7,8-Secolignans from *Schisandra wilsoniana* and Their Anti-HIV-1 Activities

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Two new 7,8-secolignans, marphenols A and B (**1** and **2**, resp.), together with a known related derivative, 7,8-secoholostylone B (**3**), were isolated from the stems of *Schisandra wilsoniana*. The structures of **1** and **2** were elucidated by spectroscopic methods, including extensive 1D- and 2D-NMR techniques. The anti-HIV-1 activities of **1**–**3** were evaluated. Compound **1** inhibited HIV-1_{IIIB}-induced syncytia formation with an EC_{50} value of 0.55 μg ml^{-1}. It reduced p24 antigen expression in acutely HIV-1_{IIIB}-infected C8166 cells and primary isolate HIV-$1_{TC\text{-}2}$-infected peripheral blood mononuclear cells (PBMCs), with EC_{50} values of 3.34 and 0.52 μg ml^{-1}, respectively. It showed no effects on the HIV-1_{IIIB} replication in chronically infected H9 cells as well as fusion inhibition.

Introduction. – Plants of the genus *Schisandra* (Schisandraceae) were used widely in traditional Chinese medicine. They were rich sources of bioactive lignans, which possessed various pharmacological effects such as antihepatitis, antitumor, and anti-HIV-1 activity [1–7].

Schisandra wilsoniana A. C. SMITH is a climbing plant mainly distributed in Heqing, Dali, and Yulong Prefectures of Yunnan Province in China. The roots, stems, leaves, and fruits of this plant have been used in folk medicine for the treatment of bellyaches, gastralgias, bones and muscles aches, cramps, as well as injuries from falls [8–10]. In our previous work, three new, highly oxygenated nortriterpenoids were isolated from this plant, which showed weak anti-HIV activities [8]. To search for further bioactive compounds from this plant, we re-examined the leaves and stems of *S. wilsoniana*, which led to the isolation of two new lignans, marphenols A and B (**1** and **2**, resp.), along with a known compound, 7,8-secoholostylone B (**3**) [11]. In addition, the anti-HIV-1 activities of **1**–**3** were evaluated. Here, their structure elucidation and biological activities are described.

Results and Discussion. – *Isolation and Characterization.* A 70% aqueous acetone extract prepared from the stems of *S. wilsoniana* was partitioned between AcOEt and H_2O. The AcOEt layer was subjected repeatedly to column chromatography on SiO_2,

[1)] These authors contributed equally to this work.

Sephadex LH-20, and *RP-18*, and semipreparative HPLC to afford compounds **1**–**3** (see *Fig. 1*), including two new 7,8-secolignans named marphenols A and B (**1** and **2**, resp.), together with a known compound, 7,8-secoholostylone B (**3**).

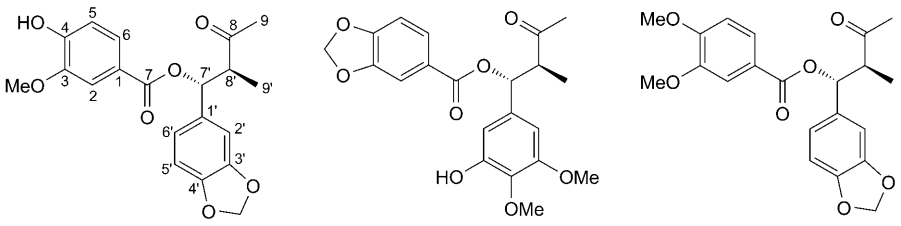

Fig. 1. *Structures of compounds* **1**–**3**

Compound **1**, obtained as white amorphous powder, was assigned the molecular formula $C_{20}H_{20}O_7$ by HR-ESI-MS (*m*/*z* 395.1112 ($[M+Na]^+$; calc. 395.1107)). The ^{1}H- and ^{13}C-NMR spectra showed signals of 20 H-atoms and 20 C-atoms, respectively, corresponding to two aromatic rings, two CO C-atoms, two Me groups, one CH group, one MeO group, and one O–CH_2–O group (*Table 1*). Comparison of 1D-NMR of **1** with those of **3** indicated that the structures of these two compounds are similar [11]. Analysis of HMBC spectrum of **1** showed that the MeO group at C(4) of the veratryl

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2** (δ *ppm*, *J* Hz) *Measured in (D$_5$)Pyridine*

Position	**1**		**2**	
	^{13}C	^{1}H	^{13}C	^{1}H
1	121.5 (*s*)		126.7 (*s*)	
2	113.4 (*d*)	7.79 (*s*)	109.5 (*d*)	7.65 (*s*)
3	153.4 (*s*)		152.2 (*s*)	
4	149.4 (*s*)		148.4 (*s*)	
5	116.3 (*d*)	7.18 (*d*, *J* = 8.1)	108.4 (*d*)	6.85 (*d*, *J* = 8.1)
6	124.6 (*d*)	7.90 (*d*, *J* = 8.2)	125.8 (*d*)	7.81 (*d*, *J* = 8.1)
7	165.4 (*s*)		164.8 (*s*)	
8	209.8 (*s*)		209.5 (*s*)	
9	29.3 (*q*)	2.32 (*s*)	29.3 (*q*)	2.26 (*s*)
1′	133.2 (*s*)		133.8 (*s*)	
2′	108.1 (*d*)	7.25 (*s*)	103.5 (*d*)	6.89 (*s*)
3′	148.4 (*s*)		152.3 (*s*)	
4′	148.1 (*s*)		137.8 (*s*)	
5′	108.4 (*d*)	6.89 (*d*, *J* = 7.9)	149.6 (*s*)	
6′	121.9 (*d*)	7.02 (*d*, *J* = 8.1)	109.6 (*d*)	7.22 (*s*)
7′	78.1 (*d*)	6.38 (*d*, *J* = 10.0)	78.5 (*d*)	6.43 (*d*, *J* = 10.0)
8′	52.2 (*d*)	3.42–3.45 (*m*)	52.2 (*d*)	3.46–3.49 (*m*)
9′	13.6 (*q*)	0.97 (*d*, *J* = 6.9)	13.8 (*q*)	1.02 (*d*, *J* = 6.9)
OCH_2O	101.7 (*t*)	5.94, 5.95 (2*s*)	102.5 (*t*)	5.99, 6.01 (2*s*)
*Me*O–C(3)	55.7 (*q*)	3.67 (*s*)		
*Me*O–C(3′)			56.0 (*q*)	3.76 (*s*)
*Me*O–C(4′)			60.3 (*q*)	3.81 (*s*)

group in **3** was replaced with a OH group in **1** (*Fig. 2*). On the other hand, a O–CH_2–O group was established to be connected with C(3′) and C(4′) by HMBCs of H-atoms of the O–CH_2–O group (δ(H) 5.94, 5.95, 2*s*) with C(3′) and C(4′), which was the same as in **3** (*Fig. 2*). The HMBCs of Me(9) (δ(H) 2.32, *s*) with C(8) and C(8′), and of Me(9′) (δ(H) 0.97, *d*, J=7.0) with C(8), C(8′), and C(7′), together with a ^{1}H,^{1}H-COSY correlation H–C(7′)/H–C(8′)/Me(9′), showed that **1** has the same five-membered side chain C(7′)–C(8′)(C(9′))–C(8)–C(9) as that in **3**. The relative configurations at C(7′) and C(8′) of **1** was deduced to be the same as those in **3** according to the coupling constant between H–C(7′) and H–C(8′) (J=10.0 Hz) in **1**, and observed ROESY correlations (*Fig. 3*). Thus, the structure of **1** was established as (–)-*rel*-(7′*R*,8′*S*)-4-hydroxy-3-methoxy-3′,4′-(methylenedioxy)-7,7′-epoxy-7,8-secolignan-7,8-dione[2]), and named as marphenol A.

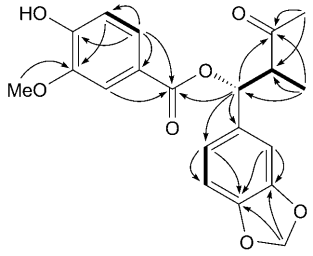

Fig. 2. *^{1}H,^{1}H-COSY* (—) *and key HMBC* (H→C) *correlations of* **1**

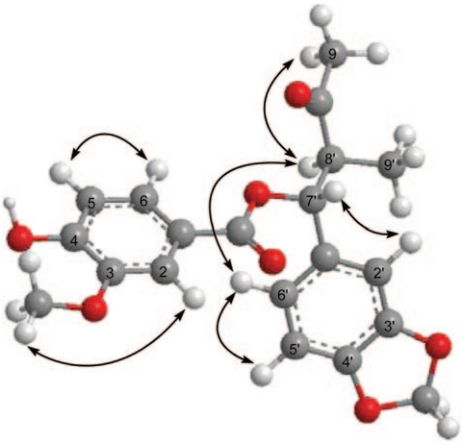

Fig. 3. *Key ROESY* (↔) *correlations of* **1**

Compound **2**, obtained as white amorphous powder, had the molecular formula $C_{21}H_{22}O_8$ as revealed by its HR-ESI-MS data (*m*/*z* 425.1210 ([M+Na]$^+$; calc. 425.1212)). The ^{1}H- and ^{13}C-NMR spectra were similar to those of **3**. Analysis of HSQC, ^{1}H,^{1}H-COSY, and HMBC spectra of **2** showed chemical-shift differences resulting from the substituents in aromatic rings. An O–CH_2–O group replaced the two MeO groups at C(3) and C(4) in **3** as indicated by HMBCs of the H-atom signals of

[2]) For systematic names, see *Exper. Part.*

the O–CH_2–O group (δ(H) 5.99, 6.01, *s*) with C(3) and C(4) in **2**. Two MeO groups were located at C(3′) and C(4′) in **2**, respectively, deduced from HMBCs of H–C(2′) with C(3′) and C(4′), and the HMBCs of the H-atoms of two MeO groups with C(3′) and C(4′). A OH group was located at C(5′), determined by downfield chemical shift of C(5′) (δ 149.6), and the HMBC correlations of H–C(6′) with C(4′) and C(5′). The relative configurations at C(7′) and C(8′) in **2** was established to be the same as those of **3** according to the coupling constant between H–C(7′) and H–C(8′) (J = 10.0 Hz). Thus, the structure of **2** was established as (–)-*rel*-(7′*R*,8′*S*)-5′-hydroxy-3′,4′-dimethoxy-3,4-(methylenedioxy)-7,7′-epoxy-7,8-secolignan-7,8-dione[2]), and named as marphenol B.

Bioassays. 1) *Anti-HIV-1 Activities in Primary Screening.* The cytotoxicities and anti-HIV-1 activities of **1**–**3** are compiled in *Table 2*. Compound **1** inhibited HIV-1-induced syncytia formation potently with a *TI* value (the ratio of CC_{50}/EC_{50}) of 18.27. Compounds **2** and **3** were less potent, with *TI* values of 4.33 and 9.19.

Table 2. *Summary of Primary Screening of Anti-HIV-1 Activities and Cytotoxicities of* **1**–**3**

Compounds	Mean ± SD[a])		*TI*
	CC_{50} [μg ml^{-1}]	EC_{50} [μg ml^{-1}]	
1	10.05 ± 3.88	0.55 ± 0.08	18.27
2	13.41 ± 4.95	1.8 ± 0.69	7.45
3	13.61 ± 1.82	2.11 ± 0.69	6.45
AZT	1219.26 ± 141.23	0.027 ± 0.002	45157.78

[a]) The data are representative of three independent experiments.

2) *Cytotoxicities.* MTT (= 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) was used to determine the cytotoxicities of the compounds. The effects of **1** on the viability of C8166, MT-4, PBMCs (peripheral blood mononuclear cells), and H9/HIV-1_{IIIB} cells were examined (*Table 3* and *Fig. 4*). Results indicated that the CC_{50} values of **1** for the cells ranged from 5.02 to 10.05 μg ml^{-1} (*Table 3*).

Table 3. *The Anti-HIV-1 Activities and Cytotoxicities of* **1** in vitro

Cells	Strains	Assays	Mean ± SD[a])		*TI*
			CC_{50} [μg ml^{-1}]	EC_{50} [μg ml^{-1}]	
C8166	HIV-1_{IIIB}	MTT/Syncytia	10.05 ± 3.88	0.55 ± 0.08	18.27
		MTT/p24	10.05 ± 3.88	3.34 ± 1.08	3.01
MT-4	HIV-1_{IIIB}	MTT	5.02 ± 0.15	1.23 ± 0.43	4.08
PBMCs	HIV-1_{TC-2}	MTT/p24	9.51 ± 2.49	0.52 ± 0.08	18.29
H9/HIV-1_{IIIB}	–	MTT/p24	7.57 ± 1.63	11.38 ± 3.05	0.67
C8166 + H9/HIV-1_{IIIB}	–	Syncytia	–	8.16 ± 1.73	–

[a]) The data are representative of three independent experiments.

3) *Anti-HIV-1 Activity of* **1**. We examined the activity of **1** to protect MT-4 cells from HIV-1-induced lytic effects. Compound **1** protected MT-4 cells from lytic effects with a *TI* value of 4.08 (*Table 3* and *Fig. 5*). Suppression of **1** on viral replication was also assessed by measuring expression of HIV-1 p24 antigen. Compound **1** reduced p24

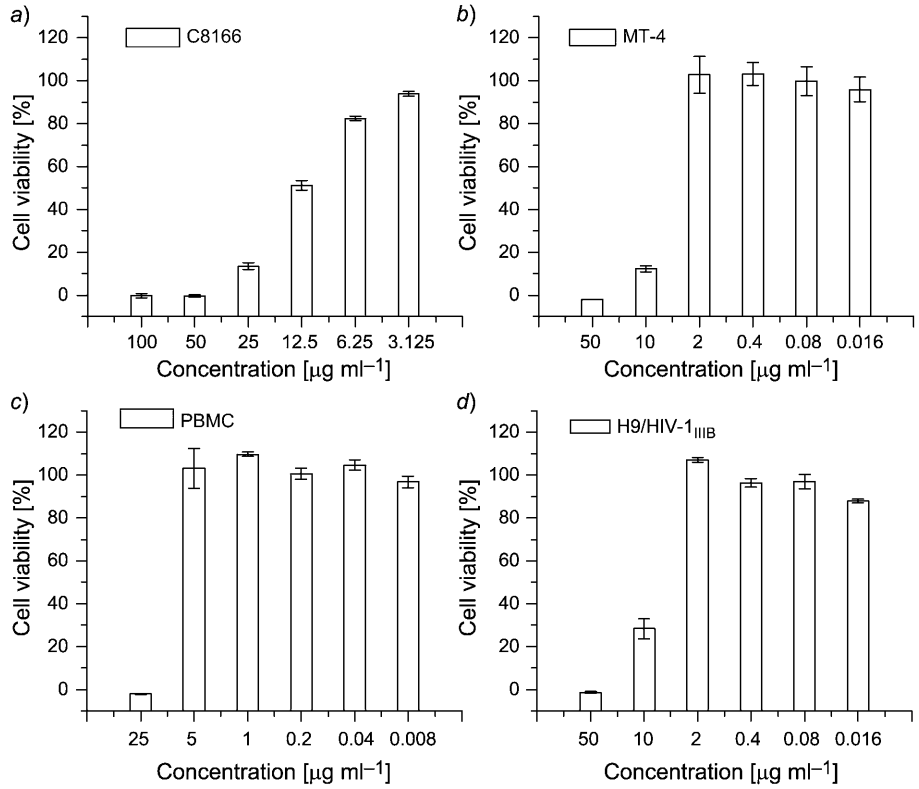

Fig. 4. *Cytotoxicities of* **1** *in different cells.* Percentage of cell viabilities was expressed as percent of control. *a*) C8166 cells, *b*) MT-4 cells, *c*) PBMCs, and *d*) H9/HIV-1_{IIIB} cells.

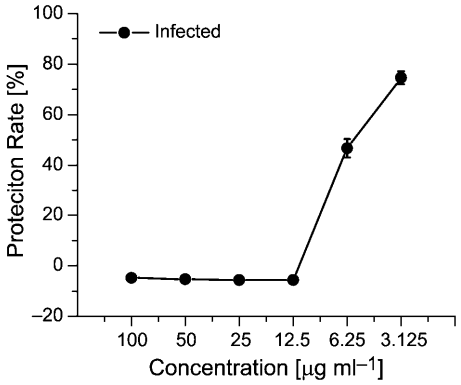

Fig. 5. *Protection of* **1** *from HIV-*1_{IIIB}*-induced cell lytic effects.* Results are expressed as percentage of cellular viability relative to control (100%).

production in acute HIV-1_{IIIB}-infected C8166 cells with an EC_{50} value of 3.34 μg ml^{-1} (*Table 3* and *Fig. 6*). It also inhibited primary isolate HIV-1_{TC-2} replication in PBMCs with an EC_{50} value of 0.52 μg ml^{-1} (*Table 3* and *Fig. 6*). Compound **1** did not inhibit p24 expression in chronically infected H9 cells (*Table 3* and *Fig. 6*). It also did not inhibit cell-to-cell fusion (*Table 3* and *Fig. 7*).

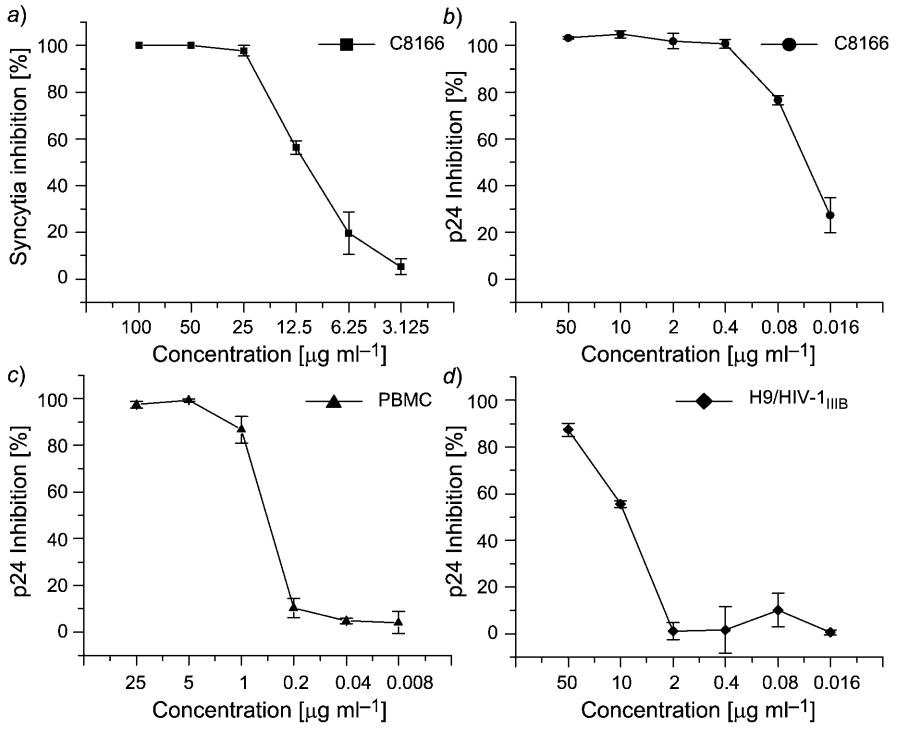

Fig. 6. *Anti-HIV-1 activities of* **1** *in different cells with HIV-1_{IIIB} or HIV-1_{TC-2}. a*) Syncytia inhibition of C8166 cells infected by HIV-1_{IIIB}, *b*) p24 inhibition of C8166 cells by HIV-1_{IIIB}, *c*) p24 inhibition of PBMCs by HIV-1_{TC-2}, and *d*) p24 inhibition of chronically infected H9/HIV-1_{IIIB} cells.

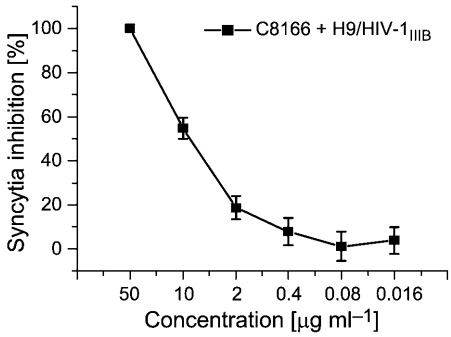

Fig. 7. *Fusion inhibition of* **1** *between normal C8166 cells and HIV-1_{IIIB} chronically infected H9 cells.* The assay was performed by counting syncytia.

Discussion. – Although current combination therapy allows viral replication to be controlled, HIV-1 is not eradicated [12]. If the treatment is stopped, residual virus present in several potential reservoirs rapidly expands allowing disease progression to be continued [13]. The plant world has supplied diverse structures possessing palliative or curative properties, and, therefore, Chinese traditional medicines are investigated by researchers worldwide [14].

Conclusions. – We reported that lignans from *S. micrantha* exhibit anti-HIV-1 activity [7]. In the present work, three lignans, **1**–**3**, were isolated from *S. wilsoniana.* In primary screening, compounds **1**–**3** showed different *TI* values (*Table 2*). If the *TI* value of a compound is higher than 10, it is subjected to further assays to ensure its anti-HIV activities. Due to this high *TI* value ($TI = 18.27$), compound **1** was selected for further investigations.

It was found that **1** processed anti-HIV-1 activities in several strains of HIV-1 in different cells (*Table 3*). These included a laboratory-adapted strain (HIV-1_{IIIB}) and a primary isolate virus (HIV-$1_{TC\text{-}2}$), three cell lines (C8166, MT-4, and H9/HIV-1_{IIIB}) and one primary cell (peripheral blood mononuclear cells (PBMCs)). The EC_{50} values of **1** on inhibiting syncytia formation and p24 antigen ranged from 0.55 to 3.34 μg ml^{-1}. The CC_{50} values of **1** were in the range of 5.02–10.05 μg ml^{-1}. The potency and toxicity have variations that may be due to difference in cell type or a reflection of the preferential actions of **1** on these cells. Compound **1** did not show inhibitory activity against chronically infected H9 cells, suggesting that the mechanism of action of **1** was not at a late step of HIV life cycle. A lack of inhibitory activity against cell-to-cell infection also suggested that **1** did not inhibit HIV entry to host cell. In summary, **1** is a new lignan inhibiting HIV-1 replication. The mechanisms of its anti-HIV activities need further investigations.

Experimental Part

General. Semiprep. HPLC: *Agilent 1100* liquid chromatograph with a *Zorbax SB-C_{18}* (9.4 mm × 25 cm) column. Prep. HPLC: *Shimadzu LC-8A* prep. liquid chromatograph with a *Zorbax SB-C_{18}* column (20 mm × 25 cm). Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qing-dao Marine Chemical, Inc.*, Qingdao, P. R. China); fractions were monitored by TLC, and spots were visualized by heating SiO_2 plates sprayed with 5% H_2SO_4 in EtOH. Optical rotations: *Horiba SEPA-300* polarimeter. UV Spectra: *Shimadzu UV-2401A* spectrophotometer. IR Spectra: *Tenor 27* spectrophotometer; KBr pellets. 1D- and 2D-NMR spectra: *DRX-500* spectrometers with TMS as internal standard. HR-ESI-MS and FAB-MS: *API QSTAR* time-of-flight spectrometer and a *VG Autospec-3000* spectrometer, resp.

Plant Material. The stems of *S. wilsoniana* were collected in Dali Prefecture of Yunnan Province, P. R. China, in July 2005. The plant material was identified by Prof. *Xi-Wen Li.* A voucher specimen (KIB 05-7-12) has been deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The air-dried and powdered stems of *S. wilsoniana* (8.0 kg) were extracted four times with 70% aq. Me_2CO (4 × 5 l, each 24 h) at r.t. and filtered to yield a filtrate, which was successively evaporated under reduced pressure and partitioned with AcOEt (3 × 4 l). The AcOEt extract (636 g) was applied to CC (SiO_2, 200–300 mesh; $CHCl_3$/MeOH gradient 20 : 1, 9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5) to give five fractions, *Frs. A–E.* The separation of *Fr. B* (45.2 g) by CC (SiO_2; PE/acetone 20 : 1–5 : 5) yielded *Fr. B1–B6. Fr. B3* (3.26 g) was purified by prep. HPLC (55% MeOH/H_2O; flow rate, 25 ml/min) to give **1** (10.8 mg; t_R 15.3 min), **2** (4.3 mg; t_R 20.4 min), **3** (26.2 mg; t_R 17.5 min).

Marlignan A (=*(−)*-rel-*(1*R,2S*)-1-(1,3-Benzodioxol-5-yl)-2-methyl-3-oxobutyl 4-Hydroxy-3-methoxybenzoate*; **1**). White amorphous powder. $[\alpha]_D^{25.8} = +30.6$ ($c = 0.36$, MeOH); UV (MeOH): 203 (5.6), 272 (3.57), 356 (1.68). IR (KBr): 3448, 3080, 2932, 2288, 1715, 1665, 1598, 1524, 1480, 1425, 1368, 1324, 1239, 1126, 1049, 1012. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 395.1112 ($[M+Na]^+$, $C_{20}H_{20}NaO_7^+$; calc. 395.1107).

Marlignan B (=*(−)*-rel-*(1*R,2S*)-1-(3-Hydroxy-4,5-dimethoxyphenyl)-2-methyl-3-oxobutyl 1,3-Benzodioxole-5-carboxylate*; **2**). White amorphous powder. $[\alpha]_D^{26.5} = +18.4$ ($c = 0.28$, MeOH). UV (MeOH): 205 (5.08), 275 (3.24), 350 (1.68). IR (KBr): 3464, 3082, 2924, 2851, 1717, 1662, 1562, 1528, 1476, 1440, 1382, 1347, 1255, 1132, 1055, 1018, 986, 874. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 425.1210 ($[M+Na]^+$, $C_{21}H_{22}NaO_8^+$; calc. 425.1212).

Reagents of Anti-HIV-1 Assays. MTT (=3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide), SDS (sodium dodecyl sulfate), DMF *Triton X-100*, Fc-specific anti-mouse IgG, phytohemagglutinin (PHA), interleukin-2 (IL-2), and AZT (=3′-azido-3′deoxythymidine; zidovudine) were purchased from *Sigma*. Horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG was purchased from *Dingguo Biotechnology Company* (P. R. China). The fetal bovine serum (FBS) was purchased from *Gibco*. *P6F4* (a p24 monoclonal antibody) and rabbit anti-p24 polyclonal antibody were prepared in our laboratory.

Cells and Viruses. Cell lines, including C8166, H9, MT-4, and H9 chronically infected with HIV-1_{IIIB}, were maintained in *Gibco* complete *RPMI-1640* (with 10% heat-inactivated FBS). PBMCs were isolated from healthy donors by *Ficoll–Hypaque* density gradient centrifugation and then incubated in complete *RPMI-1640* containing 5 μg ml^{-1} PHA and 50 U ml^{-1} IL-2 for 3 d before use. The laboratory-derived virus HIV-1_{IIIB} was obtained from *NIH AIDS Reagent Program* and *MRC AIDS Reagent Project*, UK. The primary isolate HIV-1_{TC-2} was isolated from a HIV-1-infected individual of Yunnan Province as described in [15]. The 50% HIV-1 tissue culture infectious dose ($TCID_{50}$) was determined and calculated according to the method of *Reed* and *Muench*. All the viruses were stored in small aliquots at −70°.

Cytotoxicity Assay. MTT was applied to assess the cytotoxicities of compounds [16]. Briefly, 4×10^4 cells (for PBMCs, 5×10^5 cells) were seeded per well on 96-well plate in the absence or presence of various gradient concentrations of compounds in triplicate. Then, 96-well plates were incubated for 3 d at 37°, in a 5% CO_2-humidified incubator. The supernatants were discarded, and MTT (5 mg ml^{-1} in PBS) was added to each well. After incubating 4 h, 100 μl of 50% DMF/20% SDS was added. To ensure that the crystals have completely dissolved, the plate was incubated at 37° overnight. AZT was used as the positive control. The plates were read on a *Bio-Tek Elx 800 ELISA* reader at 595/630 nm. The 50% cytotoxic concentration (CC_{50}) was calculated.

Syncytia Assay. Syncytia were counted under an inverted microscope as described in [17]. 4×10^4 C8166 cells, infected with virus HIV-1_{IIIB} at a multiplicity of infection (M.O.I.) of 0.15, were seeded on 96-well plate in the absence or presence of various gradient concentrations of compounds in triplicate. The final volume per well was 200 μl. Control assays were performed without the testing compounds in HIV-1_{IIIB}-infected and -uninfected cultures. After 3 d of culture, the cytopathic effect (CPE) was measured by counting the number of syncytia. AZT was used as the positive drug. 50% Effective concentration (EC_{50}) was calculated.

Protection Assay for HIV-1-Induced Lytic Effects. The inhibition of HIV-1-induced lytic effects in MT-4 cells was previously described [18]. Briefly, uninfected or HIV-1_{IIIB}-infected (M.O.I. = 0.75) MT-4 cells (4×10^4 cells per well) were seeded in 96-well plates with 100 μl of various gradient concentrations of compounds. Plates were incubated for 7 d at 37°, in a 5% CO_2-humidified incubator. On day 3, 100 μl of complete *RPMI-1640* media was added to each well. On day 7, the viability of cells was assessed by MTT as described above.

Inhibition of HIV-1 Replication in Acute Infection. The inhibition of compound on HIV-1 replication *in vitro* was further examined by quantification of p24 expression by ELISA as previously described in [19]. Briefly, C8166 cells (4×10^4 cells per well) were infected with HIV-1_{IIIB} (M.O.I. = 0.15) at 37°, in a 5% CO_2-humidified incubator for 2 h to allow for viral absorption. Cells were washed three times with PBS. 100 μl of cells (4×10^4 cells) were seeded with 100 μl of various gradient concentrations of compounds and incubated at 37°, in a 5% CO_2-humidified incubator for 4 d. Then, 90 μl of supertanants

were collected from each well and mixed with 10 µl of 5% *Triton X-100*. HIV-1 p24 Expression was assayed by ELISA.

Inhibition of HIV-1 Replication in PBMCs. PHA-Activated normal PBMCs were incubated with HIV-$1_{TC\text{-}2}$ (M.O.I. = 0.75) in presence of various gradient concentration of compounds at 37°, in a 5% CO_2-humidified incubator. The cells were washed three times with PBS after overnight incubation. Then, 5×10^5 cells were seeded per well with or without various gradient concentration of compound in culture medium supplemented with 50 U ml^{-1} human recombinant IL-2 for 7 d. On day 3, 100 µl of complete *RPMI-1640* media with IL-2 were added to each well. On day 7, 90 µl supernatants were collected from each well, mixed with 10 µl 5% *Triton X-100*. HIV-1 p24 Expression was assayed by ELISA [20].

Inhibition of HIV-1 Replication in Chronically Infected Cell Lines. H9 Cells chronically infected with HIV-1_{IIIB} were washed three times with PBS to remove free virus particle. 100 µl of the cells (4×10^4 cells per well) were seeded on a 96-well plate with absence or presence of 100 µl of various gradient concentrations of compounds at 37°, in a 5% CO_2-humidified incubator. On day 4, 90 µl of supertanants were collected from each well and mixed with 10 µl of 5% *Triton X-100*. HIV-1 p24 Expression was assayed by ELISA [21].

Cell-to-Cell Fusion Assay. Cell-to-cell fusion between normal C8166 cells and H9 cells chronically infected with HIV-1_{IIIB} was quantified under an inverted microscope as previously described [22]. Briefly, 3×10^4 C8166 cells were co-cultured with 1×10^4 chronically HIV-1_{IIIB}-infected H9 cells in the presence or absence of compounds with various gradient concentrations. After incubation at 37°, in a 5% CO_2-humidified incubator for 6 h, the number of syncytia was counted under an inverted microscope.

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