Bioactive Tetrahydrofuran Lignans from *Peperomia dindygulensis*

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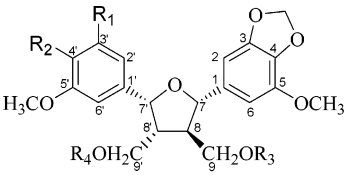
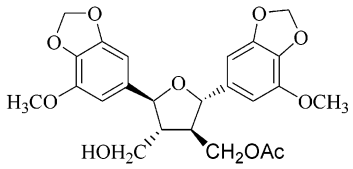
Five new tetrahydrofuran lignans (**1**−**5**), accompanied by four known compounds, were isolated from the ethyl acetate extract of *Peperomia dindygulensis*. Structures were elucidated mainly using 1D NMR, 2D NMR, and mass spectroscopic studies. The relative configurations of **1**−**5** were determined by NOE correlations. Several of the compounds showed weak growth inhibitory activity against three cell lines (WI-38, VA-13, and HepG2). Compound **5** exhibited stronger MDR (multidrug resistance) reversal activity than verapamil at 2.5 μg/mL in a cellular calcein accumulation assay. Compounds **4** and **5** showed weak inhibitory activity against induction of the intercellular adhesion molecule-1 (ICAM-1) in anti-inflammatory activity experiments.

Peperomia dindygulensis Miq. (Piperaceae) is named "shi-chan-cao" in the People's Republic of China. It grows mainly in Yunnan, Guangxi, Guangdong, Fujian, and Taiwan Provinces and traditionally has been used in folk remedies to treat stomach, mammary, liver, and esophageal cancers.[1] Four secolignans (peperomins A, B, E, and F) have been reported from plants collected in India.[2] In the present study, we report five new tetrahydrofuran lignans (**1**−**5**) from this species collected in the Yunnan Province of China. Four known compounds, 2-(3-phenylpropionyl)-1,3-cyclohexanedione (**6**), ergosta-6,22-diene-3β,5α,8α-triol,[3] 5-hydroxy-7,4′-dimethoxyflavone,[4] and 5-hydroxy-7,8,3′,4′-tetramethoxyflavone,[5] were also obtained, and the structures of the last three compounds were determined by comparisons of spectroscopic data with those of the corresponding compounds in the literature and by HREIMS measurements. Although 2-(3-phenylpropionyl)-1,3-cyclohexanedione was reported to be synthesized in a Japan patent,[6] no spectroscopic data could be found, and the structure was elucidated by using 1D and 2D NMR spectra, as well as IR and HREIMS.

The growth inhibitory activity of these compounds against three cancer cell lines and their effect on accumulation of calcein in MDR (multidrug resistance) cancer cells were tested. The anti-inflammatory activity of **1**, **4**, and **5** was also examined on the basis of inhibitory activity against induction of the intercellular adhesion molecule-1 (ICAM-1).



	R_1	R_2	R_3	R_4
1	OCH_2O		Ac	H
2	OCH_2O		H	Ac
4	OCH_3	OH	Ac	Ac
5	OCH_3	OCH_3	Ac	H



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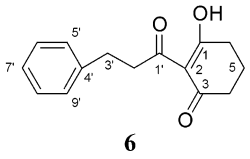

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Results and Discussion

Compound **1** had the composition $C_{24}H_{26}O_{10}$, as determined by a combination of HREIMS and ^1H and ^{13}C NMR spectra. The IR spectrum indicated the presence of hydroxyl (3630 cm^{-1}), ester carbonyl (1738 cm^{-1}), and aromatic groups (3036, 1636, and 1456 cm^{-1}). UV maxima were present at 213, 246, and 281 nm. The ^1H NMR spectrum showed two sets of tetrasubstituted aromatic ring signals [δ 6.69 (1H, d, J = 1.2 Hz, H-2) and 6.64 (1H, d, J = 1.2 Hz, H-6), 6.62 (1H, d, J = 1.0 Hz, H-2′) and 6.58 (1H, d, J = 1.0 Hz, H-6′)], two methylenedioxy groups attached to the aromatic rings [δ 5.97 (4H, s)], and two

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methoxy groups [δ 3.92 (3H, s) and 3.90 (3H, s)], which indicated the presence of two 5-methoxy-3,4-methylenedioxyphenyl groups. The ^{1}H−^{1}H COSY spectrum indicated the connections of C7−C8−C9, C7′−C8′−C9′, and C8−C8′, which were confirmed by HMBC correlations. The HMBC correlations (C-1 with H-7 and H-8, and C-1′ with H-7′ and H-8′) also showed the connections of the two phenyl groups with C-7 and C-7′, respectively. Acetyl methyl [δ 2.03 (3H, s) and 20.9 (q)] and ester carbonyl [δ 170.9 (s)] groups appeared in the ^{1}H and ^{13}C NMR spectra, which indicated that one of the oxygen atoms (9 or 9′) was acetylated. HMBC correlation between the carbonyl group and H-9 indicated that the C-9 hydroxyl group was acetylated. The degree of unsaturation was 12, and the above accounted for 11; thus, the remaining one was attributed to a tetrahydrofuran ring. ^{1}H NMR signals attributed to a tetrasubstituted furan ring were found at δ 4.55 (1H, d, J = 8.6 Hz, H-7), 2.37 (1H, m, H-8), 5.03 (1H, d, J = 7.3 Hz, H-7′), and 2.49 (1H, m, H-8′). Thus, the structure of **1** was deduced to be 7,7′-bis(5-methoxy-3,4-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran. The relative configuration 7,8-*trans*−8,8′-*trans*−7′,8′-*cis* was deduced from NOE correlations.

Compound **2** had the same molecular formula as compound **1** (HREIMS). The IR spectrum indicated hydroxyl (3650 cm^{-1}), ester carbonyl (1734 cm^{-1}), and aromatic groups (3040, 1636, 1456 cm^{-1}). The UV spectrum was similar to that of compound **1**. The ^{1}H and ^{13}C NMR data of **2** were also similar to that of **1**, except for the proton and carbon signals of C-8, C-9, C-8′, and C-9′. Downfield shifts of C-9′, H-9′a, and H-9′b and upfield shifts of C-9, H-9a, and H-9b indicated that the acetyl group was at C-9′, not C-9, a conclusion confirmed by HMBC cross-peaks between the carbonyl group and H-9′. Compound **2** had the same relative configuration as compound **1** on the basis of NOESY experiments. Thus, compound **2** was 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7,7′-bis(5-methoxy-3,4-methylenedioxyphenyl)-8-hydroxymethyl-8′-acetoxymethyltetrahydrofuran.

Compound **3** had the same molecular formula ($C_{24}H_{26}O_{10}$), and the IR and UV spectra resembled those of compounds **1** and **2**. Although **3** had ^{1}H and ^{13}C NMR signals similar to those of **1**, differences existed at C-1′, C-8′, H-7, H-7′, H-8′, and H-9′. NOE cross-peaks between H-7, H-9, and H-8′ established the relative configuration as 7,8-*trans*, 8,8′-*trans*, 7′,8′-*trans*. Thus, compound **3** was 7,8-*trans*-8,8′-*trans*-7′,8′-*trans*-7,7′-bis(5-methoxy-3,4-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran.

Compound **4** had the composition $C_{26}H_{30}O_{11}$, and the IR spectrum showed bands indicating hydroxyl, ester carbonyl, and aromatic groups. The UV spectrum exhibited absorbance typical of aromatic rings in tetrahydrofuran lignans. The ^{1}H NMR spectrum of compound **4** showed that it had one less methylenedioxy, one more methoxy methyl, and one more acetyl group than **1**. Comparison of the ^{13}C NMR chemical shifts of **4** and **1** indicated that the methylenedioxy group at C-3′, C-4′ and the hydroxylmethyl group at C-8′ in **1** were replaced by a methoxyl group, a hydroxyl group, and an acetoxymethyl group in **4**, respectively. Accordingly, **4** was deduced to be 7-(5-methoxy-3,4-methylenedioxyphenyl)-7′-(4-hydroxy-3,5-dimethoxyphenyl)-8,8′-diacetoxymethyltetrahydrofuran. The relative configuration 7,8-*trans*−8,8′-*trans*−7′,8′-*cis* was determined on the basis of NOESY correlations.

Compound **5** had the composition $C_{25}H_{30}O_{10}$, and the IR spectrum showed the presence of hydroxyl, ester carbonyl, and aromatic groups. The ^{1}H NMR spectrum was similar to that of **1**, but the spectrum of **5** showed one less methylenedioxy group and two more methoxy groups. Thus, one of the 5-methoxy-3,4-methylenedioxyphenyl groups in **1** had been substituted by a 3,4,5-trimethoxyphenyl group in **5**. This conclusion was supported by the symmetrical aromatic proton and carbon signals in the ^{1}H and ^{13}C NMR spectra of **5**. Moreover, this phenyl group was connected to C-7′ from the correlations of C-7′ with H-2 and H-6 in the HMBC spectrum of **5**. The 7,8-*trans*−8,8′-*trans*−7′,8′-*cis* configuration of **5** was elucidated on the basis of NOESY correlations. Therefore, compound **5** was determined to be 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7-(5-methoxy-3,4-methylenedioxyphenyl)-7′-(3,4,5-trimethoxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran.

The coupling constants of H-7 and H-7′ have been used to determine the relative stereochemistry of similar tetrahydrofuran rings, the larger one (about 9 Hz) indicating a *trans*-orientation between H-7 and H-8[7] and the smaller one (about 4 Hz) corresponding to the *cis*-form.[8] The coupling constants of H-7 and H-7′ of compound **3** had no evident difference from those of four other compounds, **1**, **2**, **4**, and **5**, although they had different relative configurations. Thus, application of NOESY correlations may be more accurate for the determination of the relative configurations of substituted tetrahydrofuran derivatives such as compounds **1**−**5**.

The cell growth inhibitory activity of the isolated compounds was evaluated on WI-38 cells (normal human lung cells), VA-13 cells (malignant lung tumor cells), and HepG2 cells (human liver cancer cells). Compounds **3**, **4**, 2-(3-phenylpropionyl)-1,3-cyclohexanedione, ergosta-6,22-diene-3β,5α,8α-triol, and 5-hydroxy-7,4′-dimethoxyflavone showed minimal cell growth inhibitory activity against VA-13 cells with IC_{50} values of 36.2, 47.4, 40.5, 48.1, and 38.9 μg/mL, respectively. The IC_{50} values to normal human lung cells (WI-38) were consistently greater than those of VA-13 cells. Only ergosta-6,22-diene-3β,5α,8α-triol showed weak activity to HepG2 cells, with an IC_{50} value of 47.9 μg/mL.

One of the mechanisms underlying MDR in mammalian tumor cells has been assigned to enhanced removal of drugs due to overexpression of efflux transporter proteins, such as P-glycoprotein (Pgp), the multidrug resistance proteins (MRP).[9] Thus, agents that inhibit this protein could overcome the MDR effect. Calcein AM is used as an easily operated functional fluorescent probe for this drug efflux protein.[10−12] The effects of the compounds on the cellular accumulation of calcein in MDR human ovarian cancer 2780AD cells (MDR-reversal activity) were examined by comparison with that of verapamil, a known MDR-reversal agent (Table 1). Compound **5** and ergosta-6,22-diene-3β,5α,8α-triol exhibited stronger activity toward calcein accumulation in MDR tumor cells than verapamil at 2.5 μg/mL. The above bioassay results suggested that the weak cell growth inhibitory activity of certain compounds could be enhanced by MDR-reversal agents that coexist in this plant.

Expression of an excess of intercellular adhesion molecule-1 (ICAM-1) on the surface of endothelial cells of a blood vessel plays an important role in the progress of inflammatory reaction.[13−15] The inhibitory activity of induction of ICAM-1 of compounds **1**, **4**, and **5** was examined using the human cultured cell line A549 (lung carcinoma), and the results are expressed by IC_{50} values. Preliminarily, compounds **4** and **5** showed moderate to weak inhibitory activity, and IC_{50} values were 84.4 and 189 μM when the induction of ICAM-1 was stimulated using IL-1α and 38.6 and 105 μM using TNF-α. They also showed no toxicity to A549 cells in the MTT assay. Since inflammatory reactions

Table 1. Effects of Compounds **2**, **4**, **5**, and Ergosta-6,22-diene-3β,5α,8α-triol on the Accumulation of Calcein in MDR 2780AD Cells[a]

compound	concentration, μg/mL	average of fluorescence/well ± SD[b]	% of control[c]	verapamil %[d]
control	0	4098 ± 506		
verapamil	0.25	3629 ± 113	89	100
	2.5	3909 ± 376	95	100
	25	5303 ± 300	129	100
2	0.25	3131 ± 47	76	86
	2.5	3496 ± 378	85	89
	25	3837 ± 241	94	72
4	0.25	3621 ± 174	88	100
	2.5	3879 ± 124	95	99
	25	4126 ± 310	101	78
5	0.25	3983 ± 170	97	110
	2.5	4317 ± 152	105	110
	25	5674 ± 778	138	107
ergosta-6,22-diene-3β,5α,8α-triol	0.25	3966 ± 402	97	109
	2.5	4337 ± 150	106	111
	25	3878 ± 625	95	73

[a] The amount of calcein accumulated in multidrug-resistant human ovarian cancer 2780AD cells was determined with the control in the presence of 0.25, 2.5, and 25 μg/mL test compounds. [b] The values represent means of triplicate determination. [c] The values are the relative amount of calcein accumulated in the cell compared with the control experiment. [d] The values are expressed as the relative amount of calcein accumulated in the cell as compared with that of verapamil.

are serious problems in cancer treatment, this observation could be important in future studies of the anticancer activity of *P. dindygulensis*.

Experimental Section

General Experimental Procedures. Optical rotations were determined using a Horiba SEPA-200 polarimeter, and CD spectra were recorded on a JASCO J-720W spectrometer. IR and UV spectra were recorded on a Hitachi 270-30 spectrometer in $CHCl_3$ and a JASCO V-550 UV/vis spectrophotometer in CH_3OH, respectively. ^1H and ^{13}C NMR spectra were run on a Varian UNITY-PS 500 spectrometer using $CDCl_3$ as solvent. EIMS was recorded on a JEOL LMS-FABmate instrument. HPLC separation was performed on a Hitachi L-6200 HPLC instrument with an Inertsil Prep-sil GL 10 × 250 mm column or an Inertsil Prep-ODS GL 10 × 250 mm column, using Hitachi L-7400 UV and Shodex SE-61 RI detectors.

Plant Material. The whole plant of *P. dindygulensis* was collected from Yunnan Province, People's Republic of China, in February 2002. The plant was identified by Mr. Kaijiao Jiang, Kunming Institute of Botany. A voucher specimen (PDi-2002-2) has been deposited at the Faculty of Engineering, Niigata University, Japan.

Extraction and Isolation. The dried plant material (1.75 kg) was powdered and extracted three times (4 L/each) with MeOH at room temperature with the aid of a supersonic machine, and about 105 g of residue was obtained after evaporating the MeOH. The residue was suspended in H_2O and partitioned in sequence using hexane, EtOAc, and *n*-butanol, respectively, to afford a hexane extract (40.7 g), an EtOAc extract (20.1 g), and an *n*-butanol extract (15.6 g). The EtOAc extract was separated into 12 fractions (F_1−F_{12}) by column chromatography over silica gel [7 cm i.d. column packed with silica gel (70−230 mesh, 500 g), solvent (hexane−EtOAc, gradient)]. F_4 [eluted with hexane−EtOAc (4:1), 0.45 g], F_6 [eluted with hexane−EtOAc (2:1), 2.30 g], and F_8 [eluted with hexane−EtOAc (1:5), 1.65 g] were further separated using silica gel column chromatography, normal-phase and reversed-phase HPLC methods. 5-Hydroxy-7,4′-dimethoxyflavone (3.1 mg) was obtained from F_4 using normal-phase HPLC [hexane−EtOAc (75:25)]. F_6 (2.30 g) was subjected to silica gel column chromatography using a hexane−EtOAc gradient, yielding seven subfractions (F_{6-1}−F_{6-7}). 2-(3-Phenylpropionyl)-1,3-cyclohexanedione (1.8 mg) and ergosta-6,22-diene-3β,5α,8α-triol (3.7 mg) were separated from F_{6-3} by normal-phase HPLC using hexane−EtOAc (60:40 and 70:30). F_{6-5} was separated by normal-phase HPLC [hexane−EtOAc (55:45)] to give 5-hydroxy-7,8,3′,4′-tetramethoxyflavone (2.5 mg). Compounds **1** (33.8 mg), **2** (9.3 mg), **3** (0.6 mg), **4** (5.0 mg), and **5** (1.8 mg) were isolated from F_8 (1.65 g) using silica gel column chromatography followed by normal-phase HPLC [hexane−EtOAc (55:45 and 7:3)] and reversed-phase HPLC [MeOH−H_2O (7:3)].

7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7,7′-Bis(5-methoxy-3,4-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran (1): pale yellow gum; $[\alpha]^{20}_D$ −11.0° (*c* 1.690, $CHCl_3$); UV (MeOH) λ_{max} 213, 246, 281 nm; CD (*c* 1 mM, MeOH) $[\theta]_{250}$ +1737, $[\theta]_{235}$ −2214; IR ($CHCl_3$) ν_{max} 3630, 3036, 2940, 1738, 1636, 1456, 1432, 1370, 1220, 1204, 1138, 1094, 1042 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.69 (1H, d, J = 1.2 Hz, H-2), 6.64 (1H, d, J = 1.2 Hz, H-6), 6.62 (1H, d, J = 1.0 Hz, H-2′), 6.58 (1H, d, J = 1.0 Hz, H-6′), 5.97 (4H, s, OCH_2O), 5.03 (1H, d, J = 7.3 Hz, H-7′), 4.55 (1H, d, J = 8.6 Hz, H-7), 4.25 (1H, dd, J = 6.1, 11.2 Hz, H-9a), 4.24 (1H, dd, J = 5.9, 11.2 Hz, H-9b), 3.92 (3H, s, 5-OCH_3), 3.90 (3H, s, 5′-OCH_3), 3.46 (1H, dd, J = 6.4, 11.2 Hz, H-9′a), 3.36 (1H, dd, J = 6.6, 11.2 Hz, H-9′b), 2.49 (1H, m, H-8′), 2.37 (1H, m, H-8), 2.03 (3H, s, CH_3CO); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.9 (*C*OCH_3), 149.1 (C-3,3′), 143.6 (C-5′), 143.5 (C-5), 134.9 (C-4′), 134.8 (C-4), 134.6 (C-1), 132.6 (C-1′), 106.6 (C-6), 105.6 (C-6′), 101.5 (OCH_2O), 100.5 (C-2), 100.3 (C-2′), 82.9 (C-7), 81.2 (C-7′), 64.4 (C-9), 62.9 (C-9′), 56.7 (5,5′-OCH_3), 49.8 (C-8), 49.0 (C-8′), 20.9 (*C*H_3CO); EIMS *m/z* 475 $[M + H]^+$ (16), 474 $[M]^+$ (54), 208 (95), 203 (100); HREIMS *m/z* 474.1519 (calcd for $C_{24}H_{26}O_{10}$, 474.1525).

7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7,7′-Bis(5-methoxy-3,4-methylenedioxyphenyl)-8-hydroxymethyl-8′-acetoxymethyltetrahydrofuran (2): pale yellow gum; $[\alpha]^{20}_D$ +17.8° (*c* 0.090, $CHCl_3$); UV (MeOH) λ_{max} 213, 242, 280 nm; CD (*c* 1 mM, MeOH) $[\theta]_{247}$ +2374, $[\theta]_{235}$ −115; IR ($CHCl_3$) ν_{max} 3650, 3040, 2948, 2888, 1734, 1636, 1456, 1432, 1370, 1240, 1212, 1136, 1096, 1044 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.70 (1H, d, J = 1.2 Hz, H-2), 6.66 (1H, d, J = 1.2 Hz, H-6), 6.59 (1H, s, H-2′), 6.57 (1H, s, H-6′), 5.98 (2H, s, OCH_2O), 5.97 (2H, s, OCH_2O), 5.06 (1H, d, J = 7.3 Hz, H-7′), 4.65 (1H, d, J = 8.3 Hz, H-7), 3.92 (3H, s, 5-OCH_3), 3.91 (3H, s, 5′-OCH_3), 3.85 (1H, dd, J = 9.3, 11.2 Hz, H-9′a), 3.83 (1H, dd, J = 5.1, 11.0 Hz, H-9a), 3.79 (1H, dd, J = 5.9, 11.0 Hz, H-9b), 3.75 (1H, dd, J = 8.5, 11.0 Hz, H-9′b), 2.73 (1H, m, H-8′), 2.19 (1H, m, H-8), 1.94 (3H, s, CH_3CO); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.7 (*C*OCH_3), 149.2 (C-3), 148.9 (C-3′), 143.6 (C-5), 143.5 (C-5′), 135.3 (C-1), 134.9 (C-4), 134.5 (C-4′), 132.4 (C-1′), 106.5 (C-6), 105.8 (C-6′), 101.5 (OCH_2O), 100.5 (C-2,2′), 82.5 (C-7), 81.2 (C-7′), 64.9 (C-9′), 62.7 (C-9), 56.8 (5,5′-OCH_3), 53.6 (C-8), 45.4 (C-8′), 20.8 (*C*H_3CO); EIMS *m/z* 475 $[M + H]^+$ (5), 474 $[M]^+$ (26), 203 (90), 179 (100); HREIMS *m/z* 474.1519 (calcd for $C_{24}H_{26}O_{10}$, 474.1525).

7,8-*trans*-8,8′-*trans*-7′,8′-*trans*-7,7′-Bis(5-methoxy-3,4-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran (3): pale yellow gum; $[\alpha]^{20}_D$ +20.7° (*c* 0.030, $CHCl_3$); UV (MeOH) λ_{max} 214, 245, 280 nm; CD (*c* 1 mM, MeOH) $[\theta]_{249}$ −40268; IR ($CHCl_3$) ν_{max} 3552, 3024, 2892, 1738, 1636, 1454, 1430, 1370, 1324, 1236, 1134, 1042 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.62 (2H, brs, H-2,2′), 6.60 (2H, s, H-6,6′), 5.97 (4H, s, OCH_2O), 4.95 (1H, d, J = 7.3 Hz, H-7′), 4.88 (1H, d, J = 8.1 Hz, H-7), 4.28 (1H, dd, J = 5.9, 11.5 Hz, H-9a), 4.20 (1H, dd, J = 5.4, 11.5 Hz, H-9b), 3.92 (6H, s, 5,5′-OCH_3), 3.82 (1H, dd, J = 5.1, 11.0 Hz, H-9′a), 3.76 (1H, dd, J = 5.1, 11.0 Hz, H-9′b), 2.48 (1H, m, H-8), 2.25 (1H, m, H-8′), 2.02 (3H, s, CH_3CO); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.8 (*C*OCH_3), 149.1 (C-3,3′), 143.6 (C-5,5′), 136.5 (C-1′), 136.2 (C-1), 134.8 (C-4,4′), 105.8 (C-6,6′), 101.5 (OCH_2O), 100.3 (C-2), 100.2 (C-2′), 82.9 (C-7), 82.8 (C-7′), 63.8 (C-9), 62.9 (C-9′), 56.7 (5,5′-OCH_3), 53.5 (C-8′), 50.2 (C-8), 20.8 (*C*H_3CO); EIMS *m/z* 475 $[M + H]^+$ (6), 474 $[M]^+$ (20), 206 (100), 179 (61); HREIMS *m/z* 474.1519 (calcd for $C_{24}H_{26}O_{10}$, 474.1525).

7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7-(5-Methoxy-3,4-methylenedioxyphenyl)-7′-(4-hydroxy-3,5-dimethoxyphenyl)-8,8′-diacetoxymethyltetrahydrofuran (4): pale yellow gum; $[\alpha]^{20}_D$ −23.4° (*c* 0.250, $CHCl_3$); UV (MeOH) λ_{max} 212, 244, 280 nm; CD (*c* 1 mM, MeOH) $[\theta]_{280}$ −506, $[\theta]_{247}$ +3314; IR ($CHCl_3$) ν_{max} 3552, 3032, 1734, 1622, 1458, 1430, 1370, 1326, 1236, 1116, 1040 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.70 (1H, s, H-2), 6.65 (1H, s, H-6), 6.61 (2H, s, H-2′,6′), 5.99 (2H, s, OCH_2O), 5.07 (1H, d, J = 7.1 Hz, H-7′), 4.60 (1H, d, J = 8.1 Hz, H-7), 4.28 (1H, dd, J = 5.9, 11.5 Hz, H-9a), 4.23 (1H, dd, J = 5.9, 11.5 Hz, H-9b), 3.87 (1H, dd, J = 6.1, 11.0 Hz, H-9′a), 3.72 (1H, dd, J = 8.5, 11.0 Hz, H-9′b), 3.93 (3H, s, 5-OCH_3), 3.90 (6H, s, 3′,5′-OCH_3), 2.68 (1H, m, H-8′), 2.35 (1H, m, H-8), 2.06 (3H, s, CH_3CO), 1.90 (3H, s, CH_3CO); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.8 (*C*OCH_3), 170.6 (*C*OCH_3), 149.2 (C-3), 147.0 (C-3′,5′), 143.5 (C-5), 135.0 (C-4), 134.9 (C-1), 134.1 (C-4′), 128.5 (C-1′), 106.6 (C-6), 102.8 (C-2′,6′), 101.6 (OCH_2O), 100.5 (C-2), 82.9 (C-7), 81.2 (C-7′), 64.5 (C-9′), 64.2 (C-9), 56.7 (5-OCH_3), 56.4 (3′,5′-OCH_3), 50.3 (C-8), 45.5 (C-8′), 20.8 (*C*H_3CO), 20.7 (*C*H_3CO); EIMS *m/z* 519 $[M + H]^+$ (11), 518 $[M]^+$ (38), 252 (100), 216 (55); HREIMS *m/z* 518.1789 (calcd for $C_{26}H_{30}O_{11}$, 518.1788).

7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7-(5-Methoxy-3,4-methylenedioxyphenyl)-7′-(3,4,5-trimethoxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran (5): pale yellow gum; $[\alpha]^{20}_D$ −21.8° (*c* 0.470, $CHCl_3$); UV (MeOH) λ_{max} 212, 244, 280 nm; CD (*c* 1 mM, MeOH) $[\theta]_{242}$ +3439, $[\theta]_{237}$ −1233; IR ($CHCl_3$) ν_{max} 3650, 3550, 3040, 2948, 1736, 1594, 1458, 1424, 1368, 1326, 1236, 1130, 1040 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.71 (1H, d, J = 1.2 Hz, H-2), 6.65 (1H, d, J = 1.2 Hz, H-6), 6.65 (2H, s, H-2′,6′), 5.98 (2H, s, OCH_2O), 5.08 (1H, d, J = 7.3 Hz, H-7′), 4.59 (1H, d, J = 8.3 Hz, H-7), 4.28 (1H, dd, J = 6.3, 11.5 Hz, H-9a), 4.26 (1H, dd, J = 6.3, 11.5 Hz, H-9b), 3.93 (3H, s, 5-OCH_3), 3.87 (6H, s, 3′,5′-OCH_3), 3.85 (3H, s, 4′-OCH_3), 3.48 (1H, dd, J = 6.3, 11.5 Hz, H-9′a), 3.38 (1H, dd, J = 6.3, 11.5 Hz, H-9′b), 2.54 (1H, m, H-8′), 2.40 (1H, m, H-8), 2.05 (3H, s, CH_3CO); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.9 (*C*OCH_3), 153.5 (C-3′,5′), 149.1 (C-3), 143.5 (C-5), 137.4 (C-4′), 135.0 (C-1), 134.9 (C-4), 133.7 (C-1′), 106.7 (C-6), 102.9 (C-2′,6′), 101.5 (OCH_2O), 100.5 (C-2), 82.9 (C-7), 81.4 (C-7′), 64.4 (C-9), 63.0 (C-9′), 60.9 (4′-OCH_3), 56.7 (5-OCH_3), 56.2 (3′,5′-OCH_3), 49.8 (C-8), 49.1 (C-8′), 20.9 (*C*H_3CO); EIMS *m/z* 491 $[M + H]^+$ (10), 490 $[M]^+$ (38), 224 (100), 181 (42); HREIMS *m/z* 490.1842 (calcd for $C_{25}H_{30}O_{10}$, 490.1839).

2-(3-Phenylpropionyl)-1,3-cyclohexanedione (6): pale yellow gum; UV (MeOH) λ_{max} 240, 276 nm; IR ($CHCl_3$) ν_{max} 2972, 1664, 1556, 1416, 1354, 1226, 1220, 1012 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.28 (4H, m, H-5′,6′,8′,9′), 7.18 (1H, m, H-7′), 3.36 (2H, t, J = 7.6 Hz, H-2′), 2.94 (2H, t, J = 7.6 Hz, H-3′), 2.67 (2H, ddd, J = 6.4, 6.4, 12.7 Hz, H-6), 2.48 (2H, ddd, J = 6.4, 6.8, 13.2 Hz, H-4), 1.97 (2H, m, H-5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 205.1 (C-1′), 198.2 (C-1), 195.3 (C-3), 141.0 (C-4′), 128.5, 128.4 (C-5′,6′,8′,9′), 126.0 (C-7′), 113.1 (C-2), 42.4 (C-2′), 38.7 (C-6), 33.1 (C-4), 30.5 (C-3′), 19.0 (C-5); EIMS *m/z* 245 $[M + H]^+$ (17), 244 $[M]^+$ (100), 226 (22), 153 (20), 139 (87), 112 (88), 91 (64); HREIMS *m/z* 244.1109 (calcd for $C_{15}H_{16}O_3$, 244.1099).

Cell Growth Inhibitory Activity of Compounds to WI-38, VA-13, and HepG2 in Vitro. WI-38, VA-13, and HepG2 cell lines were available from the Institute of Physical and Chemical Research (RIKEN), Tukuba, Ibaraki, Japan. WI-38 and VA-13 cells were maintained in Eagle's MEM medium (Nissui Pharmaceutical Co., Tokyo, Japan) and RITC 80-7 medium (Asahi Technoglass Co., Chiba, Japan), respectively, both supplemented with 10% (v/v) fetal bovine serum (FBS) (Filtron PTY Ltd., Australia) with 80 μg/mL kanamycin. HepG2 cells were maintained in D-MEM medium (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) FBS (Filtron PTY Ltd., Australia) with 80 μg/mL kanamycin.

Medium (100 μL) containing ca. 5000 cells (WI-38, VA-13, or HepG2) was incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 24 h in a 96-well microplate. Test samples dissolved in dimethyl sulfoxide (DMSO) were added to the medium, and incubation was continued for a further 48 h in the same conditions. Coloration substrate, WST-8 [2-(2-methyl-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt], was added to the medium. The resulting formazan concentration was determined by the absorption at 450 nm. Cell viability (%) was calculated as [(experimental absorbance − background absorbance)/(control absorbance − background absorbance) × 100]. Cell viability at different concentrations of compounds was plotted, and 50% inhibition of growth was calculated as IC_{50}.

Cellular Accumulation of Calcein. Adriamycin-resistant human ovarian cancer A2780 cells (AD10) were maintained in PRMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) FBS (Filtron PTY Ltd., Australia) with 80 μg/mL kanamycin.

Medium (100 μL) containing ca. 1×10^6 cells was incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. Test compounds were dissolved in DMSO and diluted with phosphate-buffered saline, PBS (−). Test samples of 50 μL were added to the medium and incubated for 15 min. Then, 50 μL of the fluorogenic dye calcein AM [1 μM in PBS (−)] was added to the medium, and incubation was continued for a further 60 min. After removing the supernatant, each microplate was washed with 200 μL of cold PBS (−). The washing step was repeated two times, and 200 μL of cold PBS (−) was added. Retention of the resulting calcein was measured as calcein-specific fluorescence. The absorption maximum for calcein is 494 nm, and the emission maximum is 517 nm.

Inhibitory Activity on Induction of ICAM-1. A549 cells were maintained in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) fetal calf serum (JRH Bioscience, Lenexa, KS) and a penicillin−streptomycin antibiotic mixture (Invitrogen). Mouse anti-human ICAM-1 antibody C167 was purchased from Leinco Technologies, Inc. (Ballwin, MO), and peroxidase-conjugated goat anti-mouse IgG antibody was obtained from Jackson Immuno Research Laboratories, Inc. (West Grove, PA). Recombinant IL-1α and TNF-α were provided by Dainippon Pharmaceutical Co Ltd. (Osaka Japan).

A549 cells were seeded in a microtiter plate, 2×10^4 cells/well, the day before assay. After 1 h of A549 cells with or without test compounds, 75 or 25 μL solutions of IL-1α (1 ng/mL) or TNF-α (10 ng/mL) were added to the cultures, and the cells were further incubated for 6 h. Cells were washed twice with phosphate-buffered saline (PBS) and fixed by incubation with 1% paraformaldehyde-PBS for 15 min, then washed twice with PBS. After blocking with 1% bovine serum albumin-PBS overnight, the fixed cells were treated with mouse anti-human ICAM-1 antibody for 60 min. After being washed three times with 0.02% Tween 20-PBS, the cells were treated with peroxidase-linked anti-mouse IgG antibody for 60 min. The cells were washed three times with 0.02% Tween 20-PBS. The cells were then incubated with the substrate (0.1% *o*-phenylenediamine dihydrochloride and 0.02% H_2O_2 in 0.2 M sodium citrate buffer, pH 5.3) for 20 min at 37 °C in the dark and assayed

for absorbance at 415 nm using a microplate reader. Expression of ICAM-1 was calculated as follows: Expression of ICAM-1 (% of control) = [(absorbance with sample and IL-1α/TNF-α treatment − absorbance without IL-1α/TNF-α treatment)/(absorbance with IL-1α/TNF-α treatment − absorbance without IL-1α/TNF-α treatment)] × 100.

A549 cells (2×10^4 cell/well) were seeded in a microtiter plate the day before assay and incubated in the presence or absence of test compounds for 24 h. During the last 4 h of incubation, the cells were pulsed with 500 μg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). MTT formazan was solubilized with 10% sodium dodecyl sulfate (SDS) overnight. Absorbance at 595 nm was measured. Cell viability (%) was calculated as [(experimental absorbance − background absorbance)/(control absorbance − background absorbance)] × 100.

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