7α,20-Epoxy-*ent*-kauranoids from *Isodon parvifolius*

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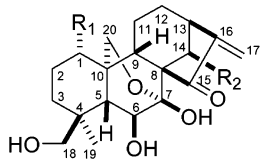
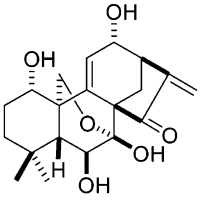
Nine new 7α,20-epoxy-*ent*-kaurane diterpenoids, parvifolines C−K (**1**−**9**), together with 12 known analogues, rabdoternin G (**10**), adenolin E (**11**), lasiodonin (**12**), lushanrubescensin F (**13**), parvifoliside (**14**), effusanin A (**15**), effusanin B (**16**), effusanin E (**17**), taibaihenryiin A (**18**), shikokianin (**19**), maoyecrystal J (**20**), and the acetonide of lasiodonin (**21**), were isolated from the leaves of *Isodon parvifolius*. The structures of compounds **1**−**9** were determined on the basis of spectroscopic methods including extensive 1D and 2D NMR analysis. The new diterpenoids (**1**−**9**) and lasiodonin (**12**) were evaluated for their inhibitory activity against A549, HT-29, and K562 cell lines.

Plants of the genus *Isodon* are phenomenal resources of *ent*-kaurane diterpenoids, some of which have antitumor, antibacterial, and anti-inflammatory activities.[1] Recently, a novel C_{19} skeleton diterpenoid (maoecrystal V)[2] and a novel asymmetric *ent*-kauranoid dimer (lushanrubescensin J)[3] were discovered. *Isodon parvifolius* (Batalin) H. Hara (Labiatae) is a perennial undershrub native to north of Sichuan Province and the Tibet Autonomous Region, People's Republic of China. Previous chemical studies of this species evidenced the presence of bioactive diterpenoids.[4−10] As a continuation of a program directed toward the isolation of new and biologically active constituents, to provide the scientific data for developing anti-inflammatory and anticancer medicines from the genus *Isodon*, we reinvestigated this plant collected in Mao County of Sichuan Province. A series of 7α,20-epoxy-*ent*-kaurane diterpenoids were isolated from the leaves of *I. parvifolius*, including nine new ones, parvifolines C−K (**1**−**9**), and 12 known diterpenoids, rabdoternin G (**10**),[11] adenolin E (**11**),[12] lasiodonin (**12**),[10] lushanrubescensin F (**13**),[13] parvifoliside (**14**),[6,7] effusanin A (**15**),[14] effusanin B (**16**),[14] effusanin E (**17**),[14,15] taibaihenryiin A (**18**),[16] shikokianin (**19**),[17,18] maoyecrystal J (**20**),[19] and acetonide of lasiodonin (**21**),[20] identified by comparing their physical and spectroscopic data with those reported in the literature. In this paper, we report the structure elucidation of the new compounds (**1**−**9**). Compounds **1**−**9** and lasiodonin (**12**) were also evaluated for inhibitory activity against A549, HT-29, and K562 cell lines.





	R_1	R_2
1	OH	H
2	OH	OH
3	OAc	OH

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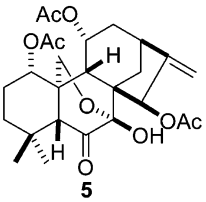

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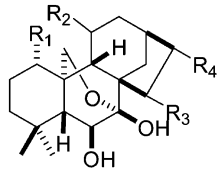
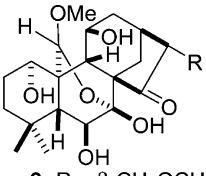
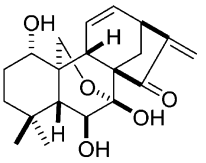


	R_1	R_2	R_3	R_4
6	OH	β-OAc	= O	= CH_2
7	OAc	β-OH	= O	= CH_2
8	OH	β-OAc	= O	β-CH_2OCH_3
11	OAc	β-OH	= O	β-CH_2OCH_3
12	OH	β-OH	= O	= CH_2
13	OH	β-OH	= O	β-CH_2OCH_3
14	OH	β-OGlc	β-OH	= CH_2
15	OH	H	= O	= CH_2
16	OAc	H	= O	= CH_2
17	OH	α-OH	= O	= CH_2
18	OH	α-OAc	= O	= CH_2
19	OAc	α-OAc	= O	= CH_2



9 R = β-CH_2OCH_3
10 R = = CH_2



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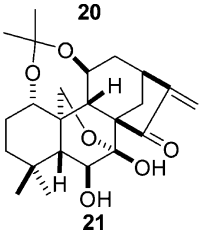

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Results and Discussion

An acetone extract of the leaves of *I. parvifolius* was partitioned successively between petroleum ether and water, ethyl acetate and water, then *n*-butanol and water. The ethyl acetate extract yielded 20 diterpenoids (**1**−**13** and **15**−**21**) by repeated silica gel chromatography, recrystallization, and high-performance liquid chromatography. Compound **14** was obtained from the *n*-butanol extract by normal-phase silica gel chromatography.

Parvifoline C (**1**), obtained as colorless crystals from MeOH, showed the sodiated molecular ion peak $[M + Na]^+$ at *m*/*z* 387.1777 in the HRESIMS, corresponding to the molecular formula $C_{20}H_{28}O_6$. This was corroborated by the ^{13}C and DEPT NMR spectra, which displayed 20 signals for the carbons of the diterpenoid skeleton.

The presence of a carbonyl group conjugated with an exocyclic methylene in **1** was suggested by the following spectroscopic data: UV (MeOH) λ_{max} (log ϵ) 241 (3.94) nm; IR (KBr) ν_{max} 1701 and 1637 cm^{-1}; ^1H NMR δ_H 5.98 and 5.30 (each 1H, s); ^{13}C NMR δ_C 211.1 (s), 154.2 (s), and 116.5 (t). In addition, on the basis of the characteristic signals of three methines [δ_C 57.5 (C-5), 52.2

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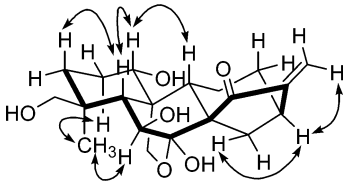

Figure 1. Key ROESY correlations of **1**.

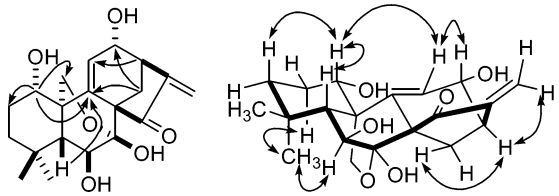

Figure 2. Key HMBC and ROESY correlations of **4**.

(C-9), and 35.2 (C-13)], three quaternary carbons [δ_C 60.5 (C-8), 41.5 (C-10), and 39.0 (C-4)], a hemiketal quarternary carbon [δ_C 95.6 (C-7)], and a noticeable oxygenated methylene [δ_C 64.2, δ_H 4.78 and 4.36 (each 1H, d, $J = 10.0$ Hz)], along with the structures of the compounds isolated previously from this plant, we assumed that **1** should be a 7β-hydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one diterpenoid, substituted by three hydroxyl groups, similar to effusanin A (**15**).[14]

The mass spectrum of compound **1** was 16 amu higher than that of **15**, indicating that there was probably one more hydroxyl group in **1**. Comparison of the NMR data between **1** and **15** confirmed the above deduction and suggested that **1** was identical to **15** except for the hydroxyl substituent at C-18. This was supported by the upfield shift of C-3 (δ_C 33.8) and C-5 (δ_C 57.5) in **1** compared with that of C-3 (δ_C 39.1) and C-5 (δ_C 61.0) in **15** due to the γ-gauche steric compression effect between the 18-OH and H-3β and H-5β. The correlations of H-1β with H-5β and H-9β and of H-2α and H-6α with Me-19 in the ROESY spectrum of **1** (Figure 1) confirmed that the hydroxyl groups of C-1 and C-6 had 1α- and 6β-orientations and another hydroxyl group was placed at C-18, respectively. Thus, compound **1** was determined to be 1α,6β,7β,-18-tetrahydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

Parvifoline D (**2**) had the molecular formula $C_{20}H_{28}O_7$, as determined by HRESIMS. Comparison of the spectroscopic data of **2** and **1** revealed similarities except for one more hydroxyl group at C-14 of **2**, causing a significant downfield chemical shift of C-8 (δ_C 62.9) and C-13 (δ_C 44.0). HMBC correlations of δ_H 5.09 (H-14) with δ_C 209.4 (C-15), 153.3 (C-16), 54.4 (C-9), and 31.0 (C-12) further supported the above assignment. The β-orientation of the 14-OH group was apparent from the ROESY correlations of H-14α with H-13α. The other substituents had the same orientations as those in **1**. Therefore, **2** was elucidated as 1α,6β,7β,14β,18-pentahydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

Parvifoline E (**3**) gave a molecular formula of $C_{22}H_{30}O_8$ by HRESIMS. The NMR data indicated that **3** was very similar to **2** except for an acetyl group in **3**. The acetyl group was placed at C-1, as established by the HMBC spectrum. All of the substituents had the same orientations as those in **2**, according to the ROESY experiment. Hence, 6β,7β,14β,18-tetrahydroxy-1α-acetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one was assigned to **3**.

Parvifoline F (**4**) was assigned the molecular formula $C_{20}H_{26}O_6$ by positive HRESIMS, possessing eight degrees of unsaturation. Its IR and NMR data suggested **4** also to be a 6β,7β-dihydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one diterpenoid, to which one trisubstituted double bond [δ_H 6.60 (1H, d, $J = 2.3$ Hz) and δ_C 147.3 (s), 123.2 (d)] and two hydroxyl groups were introduced. Analysis of the 2D NMR spectra of **4** (Figure 2) enabled us to locate the trisubstituted double bond between C-9 and C-11 and attach the other two hydroxyl groups to the C-1α and C-12α

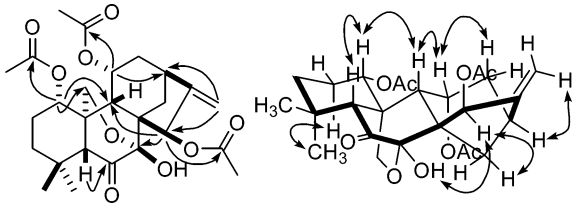

Figure 3. Key HMBC and ROESY correlations of **5**.

positions. Consequently, **4** was characterized as 1α,6β,7β,12α-tetrahydroxy-7α,20-epoxy-*ent*-kaur-9,16-dien-15-one.

Parvifoline G (**5**) was isolated as colorless crystals, whose molecular formula was inferred as $C_{26}H_{34}O_7$ by HRESIMS and NMR data. Apart from the 20 carbons belonging to the diterpenoid skeleton, there were three acetyl groups in **5**. This compound showed no characteristic absorption bands above 220 nm in its UV spectrum, but showed the presence of an exocyclic methylene moiety [δ_H 5.05, 4.80, (each 1H, br s); δ_C 154.1 (s), 110.5 (t)] and a ketone carbonyl signal δ_C [206.5 (s)] in its NMR spectra. Therefore, the carbonyl group at C-15 must be absent. Careful analysis of 2D NMR data led to the conclusion that the C-1, C-11, and C-15 positions were substituted by α-, α-, and β-acetoxyl groups, respectively, and a ketone was placed at C-6, on the basis of the HMBC correlations of H-1/C-2, C-10, C-9, and C-20, H-5/C-6, H-11/C-13 and C-9, and H-15/C-9, C-7, C-16, and C-17 and the ROESY correlations of H-1β and H-11β/H-9β and of H-15α/H-14β and OH-7β (Figure 3). The 15β-acetoxy group was confirmed by the significant upfield signal of C-9 (δ_C 47.3), which was caused by the γ-gauche steric compression effect between the 15β-acetoxy group and H-9β. Therefore, **5** was determined to be 7β-hydroxy-1α,11α,15β-triacetoxy-7α,20-epoxy-*ent*-kaur-16-en-6-one.

The ^{1}H and ^{13}C NMR data of **6** were very similar to those of lasiodonin (**12**), and the only observed difference was that **6** had an acetyl group. The acetyl group was assigned to C-11β unambiguously because the signal at δ_H 4.61 (δ_C 63.0) due to C-11 in **12** was shifted downfield to δ_H 6.04 (δ_C 67.5) in **6**, which was confirmed by the HMBC results. The stereochemistry of the remaining substituents in **6** was the same as that in **12** from a ROESY experiment. Thus, parvifoline H (**6**) was elucidated as 1α,6β,7β-trihydroxy-11β-acetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

Parvifoline I (**7**) had a positive ESIMS molecular ion the same as in **6**, observed at *m*/*z* 429 ($C_{22}H_{30}O_7Na$), also showing spectroscopic data very similar to **6**. It was presumed to be an isomer of **7** concerning the position of the acetoxyl group. The signal at δ_H 4.05 (δ_C 72.3) due to C-1 in **6** was shifted downfield to δ_H 5.44 (δ_C 76.4), suggesting the replacement of an acetoxyl group at the C-1α position in **7**. The signal at δ_H 6.04 (δ_C 67.5) due to C-11 in **6** was shifted upfield to δ_H 4.66 (δ_C 62.5), indicating the presence of a β-orientated hydroxyl group at C-11 in **7**. Eventually, **7** was identified as 6β,7β,11β-trihydroxy-1α-acetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

Parvifoline J (**8**), a white amorphous powder, had the same molecular formula ($C_{23}H_{34}O_8$) as adenolin E (**11**). There were no palpable differences in the NMR spectrum between **8** and **11**. In the same way as **6** and **7**, **8** was presumed to be an isomer of **11**, differing from each other only at the position of the acetoxyl group. In the HMBC spectrum, the correlation between H-11α and the acetoxyl group showed that the acetoxyl group was at C-11, rather than a hydroxyl group at the same position as in **11**. Thus, compound **8** was determined to be 1α,6β,7β-trihydroxy-16β-methoxymethyl-11β-acetoxy-7α,20-epoxy-*ent*-kaur-15-one.

Parvifoline K (**9**) was assigned the molecular formula $C_{22}H_{34}O_8$ by positive HRESIMS. It showed no α,β-unsaturated ketone group absorption in its UV and IR spectra. Its NMR spectra were similar to those of **10** except for the D-ring signals. The exomethylene in

10 was replaced by a methine [δ_H 2.98 (H-16); δ_C 59.3 (C-16)] and a methoxymethyl group [δ_H 3.22 (OMe), 3.77 and 3.64 (H_2-17); δ_C 58.7 (OMe) and 69.1 (C-17)] in **9**. The β-configuration of the methoxymethyl group in **9** was deduced from the abnormal upfield shift of C-12 (δ_C 30.6).[21] The above conclusion was confirmed by the ROESY correlations of H-16α/H-13α/H-14α. Therefore, the structure of **9** was represented as (20*S*)-1α,6β,7β,-11β-tetrahydroxy-16β-methoxymethyl-20-methoxy-7α,20-epoxy-*ent*-kaur-15-one.

The cytotoxicity of compounds **1**−**9** and **12** was tested against A549, HT-29, and K562 cell lines using the method described in the literature.[22] Compound **1** was the most active against K562 human leukemia cells, with an IC_{50} value of 13.8 μM. Compounds **5** and **8** were noncytotoxic in all three cell lines (IC_{50} > 100 μM). Although having a cyclopentanone conjugated with an exomethylene group, compounds **2**, **4**, **6**, and **7** were inactive in these test systems (IC_{50} > 100 μM). The above results suggest that the cyclopentanone conjugated with an exomethylene group is the active center, but the substituents in the molecule also affect their cytotoxicity.

Experimental Section

General Experimental Procedures. Melting points were obtained on an XRC-1 micro melting point apparatus and are uncorrected. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor27 spectrophotometer was used for scanning IR spectroscopy using KBr pellets. MS spectra were performed on a VG Autospec-3000 spectrometer or on a Finnigan MAT 90 instrument. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Unless specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Semipreparative HPLC was performed on an Agilent 1100 apparatus equipped with a UV detector and a Zorbax SB-C-18 (Agilent, 9.4 mm × 25 cm) column. Preparative HPLC was performed on a Shimadazu LC-8A preparative liquid chromatography apparatus with a Shimadazu PRC-ODS (K) column. Column chromatography was performed on silica gel (200−300 mesh, Qing-dao Marine Chemical Factory, Qingdao, People's Republic of China), silica gel H (60 μm, Qing-dao Marine Chemical Factory), Lichroprep RP-18 gel (40−63 μm, Merk, Darmstadt, Germany), and MCI gel (75−150 μm, Mitsubishi Chemical Corporation, Japan).

Plant Material. The leaves of *Isodon parvifolius* were collected in Mao County, north of Sichuan Province, People's Republic of China, in August 2004. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (KIB 04081802) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered leaves of *I. parvifolius* (6.0 kg) were extracted with Me_2CO (3 × 10 L, each 2 days) at room temperature and filtered. The filtrate was evaporated to give a residue, which was suspended in H_2O (4 L) and then extracted with petroleum ether (60−90 °C, 3 × 2 L), EtOAc (4 × 2.5 L), and *n*-BuOH (2 × 2 L) successively. The EtOAc extract (400 g) was decolorized on MCI gel, eluted with 90% MeOH−H_2O, to yield a yellowish gum (350 g). The gum was subjected to column chromatography over a silica gel (200−300 mesh, 2.0 kg) column, eluted with $CHCl_3$−Me_2CO (1:0−0:1 gradient system), to obtain fractions 1−5. After repeated column chromatography (silica gel, petroleum ether−Me_2CO, 20:1−4:1 gradient system), fraction 1 (8.0 g) afforded **5** (75 mg), **16** (10 mg), and **19** (200 mg). Fraction 2 (80.0 g) was applied to silica gel eluted with a petroleum ether−2-propanol (10:1−3:1) gradient to provide fractions 2a−2d. Fraction 2b (25.0 g) was further separated by RP-18 (40%−80% gradient system) and normal-phase silica gel (petroleum ether−2-propanol, 8:1) to yield **6** (10 mg), **7** (10 mg), **15** (65 mg), **17** (60 mg), **18** (13 mg), and **20** (8 mg). Fraction 2c (10.0 g) was purified over silica gel ($CHCl_3$−MeOH, 20:1), then by preparative HPLC with 65% MeOH−H_2O, to get **8** (30 mg) and **11** (40 mg), respectively. Fraction 2d provided **3** (4 mg) after being chromatographed over silica gel developing with $CHCl_3$−2-propanol (30:1), followed by semipreparative HPLC with 40% MeOH−H_2O. Compound **12** (5.0 g) was crystallized from fraction 3 (60.0 g), and the mother liquid was passed through a silica gel column, eluted with a gradient system (petroleum ether−2-propanol, 10:1−2:1), to yield three main fractions, 3a−3c. Compounds **9** and **10** were obtained from fraction 3a (0.75 g) by preparative HPLC (20% CH_3CN−H_2O). Fraction 3b (10.0 g) was purified over RP-18 (40% MeOH−H_2O) and semipreparative HPLC (13% CH_3CN−H_2O) to afford **1** (30 mg). Fraction 3c (1.0 g) was separated by a silica gel column eluted with $CHCl_3$−MeOH (15:1) to yield **13** (8 mg). Fraction 4 (60.0 g) was subjected to a silica gel column with a gradient elution (petroleum ether−2-propanol, 10:1−1:1) to obtain fractions 4a−4d. Compound **21** (30 mg) was crystallized from 4a. Compound **4** (30 mg) was isolated from 4b over a silica gel column (petroleum ether−Me_2CO, 2:1), followed by preparative HPLC (40% MeOH−H_2O). Fraction 4d (15.0 g) was subjected to RP-18 (30% MeOH−H_2O), then by preparative HPLC (20% MeOH−H_2O), to yield **2** (20 mg). The *n*-BuOH extract (200 g) was subjected to column chromatography on silica gel, eluted with $CHCl_3$−MeOH (8:1−2:1, gradient system). Compound **14** (5.0 g) was crystallized from the $CHCl_3$−MeOH (5:1) fraction directly.

Parvifoline C (1): colorless crystals (MeOH); mp 222−223 °C; $[\alpha]^{26.0}_D$ −70.9 (*c* 0.91, MeOH); UV (MeOH) λ_{max} (log ε) 241 (3.94) nm; IR (KBr) ν_{max} 3274, 2942, 2867, 1701, 1637, 1498, 1455, 1405, 1385, 1272, 1203, 1165, 1080, 1057, 997, 968, 953, 942, 913 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 7.40 (1H, d, *J* = 10.5 Hz, OH-6β), 5.98 (1H, s, H-17a), 5.30 (1H, s, H-17b), 4.78 (1H, d, *J* = 10.0 Hz, H-20a), 4.36 (2H, overlapped, H-6α and H-20b), 3.71 (3H, overlapped, H-1β and H_2-18), 2.91 (1H, d, *J* = 9.6 Hz, H-13α), 2.48−2.37 (3H, overlapped, H-11α and H_2-14), 2.20 (1H, m, H-12α), 1.95 (1H, m, H-11β), 1.91 (2H, m, H_2-2), 1.83 (1H, d, *J* = 6.8 Hz, H-5β), 1.74 (1H, br s, H-9β), 1.60 (1H, m, H-3β), 1.49 (1H, br d, *J* = 13.3 Hz, H-3α), 1.35 (1H, m, H-12β), 1.22 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; positive FABMS *m*/*z* 365 $[M + H]^+$; positive HRESIMS $[M + Na]^+$ *m*/*z* 387.1777 (calcd for $C_{20}H_{28}O_6Na$ $[M + Na]^+$, 387.1783).

Parvifoline D (2): white amorphous powder (MeOH); $[\alpha]^{28.5}_D$ −36.2 (*c* 1.08, MeOH); UV (MeOH) λ_{max} (log ε) 237 (3.76) nm; IR (KBr) ν_{max} 3405, 2938, 2872, 1709, 1641, 1450, 1209, 1080, 1058, 622 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) δ 7.34 (1H, d, *J* = 10.4 Hz, OH-6β), 6.26 (1H, s, H-17a), 5.51 (1H, s, H-17b), 5.09 (1H, s, H-14α), 4.81 (1H, d, *J* = 10.0 Hz, H-20a), 4.43 (1H, d, *J* = 10.0 Hz, H-20b), 4.35 (1H, dd, *J* = 10.4, 7.2 Hz, H-6α), 3.80 (1H, d, *J* = 11.0 Hz, H-18a), 3.70 (1H, d, *J* = 11.0 Hz, H-18b), 3.65 (1H, m, H-1β), 3.19 (1H, br d, *J* = 8.8 Hz, H-13α), 2.41 (2H, overlapped, H-11α and H-12α), 1.95 (4H, overlapped, H-9β, H_2-2 and H-11β), 1.85 (1H, d, *J* = 7.7 Hz, H-5β), 1.65 (1H, dt, *J* = 13.2, 3.3 Hz, H-3β), 1.56 (1H, d, *J* = 7.2 Hz, H-12β), 1.49 (1H, br d, *J* = 13.2 Hz, H-3α), 1.23 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 125 MHz), see Table 1; positive ESIMS *m*/*z* 403 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m*/*z* 403.1730 (calcd for $C_{20}H_{28}O_7Na$ $[M + Na]^+$, 403.1732).

Parvifoline E (3): colorless crystals (MeOH); mp 222−224 °C; $[\alpha]^{26.0}_D$ −11.2 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.83) nm; IR (KBr) ν_{max} 3420, 2949, 2874, 1733, 1711, 1641, 1453, 1375, 1243, 1078, 1059, 1033, 959, 909, 605 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) δ 6.24 (1H, s, H-17a), 5.48 (1H, s, H-17b), 5.24 (1H, s, H-14α), 4.82 (1H, dd, *J* = 11.0, 4.7 Hz, H-1β), 4.54 (1H, d, *J* = 9.9 Hz, H-20a), 4.35 (1H, d, *J* = 10.0 Hz, H-20b), 4.32 (1H, overlapped, H-6α), 3.81 (1H, d, *J* = 11.0 Hz, H-18a), 3.67 (1H, d, *J* = 11.0 Hz, H-18b), 3.16 (1H, d, *J* = 9.3 Hz, H-13α), 2.33 (1H, m, H-12α), 1.92 (2H, overlapped, H_2-11), 1.91 (3H, s, OAc), 1.88 (3H, overlapped, H-5β, H-9β, and H-2β), 1.70 (1H, m, H-3β), 1.54 (1H, m, H-2α), 1.47 (2H, overlapped, H-3α and H-12β), 1.19 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 125 MHz), see Table 1; positive ESIMS *m*/*z* 445 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m*/*z* 445.1851 (calcd for $C_{22}H_{30}O_8Na$ $[M + Na]^+$, 445.1838).

Parvifoline F (4): white amorphous powder (MeOH); $[\alpha]^{27.7}_D$ +142.7 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.96) nm, 238 (3.72); IR (KBr) ν_{max} 3397, 2947, 2905, 2877, 1710, 1643, 1456, 1394, 1368, 1272, 1164, 1149, 1092, 1077, 1037, 1020, 977, 955, 915, 698, 660, 645, 576 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 6.60 (1H, d, *J* = 2.3 Hz, H-11), 6.23 (1H, d, *J* = 12.1 Hz, OH-6β), 6.19 (1H, s, H-17a), 5.60 (1H, s, H-17b), 4.52−4.42 (4H, overlapped, H-6α, H-12β, and H_2-20), 4.14 (1H, dd, *J* = 11.7, 4.8 Hz, H-1β), 3.52 (1H, br s, H-13α), 2.85 (1H, d, *J* = 11.3 Hz, H-14α), 2.74 (1H, dd, *J* = 11.1, 4.8 Hz, H-14β), 1.85 (1H, m, H-2β), 1.82 (1H, d, *J* = 9.3 Hz, H-5β), 1.73 (1H, m, H-2α), 1.42−1.34 (2H, overlapped, H_2-3), 1.34 (3H, s, Me-18), 1.15 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 100 MHz), see Table

Table 1. ^{13}C NMR Data of Compounds **1**−**9** (δ in ppm)[a]

carbon	1	2	3	4	5	6	7	8	9
1	73.4 d	73.4 d	75.8 d	71.6 d	76.0 d	72.3 d	76.4 d	72.1 d	74.7 d
2	29.8 t	29.8 t	24.9 t	29.6 t	25.1 t	29.8 t	24.5 t	29.7 t	29.3 t
3	33.8 t	33.7 t	32.6 t	40.0 t	39.1 t	38.7 t	39.3 t	39.3 t	39.8 t
4	39.0 s	39.1 s	38.8 s	34.4 s	34.6 s	34.3 s	34.1 s	34.2 s	34.2 s
5	57.5 d	56.5 d	56.3 d	52.4 d	62.1 d	61.4 d	62.2 d	61.8 d	61.0 d
6	74.6 d	74.5 d	74.2 d	75.5 d	206.5 s	74.4 d	74.3 d	74.3 d	74.4 d
7	95.6 s	98.2 s	98.0 s	97.0 s	92.4 s	95.9 s	95.9 s	95.6 s	96.7 s
8	60.5 s	62.9 s	62.4 s	62.6 s	48.6 s	59.9 s	60.3 s	60.9 s	60.6 s
9	52.2 d	54.4 d	52.9 d	147.3 s	47.3 d	54.4 d	58.1 d	54.9 d	59.8 d
10	41.5 s	41.6 s	39.8 s	45.0 s	43.0 s	42.4 s	40.8 s	42.0 s	43.9 s
11	20.4 t	20.5 t	18.5 t	123.2 d	69.6 d	67.5 d	62.5 d	67.9 d	66.3 d
12	30.2 t	31.0 t	30.4 t	72.7 d	41.3 t	36.2 t	40.5 t	27.4 t	30.6 t
13	35.2 d	44.0 d	43.8 d	45.9 d	35.3 d	34.8 d	35.3 d	29.6 d	29.6 d
14	26.2 t	73.1 d	73.3 d	28.6 t	25.1 t	28.6 t	28.2 t	30.0 t	29.3 t
15	211.1 s	209.4 s	209.1 s	204.9 s	76.8 d	211.1 s	211.8 s	224.3 s	225.3 s
16	154.2 s	153.3 s	152.7 s	148.4 s	154.1 s	153.4 s	154.0 s	58.4 d	59.3 d
17	116.5 t	119.6 t	119.8 t	119.8 t	110.5 t	116.4 t	115.4 t	68.9 t	69.1 t
18	73.5 t	73.3 t	72.4 t	33.9 q	34.7 q	32.7 q	32.7 q	32.8 q	33.2 q
19	18.0 q	18.1 q	17.8 q	23.7 q	22.1 q	22.1 q	22.1 q	21.9 q	22.4 q
20	64.2 t	64.5 t	63.9 t	67.6 t	65.7 t	64.8 t	64.6 t	64.9 t	102.9 d
OAc			169.9 s		170.8 s	170.1 s	170.1 s	170.3 s	
			21.4 q		170.0 s	21.7 s	21.2 q	21.6 q	
					169.7 s				
					22.1 q				
					22.0 q				
					21.1 q				
OMe								58.7 q	58.7 q
									55.2 q

[a] The assignments were based on DEPT, HMQC, and HMBC experiments.

1; positive FABMS *m/z* 363 $[M + H]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 385.1627 (calcd for $C_{20}H_{26}O_6Na$ $[M + Na]^+$, 385.1627).

Parvifoline G (5): colorless crystals (MeOH); mp 158−160 °C; $[\alpha]^{28.3}_D$ −77.4 (*c* 0.53, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.43) nm; IR (KBr) ν_{max} 3445, 2955, 2924, 1752, 1730, 1375, 1360, 1257, 1227, 1113, 1061, 1040, 1028, 942, 919, 890, 605, 560 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.38 (1H, d, J = 2.1 Hz, H-15α), 5.05 (1H, s, H-17a), 4.93 (1H, d, J = 4.3 Hz, H-1β), 4.89 (1H, m, H-11β), 4.80 (1H, s, H-17b), 4.79 (1H, d, J = 9.3 Hz, H-20a), 4.33 (1H, s, OH-7β), 4.21 (1H, d, J = 9.3 Hz, H-20b), 2.75 (1H, br s, H-13α), 2.53 (1H, d, J = 12.4 Hz, H-14α), 2.40 (1H, s, H-5β), 2.32 (1H, dd, J = 15.6, 8.8 Hz, H-12α), 2.04 (1H, s, H-9β), 2.11, 2.01 and 1.94 (each 3H, s, 3 × OAc), 1.77 (1H, overlapped, H-12β), 1.75−1.64 (3H, overlapped, H_2-2 and H-14β), 1.44 (2H, overlapped, H_2-3), 1.38 (3H, s, Me-18), 1.06 (3H, s, Me-19); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive FABMS *m/z* 491 $[M + H]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 513.2106 (calcd for $C_{26}H_{34}O_7Na$ $[M + Na]^+$, 513.2100).

Parvifoline H (6): white amorphous powder (MeOH); $[\alpha]^{26.0}_D$ −18.0 (*c* 0.96, MeOH); UV (MeOH) λ_{max} (log ϵ) 230 (3.48) nm; IR (KBr) ν_{max} 3417, 2948, 2876, 1715, 1640, 1447, 1378, 1079, 1055, 970, 945 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 6.04 (1H, dd, J = 18.4, 9.0 Hz, H-11α), 5.95 (1H, s, H-17a), 5.28 (1H, s, H-17b), 4.66 (1H, d, J = 10.3 Hz, H-20a), 4.39 (1H, d, J = 10.3 Hz, H-20b), 4.24 (1H, dd, J = 10.1, 5.3 Hz, H-6α), 4.05 (1H, m, H-1β), 3.27 (1H, m, H-12α), 2.98 (1H, m, H-13α), 2.57 (1H, d, J = 12.3 Hz, H-14α), 2.33 (1H, d, J = 12.3 Hz, H-14β), 2.21 (1H, m, H-9β), 2.08 (3H, s, OAc), 1.87 (2H, m, H_2-2), 1.59 (1H, d, J = 5.3 Hz, H-5β), 1.30 (3H, overlapped, H_2-3 and H-12β), 1.13 (3H, s, Me-18), 1.05 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; positive ESIMS *m/z* 429 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 429.1891 (calcd for $C_{22}H_{30}O_7Na$ $[M + Na]^+$, 429.1889).

Parvifoline I (7): white amorphous powder (MeOH); $[\alpha]^{25.3}_D$ −15.1 (*c* 0.95, MeOH); UV (MeOH) λ_{max} (log ϵ) 234 (3.50) nm; IR (KBr) ν_{max} 3416, 2952, 1718, 1639, 1454, 1372, 1259, 1082, 1058, 973, 948 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 5.88 (1H, s, H-17a), 5.44 (1H, dd, J = 11.6, 5.0 Hz, H-1β), 5.24 (1H, s, H-17b), 4.66 (2H, overlapped, H-11α and H-20a), 4.39 (1H, d, J = 10.3 Hz, H-20b), 4.24 (1H, dd, J = 10.1, 5.3 Hz, H-6α), 3.06 (1H, dd, J = 9.9, 3.8 Hz, H-13α), 2.89 (1H, m, H-12α), 2.57 (1H, d, J = 12.3 Hz, H-14α), 2.43 (1H, J = 12.3 Hz, H-14β), 2.25 (1H, m, H-2α), 2.13 (1H, m, H-9β), 2.05 (3H, s, OAc), 1.65 (1H, d, J = 5.3 Hz, H-5β), 1.62 (1H, m, H-2β), 1.59 (1H, m, H-12β), 1.39 (2H, m, H_2-3), 1.13 (3H, s, Me-18), 1.06 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; positive ESIMS *m/z* 429 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 429.1890 (calcd for $C_{22}H_{30}O_7Na$ $[M + Na]^+$, 429.1889).

Parvifoline J (8): white amorphous powder (MeOH); $[\alpha]^{20.8}_D$ −38.2 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (3.37) nm; IR (KBr) ν_{max} 3420, 2952, 2904, 1721, 1636, 1497, 1460, 1372, 1241, 1062 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 6.02 (1H, m, H-11α), 4.58 (1H, d, J = 10.5 Hz, H-20a), 4.34 (1H, d, J = 10.5 Hz, H-20b), 4.14 (1H, dd, J = 11.0, 4.8 Hz, H-6α), 4.04 (1H, m, H-1β), 3.77 (1H, dd, J = 10.0, 4.2 Hz, H-17a), 3.63 (1H, dd, J = 9.9, 9.3 Hz, H-17b), 3.23 (3H, s, OMe), 2.90 (1H, m, H-16α), 2.83 (1H, m, H-13α), 2.75 (1H, m, H-14α), 2.46 (1H, m, H-2α), 2.37 (1H, m, H-14β), 2.06 (3H, s, OAc), 2.05 (1H, overlapped, H-9β), 1.86 (2H, overlapped, H-2β and H-12α), 1.53 (1H, d, J = 4.5 Hz, H-5β), 1.47 (1H, m, H-12β); 1.42 (2H, m, H_2-3), 1.19 (3H, s, Me-18), 1.06 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; positive ESIMS *m/z* 461 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 461.2155 (calcd for $C_{23}H_{34}O_8Na$ $[M + Na]^+$, 461.2151).

Parvifoline K (9): white amorphous powder (MeOH); $[\alpha]^{28.1}_D$ −27.0 (*c* 0.66, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (3.06) nm; IR (KBr) ν_{max} 3385, 2935, 1716, 1453, 1395, 1194, 1093, 1047, 967, 946 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) δ 6.33 (1H, d, J = 11.4 Hz, OH-6β), 5.51 (1H, s, H-20), 5.00 (1H, m, H-11α), 4.03 (1H, dd, J = 11.1, 5.6 Hz, H-6α), 3.96 (1H, br s, H-1β), 3.77 (1H, dd, J = 10.3, 4.7 Hz, H-17a), 3.64 (1H, t, J = 9.4 Hz, H-17b), 3.48 and 3.22 (each 3H, s, 2 × OMe), 2.98 (1H, m, H-16α), 2.85 (2H, overlapped, H-2α and H-14α), 2.44 (1H, dd, J = 12.2, 2.8 Hz, H-13α), 2.38 (1H, m, H-12α), 1.85 (2H, overlapped, H-2β and H-14β), 1.79 (1H, d, J = 8.9 Hz, H-9β), 1.59 (1H, t, J = 12.8 Hz, H-12β), 1.47 (1H, d, J = 5.6 Hz, H-5β), 1.42 (1H, d, J = 13.2 Hz, H-3α), 1.32 (1H, ddd, J = 12.7, 12.7, 4.1 Hz, H-3β), 1.14 (3H, s, Me-18), 0.97 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 125 MHz), see Table 1; positive FABMS *m/z* 427 $[M + H]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 449.2146 (calcd for $C_{22}H_{34}O_8Na$ $[M + Na]^+$, 449.2151).

Effusanin A (15): white amorphous powder (MeOH); ^{13}C NMR (C_5D_5N, 100 MHz) δ 210.7 (s, C-15), 154.0 (s, C-16), 115.5 (t, C-17), 95.6 (s, C-7), 74.6 (d, C-6), 73.1 (d, C-1), 63.5 (t, C-20), 61.0 (d, C-5), 60.2 (s, C-8), 51.7 (d, C-9), 41.4 (s, C-10), 39.1 (t, C-3), 34.9 (d, C-13), 33.8 (s, C-4), 33.0 (q, C-18), 30.1 (t, C-2), 29.8 (t, C-12), 26.1 (t, C-14), 21.8 (q, C-19), 20.0 (t, C-11).

Cytotoxicity Bioassay. Cytotoxicity of compounds against suspended tumor cells was determined by the trypan blue exclusion method, and against adherent cells by sulforhodamine B (SRB) assay. Cells were plated in a 96-well plate 24 h before treatment and continuously exposed to different concentrations of compounds for 72

h. After compound treatment, cells were counted (suspended cells) or fixed and stained with SRB (adherent cells) as described in the literature.[22]

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