan). PANC-1 human pancreatic cancer cell line was maintained in DMEM, and murine colon 26-L5 carcinoma cell line was maintained in RPMI 1640 medium. The other cell lines were maintained in MEM $\alpha$ . Both of these media were supplemented with 10% FBS, 0.1% NaHCO<sub>3</sub>, and 1% antibiotic antimycotic solution.

**Preferential Cytotoxic Activity against PANC-1 Cells in Nutrient-Deprived Medium (NDM).** The in vitro preferential cytotoxicity of the crude extract and the isolated compounds was determined by a previously described procedure.<sup>6</sup> Briefly, PANC-1 human pancreatic cancer cells were seeded in 96-well plates (2  $\times$  10<sup>4</sup>/well) and incubated in fresh DMEM at 37 °C under 5% CO<sub>2</sub> and 95% air for 24 h. After the cells were washed with PBS, the medium was changed to either DMEM or NDM, and serial dilutions of the test samples were added. After 24 h incubation, the cells were washed with PBS, and 100  $\mu$ L of

DMEM containing 10% WST-8 cell counting kit solution was added to the wells. After 3 h incubation, the absorbance at 450 nm was measured. Cell viability was calculated from the mean values of data from three wells by using the following equation:

$$\text{Cell viability (\%)} = \frac{[\text{Abs}_{(\text{test samples})} - \text{Abs}_{(\text{blank})}]/(\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{blank})}) \times 100$$

**Cytotoxic Activity Assay against Other Cell Lines.** Cell viability in the presence or absence of tested compounds was determined using the standard MTT assay as described previously.<sup>35</sup> In brief, exponentially growing cells were harvested and plated in 96-well plates ( $2 \times 10^3$ /well). After 24 h incubation at 37 °C under a humidified 5% CO<sub>2</sub> to allow cell attachment, the cells were treated with varying concentrations of test specimens in their respective medium (100  $\mu$ L). After 72 h incubation, 100  $\mu$ L of MTT solution (0.5 mg/mL) was added to the wells. After 2 h incubation, the formazan formed was extracted with DMSO and its amount was measured spectrophotometrically at 550 nm with a Perkin-Elmer HTS-7000 bioassay reader (Norwalk, CT). Cell viability and IC<sub>50</sub> values were calculated from the mean values of data from three wells.

**Acknowledgment.** This work was supported by a grant from the Ministry of Health and Welfare for the Third-Term Comprehensive 10-Year Strategy for Cancer Control, Japan.

**Supporting Information Available:** <sup>1</sup>H and <sup>13</sup>C NMR spectra of compounds 1–4. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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