# Bioactive Polyketides from *Peperomia duclouxii*

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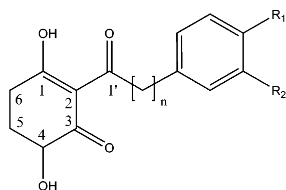
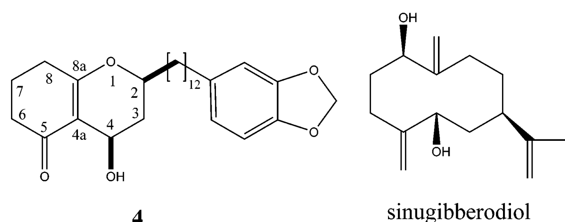
Four new compounds (**1**−**4**) were isolated along with 16 known compounds from whole plants of *Peperomia duclouxii*. The new structures were elucidated as 4-hydroxy-2-[(3,4-methylenedioxyphenyl)nonanoyl]cyclohexane-1,3-dione (**1**), 4-hydroxy-2-[(3,4-methylenedioxyphenyl)undecanoyl]cyclohexane-1,3-dione (**2**), 4-hydroxy-2-[(3,4-methylenedioxyphenyl)tridecanoyl]cyclohexane-1,3-dione (**3**), and 2-[(3,4-methylenedioxyphenyl)dodecyl]-4-hydroxy-2,3,4,6,7,8-hexahydro-2*H*-1-benzopyran-5-one (**4**), by analysis of their spectroscopic data. The known polyketides, surinone A and oleiferinone, showed cell growth inhibitory activity against the WI-38, VA-13, and HepG2 cell lines with $IC_{50}$ values that ranged from 4.4 to 9.6 μg/mL. The known sesquiterpenoid, sinugibberodiol, showed a more potent effect on calcein accumulation than verapamil at 2.5 and 25 μg/mL. Compounds **3** and **4**, surinone A, and oleiferinone showed moderate to weak inhibitory activity on the induction of the intercellular adhesion molecule-1 (ICAM-1) in the presence of IL-1α or TNF-α.

*Peperomia duclouxii* C. DC. in Lecomte (Piperaceae) is traditionally used as an anticancer agent in mainland China,[1] and a detailed introduction has been written in a previous publication.[2] Our previous investigations have afforded several lignans with cytotoxic and anti-inflammatory activities.[2−4] Presently, four new polyketides (**1**−**4**) and 16 known compounds have also been obtained from the whole plants of *P. duclouxii*. In this article, we report the structural elucidation and cytotoxic and anti-inflammatory bioactivities of the compounds isolated.




**1** $R_1,R_2 = OCH_2O$, n = 8
**2** $R_1,R_2 = OCH_2O$, n = 10
**3** $R_1,R_2 = OCH_2O$, n = 12
surinone A $R_1,R_2 = OCH_2O$, n = 14
oleiferinone $R_1 = R_2 = H$, n = 12

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## Results and Discussion

Compound **1** was assigned the molecular formula $C_{22}H_{28}O_6$ from the high-resolution EIMS. The IR spectrum showed the presence of conjugated carbonyl (1666 $cm^{-1}$) and chelated conjugated carbonyl (1566 $cm^{-1}$) groups. Three protons of an ABX system [δ 6.67 (1H, d, $J = 0.8$ Hz, H-11′), 6.71 (1H, d, $J = 8.1$ Hz, H-14′), 6.61 (1H, dd, $J = 0.8$, 8.1 Hz, H-15′)] and a methylenedioxy signal [δ 5.91 (2H, s)] in the $^1$H NMR spectrum indicated the occurrence of a 3,4-methylenedioxyphenyl group. One oxymethine [δ 4.08 (1H, dd, $J = 5.4$, 13.0 Hz, H-4)] and two methylenes [δ 2.38 (1H, dddd, $J = 5.1$, 5.1, 2.7, 13.0 Hz, H-5eq) and 1.82 (1H, dddd, $J = 6.8$, 11.6, 13.0, 13.0 Hz, H-5ax), 2.79 (1H, m, H-6a), and 2.78 (1H, m, H-6b)] were found to be connected to an $O-^4CH-^5CH_2-^6CH_2-$ moiety using the $^1H-^1H$ COSY spectrum. The enolated carbonyl (δ 197.9, C-1) was linked with the methylene at C-6 and the quaternary carbon at δ 110.3 (C-2) from the HMBC spectrum (Figure 1). Similarly, the carbonyl carbon at δ 195.6 (C-3) could be linked to C-4. The HMBC cross-peaks between the carbonyl carbon (δ 206.0, C-1′) and the protons of $^{2'}CH_2{}^{3'}CH_2$ [δ 3.07 (1H, ddd, $J = 6.1$, 8.9, 16.0 Hz, H-2′a), 2.96 (1H, ddd, $J = 6.3$, 8.8, 16.0 Hz, H-2′b), and 1.63 (2H, m, H-3′)] suggested their linkage. A long aliphatic chain was indicated from the multiple-proton signal in the range δ 1.25−1.40 in the $^1$H NMR spectrum, and it was connected to the 3,4-methylenedioxyphenylethyl [δ 1.56 (2H, m, H-8′) and 2.51 (2H, t, $J = 7.6$ Hz, H-9′)] and the acylethyl ($^{3'}CH_2-^{2'}CH_2-^{1'}CO$) groups from the $^1H-^1H$ COSY spectrum. The number of the methylenes was determined as eight from the $^{13}$C NMR spectrum and the molecular formula. The degree of unsaturation of the above moieties was also eight, so the two carbonyls (C-3 and C-1′) were connected to the quaternary carbon (C-2) to account for the remaining one degree of unsaturation. Thus, compound **1** was determined as a 4-hydroxy-2-acylcyclohexane-1,3-dione derivative and exhibited the characteristic three carbonyl carbons (C-1, C-3, and C-1′) and the quaternary carbon (C-2) of this type of compound.[5] Compound **1** was assigned as 4-hydroxy-2-[(3,4-methylenedioxyphenyl)nonanoyl]cyclohexane-1,3-dione. The 4-hydroxy group was equatorially orientated from the large coupling constant ($J = 13.0$ Hz) between H-4 and H-5ax.

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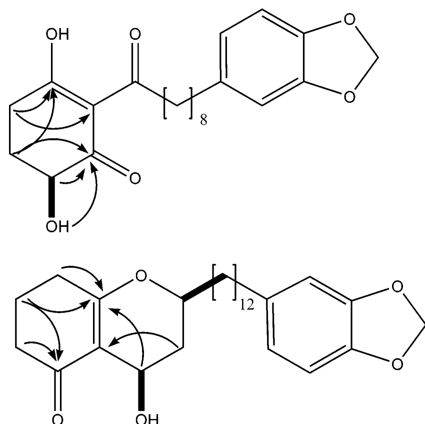

**Figure 1.** Key HMBC correlations in compounds **1** and **4**.

Compounds **2** and **3** disclosed very close $^{1}$H and $^{13}$C NMR spectra to those of compound **1**, and differences were due to the number of methylenes of their aliphatic chains ($\delta_C$ 29.2−29.6, $\delta_H$ 1.30). The molecular formulas of **2** and **3** were $C_{24}H_{32}O_6$ and $C_{26}H_{36}O_6$, respectively, from their HREIMS. Thus, compound **2** was assigned as 4-hydroxy-2-[(3,4-methylenedioxyphenyl)undecanonyl]cyclohexane-1,3-dione and compound **3** as 4-hydroxy-2-[(3,4-methylenedioxyphenyl)tridecanonyl]cyclohexane-1,3-dione.

The molecular formula of compound **4** was established as $C_{28}H_{40}O_5$ from the ion peak at *m/z* 456.2886 in the HREIMS. Similar to compounds **1**−**3**, it showed evidence for a 3,4-methylenedioxyphenylalkyl chain from the $^{1}$H NMR, $^{13}$C NMR, and $^{1}$H−$^{1}$H COSY spectra. Two moieties, $^{6}CH_2-^{7}CH_2-^{8}CH_2$ and $O-^{4}CH-^{3}CH_2-^{2}CH(O)-^{1'}CH_2-^{2'}CH_2$, were also evident from the $^{1}$H−$^{1}$H COSY spectrum (Table 2). One carbonyl carbon [$\delta$ 200.6 (C-5)] and two quaternary olefinic carbons [$\delta$ 173.3 (C-8a) and 114.7 (C-4a)] were established from the $^{13}$C NMR spectrum. The carbonyl carbon correlated with H-6 and H-7 in the HMBC spectrum (Figure 1), while C-8a showed HMBC cross-peaks with H-4, H-7, and H-8, and C-4a with H-3 and H-8. Thus, the moiety $O{=}^{5}C-^{6}CH_2-^{7}CH_2-^{8}CH_2-^{8a}C{=}^{4a}C-^{4}CH(O)-^{3}CH_2-^{2}CH(O)-^{1'}CH_2-^{2'}CH_2$ was established and confirmed by the homoallylic coupling between H-4 and H-8 in the $^{1}$H−$^{1}$H COSY spectrum. The $^{1}$H−$^{1}$H COSY spectrum also suggested the linkage of $^{2'}CH_2$ and the alkyl chain. Since two degrees of unsaturation were remaining, two alternative structures were possible, i.e., C-5 connected to C-4a, and the oxygen at C-2 to C-8a, or C-5 connected to C-8a, and the oxygen at C-2 to C-4a. The downfield chemical shift of C-8a ($\delta$ 173.3) suggested that it was substituted at the $\beta$-position of the $\alpha,\beta$-unsaturated ketone and connected to the oxygen atom. The alkyl chain contained 12 methylenes from the molecular formula; thus compound **4** was established as 2-[(3,4-methylenedioxyphenyl)-dodecyl]-4-hydroxy-2,3,4,6,7,8-hexahydro-2*H*-1-benzopyran-5-one. The NOEs between H-2, H-3a, and H-4 indicated their *cis*-configuration.

In addition to the above new compounds, two known polyketides (surinone A[5] and oleiferinone[6]), (1,3-benzodioxole)tridecanoic acid, (1,3-benzodioxole)nonanoic acid,[7] (1,3-benzodioxole)pentanoic acid, sinugibberodiol,[8] *N*-[2-(3,4-dihydroxyphenyl)ethyl]-3,4-dihydroxybenzamide, *trans*-*N*-sinapoyltyramine, *trans*-*N*-feruloyl-3-*O*-methyldopamine, *trans*-*N*-feruloyldopamine, pipercallosine, pipercallosidine, (−)-loliolide, 3-*O*-α-ionol, syringic acid, and anisic acid were also obtained from this species. Their structures were determined by comparison of NMR data with the corresponding literature values or by comparison with the authentic standard compounds.

The cytotoxic activities of the compounds isolated were evaluated on three cell lines, namely, normal lung fibroblast cells (WI-38), malignant lung tumor cells (VA-13), and liver tumor cells (HepG2). The known polyketides surinone A and oleiferinone showed cytotoxic activities against these three cell lines with $IC_{50}$ values ranging from 4.4 to 9.6 $\mu$g/mL (Table 3).

One mechanism underlying multidrug resistance (MDR) in mammalian tumor cells relates to enhanced removal of drugs due to overexpression of efflux transporter proteins, such as P-glycoprotein (Pgp), and multidrug resistance protein (MRP).[9] Thus, agents that inhibit these proteins may be able to overcome the MDR effect. The calcein derived from calcein AM by endogenous esterase can be used as a readily operated functional fluorescent probe for this drug efflux protein.[10−12] The effects of compounds **3** and **4**, surinone A, oleiferinone, loliolide, *N*-[2-(3,4-dihydroxyphenyl)-ethyl]-3,4-dihydroxybenzamide, and sinugibberodiol on the accumulation of calcein were evaluated using MDR 2780AD cells, with a known MDR reversal agent, verapamil, as positive control.

**Table 1.** NMR Spectroscopic Data for Compound **1** in $CDCl_3$[a]

| position | $\delta_C$ | $\delta_H$ (*J*, Hz) | $^{1}$H−$^{1}$H COSY | HMBC |
|---|---|---|---|---|
| 1 | 197.9 | | | H-5, H-6 |
| 2 | 110.3 | | | H-6b |
| 3 | 195.6 | | | H-4, H-5, OH-4 |
| 4 | 71.6 | 4.08 (1H, dd, 5.4, 13.0) | H-5, OH-4 | H-5, H-6, OH-4 |
| 5 | 27.1 | 2.38 (1H, dddd, 5.1, 5.1, 2.7, 13.0) | H-4, H-6 | H-4, H-6, OH-4 |
| | | 1.82 (1H, dddd, 6.8, 11.6, 13.0, 13.0) | | |
| 6 | 31.3 | 2.79 (1H, m) | H-5 | H-4, H-5 |
| | | 2.78 (1H, m) | | |
| 1′ | 206.0 | | | H-2′, H-3′ |
| 2′ | 40.2 | 3.07 (1H, ddd, 6.1, 8.9, 16.0) | H-3′ | H-3′, H-4′ |
| | | 2.96 (1H, ddd, 6.3, 8.8, 16.0) | | |
| 3′ | 24.5 | 1.63 (2H, m) | H-2′ | H-2′, H-5′ |
| 4′ | 29.3 | 1.36 (2H, m) | H-5′ | H-2′, H-3′ |
| 5′ | 29.3 | 1.31 (2H, m) | H-4′ | H-3′ |
| 6′ | 29.3 | 1.31 (2H, m) | | |
| 7′ | 29.1 | 1.31 (2H, m) | H-8′ | H-8′, H-9′ |
| 8′ | 31.7 | 1.56 (2H, m) | H-7′, H-9′ | H-7′, H-9′ |
| 9′ | 35.6 | 2.51 (2H, t, 7.6) | H-8′, H-11′, H-15′ | H-7′, H-8′, H-11′, H-15′ |
| 10′ | 136.7 | | | H-8′, H-9′, H-14′ |
| 11′ | 108.8 | 6.67 (1H, d, 0.8) | H-9′, H-15′ | H-9′, H-15′ |
| 12′ | 147.4 | | | H-11′, H-14′, $OCH_2O$ |
| 13′ | 145.4 | | | H-11′, H-14′, H-15′, $OCH_2O$ |
| 14′ | 108.0 | 6.71 (1H, d, 8.1) | H-15′ | |
| 15′ | 121.0 | 6.61 (1H, dd, 0.8, 8.1) | H-9′, H-11′, H-14′ | H-9′, H-11′ |
| $OCH_2O$ | 100.7 | 5.91 (2H, s) | | |
| OH-4 | | 4.02 (1H, brs) | H-4 | |

[a] Signals were assigned from the $^{1}$H−$^{1}$H COSY, HMQC, and HMBC spectra.

**Table 2.** NMR Spectroscopic Data for Compound **4** in $CDCl_3$[a]

| position | $\delta_C$ | $\delta_H$ ($J$, Hz) | $^1H-^1H$ COSY | HMBC |
|---|---|---|---|---|
| 2 | 77.1 | 3.99 (1H, dddd, 1.7, 5.4, 7.2, 11.2) | H-3, H-1′ | H-3, H-1′ |
| 3 | 35.1 | 2.20 (1H, ddd, 2.0, 6.8, 13.7) | H-2, H-4 | H-4 |
| | | 1.69(1H, m) | | |
| 4 | 62.1 | 4.74 (1H, dd, 6.8, 9.8) | H-3, H-8 | H-2, H-3 |
| 4a | 114.7 | | | H-3, H-8 |
| 5 | 200.6 | | | H-6, H-7 |
| 6 | 36.7 | 2.40 (1H, m) | H-7 | H-7, H-8 |
| | | 2.33 (1H, dd, 7.8, 16.7) | | |
| 7 | 20.5 | 1.97 (1H, dd, 6.8, 12.9) | H-6, H-8 | H-6, H-8 |
| | | 1.96 (1H, dd, 6.1, 12.9) | | |
| 8 | 28.4 | 2.38 (2H, m) | H-4, H-7 | H-6, H-7 |
| 8a | 173.3 | | | H-4, H-7, H-8 |
| 1′ | 34.7 | 1.74 (1H, m) | H-2, H-2′ | |
| | | 1.62 (1H, m) | | |
| 2′ | 25.0 | 1.55 (1H, m) | H-1′, H-3′ | |
| | | 1.45 (1H, m) | | |
| 3′−10′ | 29.2−29.6 | 1.27 (16H, m) | H-11′ | |
| 11′ | 31.7 | 1.55 (2H, m) | H-10′, H-12′ | H-12′ |
| 12′ | 35.7 | 2.51 (2H, t, 7.7) | H-11′, H-14′, H-18′ | H-10′, H-11′, H-14′, H-18′ |
| 13′ | 136.8 | | | H-11′, H-12′, H-17′ |
| 14′ | 108.8 | 6.67 (1H, d, 1.5) | H-12′, H-18′ | H-12′, H-18′ |
| 15′ | 147.4 | | | H-14′, H-17′, $OCH_2O$ |
| 16′ | 145.3 | | | H-14′, H-17′, H-18′, $OCH_2O$ |
| 17′ | 108.0 | 6.71 (1H, d, 7.8) | H-18′ | |
| 18′ | 121.0 | 6.61 (1H, dd, 1.5, 7.8) | H-12′, H-14′, H-17′ | H-12′, H-14′ |
| $OCH_2O$ | 100.7 | 5.91 (2H, s) | | |

[a] Signals were assigned from the $^1H-^1H$ COSY, HMQC, and HMBC spectra.

**Table 3.** Cell Growth Inhibitory Effects of Compounds from *Peperomia duclouxii* against the WI-38, VA-13, and HepG2 Cell Lines ($IC_{50}$ $\mu$g/mL)[a,b]

| compound | WI-38 | VA-13 | HepG2 |
|---|---|---|---|
| **4** | >20 | >20 | 18.2 |
| surinone A | 8.6 | 4.4 | 7.4 |
| oleiferinone | 9.6 | 5.8 | 9.2 |
| paclitaxel | 0.05 | 0.01 | 0.69 |
| adriamycin | 0.71 | 0.39 | 0.41 |

[a] Cell growth inhibitory effects on three cells were determined, and $IC_{50}$ is defined as the compound concentration causing 50% growth inhibition. [b] Loliolide, 3-*O*-α-ionol, *trans-N*-sinapoyltyramine, *trans-N*-feruloyl-3-*O*-methyldopamine, *trans-N*-feruloyldopamine, and pipercallosidine were inactive against all three cell lines ($IC_{50}$ > 20 $\mu$g/mL).

**Table 4.** Effects of Sinugibberodiol on the Accumulation of Calcein in MDR 2780AD Cells[a]

| compound | conc ($\mu$g/mL) | average fluorescence/well[b] | % of control[c] | verapamil %[d] |
|---|---|---|---|---|
| control | 0 | 3013 | | |
| verapamil | 0.25 | 3422 | 114 | 100 |
| | 2.5 | 3441 | 114 | 100 |
| | 25 | 4167 | 138 | 100 |
| sinugibberodiol | 0.25 | 3405 | 113 | 100 |
| | 2.5 | 4126 | 137 | 120 |
| | 25 | 4815 | 160 | 116 |

[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 $\mu$g/mL of each test compound. [b] Values represent the mean of triplicate determinations. [c] Values are the relative amount of calcein accumulated in the cell compared with the control experiment. [d] Values are expressed as the relative amount of calcein accumulation in the cell as compared with that of verapamil.

The known sesquiterpenoid sinugibberodiol showed a more potent activity than verapamil, at 2.5 and 25 $\mu$g/mL (Table 4).

Expression of excess amounts of ICAM-1 on the surface of endothelial cells of a blood vessel plays an important role in the progress of the inflammatory reaction.[13−15] The inhibitory effects on the induction of ICAM-1 were evaluated in the presence of IL-1α or TNF-α, using human A549 cells (human lung carcinoma), and the cell viability was measured by an MTT assay. Compounds **3** and **4**, surinone A, and oleiferinone showed moderate to weak inhibitory activity, and $IC_{50}$ values were 43.3, 65.2, 51.4, and 36.8 $\mu$g/mL when the induction of ICAM-1 was stimulated using IL-1α, and 27.2, 48.8, 33.5, and 22.9 $\mu$g/mL using TNF-α. They showed weak or no toxicity to A549 cells in the MTT assay.

## Experimental Section

**General Experimental Procedures.** Optical rotations were determined with a Horiba SEPA-200 polarimeter. UV and IR spectra were recorded on a JASCO V-550 UV/vis spectrophotometer in $CHCl_3$ and a Hitachi 270-30 spectrometer in $CHCl_3$, respectively. NMR spectra were run on a Varian UNITY-PS 500 spectrometer using $CDCl_3$ and $CD_3OD$ as solvents. HREIMS were recorded on a JEOL JMS DX-303 and a JEOL Mstation JMS-700 mass spectrometer. HPLC separations were performed on a Hitachi L-6200 HPLC instrument with an Inertsil Prep-sil GL 10 × 250 mm stainless steel column or an Inertsil Prep-ODS GL 10 × 250 mm column and monitored by a Hitachi L-7400 UV detector and a Shodex SE-61 RI detector.

**Plant Material.** The whole plants of *P. duclouxii* were collected from Lvchun, 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 (2002-2) has been deposited at the Faculty of Engineering, Niigata University, Japan.

**Extraction and Isolation.** The dried plant material (1.65 kg) was powdered and extracted four times (7.5 L/each) with MeOH at room temperature, and about 100 g of a residue was obtained after evaporating the solvents in vacuo. The residue was suspended in $H_2O$ and partitioned with hexane, EtOAc, and *n*-BuOH, respectively, to afford a hexane extract (17.3 g), an EtOAc extract (29.0 g), and a *n*-BuOH extract (15.0 g). The hexane extract was divided into four fractions ($FH_1-FH_4$) with silica gel column chromatography using a gradient of mixtures of hexane and EtOAc of increasing polarity as solvents. Fraction $FH_4$ (2.8 g) was subjected to further silica gel column chromatography to afford nine subfractions ($FH_{4-1}-FH_{4-9}$) eluting with hexane and EtOAc. Compounds **3** (33.7 mg) and **4** (9.4 mg), oleiferinone (6.9 mg), surinone A (1 mg), and pipercallosine (7.9 mg) were obtained from $FH_{4-4}$ after repeated normal-phase HPLC separations [hexane−EtOAc (8:2, 82:18, and 85:15)]. (1,3-Benzodioxole)pentanoic acid (5.4 mg), pipercallosidine (5.1 mg), and anisic acid (4.5 mg) were isolated from $FH_{4-5}$, and (−)-loliolide (2.0 mg) and 3-oxo-α-ionol (2.5 mg) from $FH_{4-7}$, using a normal-phase HPLC system. The EtOAc extract was chromatographed over a silica gel column eluting with hexane and EtOAc to give five fractions ($F_1-F_5$). Fraction $F_1$ (2.7 g) was divided into

four subfractions ($F_{1-1}$−$F_{1-4}$) by silica gel column chromatography eluting with hexane and gradient mixtures of hexane and EtOAc of increasing polarity, and sinugibberodiol (2 mg) was afforded from $F_{1-4}$. Fraction $F_2$ (3.18 g) was divided into five subfractions ($F_{2-1}$−$F_{2-5}$), and $F_{2-3}$ gave compounds **1** (2.6 mg) and **2** (4.7 mg), (1,3-benzodioxole)tridecanoic acid (2.2 mg), and (1,3-benzodioxole)nonanoic acid (1.4 mg) on repeated normal-phase HPLC [hexane−EtOAc (75:25 and 85:15)]. Fraction $F_3$ (2.92 g) was separated into five subfractions using silica gel column chromatography ($F_{3-1}$−$F_{3-5}$). *N*-[2-(3,4-Dihydroxyphenyl)ethyl]-3,4-dihydroxybenzamide (5.1 mg) was obtained from $F_{3-4}$ by normal-phase HPLC [hexane−EtOAc (1:1 and 65:35)]. Fraction $F_4$ (2.4 g) was divided into nine subfractions ($F_{4-1}$−$F_{4-9}$), and *trans-N*-feruloyl-3-*O*-methyldopamine (9.4 mg), *trans-N*-sinapoyltyramine (6.7 mg), *trans-N*-feruloyldopamine (4.9 mg), and syringic acid (8.5 mg) were purified from $F_{4-4}$, $F_{4-5}$, $F_{4-7}$, and $F_{4-8}$, respectively, on repeated normal-phase HPLC.

**4-Hydroxy-2-[(3,4-methylenedioxyphenyl)nonanoyl]cyclohexane-1,3-dione (1):** colorless gum; $[\alpha]_D^{25}$ +4.9 (*c* 0.59, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ 242 (4.22), 278 (4.37) nm; IR ($CHCl_3$) $\nu_{max}$ 2936, 2860, 1666, 1566, 1492, 1446, 1414, 1316, 1246, 1116, 1076, 1044 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) and $^{13}C$ NMR ($CDCl_3$, 125 MHz) data, see Table 1; EIMS *m/z* 389 $[M + H]^+$ (13), 388 $[M]^+$ (48), 135 (10); HREIMS *m/z* 388.1845 ($C_{22}H_{28}O_6$ requires 388.1816).

**4-Hydroxy-2-[(3,4-methylenedioxyphenyl)undecanoyl]cyclohexane-1,3-dione (2):** colorless gum; $[\alpha]_D^{25}$ +3.4 (*c* 0.29, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ 242 (3.66), 278 (3.81) nm; IR ($CHCl_3$) $\nu_{max}$ 2936, 2864, 1664, 1560, 1492, 1446, 1414, 1318, 1246, 1116, 1078, 1044, 940, 804 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 6.72 (1H, d, $J$ = 7.8 Hz, H-16′), 6.67 (1H, d, $J$ = 1.2 Hz, H-13′), 6.61 (1H, dd, $J$ = 1.2, 7.8 Hz, H-17′), 5.91 (2H, s, $OCH_2O$), 4.08 (1H, dd, $J$ = 5.4, 13.2 Hz, H-4), 4.03 (1H, brs, OH-4), 3.07 (1H, ddd, $J$ = 6.1, 8.8, 16.1 Hz, H-2′a), 2.96 (1H, ddd, $J$ = 6.2, 8.8, 16.1 Hz, H-2′b), 2.79 (1H, m, H-6a), 2.78 (1H, m, H-6b), 2.51 (2H, t, $J$ = 7.6 Hz, H-11′), 2.38 (1H, dddd, $J$ = 5.1, 5.4, 2.7, 12.9 Hz, H-5eq), 1.82 (1H, dddd, $J$ = 6.8, 11.9, 12.9, 13.2 Hz, H-5ax), 1.62 (2H, m, H-3′), 1.55 (2H, m, H-10′), 1.29 (12H, m, H-4′−H-9′); $^{13}C$ NMR ($CDCl_3$, 125 MHz) $\delta$ 206.1 (C-1′), 197.9 (C-1), 195.6 (C-3), 147.4 (C-14′), 145.3 (C-15′), 136.8 (C-12′), 121.0 (C-17′), 110.3 (C-2), 108.8 (C-13′), 108.0 (C-16′), 100.7 ($OCH_2O$), 71.6 (C-4), 40.2 (C-2′), 35.7 (C-11′), 31.7 (C-10′), 31.3 (C-6), 29.2−29.6 (C-4′-C-9′), 27.2 (C-5), 24.5 (C-3′); EIMS *m/z* 417 $[M + H]^+$ (29), 416 $[M]^+$ (100), 183 (25), 135 (100); HREIMS *m/z* 416.2216 ($C_{24}H_{32}O_6$ requires 416.2199).

**4-Hydroxy-2-[(3,4-methylenedioxyphenyl)tridecanoyl]cyclohexane-1,3-dione (3):** colorless gum; $[\alpha]_D^{25}$ −2.5 (*c* 0.37, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ 241 (3.82), 278 (4.00) nm; IR ($CHCl_3$) $\nu_{max}$ 2936, 2860, 1664, 1558, 1492, 1446, 1416, 1314, 1248, 1208, 1114, 1076, 1042, 928 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 6.71 (1H, d, $J$ = 7.8 Hz, H-18′), 6.67 (1H, d, $J$ = 1.2 Hz, H-15′), 6.61 (1H, dd, $J$ = 1.2, 7.8 Hz, H-19′), 5.91 (2H, s, $OCH_2O$), 4.08 (1H, dd, $J$ = 5.4, 13.2 Hz, H-4), 3.07 (1H, ddd, $J$ = 6.1, 8.8, 15.9 Hz, H-2′a), 2.96 (1H, ddd, $J$ = 6.4, 9.0, 15.9 Hz, H-2′b), 2.79 (1H, m, H-6a), 2.78 (1H, m, H-6b), 2.51 (2H, t, $J$ = 7.6 Hz, H-13′), 2.38 (1H, dddd, $J$ = 5.1, 5.1, 2.7, 13.0 Hz, H-5eq), 1.82 (1H, dddd, $J$ = 6.8, 11.5, 13.0, 13.0 Hz, H-5ax), 1.62 (2H, m, H-3′), 1.57 (2H, m, H-12′), 1.30 (16H, m, H-4′- H-11′); $^{13}C$ NMR ($CDCl_3$, 125 MHz) $\delta$ 206.1 (C-1′), 197.9 (C-1), 195.6 (C-3), 147.4 (C-16′), 145.3 (C-17′), 136.8 (C-14′), 121.0 (C-19′), 110.3 (C-2), 108.8 (C-15′), 108.0 (C-18′), 100.7 ($OCH_2O$), 71.6 (C-4), 40.3 (C-2′), 35.7 (C-13′), 31.8 (C-12′), 31.3 (C-6), 29.2−29.6 (C-4′-C-11′), 27.2 (C-5), 24.5 (C-3′); EIMS *m/z* 445 $[M + H]^+$ (32), 444 $[M]^+$ (100), 183 (10), 135 (63); HREIMS *m/z* 444.2508 ($C_{26}H_{36}O_6$ requires 444.2512).

**2-[(3,4-Methylenedioxyphenyl)dodecyl]-4-hydroxy-2,3,4,6,7,8-hexahydro-2*H*-1-benzopyran-5-one (4):** colorless gum; $[\alpha]_D^{25}$ +132.6 (*c* 0.58, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ 258 (3.93), 292 (sh) nm; IR ($CHCl_3$) $\nu_{max}$ 3036, 2936, 2860, 1612, 1490, 1430, 1382, 1334, 1310, 1250, 1084, 1044 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) and $^{13}C$ NMR ($CDCl_3$, 125 MHz) data, see Table 2; HREIMS *m/z* 456.2886 ($C_{28}H_{40}O_5$ requires 456.2876).

**Growth Inhibitory Activity to WI-38, VA-13, and HepG2 Cells in Vitro.** The cell lines were obtained from the Institute of Physical and Chemical Research (RIKEN), Tsukuba, 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., Brooklyn, Australia) with 80 $\mu$g/mL kanamycin. HepG2 cells were maintained in D-MEM medium (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) FBS (Filtron) with 80 $\mu$g/mL kanamycin. The activity was measured as previously described.[16]

**Cellular Accumulation of Calcein.** MDR ovarian cancer 2780AD cells (AD10) were maintained in PRMI-1640 medium (Invitrogen) supplemented with 10% (v/v) FBS (Filtron PTY Ltd.) with 80 $\mu$g/mL kanamycin. The activity was measured as previously described.[3]

**Inhibitory Activity on Induction of ICAM-1.** A549 cells were maintained in RPMI 1640 medium (Invitrogen) supplemented with heat-inactivated 10% (v/v) FBS (JRH Bioscience, Lenexa, KS), 100 U/mL penicillin G, and 100 $\mu$g/mL streptomycin. 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). Cell surface expression of ICAM-1 and cell viability on the basis of MTT assay were measured as previously described.[16]

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