Bioactive Compounds from *Peperomia pellucida*

Su Xu,[†,‡] Na Li,*[,†] Meng-Meng Ning,[†,‡] Cai-Hong Zhou,[†] Qiao-Rong Yang,[†] and Ming-Wei Wang*[,†]

The National Center for Drug Screening, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, and the Graduate School, Chinese Academy of Sciences, 189 Guo Shou Jing Road, Zhanjiang Hi-Tech Park, Shanghai 201203, People's Republic of China

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Five new compounds (**1**−**5**), including two secolignans, two tetrahydrofuran lignans, and one highly methoxylated dihydronaphthalenone, were isolated from the whole plant of *Peperomia pellucida*. These compounds were accompanied by the known peperomins A, B, C, and E, 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7,7′-bis(5-methoxy-3,4-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran, 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7-(5-methoxy-3,4-methylenedioxyphenyl)-7′-(4-hydroxy-3,5-dimethoxyphenyl)-8,8′-diacetoxymethyltetrahydrofuran, sesamin, and isoswertisin. New structures were elucidated mainly by NMR and MS techniques, and anticancer activities evaluated in HL-60, MCF-7, and HeLa cell lines. Compound **1** and peperomin E show growth inhibitory effects on the three cancer cell lines with IC_{50} values ranging between 1.4 and 9.1 and between 1.8 and 11.1 μM, respectively. Compound **2** has a weak suppressive activity on HL-60 cells (IC_{50} = 10.8 μM), while 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7,7′-bis(5-methoxy-3,4-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran exhibits estrogen-like properties (EC_{50} = 3.1 μM) in CV-1 cells transfected with human estrogen receptor (ERα).

Peperomia pellucida Kunth (Piperaceae), widely grown in southern regions of China,[1] is an herb traditionally used to treat abscesses, boils, skin wounds, trauma, and bleeding.[2] Previous chemical investigations on this plant indicated the existence of flavonoids, phytosterols, apiols, substituted styrenes, and a dimeric ArC_2 compound.[3] Anti-inflammatory,[4] bacteriacidal,[5] and analgesic activities[4,6] found in the crude extracts were also reported. To identify active components responsible for the above effects, chemical isolation and pharmacological studies were carried out. Thirteen compounds, including two new secolignans (**1**, **2**), two new tetrahydrofuran lignans (**3**, **4**), one new dihydronaphthalenone (**5**), and the known peperomins A, B, C,[7] and E,[8] 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7,7′-bis(5-methoxy-3,4-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran, 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7-(5-methoxy-3,4-methylenedioxyphenyl)-7′-(4-hydroxy-3,5-dimethoxyphenyl)-8,8′-diacetoxymethyltetrahydrofuran,[9] sesamin,[10] and isoswertisin,[11] were obtained from the EtOAc extract. New structures were elucidated on the basis of NMR and MS analysis, and the known compounds by comparison with literature data. Biological activities of four of the new compounds were evaluated in a series of cell-based assays.

Results and Discussion

The high-resolution EIMS of compound **1** gave a molecular ion peak at *m*/*z* 428.1470, in accordance with the molecular formula $C_{23}H_{24}O_8$. UV maxima were at 248 and 280 nm, and the IR spectrum showed the presence of γ-butyrolactone (1762 cm^{-1}) and aromatic ring (1635, 1591, and 1458 cm^{-1}). ^1H NMR indicated two sets of tetrasubstituted aromatic signals at δ 6.47 (1H, d, *J* = 1.2 Hz, H-2″), 6.44 (2H, s, H-2′, 6′), and 6.41(1H, d, *J* = 1.2 Hz, H-6″). Four methoxy groups [δ 3.91 (3H, s), 3.84 (6H, s), and 3.81 (3H, s)] and one methylenedioxy group [δ 5.95 (2H, m)] attached to the aromatic rings were also observed. Thus, a 5-methoxy-3,4-methylenedioxyphenyl group and a 3,4,5-trimethoxyphenyl group were established, which was verified by the ^{13}C NMR spectrum. The methine proton at δ 3.80 (1H, m, H-3) correlated with the oxymethylene [δ 4.33 (1H, dd, *J* = 7.5, 9.9 Hz, H-4a) and 4.01 (1H, dd, *J* = 3.9, 9.9 Hz, H-4b)] and the methine [δ 3.69 (1H, d, *J* = 11.4 Hz, H-5)] in the $^1H-^1H$ COSY, suggesting a linkage of $^5CH-^3CH-^4CH_2O$. The two aromatic rings were connected to the above moiety at C-5 by the HMBC correlations between C-5 and the aromatic protons. The EIMS fragment at *m*/*z* 331 also confirmed the existence of a biphenylmethyl residue. In addition to the proton and carbon signals mentioned above, ^{13}C and ^1H NMR spectra pointed to the presence of a carbonyl carbon at δ 171.0 (C-1), a quaternary olefinic carbon at δ 136.1 (C-2), a secondary olefinic carbon at δ 125.1 (C-6), and two olefinic protons at δ 6.14 (1H, d, *J* = 2.4 Hz, H-6a) and 4.87 (1H, d, *J* = 2.4 Hz, H-6b). The HMBC correlations between the carbonyl and the olefinic and oxymethylene protons (H-4a and H-4b) further suggested an α,β-unsaturated ester group. The degree of unsaturation of $C_{23}H_{24}O_8$ is 12, whereas the above moiety only accounts for 11. The remaining unsaturation degree was therefore assigned to a γ-butyrolactone ring, as confirmed by the HMBC cross-peak between the quaternary olefinic carbon (C-2) and H-5. This led to the establishment of compound **1** as 2-methylene-3-[(3′,4′,5′-trimethoxyphenyl)(5″-methoxy-3″,4″-methylenedioxyphenyl)methyl]butyrolactone. The absolute configuration at C-3 and C-5 is not clear yet.

Compound **2** has the molecular formula $C_{22}H_{24}O_8$ from HREIMS. UV maximum absorptions were at 247 and 277 nm, and the IR spectrum showed hydroxyl (3433 cm^{-1}), aromatic ring (1633, 1593, and 1452 cm^{-1}), and γ-butyrolactone (1763 cm^{-1}) frequencies. The ^1H NMR spectrum was similar to that of compound **1** and showed the disappearance of the olefinic protons in **1** and the appearance of a methyl group [δ 0.94 (3H, d, *J* = 7.2 Hz, H-6)] and a methine function [δ 2.34 (1H, m, H-2)] in **2**. This indicated the existence of a 2-methyl instead of a 2-methylene moiety in **2**. The upfield shift of H-3 confirmed this deduction. As for compound **1**, the two phenyl groups were elucidated as 5-methoxy-3,4-methylenedioxyphenyl and 3-hydroxy-4,5-dimethoxyphenyl moieties from ^1H and ^{13}C NMR spectra. Thus, compound **2** is 2-methyl-3-[(3′-hydroxyl-4′,5′-dimethoxyphenyl)(5″-methoxy-3″,4″-methlenedioxyphenyl)-methyl]butyrolactone. The absence of a NOE correlation between H-2 and H-3 in the NOESY spectrum indicated their *trans*-orientation. The absolute configuration could not be established.

A molecular formula $C_{24}H_{28}O_{10}$ was assigned to compound **3** by HREIMS. The IR spectrum suggested the presence of hydroxyl, aromatic, and carbonyl groups. 5-Methoxy-3,4-methylenedioxyphenyl and 4-hydroxy-3,5-dimethoxyphenyl groups were established from NMR spectra. The protons at δ 5.05 (1H, d, *J* = 6.9 Hz,

* To whom correspondence should be addressed. Tel: 86-21-50801313. Fax: 86-21-50800721. E-mail: nli@mail.shcnc.ac.cn (chemistry) and mwwang@siniwest.com (pharmacology).

† The National Center for Drug Screening.

‡ The Graduate School of the Chinese Academy of Sciences.

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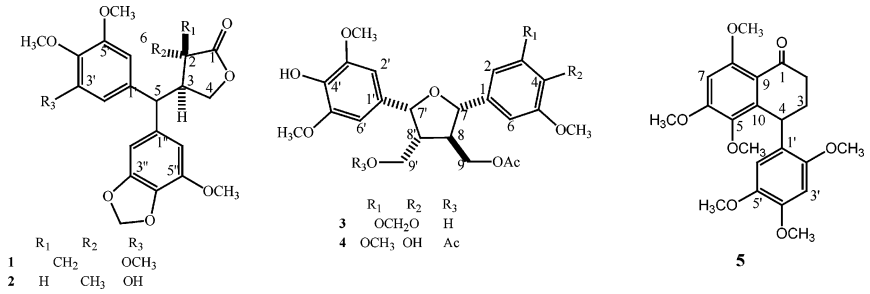

Figure 1. Structures of compounds **1**−**5**.

H-7′), 4.56 (1H, d, J = 8.1 Hz, H-7), 2.50 (1H, m, H-8′), 2.36 (1H, m, H-8), 4.24 (2H, m, H-9), 3.44 (1H, dd, J = 6.3, 11.4 Hz, H-9′a), and 3.34 (1H, dd, J = 6.6, 11.4 Hz, H-9′b) were assigned to the substructures ^{7}CH−^{8}CH−^{9}CH$_2$O, $^{7'}$CH−$^{8'}$CH−$^{9'}$CH$_2$O, and ^{8}CH−$^{8'}$CH from the ^{1}H−^{1}H COSY. HMBC cross-peaks between H-7 and C-1, C-2, and C-6 of the 5-methoxy-3,4-methylenedioxyphenyl group indicated the linkage of C-7 and this aromatic ring. Similarly, C-7′ was connected to C-1′ of the 4-hydroxy-3,5-dimethoxyphenyl group. The hydroxyl group at C-9 was acetylated on the basis of the HMBC correlation between the carbonyl (δ 171.1) and the protons at C-9. The degree of unsaturation of $C_{24}H_{28}O_{10}$ is 11, whereas the above moiety only accounts for 10; the remaining one was therefore ascribed to the presence of a tetrahydrofuran ring. Compound **3** is thus 7-(5-methoxy-3,4-methylenedioxyphenyl)-7′-(4-hydroxy-3,5-dimethoxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran. In the NOESY spectrum, the NOE cross-peaks between H-7 and H-9, H-9 and H-8′, and H-7′ and H-8′ suggested the relative configuration as 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*. The absolute configuration was not determined.

Compound **4**, $C_{26}H_{32}O_{11}$, had UV, IR, and ^{1}H and ^{13}C NMR spectra similar to those of **3**, indicating a similar tetrahydrofuran lignan type structure. Two sets of acetyl proton and carbon signals were observed in the ^{1}H and ^{13}C NMR spectra of **4**, and the carbonyl groups correlated with H-9 and H-9′ in the HMBC, respectively. Thus, **4** was an 8,8′-diacetoxymethyltetrahydrofuran lignan. The phenyl groups were established as 4-hydroxy-3,5-dimethoxyphenyl from the ^{1}H and ^{13}C NMR spectrum. It had the same relative configuration as compound **3** from the NOESY spectrum. Therefore, compound **4** was assigned as 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7,7′-(4-hydroxy-3,5-dimethoxyphenyl)-8,8′-diacetoxymethyltetrahydrofuran.

Compound **5**, $C_{22}H_{26}O_7$, exhibited a conjugated carbonyl group at 1670 cm^{-1} in the IR spectrum. Three aromatic proton singlets at δ 6.57 (H-3′), 6.48 (H-7), and 6.19 (H-6′) and six methoxy groups at δ 3.95, 3.92, 3.89, 3.86, 3.56, and 3.36 in the ^{1}H NMR and 12 aromatic carbon signals in the ^{13}C NMR indicated that it was a highly oxygenated aromatic compound. A combination of HMBC and NOESY data assigned the above signals to the two aromatic rings, a 2,4,5-trimethoxyphenyl, and a pentasubstituted benzene ring with two *o*-methoxy and one *m*-methoxy toward the aromatic methine. A CH−CH$_2$−CH$_2$ moiety evident from ^{1}H−^{1}H COSY was linked to two phenyl groups and the carbonyl carbon at δ 197.6, as revealed by the HMBC analysis (Figure 2). A six-membered ring fused with the pentasubstituted benzene ring was assumed in order to account for the degree of unsaturation of $C_{22}H_{26}O_7$. The HMBC correlation between the C-1 carbonyl carbon and the aromatic methine (H-7) further confirmed the above deduction. Thus, compound **5** is 5,6,8-trimethoxy-4-(2,4,5-trimethoxyphenyl)-3,4-dihydro-1(2*H*)-naphthalenone. This compound is laevorotatory, and the absolute configuration at C-4 was not established.

Compounds **1**, **2**, **3**, **5**, peperomins A, B, and E, and 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7,7′-bis(5-methoxy-3,4-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran were evaluated for their growth inhibitory effects on three cancer cell lines, namely, HL-60, MCF-7, and HeLa. Compound **1** and peperomin E were

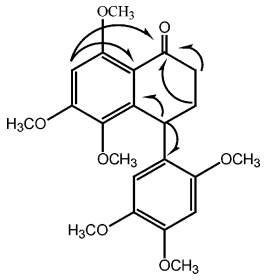

Figure 2. Key HMBC correlations in compound **5**.

efficacious in all three cell lines, while compound **2** was effective only at a high concentration (20 μM) in HL-60 cells, a result similar to the positive control, etoposide. IC$_{50}$ values of compound **1** and peperomin E calculated relative to HL-60, MCF-7, and HeLa cells ranged between 1.4 and 9.1 and between 1.8 and 11.1 μM, respectively. Compound **2** showed an IC$_{50}$ of 10.8 μM in HL-60 cells (Table 1). On the contrary, 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7,7′-bis(5-methoxy-3,4-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran promoted proliferation of MCF-7 breast cancer cells with an EC$_{50}$ of 142 nM. This compound was subjected to further evaluation for potential estrogenic properties. In CV-1 cells cotransfected with human estrogen receptor α (ERα) and a luciferase gene linked to estrogen response elements (EREs), it displayed an estrogen-like activity (EC$_{50}$ = 3.1 μM; Figure 3) that was completely blocked by an ER antagonist, raloxifene (IC$_{50}$ = 0.46 nM, data not shown). Compound **4** was not studied due to insufficient quantity of the pure material obtained. Thus, pharmacological properties of four of the five new compounds from *P. pellucida* were established, and the anticancer effects observed are of a cytotoxic nature to a large extent.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 342 polarimeter. UV and IR spectra were recorded on an Agilent DAD detector and a Perkin-Elmer 577 spectrometer with KBr disk, respectively. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AN-400 and a Varian Mercury 300 spectrometer. HREIMS were carried out on a Finigan MAT 95 instrument. HPLC analysis was performed on an HP 1100 series with DAD detector using a Zorbax ODS column (4.6 × 250 mm, 5 μm). HPLC preparations were carried out on a Waters HPLC instrument with a Kromasil SIL column (10 × 250 mm, 5 μm) and a Zorbax SB-C18 column (9.4 × 250 mm, 5 μm). Silica gel (200−300 mesh, Shanghai Chemical Reagents Co.), reversed-phase silica gel (ODS) (20−45 μm, Fuji Silysia Chemical Co., Ltd.), and Pharmadex LH-20 (Amersham Biosciences) were used for column chromatography. Silica gel 60 F$_{254}$ plates (Qingdao Ocean Chemical Industry Co.) were used for TLC.

Plant Material. *Peperomia pellucida* was collected from Xishuangbanna of Yunnan Province, China, in June 2004, and was identified by Mr. Hongbo He, Yunnan Branch, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences. A voucher specimen (PP-2004-6) has been deposited in the National Center for Drug Screening, Shanghai, China.

Table 1. Inhibition of Cancer Cell Proliferation by Active Constituents from *P. pellucida*

	IC_{50} (μM)[a]			growth inhibition (%)[b]		
	HL-60	MCF-7	HeLa	HL-60	MCF-7	HeLa
1	1.4 ± 0.2	3.8 ± 1.1	9.1 ± 0.3	92 ± 8	91.0 ± 0.8	96 ± 1
2	10.8 ± 1.1	ND[c]	ND	92 ± 4	53.2 ± 6.1	10 ± 3
5	ND	ND	ND	<5	37.6 ± 1.0	<5
peperomin A	ND	ND	ND	17 ± 4	27.7 ± 2.5	<5
peperomin E	1.8 ± 0.8	3.9 ± 0.2	11.1 ± 0.4	93 ± 8	90.7 ± 0.9	95 ± 2
etoposide	0.21 ± 0.01	0.19 ± 0.03	3.6 ± 0.5	93.5 ± 4.2	73.5 ± 1.0	85.3 ± 7.3

[a] IC_{50} was calculated as the concentration of ligand required to give half-maximal inhibition on the cell growth, and values represented are means ± SD of three independent experiments. [b] Cell growth inhibition was evaluated at a concentration of 20 μM for each compound, and values given are means ± SD of three independent experiments. [c] Not determined.

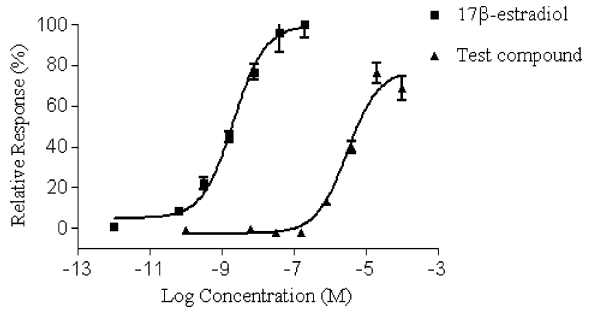

Figure 3. Induction of luciferase activity in CV-1 cells by 17β-estradiol and the test compound bis(5-methoxy-3,4-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran. Agonist efficacy was estimated relative to 17β-estradiol (100%; positive control). EC_{50} value (3.1 μM) was calculated as the concentration of ligand required to give half-maximal activation. Data plotted are representative of three independent experiments (mean ± SD).

Extraction and Isolation. The whole dried plant (500 g) was powdered and extracted with EtOH (2.5 L × 3) at room temperature with the aid of a supersonic machine and yielded about 32 g of residue after evaporating the solvents in vacuo. The residue was suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively, to afford 10.0 g of petroleum ether extract, 11.5 g of EtOAc extract, and 4.1 g of *n*-BuOH extract. The EtOAc extract was subjected to silica gel column chromatography, eluting with a gradient of petroleum ether and EtOAc, and seven fractions (F_1−F_7) were obtained. F_1 (1.4 g) afforded 43 mg of sesamin by silica gel column chromatography followed by a normal-phase HPLC preparation (hexane−EtOAc, 9:1). F_2 (1.2 g) was chromatographed in sequence on a silica gel column, a normal-phase HPLC preparation (hexane−EtOAc, 6:4), a reversed-phase HPLC preparation (MeOH−H_2O, 6:4), and PTLC (benzene−EtOAc, 70:3) to afford peperomins A (18 mg) and E (6 mg). F_3 (600 mg) was separated with a normal-phase HPLC (hexane−EtOAc, 6:4) to give two subfractions (F_{3-1} and F_{3-2}). Peperomin B (29 mg) and compounds **1** (5 mg) and **5** (12 mg) were obtained from F_{3-1} (445 mg) by a further reversed-phase HPLC (MeOH−H_2O, 48:52). 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7,7′-Bis(5-methoxy-3,4-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran (20 mg) and 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7-(5-methoxy-3,4-methylenedioxyphenyl)-7′-(4-hydroxy-3,5-dimethoxyphenyl)-8,8′-diacetoxymethyltetrahydrofuran (1 mg) were purified by a reversed-phase HPLC column (MeOH−H_2O, 1:1) from F_{3-2} (129 mg). F_4 (400 mg) was subjected to a reversed-phase HPLC column (MeOH−H_2O, 55:45) and a Pharmadex LH-20 column (MeOH) successively, to afford peperomin C (43 mg) and compound **2** (19 mg). F_5 (200 mg) was separated with a normal-phase HPLC (hexane−EtOAc, 1:1) and a reversed-phase HPLC (MeOH−H_2O, 1:1) to yield compounds **3** (24 mg) and **4** (1 mg). The *n*-BuOH extract was divided into five fractions (B_1−B_5) using silica gel column chromatography, eluted with a gradient of $CHCl_3$ and MeOH. Isoswertisin (21 mg) was obtained by silica gel column chromatography ($CHCl_3$−MeOH, 5:1) followed by a reversed-phase HPLC column (MeOH−H_2O, 1:3) from B_3 (700 mg).

2-Methylene-3-[(3′,4′,5′-trimethoxyphenyl)(5″-methoxy-3″,4″-methylenedioxyphenyl)methyl]butyrolactone (1): colorless gum; $[\alpha]_D^{20}$ −5.0 (*c* 0.175, acetone); UV (MeOH−H_2O) λ_{max} 248, 280 nm; IR (KBr) ν_{max} 2919, 1762, 1635, 1591, 1508, 1458, 1327, 1244, 1128, and 1041 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.47 (1H, *J* = 1.2 Hz, H-2″), 6.44 (2H, s, H-2′, 6′), 6.41 (1H, d, *J* = 1.2 Hz, H-6″), 6.14 (1H, d, *J* = 2.4 Hz, H-6a), 5.95 (2H, m, OCH_2O), 4.87 (1H, d, *J* = 2.4 Hz, H-6b), 4.33 (1H, dd, *J* = 7.5, 9.9 Hz, H-4a), 4.01 (1H, dd, *J* = 3.9, 9.9 Hz, H-4b), 3.91 (3H, s, 5″-OCH_3), 3.80 (1H, overlapped, H-3), 3.84 (6H, s, 3′,5′-OCH_3), 3.81 (3H, s, 4′-OCH_3), 3.69 (1H, d, *J* = 11.4 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0 (C, C-1), 153.6 (C, C-3′,5′), 149.8 (C, C-3″), 143.9 (C, C-5″), 137.5 (C, C-1′), 137.1 (C, C-4′), 136.3 (C, C-1″), 136.1 (C, C-2), 134.6 (C, C-4″), 125.1 (CH_2, C-6), 108.5 (CH, C-6″), 105.5 (CH, C-2′,6′), 101.8 (CH_2, OCH_2O), 101.4 (CH, C-2″), 69.9 (CH_2, C-4), 61.1 (CH_3, 5″-OCH_3), 57.2 (CH_3, 4′-OCH_3), 56.2 (CH_3, 3′,5′-OCH_3), 55.8 (CH, C-5), 42.8 (CH, C-3); EIMS *m*/*z* 428 $[M]^+$ (6), 331 (100), 301 (7), 251 (7), 149 (15); HREIMS *m*/*z* 428.1470 (calcd for $C_{23}H_{24}O_8$, 428.1471).

2,3-*trans*-2-Methyl-3-[(3′-hydroxyl-4′,5′-dimethoxyphenyl)(5″-methoxy-3″,4″-methylenedioxyphenyl)methyl]butyrolactone (2): colorless gum; $[\alpha]_D^{20}$ +19.5 (*c* 0.615, acetone); UV (MeOH−H_2O) λ_{max} 247, 277 nm; IR (KBr) ν_{max} 3433, 2937, 1763, 1633, 1593, 1508, 1452, 1431, 1346, 1198, 1094, 1041, 926, and 710 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.52 (1H, *J* = 1.5 Hz, H-2′), 6.47 (1H, *J* = 1.2 Hz, H-2″), 6.40 (1H, *J* = 1.2 Hz, H-6″), 6.32 (1H, d, *J* = 1.5 Hz, H-6′), 5.92 (2H, m, OCH_2O), 4.29 (1H, dd, *J* = 7.5, 9.3 Hz, H-4a), 3.89 (3H, s, 5″-OCH_3), 3.85 (3H, s, 4′-OCH_3), 3.83 (3H, s, 5′-OCH_3), 3.80 (1H, overlapped, H-4b), 3.56 (1H, d, *J* = 11.4 Hz, H-5), 2.87 (1H, m, H-3), 2.34 (1H, m, H-2), 0.94 (3H, d, *J* = 7.2 Hz, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 180.0 (C, C-1), 152.7 (C, C-5′), 149.7 (C, C-3′), 149.6 (C, C-3″), 143.8 (C, C-5″), 138.4 (C, C-1′), 136.3 (C, C-1″), 134.8 (C, C-4′), 134.7 (C, C-4″), 107.9 (CH, C-6″), 107.1 (CH, C-2′), 104.0 (CH, C-6′), 101.7 (CH_2, OCH_2O), 101.4 (CH, C-2″), 70.5 (CH_2, C-4), 61.2 (CH_3, 4′-OCH_3), 57.1 (CH_3, 5″-OCH_3), 56.3 (CH, C-5), 56.1 (CH_3, 5′-OCH_3), 47.3 (CH, C-3), 40.4 (CH, C-2), 16.0 (CH_3, C-6); EIMS *m*/*z* 416 $[M]^+$ (16), 347 (11), 317 (100), 287 (3); HREIMS *m*/*z* 416.1491 (calcd for $C_{22}H_{24}O_8$, 416.1471).

7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7-(5-Methoxy-3,4-methylenedioxyphenyl)-7′-(4-hydroxy-3,5-dimethoxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran (3): colorless amorphous powder; $[\alpha]_D^{20}$ −8.1 (*c* 0.630, acetone); UV (MeOH−H_2O) λ_{max} 239, 276 nm; IR (KBr) ν_{max} 3446, 3010, 2941, 1734, 1635, 1614, 1514, 1456, 1321, 1238, 1115, 1040, 837 and 754 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.69 (1H, s, H-2), 6.64 (3H, s, H-6, 2′, 6′), 5.96 (2H, m, OCH_2O), 5.65 (1H, brs, OH), 5.05 (1H, d, *J* = 6.9 Hz, H-7′), 4.56 (1H, d, *J* = 8.1 Hz, H-7), 4.24 (2H, m, H-9), 3.90 (3H, s, 5-OCH_3), 3.87 (6H, s, 3′,5′-OCH_3), 3.44 (1H, dd, *J* = 6.3, 11.4 Hz, H-9′a), 3.34 (1H, dd, *J* = 6.6, 11.4 Hz, H-9′b), 2.50 (1H, m, H-8′), 2.36 (1H, m, H-8), 2.02 (3H, s, CH_3CO); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.1 (C, *C*OCH_3), 149.3 (C, C-3), 147.4 (C, C-3′, 5′), 143.6 (C, C-5), 135.1 (C, C-1, 4), 134.3 (C, C-4′), 129.2 (C, C-1′), 106.9 (CH, C-6), 102.8 (CH, C-2′, 6′), 101.7 (CH_2, OCH_2O), 100.7 (CH, C-2), 83.1 (CH, C-7), 81.6 (CH, C-7′), 64.6 (CH_2, C-9), 63.1 (CH_2, C-9′), 56.8 (CH_3, 5-OCH_3), 56.5 (CH_3, 3′,5′-OCH_3), 49.9 (CH, C-8), 49.2 (CH, C-8′), 21.0 (CH_3, *C*H_3CO); EIMS *m*/*z* 476 $[M]^+$ (63), 338 (39), 210 (94), 206 (31), 203 (72), 198 (37), 181 (65), 179 (100); HREIMS *m*/*z* 476.1671 (calcd for $C_{24}H_{28}O_{10}$, 476.1683).

7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-7,7′-(4-Hydroxyl-3,5-dimethoxyphenyl)-8,8′-diacetoxymethyltetrahydrofuran (4): colorless amorphous powder; UV (MeOH−H_2O) λ_{max} 239, 271 nm; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.72 (2H, s, H-2, 6), 6.62 (2H, s, H-2′, 6′), 5.56 (1H, brs, OH), 5.52 (1H, brs, OH), 5.10 (1H, d, *J* = 6.9 Hz, H-7′), 4.62 (1H, d, *J* = 7.8 Hz, H-7), 4.27 (2H, m, H-9), 3.92 (6H, s, OCH_3), 3.88 (6H, s, OCH_3), 3.82 (2H, m, H-9′), 2.70 (1H, m, H-8′), 2.38 (1H, m, H-8),

2.04 (3H, s, CH_3CO-9), 1.90 (3H, s, CH_3CO-9′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.2 (C, 2 × $COCH_3$), 147.4 (C, C-3, 5), 147.2 (C, C-3′, 5′), 134.8 (C, C-4, 4′), 131.5 (C, C-1), 129.2 (C, C-1′), 103.5 (CH, C-2, 6), 102.9 (CH, C-2′, 6′), 83.3 (CH, C-7), 81.3 (CH, C-7′), 64.7 (CH_2, C-9′), 64.6 (CH_2, C-9), 56.5 (CH_3, 4 × OCH_3), 50.3 (CH, C-8), 45.7 (CH, C-8′), 21.1 (CH_3, CH_3CO), 20.9 (CH_3, CH_3CO); EIMS *m*/*z* 520 $[M]^+$ (5), 430 (16), 331 (100), 252 (6), 197 (6), 181 (6); HREIMS *m*/*z* 520.1940 (calcd for $C_{26}H_{32}O_{11}$, 520.1945).

5,6,8-Trimethoxy-4-(2,4,5-trimethoxyphenyl)-3,4-dihydro-1(2*H*)-naphthalenone (5): yellowish gum; $[\alpha]_D^{20}$ −3.0 (*c* 0.175, acetone); UV (MeOH−H_2O) λ_{max} 234, 280, 324 nm; IR (KBr) ν_{max} 2937, 2843, 1670, 1591, 1508, 1458, 1392, 1321, 1209, 1080, 1036, 955, and 810 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.57 (1H, s, H-3′), 6.48 (1H, s, H-7), 6.19 (1H, s, H-6′), 5.03 (1H, m, H-4), 3.95 (3H, s, 8-OCH_3), 3.92 (3H, s, 6-OCH_3), 3.89 (3H, s, 2′-OCH_3), 3.86 (3H, s, 4′-OCH_3), 3.56 (3H, s, 5′-OCH_3), 3.36 (3H, s, 5-OCH_3), 2.43 (2H, m, H-2), 2.16 (2H, m, H-3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 197.6 (C, C-1), 158.5, 157.8 (C, C-6, 8), 151.7 (C, C-2′), 148.5 (C, C-4′), 142.7 (C, C-5′), 142.1 (C, C-10), 139.3 (C, C-5), 123.1 (C, C-1′), 116.6 (C, C-9), 114.4 (CH, C-6′), 98.3 (CH, C-3′), 95.9 (CH, C-7), 60.7 (CH_3, 5-OCH_3), 57.3 (CH_3, 5′-OCH_3), 56.7 (CH_3, 2′-OCH_3), 56.6 (CH_3, 8-OCH_3), 56.4 (CH_3, 4′-OCH_3), 55.9 (CH_3, 6-OCH_3), 35.9 (CH_2, C-2), 32.5 (CH, C-4), 27.6 (CH_2, C-3); EIMS *m*/*z* 402 $[M]^+$ (100), 387 (9), 371 (16); HREIMS *m*/*z* 402.1704 (calcd for $C_{22}H_{26}O_7$, 402.1679).

Cell Proliferation Assay. HL-60 and MCF-7 were derived from human acute promyelocytic leukemia and breast cancer, respectively. HeLa is a human cervical cancer cell line. MCF-7 and HeLa cells were maintained in low-glucose DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS). HL-60 cells were maintained in RPMI1640 medium supplemented with 10% (v/v) FBS.

Medium (90 μL) containing ca. 2000 MCF-7 cells and 3000 HeLa cells was incubated at 37 °C in a humidified atmosphere of 5% CO_2 overnight in a 96-well microtiter plate. For MCF-7 cells, test samples (10 μL) were added to the medium and incubated for 72 h. Following change of medium and samples, incubation was continued for an additional 45 h. A total of 69 incubation hours were executed for HeLa cells without change of medium and samples. Coloration substrate, MTT (methyl thiazol tetrazolium), was added to the medium followed by further incubation for 3 h. The medium was then displaced by 150 μL of DMSO and absorbance at 560 nm with a 690 nm reference measured.

Medium (90 μL) containing ca. 10000 HL-60 cells was incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 4 h in a 96-well microtiter plate. Test samples (10 μL) were added to the medium, and incubation was continued for 69 h. Coloration substrate, cell counting kit-8 (CCK-8), was added to the medium followed by further incubation for 3 h. Absorbance at 450 nm with a 600 nm reference was measured thereafter.

Cell viability (%) was assessed as [(experimental absorbance − background absorbance)/(control absorbance − background absorbance)] × 100, and IC_{50} was calculated as the concentration of ligand required to give half-maximal inhibition on the cell growth.

Cotransfection Assay. CV-1 cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 for 24 h in a 6 cm plate. Human estrogen receptor α (ERα) gene contained in pcDNA3.1 (pcDNA3.1-hERα) and a luciferase report gene plasmid (ERE-MMTV-Luc) were cotransfected into the cells followed by additional 8 h incubation. The transfected cells were seeded onto a 96-well microtiter plate (10 000/well) and different concentrations of the test compound added. For blocking studies, raloxifene, an ER antagonist, was added 0.5 h before the introduction of estradiol (1 nM, positive control) or the test compound (20 μM). After a 24 h incubation period, the expressed luciferase activity was determined in a Wallac 1420 multilabel counter (Victor^2, Perkin-Elmer) using the Steady-Glo luciferase assay solutions from Promega.

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