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Brandisianins A−F, Isoflavonoids Isolated from *Millettia brandisiana* in a Screening Program for Death-Receptor Expression Enhancement Activity

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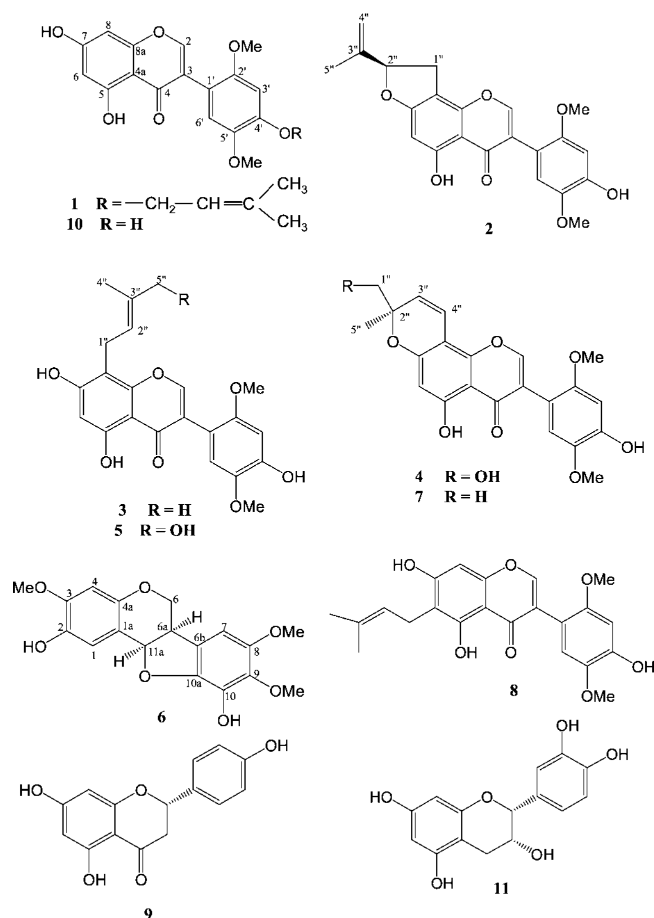
In a screening study for natural products with tumor-selective apoptosis-inducing properties, six new isoflavonoids (**1**−**6**), named brandisianins A−F, respectively, have been isolated from a MeOH extract of the dried leaves of *Millettia brandisiana*, together with five known compounds. The structures of the new compounds were elucidated by spectroscopic data interpretation. Among these compounds, brandisianin D (**4**) exhibited death-receptor 5 expression enhancement activity in a luciferase assay based in DLD-1/*Sac*I cells. The results suggest that brandisianin D (**4**) might overcome TRAIL-resistance by an increase in DR5 expression.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) can selectively induce apoptosis in a wide range of cancer cell types, but is not cytotoxic to most normal cells.[1–4] Therefore, recently TRAIL is now of wide interest for cancer drug discovery worldwide. It has been reported that TRAIL binds to death receptors such as DR5 (death receptor 5 = TRAIL-R2) or DR4 (death receptor 4 = TRAIL-R1), resulting in the activation of caspase-signaling pathways leading to apoptosis.[5] However, recently it has become known that considerable numbers of cancer cells are known to be resistant to apoptosis induced by TRAIL.[6] Therefore, for the clinical use of TRAIL in cancer therapy, it is extremely important to overcome TRAIL resistance. Since in many tumor cells, inactivation of DR5 is frequently observed in vivo and in vitro, enhancement of DR5 expression may contribute to the tumor-selective induction of apoptosis mediated by TRAIL.[7] Although there are a few compounds reported with DR5 expression enhancing activity, further study is required to find compounds with effective and selective activity.[8–11] During our investigations in searching for bioactive natural products from various sources, we have initiated a screening program for DR5 expression enhancement activity using a luciferase assay system in DLD-1/*Sac*I cells.[12] With this system, we have examined more than 100 MeOH extracts of medicinal plants collected in Thailand and found that a MeOH extract of leaves of *Millettia brandisiana* Kurz was potently active in this screening system. Bioassay-guided fractionation of *M. brandisiana* led to the isolation and identification of 11 compounds including six new isoflavonoids, brandisianins A−F (**1**−**6**), together with five known compounds, 4′-demethyltoxicarol isoflavone (**7**),[13] viridiflorin (**8**),[14] naringenin (**9**),[15] olibergin A (**10**),[16] and (−)-epicatechin (**11**).[17] The structures of the new compounds were elucidated by means of spectroscopic analysis, and their isolates were evaluated biologically.



Results and Discussion

To search for natural products with DR5 expression enhancement activity, we adopted a luciferase assay system based on DLD-1/*Sac*I cells. DLD-1/*Sac*I cells are a human colon cancer cell line stably transfected with the pDR5/*Sac*I plasmid, which contains the human DR5 promoter sequence and a luciferase gene.[12] In this screening system using DLD-1/*Sac*I cells, the up-regulation of the DR5 promoter was assessed by luminescence depending on luciferase gene (LUC) expression. The MeOH extract of *M. brandisiana* was successively partitioned between hexane, EtOAc, and *n*-BuOH, and these extracts, along with the aqueous layer, were evaluated for DR5-LUC expression activation. DR5-LUC expression was found to be strongly induced by the EtOAc-soluble partition, which was subjected to column chromatography followed by repeated reversed-phase HPLC to yield brandisianins A−F (**1–6**) and five known compounds (**7–11**).

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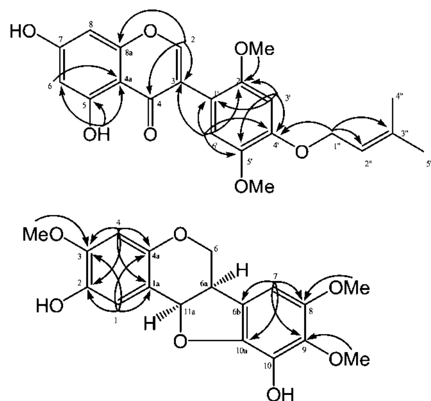

Figure 1. Key HMBC correlations of **1** and **6**.

Brandisianin A (**1**) was shown to have a molecular formula of $C_{22}H_{22}O_7$ on the basis of the HRFABMS data (*m/z* 437.1004, [M + K]$^+$, Δ +0.1 mmu). The IR absorption bands at 1658 and 3263 cm^{-1}, due to a conjugated carbonyl and hydroxyl group, respectively, as well as the UV absorptions at λ_{max} 296 and 260 nm were suggestive of the flavone or isoflavone nature of **1**. The ^{1}H and ^{13}C NMR spectra of **1** showed low-field resonances due to an sp^2 methine at δ_H 7.85 (1H, s) and δ_C 154.8, which were characteristic of H-2 and C-2, respectively, on an isoflavone nucleus.[18] The ^{1}H NMR spectra further revealed the signals of two *meta*-coupled aromatic hydrogens (δ_H 6.35 and 6.28, each 1H, d, J = 2.0 Hz), two singlet hydrogens (δ_H 6.63 and 6.87, each 1H, s), two methoxy groups (δ_H 3.76 and 3.84, each 3H, s), and one prenyl unit (δ_H 1.76 and 1.78 each 3H, s; δ_H 5.52, 1H, t, J = 6.4 Hz; δ_H 4.63, 2H, d, J = 6.4 Hz). Considering the molecular formula, it was suggested that the two hydroxy groups were attached to the isoflavone nucleus, and the HMBC spectroscopic data (Figure 1) showed the positions of the substituents: two methoxy groups at C-2′ and C-5′ (OMe-2′/C-2′ and OMe-5′/C-5′), two hydrogens at C-6 (H-6/C-4a) and C-8, two hydroxy groups at C-5 (OH-5/C-4a, 5, 6) and C-7, and the prenyl unit at C-4′ (H$_2$-1″/C-4′). In addition, in a NOE experiment, irradiation of H-3′ (δ_H 6.63) caused a NOE at OMe-2′ and H$_2$-1. From these observations, the structure of brandisianin A was concluded as **1**.

Brandisianin B (**2**) was optically active, and its molecular formula was determined as $C_{22}H_{20}O_7$ from the HRFABMS data (*m/z* 396.1212, [M]$^+$, Δ +0.3 mmu). The presence of an isoflavone framework was suggested from the IR and UV absorptions as well as from the ^{1}H and ^{13}C NMR signals for H-2 (δ_H 7.84, 1H, s) and C-2 (δ_C 154.3), which were also observed in **1**. The ^{1}H and ^{13}C NMR spectra revealed the presence of two methoxy groups and two hydroxy groups. In place of the prenyl unit as observed in **1**, signals due to an sp^3 methylene, sp^3 oxymethine, sp^2 methylene, sp^2 quaternary carbon, and a methyl group were observed, constructing a 2″-isopropenyldihydrofuran unit. This unit was shown to be attached at the C-7 and C-8 positions, which were deduced from the HMBC correlations from H$_2$-1″ to C-7, C-8, and C-8a. The absolute configuration of C-2″ was elucidated on the basis of the CD spectra of the osmate ester/pyridine complex of **2**. The absolute stereochemistry at the 2″-position of the 2″-isopropenyldihydrofurano unit contained in rotenone and related natural products was elucidated by the sign of the CD Cotton effect of the osmate ester/pyridine complex (i.e., positive Cotton effect for 2″*S*-configuration and negative for 2″*R*).[19] The CD of the osmate ester/pyridine complex of **2** showed a negative Cotton effect at 468 nm ([θ] −4791), implying a 2″*R*- configuration for **2**. Thus, the structure of brandisianin B was revealed as **2**.

Brandisianin C (**3**) was shown to have the molecular formula $C_{22}H_{22}O_7$ from its HRFABMS (*m/z* 398.1370, [M]$^+$, Δ +0.4 mmu), which was the same as that of **1**. The ^{1}H and ^{13}C NMR data closely resembled those of **1**, except that **3** showed five sp^2 methine signals, while **1** showed six. Signals for a prenyl group were also observed for **3** as in **1**, but the signals for the methylene group (C-1″ position) of **3** resonated at a higher field (δ_H 3.44, 2H, d, J = 6.8 Hz) than that of **1** (δ_H 4.63, 2H, d, J = 6.4 Hz). Thus, it was indicated that the prenyl group was attached not on an oxygen but directly on a carbon. In the HMBC spectrum of **3**, the H$_2$-1″ resonance showed correlations with C-7, C-8, and C-8a, implying that the prenyl group is attached to the C-8 position. In the B-ring of **3**, two methoxy groups could be located at the C-2′ and C-5′ positions and a hydroxyl group was attached at C-4′, which was suggested from the HMBC correlations (H-3′/C-1′, C-2′, C-4′, and C-5′, H-6′/C-2′, C-4′, and C-5′, and MeO-2′/C-2′, and MeO-5′/C-5′). From these results, the structure of brandisianin C was concluded as **3**.

The molecular formula of brandisianin D (**4**) was established as $C_{22}H_{20}O_8$ from the HRFABMS data (*m/z* 413.1227, [M + H$^+$], Δ −0.9 mmu). The mass spectra exhibited a base peak at *m/z* 381 corresponding to a [M − CH$_2$OH]$^+$ ion.[20] The ^{1}H and ^{13}C NMR signals of **4** were almost identical to those of 4′-demethyltoxicarolisoflavone (**7**), previously isolated from *Tephrosia polyphylla*,[13] expect that **7** had two methyl groups resonances (δ_H 1.47, 6H, s), but **4** showed signals for one methyl group (δ_H 1.42, 3H, s) and one oxymethylene group (δ_H 3.70, 3.68, each 1H, br s; δ_C 68.8). Thus, one of the geminal dimethyl groups at the C-1″ position of **7** was replaced by a hydroxymethyl group for **4**. The absolute configuration at the C-2″ position of **4** was suggested as follows. The CD curve of **4** showed a positive Cotton effect at 272 nm

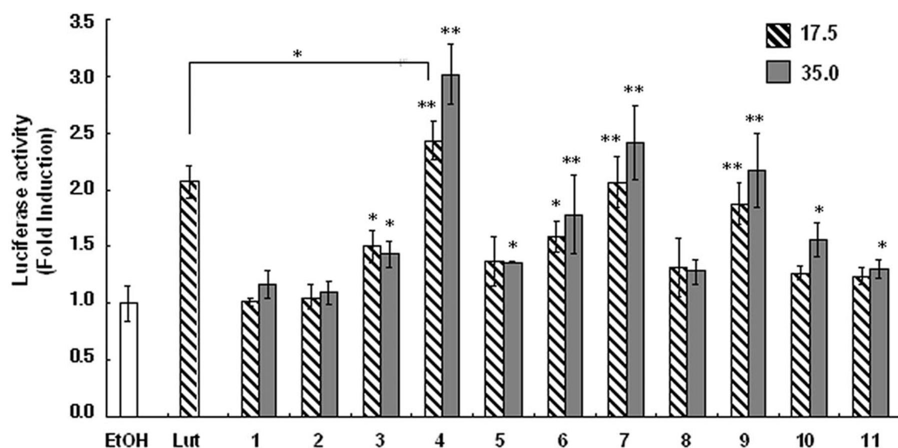

Figure 2. Activation of DR5 promoter activity by the flavonoids **1**−**11**, luteolin (positive control: Lut), and EtOH (negative control). All samples were tested at 17.5 and 35.0 μM. The bar represents the means (n = 3 ± SD). Significant differences in activation of DR5 promoter activity were shown at p < 0.05 (*), p < 0.01 (**), when compared to the positive and negative control.

Table 1. ^1H NMR Data of Compounds **1**−**5** in $CDCl_3$[a]

position	**1**[c]	**2**[d]	**3**[b,c]	**4**[d]	**5**[b,d]
2	7.85 (s)	7.84 (s)	8.12 (s)	7.92 (s)	8.13 (s)
OH-5	12.91 (s)	13.11 (s)	12.99 (s)	13.00 (s)	13.00 (s)
6	6.28 (d, 2.0)	6.34 (s)	6.36 (s)	6.31 (s)	6.37 (s)
OH-7			8.00 (s)		
8	6.35 (d, 2.0)				
3′	6.63 (s)	6.66 (s)	6.62 (s)	6.66 (s)	6.62 (s)
OH-4′		5.75 (br s)			
6′	6.87 (s)	6.87 (s)	6.97 (s)	6.88 (s)	6.97 (s)
OMe-2′	3.76 (3H, s)	3.74 (3H, s)	3.71 (3H, s)	3.74 (3H, s)	3.71 (3H, s)
OMe-5′	3.84 (3H, s)	3.87 (3H, s)	3.80 (3H, s)	3.87 (3H, s)	3.79 (3H, s)
1″	4.63 (2H, d, 6.4)	3.06 (dd, 7.6, 15.1) 3.42 (dd, 9.5, 15.1)	3.44 (2H, d, 6.8)	3.68 (br s) 3.70 (br s)	3.49 (2H, d, 7.0)
2″	5.52 (t, 6.4)	5.35 (dd, 7.6, 9.5)	5.24 (m)		5.50 (m)
3″				5.56 (d, 10.0)	
4″	1.76 (3H, s)	4.96 (br s) 5.11 (br s)	1.65 (3H, s)	6.86 (d, 10.0)	1.80 (3H, s)
5″	1.78 (3H, s)	1.78 (3H, s)	1.79 (3H, s)	1.42 (3H, s)	3.89 (2H, br s)

[a] Chemical shifts (δ) are in ppm and *J* in Hz. [b] Measured in CD_3COCD_3. [c] Recorded at 400 MHz. [d] Recorded at 500 MHz.

(MeOH, [θ] +2490) due to the styrene chromophore, implying that **4** has a 2″*S*-configuration, on the basis of Kikuchi's method for chromenol ring derivatives.[21] It was assumed that the hydroxymethyl group (C-1″) of **4**, which is larger than the methyl substituent (C-5″), adopted a pseudoequatorial orientation.

Brandisianin E (**5**) was shown to have the molecular formula $C_{22}H_{22}O_8$ from the HRFABMS data (*m/z* 414.1318, $[M]^+$, Δ +0.3 mmu). The ^1H and ^{13}C NMR signals were almost identical to those of **3** expect for the presence of an sp^3 oxymethylene group (δ_H 3.89, 2H, br s; δ_C 68.1). The C-5″ methyl group of **3** was replaced by a hydroxymethyl group for **5**, which was indicated by the HMBC correlations (H_2-5″/C-2″, C-3″, and C-4″, H_3-4″/C-2″, C-3″, and C-5″). In addition, irradiation of H_2-1″ (δ_H 3.49, 2H, d, J = 7.0 Hz) showed a NOE correlation with H_3-4″ and suggested the 2″*E*-geometry. Thus, the structure of brandisianin E was revealed to be **5**.

The molecular formula of brandisianin F (**6**) was determined to be $C_{18}H_{18}O_7$ on the basis of the HRFABMS data (*m/z* 346.1041, $[M]^+$, Δ −1.2 mmu). The ^1H and ^{13}C NMR spectra of **6** showed signals for one oxymethylene (δ_H 4.22, 1H, dd, J = 4.5, 11.0 Hz; δ_H 3.65, 1H, t, J = 11.0 Hz) and two methines [δ_H 3.49 (1H, m) and δ_H 5.43 (1H, d, J = 7.0 Hz)], and these signals were characteristic of a pterocarpan skeleton.[22] The ^1H and ^{13}C NMR of **6** also revealed signals for three methoxy groups [δ_H 3.86, 3.90, and 3.99 (each 3H, s)] and three sp^2 methines [δ_H 6.46, 6.57, and 7.06 (each 1H, s)]. Considering the molecular formula, it could be suggested that three methoxy and two hydroxy groups were attached to the pterocarpan nucleus. The HMBC spectroscopic data (Figure 1) indicated the positions of the three methoxy groups on C-3, C-8, and C-9 (OMe-3/C-3, OMe-8/C-8, and OMe-9/C-9) and three hydrogens on C-1 (H-1/C-2, C-3, C-4a, and C-11a), C-4 (H-4/C-2, C-3, C-1a, and C-4a), and C-7 (H-7/C-9 and C-10a). Thus, the two hydroxy groups had to be located on the C-2 and C-10 positions. In a NOE experiment, irradiating H-7 resulted in enhancement of the signals for H-6a and OMe-8, and irradiation of the OMe-3 signal caused an enhancement at H-4. Protons H-6a and H-11a were suggested to be *cis*-diaxial from the coupling constant (J = 7.0 Hz) and from comparison with the literature values[23] (J = 6.6 Hz for *cis* and 13.4 Hz for *trans*). Their absolute configurations were deduced as (*R*, *R*) from the negative Cotton effect of the CD curve (MeOH, [θ] −29370) at 234 nm by comparison with the CD data of related known compounds.[24]

The isolated flavonoids (**1**–**11**) were evaluated for DR5 promoter activity using the luciferase assay using DLD-1/*Sac*I cells. The activity was determined under the conditions at which luteolin did not inhibit the growth of cells due to cytotoxic effects. The cytotoxic activities of isolates against HeLa and DLD-1 cells are summarized in Table 3. Among these flavonoids, brandisianin D (**4**) and 4′-demethyltoxicarolisoflavone (**7**), which both possess a pyran ring attached on the C-7/C-8 positions, showed 3.0- and 2.4-fold activation of DR5 promoter activity, respectively, compared with control cells at a concentration of 35.0 μM. Moreover, brandisianin D (**4**) showed more potent activity than that of luteolin at 17.5 μM (Figure 2). The results of this study suggest that brandisianin D (**4**) might overcome TRAIL resistance by an increase in DR5 expression. Although it is known that some natural products[8–10] or synthetic small molecules[11] exhibited DR5 promotion activity,

Table 2. ^{13}C NMR Data of Compounds **1**−**5** in $CDCl_3$[a]

position	**1**[c]	**2**[d]	**3**[b,c]	**4**[d]	**5**[b,d]
2	154.8	154.3	156.0	154.8	156.0
3	120.7	120.3	121.2	120.4	121.3
4	180.8	180.8	181.8	180.9	181.8
4a	106.2	106.0	106.1	106.4	106.1
5	162.8	163.5	161.3	162.4	161.4
6	99.4	94.3	99.4	100.0	99.4
7	162.2	166.2	162.4	158.9	162.5
8	94.0	102.8	107.2	100.9	106.9
8a	158.0	152.8	156.2	152.2	156.3
1′	110.7	110.0	111.1	109.9	111.1
2′	151.8	152.3	153.5	152.2	153.5
3′	99.9	100.0	101.2	99.9	101.2
4′	149.4	146.8	148.6	146.9	148.6
5′	143.5	140.3	141.9	140.3	141.8
6′	115.3	114.4	116.8	114.3	116.8
OMe-2′	56.6	56.4	56.5	56.4	56.5
OMe-5′	56.7	56.6	57.2	56.6	57.2
1″	66.1	30.8	22.0	68.8	21.5
2″	119.8	87.9	123.2	81.1	122.5
3″	137.9	143.0	131.9	123.3	136.4
4″	18.3	112.9	17.9	117.3	13.8
5″	25.8	16.9	25.8	23.0	68.1

[a] Chemical shifts (δ) are in ppm and *J* in Hz. [b] Measured in CD_3COCD_3. [c] Recorded at 400 MHz. [d] Recorded at 500 MHz.

Table 3. Cytotoxic Activities of Compounds **1**−**11** against the HeLa and DLD-1 Cell Lines (IC_{50}, μM)

compound	HeLa	DLD-1
1	>25	>25
2	9.7	>25
3	19.1	>25
4	>25	>25
5	21.7	>25
6	>25	>25
7	>25	>25
8	>25	>25
9	>25	>25
10	>25	>25
11	>25	>25

this is the first report that isoflavonoids showed enhancement of DR5 expression.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter. IR spectra were measured on KBr disks or ATR in a JASCO FT-IR 230 spectrophotometer. UV spectra were obtained on a Shimadzu UV mini-1240 spectrometer. CD spectra were obtained in a JASCO J-720WI spectropolarimeter. The NMR spectra were recorded on JEOL A 400 and A 500 spectrometers with a deuterated solvent, the chemical shift of which was used as an internal standard. EIMS was measured on a JEOL GC-Mate spectrophotometer, and high-resolution fast-atom bombardment mass spectra (HRFABMS) were measured on a JEOL HX-110A spectrometer.

Plant Material. Leaves of *M. brandisiana* were collected in Khon Kaen, Thailand, in November 1999 and were identified by T.K. A voucher specimen (6-220) is maintained at the Graduate School of Pharmaceutical Science, Chiba University.

Luciferase Assay to Assess the Enhancement of DR5 Promoter Activation. DLD-1/*Sac*I cells were seeded in a 24-well culture plate (2×10^5 cells per well) in 1 mL of RPMI medium containing 10% FBS. Cells were incubated at 37 °C in a 5% CO_2 incubator for 24 h. Then the cells were treated with the test samples (100 μg/mL). After 24 h of incubation, cells were rinsed with PBS and 100 μL of 1× lysis reagent (Promega) was added to each well. Forty microliters of cell lysate was transferred to a 96-well microtiter plate. Then 200 μL of luciferase assay substrates (Promega) was added to each well, and luminescence was measured in a Luminoskan Ascent Luminometer (Thermo Electron Corporation). The enhancement of DR5 promoter activity was evaluated by relative light intensity compared with that of the control (cells treated with EtOH). Luteolin, which is known to activate the DR5 promoter, was used as a positive control at a concentration of 17.5 μM.

Cytotoxicity Testing. Cytotoxicity assays of compounds **1**–**11** were performed using the FMCA method.[25]

Extraction and Isolation. The leaves of *M. brandisiana* (220 g) were extracted with MeOH. After removal of chlorophylls from the extract by Diaion HP20 column chromatography (43 × 400 mm), the fraction (23.7 g) eluted with 100% MeOH was suspended in water (300 mL) and successively partitioned against hexane (300 mL × 3), EtOAc (300 mL × 3), and *n*-BuOH (300 mL × 3). Since the EtOAc extract (4.9 g) showed the most potent DR5 expression activation (2.5-fold at 100 μg/mL), this extract (4.9 g) was subjected to silica gel column chromatography (30 × 350 mm), eluted with increasing concentrations of acetone in hexane, to afford three fractions (1A−1C). Fractions 1A (2.4 g) and 1B (0.3 g) showed potent DR5 expression activation (2.4- and 6.8-fold, respectively, at 100 μg/mL). Fraction 1A [eluted with hexane/acetone (3:1 to 1:9)] was then purified by reversed-phase HPLC (YMC-Pack Pro C_{18}, 250 × 10 mm) eluting with 77% MeOH to give compounds **1** (13.1 mg), **2** (23.4 mg), and **7** (27.5 mg). Fraction 1B, eluted with acetone, was further subjected to silica gel chromatography (1.6 × 500 mm), eluted with 50−100% acetone in hexane and 100% MeOH, to afford seven fractions, 2A−2G. Fraction 2A (27.9 mg), eluted with 50% acetone in hexane, was further purified by reversed-phase HPLC (YMC J'sphere ODS-M80, 250 × 10 mm), eluting with 75% MeOH, to give compounds **3** (10.0 mg) and **8** (1.0 mg). Fraction 2B (48.0 mg), eluted with 75% acetone in hexane, was subjected to reversed-phase HPLC (YMC J'sphere ODS-M80, 250 × 10 mm), eluting with 58% MeOH, to yield compounds **4** (2.4 mg), **6** (3.0 mg), **9** (1.6 mg), and **10** (4.6 mg). Fraction 2E (88.4 mg), eluted with 88% acetone in hexane, was purified by normal-phase HPLC (Inertsil Diol, 250 × 6 mm), eluting with hexane/2-propanol (52:48), to yield compound **11** (57.0 mg). Compound **5** (3.7 mg) was obtained from fraction 2D (25.7 mg), eluted with 83% acetone in hexane, by purification with reversed-phase HPLC (YMC J'sphere ODS-M80, 250 × 10 mm), eluting with 56% MeOH.

Brandisianin A (1): white powder; IR (KBr) υ_{max} 3263, 2931, 1658, 1207, 1024, 816 cm^{-1}; UV (MeOH) λ_{max} (log ε) 260 (4.3), 296 (4.1) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 398 [M]$^+$ (11), 396 (9), 331 (20), 330 (100), 315 (20), 301 (12), 299 (19), 287 (12), 207 (30), 153 (19), 135 (13), 105 (17); HRFABMS *m/z* 437.1004 [M + K]$^+$ (calcd for $C_{22}H_{22}O_7K$, 437.1003).

Brandisianin B (2): white powder; $[\alpha]^{25}_D$ −4.8 (*c* 1.0, $CHCl_3$); IR (KBr) υ_{max} 3545, 2937, 1658, 1163, 1041, 825 cm^{-1}; UV (MeOH) λ_{max} (log ε) 264 (4.4), 295 (4.0) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 396 [M]$^+$ (100), 381 (27), 365 (38), 219 (21), 177 (17), 169 (11), 163 (12), 135 (11); HRFABMS *m/z* 396.1212 [M]$^+$ (calcd for $C_{22}H_{20}O_7$, 396.1209).

Brandisianin C (3): pale yellow powder; IR (KBr) υ_{max} 3469, 2925, 1655, 1198, 825 cm^{-1}; UV (MeOH) λ_{max} (log ε) 264 (4.3), 293 (3.9) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 398 [M]$^+$ (100), 383 (32), and 367 (11); HRFABMS *m/z* 398.1370 [M]$^+$ (calcd for $C_{22}H_{22}O_7$, 398.1366).

Brandisianin D (4): pale yellow powder; $[\alpha]^{23}_D$ −11.1 (*c* 0.1, MeOH); IR (ATR) υ_{max} 3346, 1642 cm^{-1}; UV (MeOH) λ_{max} (log ε) 269 (4.5), 300 (4.0) nm; CD (MeOH) λ_{max} ([θ]) 259 (704), 272 (2493) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 412 [M]$^+$ (14), 394 (14), 382 (25), 381 (100), 351 (23); HRFABMS *m/z* 413.1227 [M + H$^+$] (calcd for $C_{22}H_{21}O_8$, 413.1236).

Brandisianin E (5): white powder; IR (ATR) υ_{max} 3355, 2926, 1651, 1196 cm^{-1}; UV (MeOH) λ_{max} (log ε) 265 (4.4), 296 (4.1) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 414 [M]$^+$ (23), 396 (68), 381 (45), 365 (23), 110 (20), 83 (100); HRFABMS *m/z* 414.1318 [M]$^+$ (calcd for $C_{22}H_{22}O_8$, 414.1315).

Brandisianin F (6): pale yellow oil; $[\alpha]^{21}_D$ −164.3 (*c* 0.1, $CHCl_3$); IR (ATR) υ_{max} 3451, 2939, 1467, 1095 cm^{-1}; UV (MeOH) λ_{max} (log ε) 231 (4.1), 298 (4.0) nm; CD (MeOH) λ_{max} ([θ]) 234 (−29 370), 287 (−8580), 305 (16 830) nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06 (1H, s, H-1), 6.57 (1H, s, H-7), 6.46 (1H, s, H-4), 5.43 (1H, d, J = 7.0 Hz, H-11a), 4.22 (1H, dd, J = 4.5, 11.0 Hz, H-6), 3.99 (3H, s, OMe-8), 3.90 (3H, s, OMe-9), 3.86 (3H, s, OMe-3), 3.65 (1H, t, J = 11.0 Hz, H-6), 3.49 (1H, m, H-6a); ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.4 (C-4a), 148.1 (C-3), 144.0 (C-10a), 143.7 (C-10), 140.7 (C-2), 138.8 (C-9), 137.9 (C-8), 123.2 (C-6b), 115.3 (C-1), 112.1 (C-1a), 104.6 (C-7), 100.2 (C-4), 78.4 (C-11a), 66.6 (C-6), 61.6 (OMe-9), 60.5 (OMe-8), 56.2 (OMe-3), 40.8 (C-6a); EIMS *m/z* 346 [M]$^+$ (100), 332 (18), 331 (97), 316 (11), 279 (15), 194 (13), 173 (10), 167 (25), 158 (11), 149 (66); HRFABMS *m/z* 346.1041 [M]$^+$ (calcd for $C_{18}H_{18}O_7$, 346.1053).

CD Measurements of Osmate Esters−Pyridine Complex of 2. Compound **2** (475 μg, 1.2 μmol) was dissolved in CH_2Cl_2 (63 μL) containing 25 μmol (2 μL) of pyridine, and the resulting solutions were then treated with OsO_4 [1.4 μmol (356 μg) in 10 μL of CH_2Cl_2] for about 30 min at rt. The mixture was diluted with CH_2Cl_2 to give a final volume of 2.8 mL. The CD spectra of the resulting osmate ester/pyridine complex showed a negative Cotton effect at 468 nm ([θ] −4791). For comparison, rotenone with the *R*-configuration was treated by the same procedures to give a corresponding osmate ester/pyridine complex, which showed a negative Cotton effect at 473 nm ([θ] −6304).[26]

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References and Notes

(1) Wiley, S. R.; Schooley, K.; Smolak, P. J.; Din, W. S.; Huang, C. P.; Nicholl, J. K.; Sutherland, G. R.; Smith, T. D.; Rauch, C.; Smith, C. A.; Goodwin, R. G. *Immunity* **1995**, *3*, 673–682.
(2) Pitti, R. M.; Marsters, S. A.; Ruppert, S.; Donahue, C. J.; Moore, A.; Ashkenazi, A. *J. Biol. Chem.* **1996**, *271*, 12687–12690.
(3) Walczak, H.; Miller, R. E.; Ariail, K.; Gliniak, B.; Griffith, T. S.; Kubin, M.; Chin, W.; Jones, J.; Woodward, A.; Le, T.; Smith, C.; Smolak, P.; Goodwin, R. G.; Rauch, C. T.; Schuh, J. C.; Lynch, D. H. *Nat. Med.* **1999**, *5*, 157–163.
(4) Ashkenazi, A.; Pai, R. C.; Fong, S.; Leung, S.; Lawrence, D. A.; Marsters, S. A.; Blackie, C.; Chang, L.; McMurtrey, A. E.; Hebert, A.; DeForge, L.; Koumenis, I. L.; Lewis, D.; Harris, L.; Bussiere, J.; Koeppen, H.; Shahrokh, Z.; Schwall, R. H. *J. Clin. Invest.* **1999**, *104*, 155–162.
(5) LeBlanc, H. N.; Ashkenazi, A. *Cell Death Differ.* **2003**, *10*, 66–75.
(6) Zhang, L.; Fang, B. *Cancer Gene Ther.* **2005**, *12*, 228–237.
(7) Wang, S.; El-Deiry, W. S. *Cancer Res.* **2004**, *64*, 6666–6672.
(8) Horinaka, M.; Yoshida, T.; Shiraishi, T.; Nakata, S.; Wakada, M.; Nakanishi, R.; Nishino, H.; Matsui, H.; Sakai, T. *Oncogene* **2005**, *24*, 7180–7189.

(9) Hasegawa, H.; Yamada, Y.; Komiyama, K.; Hayashi, M.; Ishibashi, M.; Yoshiba, T.; Sakai, T.; Koyano, T.; Kam, T. S.; Murata, K.; Sugahara, K.; Tsuruda, K.; Akamatsu, N.; Tsukasaki, K.; Masuda, M.; Takasu, N.; Kamihira, S. *Blood* **2006**, *107*, 679–688.
(10) Jung, E. M.; Lim, J. H.; Lee, T. J.; Park, J. W.; Choi, K. S.; Kwon, T. K. *Carcinogenesis* **2005**, *26*, 1905–1913.
(11) Liu, X.; Yue, P.; Chen, S.; Hu, L.; Lonial, S.; Khuri, F.; Sun, S. Y. *Cancer Res.* **2007**, *67*, 4981–4988.
(12) Yoshida, T.; Maeda, A.; Tani, N.; Sakai, T. *FEBS Lett.* **2001**, *507*, 381–385.
(13) Dagne, E.; Mammo, W.; Sterner, O. *Phytochemistry* **1992**, *31*, 3662–3663.
(14) Gomez, F.; Calderon, J. S.; Quijano, L.; Dominguez, M.; Rios, T. *Phytochemistry* **1985**, *24*, 1126–1128.
(15) Osawa, K.; Yasuda, H.; Maruyama, T.; Morita, H.; Takeya, K.; Itokawa, H. *Chem. Pharm. Bull.* **1992**, *40*, 2970–2974.
(16) Ito, C.; Itoigawa, M.; Kanematsu, T.; Ruangrungsi, N.; Mukainaka, T.; Tokuda, H.; Nishino, H.; Furukawa, H. *Phytochemistry* **2003**, *64*, 1265–1268.
(17) Shen, C. C.; Chang, Y. S.; Ho, L. K. *Phytochemistry* **1993**, *34*, 843–845.
(18) Ito, C.; Itoigawa, M.; Kojima, N.; Tokuda, H.; Hirata, T.; Nishino, H.; Furukawa, H. *J. Nat. Prod.* **2004**, *67*, 1125–1130.
(19) Tahara, S.; Ingham, J. L.; Mizutani, J. *Agric. Biol. Chem.* **1987**, *51*, 211–216.
(20) Tanaka, H.; Tanaka, T.; Etoh, H.; Watanabe, N.; Ahmad, M.; Qurashi, I.; Khan, M. R. *Heterocycles* **1998**, *48*, 2661–2667.
(21) Ishibashi, M.; Ohizumi, Y.; Cheng, J. F.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1988**, *53*, 2855–2858.
(22) Sakurai, Y.; Sakurai, N.; Taniguchi, M.; Nakanishi, Y.; Bastow, K. F.; Wang, X.; Cragg, G. M.; Lee, K. H. *J. Nat. Prod.* **2006**, *69*, 397–399.
(23) Kiss, L.; Kurtan, T.; Antus, S.; Benyei, A. *Chirality* **2003**, *15*, 558–563.
(24) Palazzino, G.; Rasoanaivo, P.; Federici, E.; Nicoletti, M.; Galeffi, C. *Phytochemistry* **2003**, *63*, 471–474.
(25) Ohtsuki, T.; Sato, M.; Koyano, T.; Kowithayakorn, T.; Kawahara, N.; Goda, Y.; Ishibashi, M. *Bioorg. Med. Chem.* **2006**, *14*, 659–665.
(26) Buchi, G.; Crombie, L.; Godin, P. J.; Kaltenbronn, J. S.; Siddalingaiah, K. S.; Whiting, D. A. *J. Chem. Soc.* **1961**, *284*, 3–2860.

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