Bioactive Lignans from *Peperomia heyneana*

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Received October 23, 2006

Four new compounds, including three secolignans (**1**−**3**) and one tetrahydrofuran lignan (**4**), were isolated from the petroleum ether and EtOAc fractions of *Peperomia heyneana*. These compounds were accompanied by eight known secolignans, one known tetrahydrofuran lignan, one known cyclohexenone, and one known amide. The structures were elucidated mainly by 1D and 2D NMR and MS experiments, and the relative configurations by NOE techniques. Five compounds were evaluated for their inhibitory activities against HIV-1 in infected C8166 cells.

Peperomia heyneana, widely growing in the southern regions of China,[1] is traditionally used to dissipate blood stasis and stop bleeding.[2] In order to identify its active components, chemical isolation and pharmacological studies were carried out. Fifteen compounds, including three new secolignans (**1**−**3**), one new tetrahydrofuran lignan (**4**), and 11 known compounds, were isolated from the petroleum ether and EtOAc extracts. The structures of the new compounds were elucidated on the basis of NMR and MS analyses, and known compounds by comparison with literature data. Anti-HIV activities of five compounds were evaluated.

The high-resolution EIMS of compound **1** gave a molecular ion peak at *m*/*z* 418.1614, in accordance with the molecular formula $C_{22}H_{26}O_8$. UV maxima were at 247 and 276 nm, and the IR spectrum showed the presence of hydroxy (3415 cm^{-1}), aromatic ring (1634, 1593, and 1460 cm^{-1}), and γ-butyrolactone (1767 cm^{-1}) functional groups. The ^1H NMR spectrum showed four aromatic protons [δ 6.57 (1H, brs, H-2′), 6.34 (1H, brs, H-6′), and 6.45 (2H, s, H-2″, 6″)], four methoxy groups [δ 3.85 (3H, s), 3.83 (6H, s), and 3.79 (3H, s)], and two phenolic hydroxy groups [δ 5.68 (1H, brs) and 5.53 (1H, brs)], which indicated the existence of a symmetrical and an unsymmetrical tetrasubstituted phenyl group. The HMBC correlations between H-2″(6″) and C-3″(5″), C-4″, between the methoxy at δ 3.83 and C-3″(5″), and between the methoxy at δ 3.79 and C-4″ suggested the presence of a 3″,4″,5″-trimethoxyphenyl group. Thus, the unsymmetrical aromatic moiety was 3′,4′-dihydroxy-5′-methoxyphenyl. The remaining oxymethylene [δ 4.30 (1H, dd, J = 8.1, 9.0 Hz, H-5a) and 3.80 (1H, overlapped, H-5b)], three methines [δ 3.57 (1H, d, J = 11.4 Hz, H-6), 2.89 (1H, m, H-4), and 2.34 (1H, m, H-3)], and a methyl resonance [δ 0.92 (3H, d, J = 7.2 Hz, H-7)] were ascribed to a $^6CH-^4CH(-^3CH-^7CH_3)-^5CH_2O$ fragment by the $^1H-^1H$ COSY spectrum. The two aromatic rings were connected to the above moiety at C-6 by the HMBC cross-peaks between C-6 and the aromatic protons. The EIMS base peak at *m*/*z* 319 (diphenylmethyl) further confirmed their linkages. A γ-butyrolactone ring was deduced from the HMBC cross-peaks between H-3, H-5a, H-7, and the lactone carbonyl [δ 179.9 (C-2)]. The NOE correlation between H-4 and CH_3-7 indicated *trans* orientation of H-3 and H-4. Thus, compound **1** is 3,4-*trans*-3-methyl-4-[(3,4-dihydroxy-5-methoxyphenyl)(3,4,5-trimethoxyphenyl)methyl]butyrolactone.

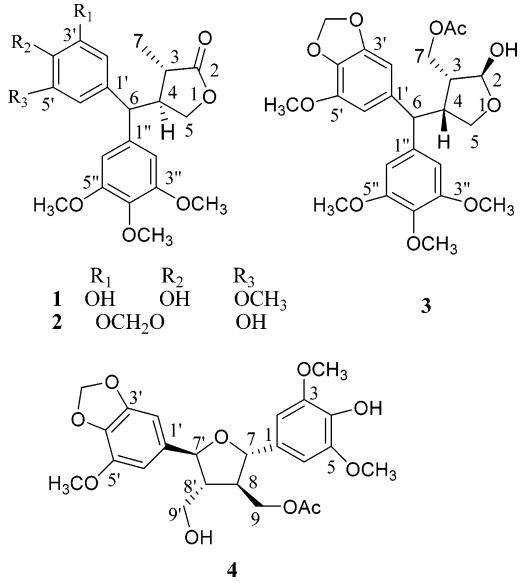

Figure 1. Structures of compounds **1**−**4**.

Compound **2** had the molecular formula $C_{22}H_{24}O_8$ from the HREIMS. UV absorption maxima were at 250 and 278 nm. The peaks at 1767 and 931 cm^{-1} in the IR spectrum were attributed to γ-butyrolactone and methylenedioxy groups, respectively. The ^1H NMR spectrum was similar to that of **1**, and the evident difference was the appearance of the methylenedioxy resonances and the disappearance of the phenolic hydroxy proton and an *O*-methyl group. The aromatic moieties were determined to be 5′-hydroxy-3′,4′-methylenedioxyphenyl and 3″,4″,5″-trimethoxyphenyl by the HMBC spectrum, which were confirmed by the EIMS base peak at *m*/*z* 317. The *trans* configuration of H-3 and H-4 was deduced from the NOESY spectrum. Thus, compound **2** is 3,4-*trans*-3-methyl-4-[(5-hydroxy-3,4-methylenedioxyphenyl)(3,4,5-trimethoxyphenyl)methyl]butyrolactone.

Compound **3** exhibited a molecular ion at *m*/*z* 490.1845 in the HREIMS, consistent with the molecular formula $C_{25}H_{30}O_{10}$. The IR spectrum indicated the presence of hydroxy (3427 cm^{-1}), aromatic ring (1633, 1591, and 1452 cm^{-1}), and ester carbonyl (1735 cm^{-1}) groups. The ^1H and ^{13}C NMR were similar to those of compounds **1** and **2**, and the significant difference was that the carbonyl at C-2 in **1** and **2** was replaced by a hemiacetal group in compound **3**. H-2 correlated with C-3, C-4, C-5, and C-7 in the HMBC spectrum. In addition, the methyl at C-3 was replaced by an acetylated hydroxymethyl group in compound **3**, which caused

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10.1021/np0605236 CCC: $37.00
Published on Web 02/10/2007

Table 1. Anti-HIV-1 Activity of Selected Compounds from *Peperomia heyneana*

compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)	selectivity index[c]
peperomin A	5.3	43.2	8.2
peperomin B	5.4	49.1	9.1
peperomin C	42.6	192.3	4.5
2-methyl-3-[(3,4,5-trimethoxyphenyl)-(3-hydroxy-4,5-dimethoxyphenyl)methyl]-butyrolactone	27.3	140.4	5.1
7,8-*trans*-8,8′ -*trans*-7′,8′-*cis*-7-(5-methoxy-3,4-methylenedioxyphenyl)-7′-(4′-hydroxy-3′,5′-dimethoxyphenyl)-8-acetoxymethyl-8′hydroxy-methyltetrahydrofuran	49.8	229.4	4.6
AZT	0.017	4552.1	$>10^5$

[a] EC_{50} was calculated as the concentration that reduced by 50% the production of syncytium in infected C8166 cells. [b] CC_{50} was the concentration that causes the reduction of viable cells by 50% compared to uninfected C8166 cells. [c] Selectivity index = CC_{50}/EC_{50}.

the downfield shifts of H-3, H-4, H-7, C-3, and C-7. The ^{1}H NMR spectrum showed the presence of 5′-methoxy-3′,4′-methylenedioxyphenyl and 3″,4″,5″-trimethoxyphenyl groups, which were connected at C-6 from the HMBC data and EIMS base peak at *m*/*z* 331. Thus, compound **3** was deduced as 3-acetoxymethyl-4-[(5-methoxy-3,4-methylenedioxyphenyl)(3,4,5-trimethoxyphenyl)methyl]tetrahydrofuran-2-ol. The H-2 singlet suggested that the dihedral angle of $H_2-C_2-C_3-H_3$ approximated 90°. It was not possible to determine the relative orientation of H-3 and H-4 from the NOESY spectrum due to the overlap of H-4, H-5b, and H-6 in $CDCl_3$. The proton resonances were well resolved in C_6D_6, and the NOE correlation between H-3 and H-4 indicated their *cis* orientation. Thus, the relative configuration of compound **3** is 2,3-*trans*-3,4-*cis*.

A molecular formula of $C_{24}H_{28}O_{10}$ was ascribed to compound **4** from the HREIMS. The IR spectrum showed bands of hydroxy and ester carbonyl groups at 3427 and 1736 cm^{-1}. The presence of a 4-hydroxy-3,5-dimethoxyphenyl and a 5′-methoxy-3′,4′-methylenedioxyphenyl group was deduced from the ^{1}H NMR and HMBC spectra. The protons at δ 4.88 (1H, d, J = 8.7 Hz, H-7), 4.97 (1H, d, J = 8.1 Hz, H-7′), 4.23 (2H, m, H-9), 3.82 (1H, m, H-9′a), 3.77 (1H, m, H-9′b), 2.51 (1H, m, H-8), and 2.27 (1H, m, H-8′) were assigned to the substructures of $O^7CH-^8CH-^9CH_2O$, $O^{7'}CH-^{8'}CH-^{9'}CH_2O$, and $^8CH-^{8'}CH$ from $^1H-^1H$ COSY data, which were further confirmed by the HMBC spectrum. Moreover, the HMBC correlations between C-1 and H-7, H-8 and between C-1′ and H-7′, H-8′ indicated the linkage of the phenyl groups at C-7 and C-7′, respectively. The hydroxy group at C-9 was acetylated on the basis of the HMBC correlation between the ester carbonyl (δ 171.1) and H-9. The degree of unsaturation was 11, and the above residue accounted for 10; thus the remaining unsaturation degree was attributed to a tetrahydrofuran ring. The structure of **4** was deduced to be 7-(4-hydroxy-3,5-dimethoxyphenyl)-7′-(5′-methoxy-3′,4′-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran. The NOE cross-peaks between H-7, H-9, and H-8′ and between H-7′, H-9′, and H-8 suggested the relative configuration as 7,8-*trans*-8,8′-*trans*-7′,8′-*trans*. The absolute configuration was not determined.

In addition to the new compounds, 11 known compounds were also obtained: peperomins A, B, C,[3] and F,[4] 2-methyl-3-[(3,4,5-trimethoxyphenyl)(3-hydroxy-4,5-dimethoxyphenyl)methyl]butyrolactone, 2-methylene-3-[(5-methoxy-3,4-methylenedioxyphenyl)(4-hydroxy-3,5-dimethoxyphenyl)methyl]butyrolactone, 2-methyl-3-[(3,4,5-trimethoxyphenyl)-(4-hydroxy-3,5-dimethoxyphenyl)methyl]butyrolactone, 2-acetoxymethyl-3-[(5-methoxy-3,4-methylene-dioxyphenyl)(4-hydroxy-3,5-dimethoxyphenyl)methyl]-butyrolactone,[5] 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,[6] 3-oxo-α-ionol,[7] and *N*-*trans*-feruloyltyramine.[8]

It was reported previously that lignans possess certain anti-HIV activities.[9] Thus, peperomins A, B, and C, 2-methyl-3-[(3,4,5-trimethoxyphenyl)(3-hydroxy-4,5-dimethoxyphenyl)methyl]butyrolactone, and 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 were evaluated in cell-based assay systems to examine their effects on HIV-1. As shown in Table 1, peperomins A and B showed moderate inhibitory effects on HIV-1 IIIB growth in C8166 cells (i.e., cytopathic effect, CPE), with EC_{50} values around 5 μM, while the three other compounds displayed much lower activities (EC_{50} > 20 μM). It appeared that the observed bioactivity was somewhat related to the cytotoxicity expressed as CC_{50} of these compounds, as determined in parallel by the MTT method.[10] This led to low selectivity indices (i.e., therapeutic index, TI < 10 calculated by dividing CC_{50} by EC_{50}). Obviously, substantial structural modification would be required if this class of lignans were selected for anti-HIV-1 drug development. Nevertheless, this is the first demonstration regarding the anti-HIV activity *in vitro* of secolignans.

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 disks, respectively. NMR spectra were obtained on Bruker AN-400, Varian Mercury 300, and Varian Inova 600 spectrometers. 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 (CC). Silica gel 60 F_{254} plates (Qingdao Ocean Chemical Industry Co.) were used for TLC analysis.

Plant Material. *P. heyneana* Miq. 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 (PH-2004-6) has been deposited in the National Center for Drug Screening, Shanghai, China.

Extraction and Isolation. The whole plant material (745 g) was powdered and extracted with EtOH (3 × 3.5 L/each) at room temperature with the aid of an ultrasonic machine, and about 73 g of residue was obtained after evaporating the solvents *in vacuo*. The residue was suspended in H_2O and partitioned in sequence with petroleum ether, EtOAc, and *n*-BuOH to afford a petroleum ether extract (26.6 g), an EtOAc extract (13.4 g), an *n*-BuOH extract (11.6 g), and a H_2O extract (19.3 g). The petroleum ether extract was subjected to silica gel CC eluting with a gradient of petroleum ether and acetone, and seven fractions ($F_{1-1}-F_{1-7}$) were obtained. The EtOAc extract was chromatographed over a silica gel column eluting with CH_2Cl_2 and MeOH and gave five fractions ($F_{2-1}-F_{2-5}$). F_{1-5} (1.0 g) was combined into F_{2-1} (7.9 g) by TLC analysis and named F_3 (8.9 g). F_3 was further separated into 13 fractions ($F_{3-1}-F_{3-13}$) by CC. Peperomins A (200

mg), B (700 mg), and C (30 mg) were isolated from F_{3-7} (2.2 g), and 3-oxo-α-ionol (10 mg) was isolated from F_{3-5} (400 mg) by ODS CC. Compound **2** (4 mg), peperomin F (70 mg), 2-methyl-3-[(3,4,5-trimethoxyphenyl)(3-hydroxy-4,5-dimethoxyphenyl)methyl]butyrolactone (11 mg), 2-methylene-3-[(5-methoxy-3,4-methylenedioxyphenyl)-(4-hydroxy-3,5-dimethoxyphenyl)methyl]butyrolactone (1 mg), and 2-methyl-3-[(3,4,5-trimethoxyphenyl)(4-hydroxy-3,5-dimethoxyphenyl)-methyl]butyrolactone (2 mg) were obtained from F_{3-9} (830 mg) by ODS or Pharmadex LH-20 CC. Compound **3** (4 mg) and 2-acetoxymethyl-3-[(5-methoxy-3,4-methylenedioxyphenyl)(4-hydroxy-3,5-dimethoxyphenyl)methyl]butyrolactone (3 mg) were isolated from F_{3-10} by ODS CC. F_{3-11} afforded compounds **1** (3 mg), **4** (5 mg), 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 (40 mg), and *N*-*trans*-feruloyltyramine (7 mg) using ODS CC.

3,4-*trans*-3-Methyl-4-[(3,4-dihydroxy-5-methoxyphenyl)(3,4,5-trimethoxyphenyl)methyl]butyrolactone (1): colorless gum; $[\alpha]_D^{20}$ +40.0 (*c* 0.270, CH_3OH); UV (CH_3OH-H_2O) λ_{max} 247, 276 nm; IR (KBr) ν_{max} 3415, 2937, 1767, 1634, 1593, 1516, 1460, 1327, 1240, 1126, and 1016 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.57 (1H, brs, H-2′), 6.45 (2H, s, H-2″, 6″), 6.34 (1H, brs, H-6′), 5.68 (1H, brs, OH), 5.53 (1H, brs, OH), 4.30 (1H, dd, J = 8.1, 9.0 Hz, H-5a), 3.85 (3H, s, 5′-OCH_3), 3.83 (6H, s, 3″, 5″-OCH_3), 3.79 (3H, s, 4″-OCH_3), 3.80 (1H, overlapped, H-5b), 3.57 (1H, d, J = 11.4 Hz, H-6), 2.89 (1H, m, H-4), 2.34 (1H, m, H-3), 0.92 (3H, d, J = 7.2 Hz, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.9 (C, C-2), 153.4 (C, C-3″, 5″), 147.1 (C, C-5′), 144.2 (C, C-3′), 137.9 (C, C-1″), 137.0 (C, C-4″), 133.3 (C, C-1′), 131.5 (C, C-4′), 107.2 (CH, C-2′), 104.5 (CH, C-2″, 6″), 102.9 (CH, C-6′), 70.5 (CH_2, C-5), 60.9 (CH_3, 4″-OCH_3), 56.2 (CH_3, 5′,3″,5″-OCH_3; CH, C-6), 47.1 (CH, C-4), 40.2 (CH, C-3), 15.8 (CH_3, C-7); EIMS *m*/*z* 418 $[M]^+$ (17), 320 (23), 319 (100), 150 (2); HREIMS *m*/*z* 418.1614 (calcd for $C_{22}H_{26}O_8$, 418.1628).

3,4-*trans*-3-Methyl-4-[(5-hydroxy-3,4-methylenedioxyphenyl)-(3,4,5-trimethoxyphenyl)methyl]butyrolactone (2): colorless gum; $[\alpha]_D^{20}$ +35.4 (*c* 0.130, CH_3OH); UV (CH_3OH-H_2O) λ_{max} 250, 278 nm; IR (KBr) ν_{max} 3419, 2937, 1767, 1637, 1591, 1506, 1458, 1425, 1373, 1327, 1240, 1184, 1128, 1068, 1035, 1016, and 931 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.44 (1H, d, J = 1.5 Hz, H-6′), 6.44 (2H, s, H-2″, 6″), 6.41 (1H, d, J = 1.5 Hz, H-2′), 5.94 (2H, s, OCH_2O), 5.42 (1H, brs, OH), 4.31 (1H, dd, J = 7.5, 9.6 Hz, H-5a), 3.84 (6H, s, 3″,5″-OCH_3), 3.80 (3H, s, 4″-OCH_3), 3.80 (1H, overlapped, H-5b), 3.58 (1H, d, J = 11.4 Hz, H-6), 2.89 (1H, m, H-4), 2.34 (1H, m, H-3), 0.94 (3H, d, J = 7.2 Hz, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.8 (C, C-2), 153.4 (C, C-3″, 5″), 149.2 (C, C-3′), 139.4 (C, C-5′), 137.6 (C, C-1″), 137.1 (C, C-4″), 136.3 (C, C-1′), 133.1 (C, C-4′), 110.1 (CH, C-6′), 104.6 (CH, C-2″, 6″), 101.6 (CH_2, OCH_2O), 100.9 (CH, C-2′), 70.4 (CH_2, C-5), 60.9 (CH_3, 4″-OCH_3), 56.2 (CH_3, 3″,5″-OCH_3; CH, C-6), 47.0 (CH, C-4), 40.2 (CH, C-3), 15.9 (CH_3, C-7); EIMS *m*/*z* 416 $[M]^+$ (18), 318 (24), 317 (100), 161 (11), 150 (20); HREIMS *m*/*z* 416.1480 (calcd for $C_{22}H_{24}O_8$, 416.1471).

2,3-*trans*-3,4-*cis*-3-Acetoxymethyl-4-[(5-methoxy-3,4-methylenedioxyphenyl)(3,4,5-trimethoxyphenyl)methyl]tetrahydrofuran-2-ol (3): colorless gum; $[\alpha]_D^{20}$ −68.6 (*c* 0.185, CH_3OH); UV (CH_3OH-H_2O) λ_{max} 250, 280 nm; IR (KBr) ν_{max} 3427, 2939, 1735, 1633, 1591, 1508, 1452, 1427, 1327, 1367, 1246, 1128, 1089, 1041, 1005, and 924 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.55 (2H, H-2″, 6″), 6.45 (1H, d, J = 1.5 Hz, H-2′), 6.39 (1H, d, J = 1.5 Hz, H-6′), 5.91 (1H, d, J = 1.5 Hz, OCH_2O), 5.90 (1H, d, J = 1.2 Hz, OCH_2O), 5.45 (1H, s, H-2), 4.06 (1H, dd, J = 4.5, 10.8 Hz, H-7a), 3.93 (1H, overlapped, H-7b), 3.88 (1H, overlapped, H-5a), 3.88 (3H, s, 5′-OCH_3), 3.85 (6H, s, 3″,5″-OCH_3), 3.79 (3H, s, 4″-OCH_3), 3.59 (3H, overlapped, H-4, 5b, 6), 2.43 (1H, m, H-3), 1.99 (3H, s, CH_3CO); ^1H NMR (C_6D_6, 600 MHz) δ 6.64 (2H, s, H-2″, 6″), 6.51(1H, d, J = 1.5 Hz, H-2′), 6.48 (1H, d, J = 1.5 Hz, H-6′), 5.48 (1H, s, H-2), 5.29 (1H, d, J = 1.5 Hz, OCH_2O), 5.26 (1H, d, J = 1.2 Hz, OCH_2O), 4.32 (1H, dd, J = 4.4, 11.2 Hz, H-7a), 4.14 (2H, overlapped, H-7b, 5a), 3.82 (1H, m, H-4), 3.76 (3H, s, 4″-OCH_3; 1H, m, H-5b), 3.63 (1H, d, J = 12.2 Hz, H-6), 3.50 (3H, s, 5′-OCH_3), 3.40 (6H, s, 3″,5″-OCH_3), 2.65 (1H, m, H-3), 1.55 (3H, s, CH_3CO); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.1 (C, *C*OCH_3), 153.6 (C, C-3″, 5″), 149.4 (C, C-3′), 143.6 (C, C-5′), 138.0 (C, C-1′, 1″), 137.0 (C, C-4″), 134.0 (C, C-4′), 107.2 (CH, C-6′), 104.4 (CH, C-2″, 6″), 101.6 (CH_2, OCH_2O), 101.2 (CH, C-2, 2′), 71.8 (CH_2, C-5), 61.2 (CH_2, C-7), 60.9 (CH_3, 4″-OCH_3), 57.0 (CH_3, 5′-OCH_3), 56.4 (CH_3, 3″,5″-OCH_3), 50.9 (CH, C-6), 46.4 (CH, C-3), 42.3 (CH, C-4), 21.1 (CH_3, *C*H_3CO); EIMS *m*/*z* 490 $[M]^+$ (13), 412 (10), 332 (21), 331 (100), 165 (5); HREIMS *m*/*z* 490.1845 (calcd for $C_{25}H_{30}O_{10}$, 490.1839).

7,8-*trans*-8,8′-*trans*-7′,8′-*trans*-7-(4-Hydroxy-3,5-dimethoxyphenyl)-7′-(5′-methoxy-3′,4′-methylenedioxyphenyl)-8-acetoxymethyl-8′-hydroxymethyltetrahydrofuran (4): colorless gum; $[\alpha]_D^{20}$ −42.4 (*c* 0.162, CH_3OH); UV (CH_3OH-H_2O) λ_{max} 245, 280 nm; IR (KBr) ν_{max} 3427, 2939, 1736, 1635, 1614, 1514, 1464, 1431, 1367, 1325, 1240, 1117, 1040, and 833 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.64 (2H, s, H-2, 6), 6.63 (1H, d, J = 1.2 Hz, H-2′), 6.60 (1H, d, J = 1.2 Hz, H-6′), 5.96 (2H, s, OCH_2O), 5.56 (1H, s, 4-OH), 4.97 (1H, d, J = 8.1 Hz, H-7′), 4.88 (1H, d, J = 8.7 Hz, H-7), 4.23 (2H, m, H-9), 3.90 (3H, s, 5′-OCH_3), 3.89 (6H, s, 3,5-OCH_3), 3.82 (1H, m, H-9′a), 3.77 (1H, m, H-9′b), 2.51 (1H, m, H-8), 2.27 (1H, m, H-8′), 1.98 (3H, s, CH_3CO); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.1 (C, *C*OCH_3), 149.3 (C, C-3′), 147.3 (C, C-3, 5), 143.8 (C, C-5′), 136.9 (C, C-1′), 135.0 (C, C-4′), 134.5 (C, C-4), 132.6 (C, C-1), 105.9 (CH, C-6′), 103.1 (CH, C-2, 6), 101.7 (CH_2, OCH_2O), 100.4 (CH, C-2′), 83.8 (CH, C-7), 82.9 (CH, C-7′), 64.0 (CH_2, C-9), 62.2 (CH_2, C-9′), 56.9 (CH_3, 5′-OCH_3), 56.5 (CH_3, 3,5-OCH_3), 53.7 (CH, C-8′), 50.1 (CH, C-8), 21.0 (CH_3, *C*H_3CO); EIMS *m*/*z* 476 $[M]^+$ (40), 331 (15), 317 (45), 252 (24), 208 (100), 203 (23), 207 (33), 191 (40); HREIMS *m*/*z* 476.1691 (calcd for $C_{24}H_{28}O_{10}$, 476.1683).

Anti-HIV Assay. Cytopathic Effect Assay. In the presence of 100 μL of various concentrations of test compounds, 50 μL of C8166 cells (8×10^5/mL) was seeded and then 50 μL of HIV-1 IIIB added (1300 $TCID_{50}$/well). After 3 days of culture at 37 °C in a humidified atmosphere of 5% CO_2, CPE was measured by counting the number of syncytia (multinucleated giant cells) in each well under an inverted microscope. Percentage inhibition on syncytial cell formation was estimated by comparison of syncytial cells (%) in treated wells with that in control wells to calculate the 50% effective concentration (EC_{50}).

Cytotoxicity Assay. The cytotoxicity of test compounds on C8166 cells was assessed by the MTT method. One hundred microliters of cells (4×10^5/mL) was seeded on a microtiter plate, and then 100 μL of various concentrations of test compounds added and incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 72 h. The supernatants were discarded, and MTT (methyl thiazole tetrazolium) (5 mg/mL in PBS) was added to each well. Following 4 h incubation, 100 μL of 50% DMF−20% SDS was added. After the formazan was dissolved completely, the plates were read on a Bio-Tek Elx 800 ELISA reader at 595/630 nm. The cytotoxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was calculated from the dose−response curve.

Acknowledgment. We are indebted to Dr. Dale E. Mais for his critical review of the manuscript. This work was supported by Shanghai Municipality Science and Technology Development Fund (05dZ22914 and 06DZ22907) and the Ministry of Science and Technology (2004CB518902).

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NP0605236