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Stelleralides D–J and Anti-HIV Daphnane Diterpenes from *Stellera chamaejasme*

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Abstract

Bioassay-guided fractionation of a petroleum ether extract of the roots of *Stellera chamaejasme* led to the isolation of seven new (stelleralides D–J, **1–7**) and 12 known (**8–19**) daphnane diterpenoids. The structures and relative configurations of **1–7** were established on the basis of extensive spectroscopic analysis, including HRESIMS and comprehensive NMR techniques. All isolates were evaluated for anti-HIV activity in MT4 cells. All compounds tested, except **2**, showed anti-HIV activity, and, especially, five 1 α -alkyldaphnane diterpenoids (**3**, **4**, **5**, **10**, and **11**) exhibited extremely potent anti-HIV activity, with EC₅₀ values of 0.06–1.1 nM and selectivity index values of more than 10 000.

Graphical Abstract

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Notes

The authors declare no competing financial interest.

Supporting Information

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¹H NMR, ¹³C NMR, ¹H–¹H COSY, HSQC, HMBC, NOESY, CD, and IR spectra for compounds **1–7** (PDF)

The rapid, worldwide spread of acquired immunodeficiency syndrome (AIDS) has prompted an intense research effort to discover compounds that can inhibit effectively the human immunodeficiency virus (HIV).^{1,2} Indeed, highly active antiretroviral therapy (HAART), which combines three to four antiretrovirals, can effectively control plasma viremia in many patients, although the virus is suppressed rather than truly eradicated.^{3–5} Thus, a major goal of current AIDS therapy continues to be the development of new anti-HIV compounds as well as drug regimens for eradication of the AIDS virus.

Stellera chamaejasme L. (Thymelaeaceae), a toxic perennial herb, is distributed prevalently in northern and southwestern mainland China and Nepal. Its dried roots, named “Rui-Xiang-Lang-Du” in traditional Chinese medicine, have long been used for the treatment of stubborn skin ulcers, tinea, scabies, tuberculosis, and chronic tracheitis.⁶ Previous chemical investigations on the roots of this plant have led to the isolation of diverse secondary metabolites, including highly functionalized daphnane diterpenoids,^{7–9} tiglane diterpenoids,¹⁰ lignans,^{11,12} biflavonoids,^{13–16} coumarins,^{17,18} and sesquiterpenoids.^{11,19} In particular, certain daphnane and tiglane diterpenoids have shown extremely potent anti-HIV activity at low nanomolar concentrations with relatively low cytotoxicity.^{7,10} However, these compounds are very difficult to synthesize because of their complex structure. Accordingly, further analysis of the plant material was conducted to acquire a significant quantity of several known, as well as new, anti-HIV diterpenoids from *S. chamaejasme*.

Consequently, the current research on anti-HIV diterpenoids from *S. chamaejasme* led to the isolation of 19 daphnane diterpenoids, including seven new [stelleralides D–J (**1**–**7**)] and 12 known compounds [stelleralide C (**8**),⁷ pimelotide A (**9**),²⁰ gnidimacrin (**10**),⁷ pimelea factor P2 (**11**),⁷ wikstroelide F (**12**),⁷ wikstroelide A (**13**),²¹ wikstroelide B (**14**),²² wikstrotoxin A (**15**),²³ huratoxin (**16**),⁷ wikstroelide M (**17**),²² wikstroelide J (**18**),²² and simplexin (**19**)⁷]. Herein, are described the isolation and structural elucidation of the new compounds together with the anti-HIV activity of all daphnane diterpenoids isolated.

RESULTS AND DISCUSSION

In a bioassay-guided fractionation of an ethanolic extract ($EC_{50} = 0.42 \pm 0.08 \mu\text{g/mL}$, selectivity index (SI) 23) of the roots of *S. chamaejasme*, three solvent fractions (petroleum ether, EtOAc, *n*-BuOH) were obtained. The petroleum ether-soluble fraction exhibited promising anti-HIV activity with an EC_{50} value of $0.06 \pm 0.007 \mu\text{g/mL}$ (SI 320), while the EtOAc-soluble fraction had a higher EC_{50} value of $1.26 \pm 0.19 \mu\text{g/mL}$ (SI 15), and the *n*-BuOH-soluble fraction showed no anti-HIV activity. The active fraction was separated by repeated column chromatography and preparative HPLC to afford 19 daphnane diterpenoids.

Compound **1** was obtained as a colorless oil. Its molecular formula was determined as $C_{53}H_{76}O_{12}$ based on an HRESIMS ion at m/z 927.5235 $[M + Na]^+$ (calcd for $C_{53}H_{76}O_{12}Na$, 927.5229), indicating a hydrogen deficiency index (HDI) of 16. The IR spectrum revealed the presence of hydroxy (3478 cm^{-1}), carbonyl (1799 and 1718 cm^{-1}), and aromatic groups (1648 and 1452 cm^{-1}). The ^1H NMR data (Table 1) of **1** indicated two tertiary methyl (δ_{H} 1.73 and 1.79, each 3H, s), one secondary methyl (δ_{H} 1.02, 3H, d, $J = 6.4$ Hz), one primary methyl (δ_{H} 0.86, 3H, t, $J = 8.0$ Hz), two oxygenated methylenes (δ_{H} 4.09, 4.54, 4.68, and 4.97), three oxygenated methines (δ_{H} 3.44, 4.35, and 4.82), a terminal double bond (δ_{H} 5.05 and 4.94), and five aromatic [δ_{H} 7.57 (1H, dd, $J = 8.4, 1.2$ Hz), 7.46 (2H, dd, $J = 8.0, 8.0$ Hz), 8.04 (2H, dd, $J = 8.4, 1.2$ Hz)] protons. Analysis of the ^{13}C NMR and DEPT spectra of **1** established the presence of daphnane ketal-lactone skeleton resonances²⁰ including a characteristic quaternary carbon resonance at δ_{C} 120.4 (C-1'), an acetal carbon resonance at δ_{C} 112.1 (C-2), a lactone carbonyl carbon resonance at δ_{C} 173.9 (C-3), and a methyl carbon resonance at δ_{C} 19.6 (C-19). Comparison of the ^1H and ^{13}C NMR data (Table 1) of **1** with those of stelleralide C (**8**)⁷ implied the similarity of essential structural signals. Additionally, the presence of a fatty acid was suggested [δ_{C} 173.8 (C-1'''), 34.1 (C-2'''), 24.7 (C-3'''), 29.0–29.8 (C-4'''–C-13'''), 31.9 (C-14'''), 22.7 (C-15'''), 14.1 (C-16''')]. On the basis of the

molecular formula, the fatty acid was proposed as palmitic acid. The linkage of palmitic acid at C-20 of the diterpenoid skeleton was confirmed by the HMBC correlation between H₂-20 (δ_{H} 4.09 and 4.68) and the carbonyl carbon (δ_{C} 173.8) of palmitic acid (Figure 1) as well as the downfield chemical shift of C-20 from δ_{C} 63.0 in **8** to δ_{C} 63.9 in **1**. Other correlations in the HMBC spectrum confirmed the connectivities in this compound.

The relative configuration of **1** was mainly established by comparison of its spectroscopic data with those of stelleramide C (**8**), as well as NOESY results (Figure 1) and molecular modeling calculations. The NOESY correlations of H-1/H-19, H-10'/H-1, H-7/H-8, and H-8/H-14 indicated that these protons are cofacial and β -oriented, while the correlations of H-10/H-9', H-9'/H₂-18, and H-5/H-10 suggested that these protons are α -oriented. Thus, the structure of **1** (stelleramide D) was established as shown.

The molecular formula of compound **2**, obtained as a colorless oil, was assigned as C₅₅H₇₆O₁₂ according to its HRESIMS (m/z 951.5184 [M + Na]⁺, calcd for C₅₅H₇₆O₁₂Na, 951.5229), with an HDI of 18. The IR spectrum indicated the presence of hydroxy (3459 cm⁻¹), carbonyl (1797 and 1717 cm⁻¹), and aromatic (1455 cm⁻¹) groups. On the basis of the ¹H and ¹³C NMR spectra (Table 1), it was evident that **2** has the same daphnane ketal-lactone skeleton as **1**. The key difference between the two compounds was in the lipid motif. The signals due to olefin protons (δ_{H} 5.32, 5.33, 5.35, and 5.37) were consistent with the presence of an unsaturated fatty acid. The lipid group was defined as linoleic acid by analysis of the appropriate carbon signals [δ_{C} 174.0 (C-1'''), 33.9 (C-2'''), 24.7 (C-3'''), 29.0–29.8 (C-4'''–C-7'''), 27.1 (C-8''', C-14'''), 127.9 (C-9'''), 130.2 (C-10'''), 25.6 (C-11''', C-15'''), 130.1 (C-12'''), 128.1 (C-13'''), 31.7 (C-16'''), 22.5 (C-17'''), and 14.0 (C-18''')]. In order to confirm the structure of the linoleic acid moiety, a GC-MS experiment²⁴ was conducted (see Experimental Section). The attachment of the linoleic acid group to C-20 was assigned by the key HMBC correlations from H₂-20 (δ_{H} 4.09 and 4.68) to C-1''' (δ_{C} 174.0). The relative configurations of **2** were determined to be the same as those of **1** by analysis of its NOESY spectrum and molecular modeling, with β -orientations of H-1, H-7, H-8, H-14, H₃-19, and H-10' and α -orientations of H-5, H-10, H₂-18, and H-9'. The structure of **2** (stelleramide E) was thus established as shown.

Compound **3** was isolated as a white, amorphous powder. Its HRESIMS exhibited a pseudomolecular peak at m/z 855.3557 [M + Na]⁺ (calcd for C₄₆H₅₆O₁₄Na, 855.3562), corresponding to the molecular formula C₄₆H₅₆O₁₄. In accordance with the molecular formula, 46 carbon signals were evident in the ¹³C NMR spectrum. The ¹H and ¹³C NMR spectra of **3** indicated the presence of four methyl groups [δ_{C} 18.9, δ_{H} 1.80 (3H, s); δ_{C} 14.5, δ_{H} 1.18 (3H, d, J = 6.6 Hz); δ_{C} 18.5, δ_{H} 1.22 (3H, d, J = 7.2 Hz); δ_{C} 21.1, δ_{H} 2.10 (3H, s)], a terminal double bond [δ_{C} 145.4, 111.9; δ_{H} 5.15 (1H, brs), 4.92 (1H, dd, J = 1.2, 1.2 Hz)], two hydroxymethyls [δ_{C} 66.8, δ_{H} 4.85 (1H, dd, J = 10.2, 3.0 Hz), 4.08 (1H, dd, J = 10.2, 10.2 Hz); δ_{C} 65.8, δ_{H} 3.83 (2H, d, J = 7.2 Hz)], two benzoyl moieties (δ_{C} 166.1, 168.4, 129.3, 130.8, 130.2 $\times$ 2, 129.6 $\times$ 2, 128.5 $\times$ 2, 128.4 $\times$ 2, 132.8, 133.6), an acetyl carbonyl resonance (δ_{C} 170.9), and, especially, a typical quaternary carbon resonance at δ_{C} 118.4 (C-1'), signifying that compound **3** is a 1 α -alkyldaphnane derivative.^{25,26} The NMR spectra of **3** resembled closely those of stelleramide A,⁷ suggesting that the two compounds have the same molecular skeleton and substituent groups. However, detailed comparison of their ¹H

and ^{13}C NMR data (Table 2) showed that an additional benzoyloxy group [δ_{C} 166.1 (Bz-CO), 130.8 (Bz-1''), 129.6 (Bz-2'' and 6''), 128.4 (Bz-3'' and 5''), and 132.8 (Bz-4'') and δ_{H} 8.06 (H-2'' and 6''), 7.45 (H-3'' and 5''), and 7.56 (H-4'')] is present in **3**. This conclusion was also supported by the molecular formula of **3**, which was 120 mass units greater than that of stelleralide A. The structure of **3** was fully determined by 2D NMR spectroscopic analysis. The HMBC correlations (Figure 1) from H-10' (δ_{H} 1.22) to C-1 (δ_{C} 49.2) and C-9' (δ_{C} 27.9) and from H-7' (δ_{H} 5.34) to C-5' (δ_{C} 19.3), C-9' (δ_{C} 27.9), and a carbonyl group (δ_{C} 166.1) suggested that the benzoyloxy group is attached at C-7'. The relative configuration of **3** was established by its NOESY spectrum (Figure 1), comparison of its NMR data with those of stelleralide A, and molecular modeling and determined to be the same as that of stelleralide A with α -orientations of H-3, H-5, H-10, H-2', H-7', and H-10' and β -orientations for H-1, H-8, H-7, H-14, and H-19. Consequently, compound **3** (stelleralide F) was assigned as shown.

Compound **4** was assigned the molecular formula $\text{C}_{51}\text{H}_{48}\text{O}_{14}$ based on a positive HRESIMS $[\text{M} + \text{Na}]^+$ ion at m/z 917.3694. The ^1H and ^{13}C NMR spectra of **4** and **3** were similar, suggesting that **4** is also a 1 α -alkyldaphnane derivative. Detailed comparison of the ^1H and ^{13}C NMR data (Table 2) of **4** with those of **3** indicated the only difference to be an acetyloxy group at C-18 in **3** compared with a benzoyloxy group in **4**. This assignment was further confirmed by the HMBC correlation between H₂-18 (δ_{H} 4.41 and 5.11) and the benzoyloxy carbonyl (δ_{C} 166.5). The relative configuration of **4** was assigned as being the same as that of **3** based on comparison of their NMR data, NOESY experiments, and molecular modeling. Therefore, the structure of **4** (stelleralide G) was determined as shown.

Stelleralide H (**5**) was found to have the same molecular formula, $\text{C}_{44}\text{H}_{54}\text{O}_{12}$, as gnidimacrin (**10**), as determined by its HRESIMS. Detailed comparison of the NMR data of **5** with those of **10** suggested that these compounds are structural analogues, differing only in the location of the benzoyl group. The benzoyl group was located at C-20 in **5** rather than at C-3 in **10** based on the HMBC correlation between H₂-20 (δ_{H} 4.38) and its corresponding benzoyl carbonyl (δ_{C} 167.5). The configuration of **5** was elucidated by NOESY correlations to be the same as that of **10**. Thus, the structure of **5** (stelleralide H) was established as shown.

Stelleralide I (**6**) was obtained as a white, amorphous powder, and its molecular formula was deduced as $\text{C}_{35}\text{H}_{50}\text{O}_9$ from an HRESIMS ion at m/z 637.3349 (calcd for $\text{C}_{35}\text{H}_{50}\text{O}_9\text{Na}$, 637.3347). The ^1H and ^{13}C NMR data (Table 3) of **6** were found to be closely similar to those of wikstroelide B (**14**), a daphnanetoxin diterpene. The main difference between them was the presence of a hydroxy group at C-12 in **6** rather than an acetyloxy group in **14**. This assignment was determined from the shifts in two carbon resonances: C-12 was shifted upfield from δ_{C} 78.2 in **14** to 77.6 in **6**, and C-13 was shifted downfield from δ_{C} 83.4 in **14** to δ_{C} 85.0 in **6**. The location of the hydroxy group at C-12 was confirmed by the HMBC correlations from H-12 (δ_{H} 3.94, 1H, brs) to C-9 (δ_{C} 78.5), C-11 (δ_{C} 44.9), C-13 (δ_{C} 85.0), C-14 (δ_{C} 80.6), and C-15 (δ_{C} 144.7). In addition, in the NOESY spectrum, H-12 correlated with H-18 (δ_{H} 1.23, 3H, d, $J = 7.6$ Hz), indicating that H-12 is α -oriented. Hence, the structure of compound **6** was fully established as shown.

Stelleralide J (**7**) was assigned a molecular formula of $C_{34}H_{52}O_{10}$, according to the HRESIMS (m/z 643.3455 $[M + Na]^+$, calcd for $C_{34}H_{52}O_{10}$, 643.3453). On the basis of its 1H and ^{13}C NMR data (Table 3), **7** was considered to be a structural analogue containing 18 more mass units (H_2O) than wiktstroelide M (**16**). A direct comparison of their ^{13}C NMR spectra showed that the carbon resonances at δ_C 61.9 (C-6) and 63.6 (C-7) in **16** were shifted downfield to δ_C 76.8 and 79.1 in **7**, suggesting that the 6,7-epoxide moiety in **16** is replaced by two hydroxy groups in **7**. This deduction was confirmed from the HSQC and HMBC spectra, particularly HMBC correlations (Figure 1) observed between H-7 (δ_H 4.31) and C-5 (δ_C 72.4), C-9 (δ_C 76.3), and C-20 (δ_C 67.0), as well as H-8 (δ_H 3.29) and C-6 (δ_C 76.8) (Figure 1). In the NOESY spectrum (Figure 1) of **7**, H-8 (δ_H 3.29) showed significant correlations with H-7 (δ_H 4.31) and H-14 (δ_H 5.65), suggesting that H-7, H-8, and H-14 have β -orientations. Correlation of H-7 (δ_H 4.31) and H₂-20 (δ_H 3.77) indicated that the hydroxymethyl at C-6 is also β -oriented, while the hydroxy at C-6 was α -oriented (Figure 1). A correlation was present between H-5 (δ_H 3.83) and H-10 (δ_H 3.63), but not between H₃-18 (δ_H 1.06) and H-8 (δ_H 3.29), which indicated that H-5, H-10, and H₃-18 have α -orientations. Consequently, compound **7** (Figure 1) was elucidated as shown.

The isolated compounds (**1–19**) were evaluated for anti-HIV activity against NL4-3 virus replication in MT4 lymphocytes. Cytotoxicity [50% toxic concentration (CC_{50})] was also assessed, and the results are summarized in Table 4. The 1 α -alkyldaphnane diterpenoids (**3**, **4**, **5**, **10**, and **11**) exhibited the most potent anti-HIV-1 activity, with EC_{50} values of 0.06–1.1 nM (SI > 10 000). The daphnanetoxin diterpenes (**6**, **7**, **13–19**) and compound **12** displayed lower, but still potent anti-HIV-1 effects (EC_{50} 2.6–120 nM, SI = 100–6000). The least potent compounds were **1**, **2**, **8**, and **9**, which contain a 2,4-epoxide moiety and lactone ring (SI < 50).

Structurally, the main difference between the most potent (**3–5**, **10**, **11**) and the least potent (**1**, **2**, **8**, **9**) compounds is in ring A. These results indicated that the nature of ring A appears to be responsible for the enhanced anti-HIV activity. In addition, compounds **3–5**, **10**, and **11** with a cyclopentane ring A were more potent than **6**, **7**, and **12–19** with a cyclopentenone or cyclopentanone ring A, suggesting the importance of a cyclopentane ring A for optimal anti-HIV activity.

On the basis of their significant anti-HIV activity, compounds **3–5**, **10**, and **11** should be investigated in greater detail to develop a deeper understanding of their anti-HIV characteristics and potential. The new compounds likely share the same mechanisms of action as previously investigated daphnane- and tiglane-type diterpenes.^{7,10,27}

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were measured on an Autopol V Plus instrument (Rudolph, Hackettstown, NJ, USA). UV spectra were recorded in MeOH using a Lambda 25 spectrophotometer (PerkinElmer, Wellesley, MA, USA). CD spectra were obtained on a JASCO J-715 spectrometer. IR spectra were measured on a PE Spectrum RXI spectrophotometer (PerkinElmer) using KBr pellets. NMR spectroscopic data were recorded at room

temperature on Bruker AMX-400 MHz and AMX-600 MHz instruments in CDCl₃ with TMS as an internal standard. Standard pulse sequences were employed for the measurement of 2D NMR spectra (¹H–¹H COSY, HSQC, HMBC, and NOESY). ESIMS analysis were carried out on a Dionex Ultimate 3000 UPLC instrument with an LTQ Velos Pro MS spectrometer (Thermo Fisher Scientific, USA). HRESIMS were acquired with a Bruker Daltonics APEXIII 7.0 TESLA FTMS system (Bruker Daltonics, Billerica, MA, USA). Analytical HPLC was carried out on an Agilent 1200 series LC instrument with a DAD detector (Agilent Technologies, Palo Alto, CA, USA) and a Symmetry C₁₈ column (4.6 × 250 mm, 5 μm). Preparative HPLC was performed on an Agilent 1100 (Agilent Technologies) and a YMC-Pack Pro C₁₈ RS column (20 × 250 mm, 5 μm). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., Qingdao, People's Republic of China), Sephadex LH-20 (25–100 μm, Pharmacia, Germany), and RP-C₁₈ (30–50 μm, Fuji Silysia Chemical Co. Ltd., Aichi, Japan) were used for column chromatography (CC). The fractions were monitored by TLC (HSGF 254, Yantai, People's Republic of China), and detection was achieved by 10% H₂SO₄ in EtOH. All solvents used for CC were of analytical grade (Shanghai Chemical Reagents Co. Ltd., Shanghai, People's Republic of China), and solvents used for HPLC were of HPLC grade. Linoleic acid was obtained from Sigma-Aldrich Company Ltd., Gillingham, United Kingdom.

Plant Material

The roots of *S. chamaejasme* (4 years old) were purchased from Baotou, Inner Mongolia, People's Republic of China, in August 2011 and were authenticated by one of the authors (D.-F.C.). A voucher specimen (DFC-YM-SC-2011-08) is deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, People's Republic of China.

Extraction and Isolation

The dried roots of *S. chamaejasme* were ground into a powder (40 kg), which was percolated with 95% aqueous EtOH. After removal of the solvent under vacuum, the residue was suspended in H₂O and successively extracted with petroleum ether, EtOAc, and *n*-BuOH. The petroleum ether-soluble fraction (350 g) was subjected to VLC on silica gel using a stepwise gradient elution of petroleum ether–Me₂CO (30:1, 20:1, 10:1, 5:1, 2:1, and 1:1) to afford five subfractions (Fr.A–Fr.E). Fr.C (40 g) was passed through a silica gel column eluted with petroleum ether–EtOAc (30:1 to 10:1) to give seven fractions (Fr.C1–Fr.C7). Fr.C2 (7 g) was applied to CC on Sephadex LH-20 (*n*-hexane–CH₂Cl₂–MeOH, 5:4:1) to obtain six fractions (Fr.C2a–Fr.C2f). Fr.C2b (120 mg) was purified by preparative HPLC (10 mL/min, 50 min 85–95% MeCN–H₂O gradient elution) to yield **1** (10 mg) and **2** (8 mg). Fr.C2f (400 mg) was separated by preparative HPLC (10 mL/min, 50 min 70–95% MeCN–H₂O gradient elution) to acquire **12** (15 mg), **13** (35 mg), and **16** (40 mg). Fr.C3 (5 g) was chromatographed on an RP-C₁₈ silica gel column (MeOH–H₂O, 70:30 to 100:0) to give five fractions (Fr.C3a–Fr.C3e). Fr.C3d (1.2 g) was separated on a preparative HPLC column (10 mL/min, 75% MeCN–H₂O isocratic elution) to obtain **3** (8 mg), **4** (5 mg), **9** (20 mg), **10** (15 mg), and **19** (25 mg). Separation of Fr.D (30 g) by MPLC (petroleum ether–EtOAc, 15:1 to 0:1) gave six fractions (Fr.D1–Fr.D6). Fr.D3 was purified by passage over Sephadex LH-20 (CHCl₃–MeOH, 1:1) and then by preparative HPLC (CH₃CN–H₂O,

eluting from 65:35 to 90:10 for 40 min with a flow rate of 10 mL/min) to afford **6** (4 mg), **7** (7 mg), **8** (25 mg), **17** (12 mg), and **15** (9 mg). Using the same purification procedures, Fr.D4 afforded **5** (9 mg), **11** (11 mg), **18** (7 mg), and **14** (20 mg).

Stelleralide D (1): colorless oil; $[\alpha]_D^{20} +5.3$ (c 0.10, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ (log ϵ) 233 (4.38) nm; CD (MeOH, nm) $\lambda_{\max}$ (ϵ) 208 (4.06), 228 (7.70); IR (KBr) $\nu_{\max}$ 3478, 2924, 2854, 1799, 1718, 1648, 1452, 1396, 1270, 713 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃) data, see Table 1; HRESIMS m/z 927.5235 [M + Na]⁺ (calcd for C₅₃H₇₆O₁₂Na, 927.5229).

Stelleralide E (2): colorless oil; $[\alpha]_D^{20} -4.8$ (c 0.10, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ (log ϵ) 233 (4.16) nm; CD (MeOH, nm) $\lambda_{\max}$ (ϵ) 208 (4.67), 228 (7.78); IR (KBr) $\nu_{\max}$ 3459, 2923, 2854, 2359, 2341, 1797, 1717, 1455, 1395, 1173, 717 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃) data, see Table 1; HRESIMS m/z 951.5184 [M + Na]⁺ (calcd for C₅₅H₇₆O₁₂Na, 951.5229).

Stelleralide F (3): white, amorphous powder; $[\alpha]_D^{20} -7.7$ (c 0.10, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 233 (4.02) nm; CD (MeOH, nm) $\lambda_{\max}$ (ϵ) 215 (1.11), 236 (-1.14), 273 (0.87); IR (KBr) $\nu_{\max}$ 3463, 2925, 2860, 2360, 2340, 1731, 1712, 1457, 1384, 1279, 1025, 713 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃) data, see Table 2; HRESIMS m/z 855.3557 [M + Na]⁺ (calcd for C₄₆H₅₆O₁₄Na, 855.3562).

Stelleralide G (4): white, amorphous powder; $[\alpha]_D^{20} -16.7$ (c 0.10, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 233 (4.15) nm; CD (MeOH, nm) $\lambda_{\max}$ (ϵ) 215 (1.07), 237 (-7.42); IR (KBr) $\nu_{\max}$ 3453, 2934, 2854, 1715, 1451, 1384, 1275, 1025, 712 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃) data, see Table 2; HRESIMS m/z 917.3694 [M + Na]⁺ (calcd for C₅₁H₄₈O₁₄Na, 917.3719).

Stelleralide H (5): white, amorphous powder; $[\alpha]_D^{20} +4.6$ (c 0.10, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 233 (4.31) nm; CD (MeOH, nm) $\lambda_{\max}$ (ϵ) 215 (4.07), 236 (-5.42); IR (KBr) $\nu_{\max}$ 3446, 2927, 2858, 1716, 1451, 1272, 1112, 1023, 711 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃) data, see Table 2; HRESIMS m/z 775.3693 [M + H]⁺ (calcd for C₄₄H₅₄O₁₂ 775.3688).

Stelleralide I (6): white, amorphous powder; $[\alpha]_D^{20} +21.6$ (c 0.10, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 256 (3.98) nm; CD (MeOH, nm) $\lambda_{\max}$ (ϵ) 226 (-12.1), 242 (5.82); IR (KBr) $\nu_{\max}$ 3435, 2920, 2843, 1709, 1616, 1463, 1375, 1019 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) and ¹³C NMR (100 MHz, CDCl₃) data, see Table 3; HRESIMS m/z 637.3349 [M + Na]⁺ (calcd for C₃₅H₅₀O₉Na, 637.3347).

Stelleralide J (7): white, amorphous powder; $[\alpha]_D^{20} +2.2$ (c 0.12, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 256 (4.01) nm; CD (MeOH, nm) $\lambda_{\max}$ (ϵ) 243 (-1.69), 268 (3.08); IR (KBr) $\nu_{\max}$ 3405, 2925, 2854, 2361, 1701, 1639, 1006 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) and ¹³C

NMR (100 MHz, CDCl₃) data, see Table 3; HRESIMS m/z 643.3455 [M + Na]⁺ (calcd for C₃₄H₅₀O₁₀Na, 643.3453).

Base Hydrolysis of **2** and GC-MS Analysis

Compound **2** (2 mg) in CH₂Cl₂ (2 mL) was allowed to stand at room temperature for 3 h with 0.05 M NaOMe in MeOH (0.5 mL). The mixture was neutralized, followed by addition of 10 mL of 14% BF₃–MeOH, and was heated at 80 °C for 5 min. Hexane (3 mL) was added to the above mixture through the top of the condenser, and heating was continued for 2 min. After dilution with a saturated NaCl solution, the organic layer was collected and evaporated to dryness using N₂. The residue was redissolved in hexane and analyzed by GC-MS (Shimadzu, GCMS-QP2010 Ultra) using an Inertcap column (0.25 mm × 30 m) under the following conditions [injector temperature, 250 °C; initial temperature, 80 °C (1 min), increased at 25 °C/min to 230 °C, held for 10 min; carrier gas, He operated in the splitless mode; injection size, 0.2 µL; MS conditions: EI voltage, 70 eV; scanned-mass range, m/z 50–1000]. Identification of linoleic acid was carried out for **2**, giving a peak at 12.23 min. With authentic linoleic acid, a peak was detected at 12.24 min.

Anti-HIV Assays

HIV-1 NL4-3 (multiplicity of infection = 0.001) was used to infect MT4 cells in the presence of various concentrations of compounds. Fresh medium, which contained appropriate concentrations of the compounds, was added to the culture 48 h after infection to maintain normal cell growth. Virus replication was analyzed on day 4 postinfection using p24 ELISA kits from PerkinElmer. The compound concentration that inhibited HIV-1 replication by 50% (EC₅₀) was calculated by using the biostatistics software CalcuSyn (Biosoft).

Cytotoxicity Assays

Cytotoxicity of the purified compounds toward MT4 cells was determined by using a cell viability kit provided by Promega. The CellTiter-Glo luminescent cell viability assay is a simple method of determining the viability of the cells in culture based on quantitation of ATP in metabolically active cells. The CellTiter-Glo reagent was added to MT4 cells that were cultured parallel to the antiviral assays. The cytotoxic concentration that caused the reduction of viable cells by 50% (CC₅₀) was calculated from the dose–response curve using CalcuSyn.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Das Neves J, Amiji MM, Bahia MF, Sarmiento B. *Adv Drug Delivery Rev.* 2010; 62:458–477.
2. Bedoya LM, Marquez N, Martinez N, Gutierrez-Eisman S, Alvarez A, Calzado MA, Rojas JM, Appendino G, Munoz E, Alcamí J. *Biochem Pharmacol.* 2009; 77:965–978. [PubMed: 19100719]
3. Alexaki A, Liu Y, Wigdahl B. *Curr HIV Res.* 2008; 6:388–400. [PubMed: 18855649]
4. Marsden MD, Zack JA. *Future Virol.* 2010; 5:97–109. [PubMed: 21318097]
5. Finzi D, Hermankova M, Carruth LM, Buck C. *Science.* 1997; 278:1295–1300. [PubMed: 9360927]
6. Xu ZH, Qin GW, Li XY, Xu RS. *Acta Pharm Sin.* 2001; 36:668–671.
7. Asada Y, Sukemori A, Watanabe T, Malla KJ, Yoshikawa T, Li W, Koike K, Chen CH, Akiyama T, Qian KD, Nakagawa-Goto K, Morris-Natschke SL, Lee KH. *Org Lett.* 2011; 13:2904–2907. [PubMed: 21561135]
8. Niwa M, Takamizawa H, Tatematsu H, Hirata Y. *Chem Pharm Bull.* 1982; 30:4518–4520.
9. Jiang ZH, Tanaka T, Sakamoto T, Kouno I, Duan JA, Zhou RH. *Chem Pharm Bull.* 2002; 50:137–139. [PubMed: 11824576]
10. Asada Y, Sukemori A, Watanabe T, Malla KJ, Yoshikawa T, Li W, Kuang XZ, Koike K, Chen CH, Akiyama T, Qian KD, Lee KH. *J Nat Prod.* 2013; 76:852–857. [PubMed: 23611151]
11. Qiao LR, Yang L, Zou JH, Li L, Sun H, Si YK, Zhang D, Chen XG, Dai JG. *Planta Med.* 2012; 78:711–719. [PubMed: 22441834]
12. Tatematsu H, Kurokawa M, Niwa M. *Chem Pharm Bull.* 1984; 32:1612–1613.
13. Yang G, Liao Z, Xu Z, Zhang H, Chen D. *Chem Pharm Bull.* 2005; 53:776–779. [PubMed: 15997133]
14. Liu GQ, Tatematsu H, Kurokawa M, Niwa M, Hirata Y. *Chem Pharm Bull.* 1984; 32:362–365.
15. Niwa M, Liu GQ, Tatematsu H, Hirata Y. *Tetrahedron Lett.* 1984; 25:3735–3738.
16. Yan ZQ, Guo HR, Yang JY, Liu Q, Jin H, Xu R, Cui HY, Qin B. *Phytochemistry.* 2014; 106:61–68. [PubMed: 25096753]
17. Yang GH, Chen DF. *Chin J Nat Med.* 2006; 4:425–427.
18. Tikhomrova LI, Markova LP, Tumbaa H. *Khim Prirod Soedin.* 1974; 3:402–403.
19. Liu LP, Han K, Chen W, Zhang YY, Tong LJ, Peng T, Xie H, Ding J, Wang HB. *Bioorg Med Chem.* 2014; 22:4198–4203. [PubMed: 24931277]
20. Hayes PY, Chow S, Somerville MJ, Fletcher MT, De Voss JJ. *J Nat Prod.* 2010; 73:1907–1913. [PubMed: 21049973]
21. Abe F, Iwase Y, Yamauchi T, Kinjo K, Yaga S, Ishii M, Iwahana M. *Phytochemistry.* 1998; 47:833–837. [PubMed: 9542174]
22. Abe F, Iwase Y, Yamauchi T, Kinjo K, Yaga S. *Phytochemistry.* 1997; 44:643–647.
23. Jolad SD, Hoffmann JJ, Timmermann BN, Schram KH, Cole JR. *J Nat Prod.* 1983; 46:675–680.
24. Metcalfe LD, Schmitz AA. *Anal Chem.* 1961; 33:363–364.
25. Wang HB, Liu LP, Wang XY. *Magn Reson Chem.* 2013; 51:580–592. [PubMed: 23813597]
26. Liao SG, Chen HD, Yue JM. *Chem Rev.* 2009; 109:1092–1140. [PubMed: 19182998]
27. Huang L, Ho P, Yu J, Zhu L, Lee KH, Chen CH. *PLoS One.* 2011; 6:e26677. [PubMed: 22039528]

Figure 1.

Key ^1H - ^1H COSY, HMBC, and NOESY correlations of **1**, **3**, and **7**.

Table 1¹H (600 MHz) and ¹³C (150 MHz) NMR Spectroscopic Data for Compounds 1 and 2 in CDCl₃

position	1^a		2^b	
	δ_{H} [J in (Hz)]	δ_{C}	δ_{H} [J in (Hz)]	δ_{C}
1	2.86 (1H, dd, 14.0, 4.0)	54.5	2.85 (1H, dd, 12.0, 4.0)	54.5
2		112.1		112.1
3		173.9		173.9
4		86.5		86.5
5	4.82 (1H, brs)	69.3	4.82 (1H, brs)	69.3
6		57.1		57.1
7	3.44 (1H, brs)	59.3	3.43 (1H, brs)	59.3
8	3.26 (1H, dd, 2.8, 1.6)	34.8	3.26 (1H, dd, 2.8, 1.6)	34.8
9		80.1		80.1
10	2.89 (1H, d, 4.0)	54.8	2.89 (1H, d, 4.2)	54.8
11	2.19 (1H, t, 6.8, 6.8)	42.8	2.19 (1H, t, 6.8, 7.2)	42.8
12	2.08 (1H, dd, 11.2, 6.8)	32.6	2.08 (1H, dd, 11.2, 6.8)	32.6
	2.32 (1H, dd, 11.2, 6.8)		2.32 (1H, dd, 11.2, 6.8)	
13		83.4		83.4
14	4.35 (1H, s)	80.6	4.35 (1H, s)	80.6
15		145.7		145.7
16	4.94 (1H, dd, 1.8, 1.8)	111.6	4.94 (1H, dd, 2.0, 2.0)	111.6
	5.05 (1H, s)		5.03 (1H, s)	
17	1.79 (3H, s)	18.8	1.79 (3H, s)	18.7
18	4.54 (1H, dd, 12.4, 6.8)	69.5	4.54 (1H, dd, 12.4, 6.8)	69.5
	4.97 (1H, d, 12.5)		4.97 (1H, d, 12.5)	
19	1.73 (3H, s)	19.6	1.74 (3H, s)	19.5
20	4.09, 4.68 (2H, d, 12.5)	63.9	4.09, 4.68 (2H, d, 12.5)	63.9
1'		120.4		120.4
2'	2.10 (1H, ddd, 12.0, 6.0, 3.0)	31.3	2.10 (1H, ddd, 12.0, 6.0, 3.0)	31.3
	1.93 (1H, ddd, 12.0, 5.6, 3.0)		1.93 (1H, ddd, 12.0, 5.6, 3.0)	
3'	1.56, 1.76 (2H, m)	22.2	1.56, 1.76 (2H, m)	22.1
4'	1.27, 1.62 (2H, m)	25.1	1.27, 1.62 (2H, m)	25.1
5'	1.17, 1.48 (2H, m)	24.2	1.17, 1.48 (2H, m)	24.2
6'	1.27, 1.52 (2H, m)	26.6	1.27, 1.52 (2H, m)	26.6
7'	1.11, 1.52 (2H, m)	26.9	1.11, 1.52 (2H, m)	26.9
8'	0.87, 2.27 (2H, m)	32.6	0.87, 2.31 (2H, m)	32.6
9'	1.13 (1H, m)	37.8	1.13 (1H, m)	37.8
10'	1.02 (3H, d, 6.4)	19.2	1.02 (3H, d, 6.4)	19.2
Bz-CO		166.4		166.4
Bz-1''		130.0		130.0
Bz-2'', 6''	8.04 (2H, dd, 8.4, 1.2)	129.5	8.04 (2H, dd, 11.2, 1.2)	129.5
Bz-3'', 5''	7.46 (2H, dd, 8.0, 8.0)	128.5	7.45 (2H, dd, 7.6, 7.6)	128.5

position	1^a	2^b	
	δ_{H} [<i>J</i> in (Hz)]	δ_{C}	δ_{H} [<i>J</i> in (Hz)]
Bz-4''	7.57 (1H, dd, 7.2, 1.2)	133.2	7.57 (1H, dd, 7.6, 1.2)

^aData for palmitic acid group: δ_{H} 2.37 (2H, t, 8.0, H-2'''), 1.62 (2H, m, H-3'''), 1.23–1.30 (24H, m, H-4'''–H-15'''), 0.86 (3H, t, 8.0, H-16'''); δ_{C} 173.8 (C-1'''), 34.1 (C-2'''), 24.7 (C-3'''), 29.0–29.8 (C-4'''–C-13'''), 31.9 (C-14'''), 22.7 (C-15'''), 14.1 (C-16''').

^bData for linoleic acid group: δ_{H} 2.38 (2H, t, 8.0, H-2'''), 1.62 (2H, m, H-3'''), 1.23–1.30 (12H, m, H-4'''–H-7''', H-16''', and H-17'''), 2.04 (4H, m, H-8''', H-14'''), 5.32 (1H, m, H-9'''), 5.37 (1H, m, H-10'''), 2.76 (4H, t, 5.2, H-11''', H-15'''), 5.35 (1H, m, H-12'''), 5.33 (1H, m, H-13'''), and 0.88 (3H, t, 6.8, H-18'''); δ_{C} 174.0 (C-1'''), 33.9 (C-2'''), 24.7 (C-3'''), 29.0–29.8 (C-4'''–C-7'''), 27.1 (C-8''', C-14'''), 127.9 (C-9'''), 130.2 (C-10'''), 25.6 (C-11''', C-15'''), 130.1 (C-12'''), 128.1 (C-13'''), 31.7 (C-16'''), 22.5 (C-17'''), and 14.0 (C-18''').

Table 2
¹H (600 MHz) and ¹³C (150 MHz) NMR Spectroscopic Data for Compounds 3–5 in CDCl₃

position	4 ^a		4 ^b		5 ^c	
	δ _H [J in (Hz)]	δ _C	δ _H [J in (Hz)]	δ _C	δ _H [J in (Hz)]	δ _C
1	2.84 (1H, dd, 11.4, 11.4)	49.2	3.00 (1H, dd, 11.2, 11.2)	49.2	2.68 (1H, dd, 12.0, 12.0)	48.1
2	1.81 (1H, m)	37.4	1.87 (1H, m)	37.3	1.71 (1H, m)	37.4
3	4.90 (1H, d, 4.8)	82.4	4.96 (1H, d, 5.2)	82.3	3.81 (1H, d, 7.2)	79.5
4		79.4		79.5		78.5
5	4.06 (1H, d, 4.0)	73.5	4.12 (1H, d, 4.2)	73.2	3.79 (1H, s)	70.9
6		60.4		60.8		60.3
7	3.36 (1H, brs)	63.5	3.40 (1H, brs)	63.5	3.36 (1H, brs)	63.5
8	3.01 (1H, d, 1.8)	36.6	3.06 (1H, d, 1.8)	36.6	3.09 (1H, d, 1.8)	36.6
9		81.3		81.3		80.9
10	2.99 (1H, d, 12.0)	48.1	3.01 (1H, d, 12.0)	48.1	2.92 (1H, d, 14.4)	47.9
11	2.65 (1H, t, 7.8)	40.9	2.85 (1H, t, 7.8)	40.9	2.76 (1H, t, 7.8)	41.1
12	1.88 (1H, m)	29.2	1.98 (1H, m)	29.2	2.03 (1H, m)	29.5
	2.21 (1H, d, 12.0)		2.35 (1H, d, 14.8)		2.32 (1H, d, 14.4)	
13		84.4		84.5		84.5
14	4.37 (1H, d, 2.4)	81.3	4.43 (1H, d, 2.8)	81.3	4.40 (1H, d, 2.4)	81.4
15		145.4		145.4		145.6
16	4.93 (1H, dd, 1.2, 1.2)	111.9	4.98 (1H, dd, 1.2, 1.2)	111.7	4.97 (1H, 1.2, 1.2)	111.9
	5.15 (1H, s)		5.21 (1H, s)		5.20 (1H, s)	
17	1.80 (3H, s)	18.9	1.84 (3H, s)	18.8	1.84 (3H, s)	18.9
18	4.85 (1H, dd, 10.2, 3.0)	66.8	4.41 (1H, dd, 10.8, 3.8)	67.2	4.16 (1H, d, 12.0)	67.6
	4.08 (1H, dd, 10.2, 10.2)		5.11 (1H, dd, 8.0, 8.0)		5.01 (1H, d, 12.0)	
19	1.18 (3H, d, 6.6)	14.5	1.23 (3H, d, 6.8)	14.5	1.16 (3H, d, 6.0)	14.5
20	3.83 (2H, d, 7.2)	65.8	3.87 (2H, d, 7.2)	65.8	4.38 (1H, d, 10.2)	68.2
					4.91 (1H, d, 10.2)	
1'		118.4		118.4		118.5
2'	3.89 (1H, dd, 8.4, 3.0)	70.6	3.95 (1H, dd, 7.6, 3.0)	70.5	3.90 (1H, dd, 10.8, 3.6)	70.8
3'	1.56, 1.66 (1H, m)	28.6	1.59, 2.42 (1H, m)	28.6	1.71, 1.53 (1H, m)	28.5

position	δ^a		q^b		5^c	
	δ_H [J in (Hz)]	δ_C	δ_H [J in (Hz)]	δ_C	δ_H [J in (Hz)]	δ_C
4'	1.30, 1.66 (1H, m)	24.6	1.36, 1.66 (1H, m)	24.4	1.27, 1.58 (1H, m)	25.3
5'	1.40, 1.53 (2H, m)	19.3	1.48, 1.58 (2H, m)	19.3	1.20, 1.32 (2H, m)	24.2
6'	1.66, 1.87 (2H, m)	28.7	1.71, 1.93 (2H, m)	28.7	1.36 (2H, m)	23.6
7'	5.35 (1H, dd, 13.6, 6.8)	73.4	5.39 (1H, dd, 13.6, 6.8)	73.4	1.24, 1.34 (1H, m)	23.1
8'	1.53, 1.96 (1H, m)	27.6	1.58, 2.01 (1H, m)	27.6	0.99, 1.59 (1H, m)	22.8
9'	2.31 (1H, m)	27.9	2.43 (1H, m)	28.1	2.28 (1H, m)	27.3
10'	1.22 (3H, d, 7.2)	18.5	1.30 (3H, d, 7.2)	18.5	0.99 (3H, d, 7.2)	18.2

^aData for benzoyl and acetyl groups: δ_H 8.19 (2H, dd, 7.8, 1.2, H-2'', H-6''), 7.48 (2H, dd, 6.8, 7.8, H-3'', 5''), 7.62 (1H, dd, 6.8, 1.2, H-4''), 8.06 (2H, dd, 10.4, 1.2, H-2''', H-6'''), 7.45 (2H, dd, 10.4, 7.8, H-3''', 5'''), 7.56 (1H, dd, 7.8, 1.0, H-4'''), 2.10 (3H, s, Ac-Me); δ_C 166.1, 168.4 (Bz-CO), 130.8 (C-1''), 130.2 (C-2'', 6''), 128.5 (C-3'', 5''), 133.6 (C-4''), 129.3 (C-1'''), 129.6 (C-2''', 6'''), 128.4 (C-3''', 5'''), 132.8 (C-4'''), 170.9 (Ac-CO), 21.1 (Ac-Me).

^bData for benzoyl group: δ_H 8.19 (2H, dd, 12, 1.8, H-2'', H-6''), 7.39 (2H, dd, 12.0, 10.8, H-3'', 5''), 7.62 (1H, dd, 10.8, 1.8, H-4''), 8.09 (2H, dd, 10.8, 2.4, H-2''', H-6'''), 7.48 (2H, dd, 10.8, 11.4, H-3''', 5'''), 7.58 (1H, dd, 11.4, 2.4, H-4'''), 8.16 (2H, dd, 10.8, 1.8, H-2''', H-6'''), 7.51 (2H, dd, 10.8, 12, H-3''', 5'''), 7.54 (1H, dd, 12.0, 1.8, H-4'''), δ_C 166.1, 166.5, 168.4 (Bz-CO), 129.3 (C-1''), 129.9 (C-2'', 6''), 128.4 (C-3'', 5''), 133.5 (C-4''), 130.8 (C-1'''), 129.4 (C-2''', 6'''), 128.5 (C-3''', 5'''), 132.8 (C-4'''), 130.5 (C-1'''), 129.4, (C-2''', 6'''), 128.6 (C-3''', 5'''), 133.0 (C-4''').

^cData for benzoyl group: δ_H 8.08 (4H, dd, 7.8, 1.2, H-2'', 6'', 2'', 6''), 7.46 (4H, dd, 7.8, 7.2, H-3'', 5'', 3'', 5''), 7.58 (2H, dd, 7.2, 1.2, H-4'', 4''), δ_C 166.5, 167.1 (Bz-CO), 129.6 (C-1''), 129.9 (C-2'', 6''), 128.4 (C-3'', 5''), 133.1 (C-4''), 130.1 (C-1'''), 129.7 (C-2''', 6'''), 128.4, 128.4 (C-3''', 5'''), 133.1 (C-4''').

Table 3¹H (400 MHz) and ¹³C (100 MHz) NMR Spectroscopic Data for Compounds 6 and 7 in CDCl₃

position	6		7	
	δ_{H} [J in (Hz)]	δ_{C}	δ_{H} [J in (Hz)]	δ_{C}
1	7.59 (1H, brs)	160.9	7.73 (1H, s)	162.4
2		136.6		134.8
3		209.8		209.5
4		72.5		74.8
5	4.25 (1H, s)	72	3.83 (1H,s)	72.4
6		60.6		76.8
7	3.55 (1H, d, 1.8)	64.3	4.31 (1H, s)	79.1
8	3.78 (1H, brs)	34.9	3.29 (1H, brs)	40.1
9		78.5		76.3
10	3.85 (1H, t, 2.8)	47.6	3.63 (1H, d, 5.0)	53.3
11	2.49 (1H, dd, 14.0, 6.8)	44.9	2.24 (1H, m)	36.5
12	3.94 (1H, brs)	77.6	2.14 (2H, m)	37.5
13		85.0		74.4
14	4.74 (1H, d, 1.8)	80.6	5.65 (1H, s)	79.8
15		144.7		145.3
16	5.14 (2H, d, 10.8)	112.9	5.06 (1H, s), 5.17 (1H, s)	114.3
17	1.89 (3H, s)	18.9	1.88 (3H, s)	18.9
18	1.23 (3H, d, 7.6)	18.6	1.06 (3H, d, 8.5)	17.6
19	1.82 (3H, s)	9.9	1.80 (3H, s)	9.8
20	3.78 (1H, d, 12.0)	65.0	3.77 (2H, d, 11.2)	67.0
	3.94 (1H, d, 12.0)			
1'		116.8		168.6
2'	5.64 (1H, d, 15.2)	139.1	5.90 (1H, d, 16.0)	118.6
3'	6.67 (1H, dd, 15.2, 10.4)	122.7	7.35 (1H, dd, 14.0, 7.6)	146.8
4'	5.86 (1H, dd, 14.4, 8.0)	128.6	7.35 (1H, dd, 14.0, 7.6)	128.2
5'	5.86 (1H, dd, 14.4, 8.0)	134.9	6.21 (1H, dd, 14.0, 9.2)	146.3
6'	2.11 (2H, dd, 14.4, 7.2)	32.7	1.29 (2H, m)	31.9
7'	1.27 (2H, m)	29.2	1.30 (2H, m)	29.2
8'	1.28 (2H, m)	29.2	1.30 (2H, m)	29.2
9'	1.28 (2H, m)	29.3	1.30 (2H, m)	29.3
10'	1.29 (2H, m)	29.3	1.30 (2H, m)	29.4
11'	1.30 (2H, m)	29.5	1.30 (2H, m)	29.5
12'	1.30 (2H, m)	29.5	1.30 (2H, m)	31.8
13'	1.31 (2H, m)	31.9	1.30 (2H, m)	22.6
14'	1.31 (2H, m)	22.6	0.89 (3H, d, 6.4)	14.1
15'	0.90 (3H, t, 7.2)	14.1		

Table 4Anti-HIV and Cytotoxicity Activities of Compounds 1–19^a

compound	anti-HIV	cytotoxicity	selectivity index
	EC ₅₀ (μM)	CC ₅₀ (μM)	CC ₅₀ /EC ₅₀
1	0.59 ± 0.18	11.1	18
2	6.71 ± 2.46	10.8	1
3	0.00093 ± 0.00025	12	12903
4	0.00073 ± 0.00032	11.2	15342
5	0.00098 ± 0.00037	12.9	13163
6	0.12 ± 0.038	16.3	135
7	0.044 ± 0.015	4.4	100
8	0.33 ± 0.12	13.8	41
9	1.12 ± 0.30	18.3	16
10	0.00006 ± 0.00002	1.29	21500
11	0.0011 ± 0.0004	15.7	14272
12	0.048 ± 0.0180	15.3	318
13	0.012 ± 0.0041	15.6	1300
14	0.039 ± 0.0082	15.3	392
15	0.013 ± 0.0041	16.7	1284
16	0.0026 ± 0.0009	14	5384
17	0.059 ± 0.0150	16.6	281
18	0.044 ± 0.0118	15.2	345
19	0.047 ± 0.0148	18.8	400
AZT	0.032 ± 0.0082	3.74	116

^aThe values are means ± SD (*n* = 3). AZT (zidovudine) was used as a positive control.