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Cytotoxic *ent*-Kaurane Diterpenoids from *Isodon wikstroemioides*

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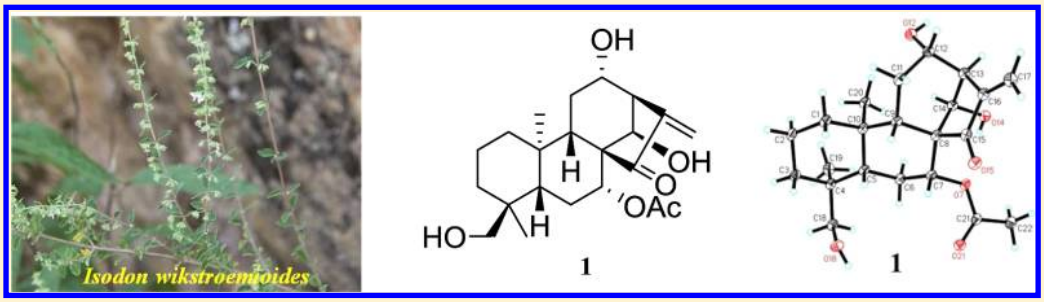
Supporting Information



ABSTRACT: Phytochemical investigation of EtOAc extracts of the aerial parts of *Isodon wikstroemioides* afforded 18 new *ent*-kaurane diterpenoids (wikstroemioidins E−V, **1**−**18**), along with 17 known analogues (**19**−**35**). The absolute configurations of **1** and **16** were confirmed by single-crystal X-ray diffraction analysis. The isolates were screened against five human tumor cell lines; compounds **3**, **4**, **9**, **11**−**13**, **23**, **25**−**28**, and **33** exhibited significant cytotoxic activity against all five, with IC_{50} values ranging from 0.4 to 5.1 μM. In addition, 17 of the isolates strongly inhibited nitric oxide production in LPS-activated RAW264.7 macrophages.

The *ent*-kaurane diterpenoids are a large group of compounds isolated from the genus *Isodon*, which includes approximately 150 species and is distributed all over the world. These diterpenoids have attracted considerable attention due to their diverse structures and interesting biological properties,[1−3] and many new *ent*-kauranoids have recently been isolated.[4] In addition, due to their potent antitumor activity, low toxicity, and compelling structures with several vicinal stereogenic centers, some such as maoecrystal V[5,6] and maoecrystal Z[7,8] have been selected as targets for total synthesis.

Isodon wikstroemioides (Hand.−Mazz.) H. Hara (Lamiaceae) is a perennial herb that is primarily distributed in the northwestern regions of Yunnan Province and the western district of the Sichuan Region in the People's Republic of China.[9] Previous phytochemical investigations of *I. wikstroemioides* plants indigenous to the Deqin Prefecture of Yunnan Province resulted in the isolation of eight 7,20-epoxy-*ent*-kauranoids and one 6,7-*seco*-7,20-olide-*ent*-kauranoid.[10] Because plants from the genus *Isodon* often produce secondary metabolites with different structures and biological activities when grown under different ecological conditions,[11−14] a study of *I. wikstroemioides* plants collected from the Ranwu Prefecture of Sichuan Province was undertaken in the hopes of identifying further novel bioactive diterpenoids with interesting structures and biological activities. These studies resulted in the isolation of 15 new C-20-nonoxygenated-*ent*-kaurane diterpenoids, wikstroemioidins E−S (**1**−**15**), three new C-20-oxygenated-nonepoxy-*ent*-kaurane diterpenoids, wikstroemioidins T−V (**16**−**18**), and 17 known compounds (**19**−**35**). All of the isolates other than **10** and **15** were screened against the HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human tumor cell lines to determine their cytotoxic activity. In addition, their ability to inhibit LPS-induced NO production in RAW264.7 macrophages was also tested. Herein, the isolation, structure elucidation, and biological activities of these compounds as well as the results of a preliminary structure−activity relationship study are described.

RESULTS AND DISCUSSION

A 70% aqueous acetone extract of the air-dried and powdered aerial parts of *I. wikstroemioides* (7.5 kg) was partitioned between EtOAc and H_2O to afford an EtOAc extract (380 g). The EtOAc extract was subjected to column chromatography over silica gel, MCI CHP-20 gel, and Lichroprep RP-18, after which it was further purified by semipreparative HPLC to afford 18 new *ent*-kauranoids that have been named wikstroemioidins E−V (**1**−**18**) and 17 known compounds, namely, nervonin G (**19**),[15] adenanthins J and K (**20** and **21**),[16] nervonin E (**22**),[15] adenanthin (**23**),[17] isoscoparins E and L (**24** and **25**),[18] excisanin C (**26**),[19] albopilosin A (**27**),[20] phyllostachysin F (**28**),[21] rabdokunmins A, B, and D (**29**−

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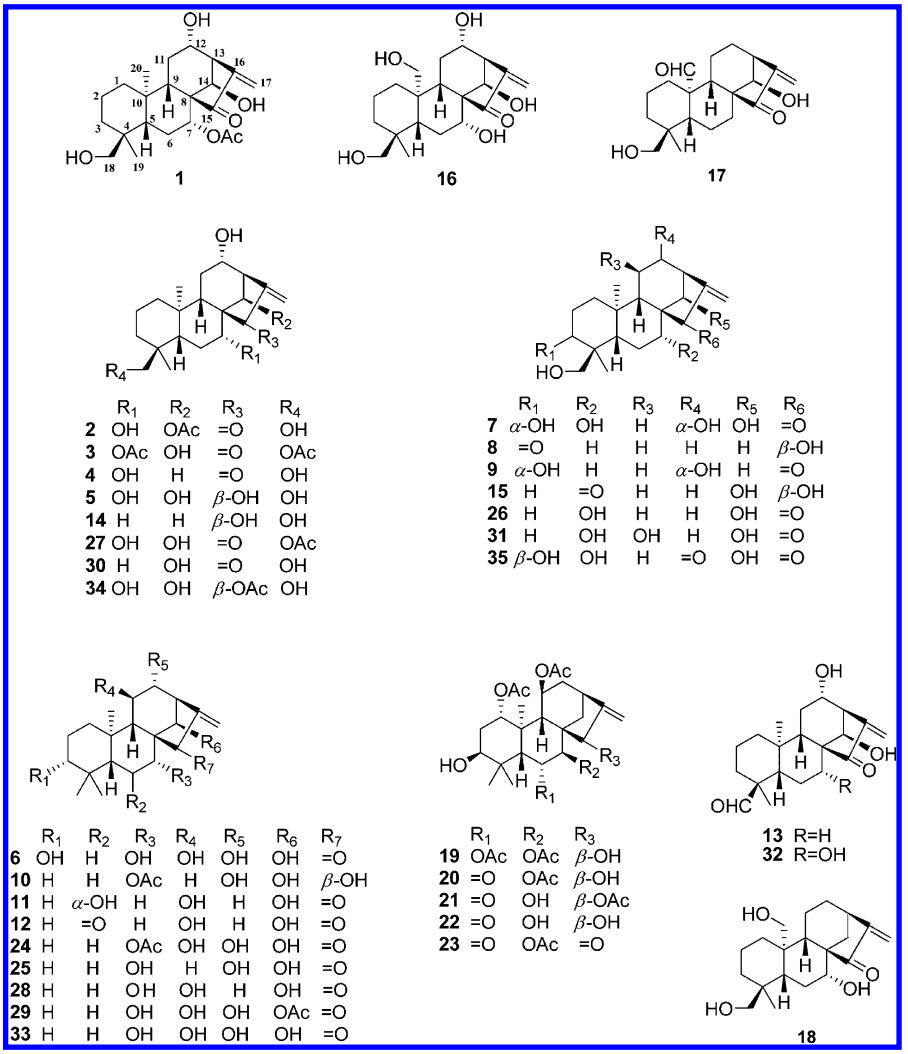
Chart 1



31),[22] macrocalyxin C (**32**),[23] rabdoloxin B (**33**),[24] albopilosin B (**34**),[25] and 3β,7α,14β,18-tetrahydroxy-*ent*-kaur-16-ene-12,15-dione (**35**).[26] The structures of the known compounds were determined by comparing their spectroscopic data to literature values.

On the basis of HREIMS ([M]$^+$ 392.2183, calcd 392.2199) and ^{13}C NMR data, wikstroemioidin E (**1**) has the molecular formula $C_{22}H_{32}O_6$, indicating seven indices of hydrogen deficiency. Its IR absorption bands at 3466, 1719, and 1646 cm^{-1} indicate the presence of hydroxy, carbonyl, and alkenyl groups. Its ^{1}H NMR data (Table 1) show characteristic resonances of two methyl groups and an acetyl group, while its ^{13}C NMR and DEPT data (Table 2) feature 22 carbon resonances including two methyl groups, an olefinic group, an acetoxy group, and six methylene (one oxygenated), six methine (three oxygenated), and four quaternary carbons (one carbonyl). Protons were assigned to related carbon resonances via the ^{1}H NMR and HSQC spectroscopic data. On the basis of its NMR data and the structures of the diterpenoids previously isolated from *I. wikstroemioides*, compound **1** was tentatively assigned as an *ent*-kauranoid. The ^{1}H−^{1}H COSY data (Figure 1) of **1** exhibit H_2-1/H_2-2/H_2-3, H-5/H_2-6/H-7, and H-9/H_2-11/H-12/H-13/H-14 correlations. Similarly, its HMBC data (Figure 1) indicate that H-5 (δ_H 1.75) correlates with C-3, C-6, C-9, C-18, C-19, and C-20, while H-13 (δ_H 3.62) correlates with C-8, C-11, C-14, C-15, and C-17. These results indicated that **1** is a C-20-nonoxygenated-*ent*-kauranoid. The presence of an acetoxy group at C-7 and three hydroxy groups at C-12, C-14, and C-18 is supported by the HMBC correlations of H-7 (δ_H 6.07) with C-6, C-8, C-14, C-15, and an acetyl carbonyl, of H-12 (δ_H 4.35) with C-9 and C-14, and of H_2-18 (δ_H 3.60 and 3.39) with C-4, C-5, and C-19.

The relative configuration of **1** was determined by analyzing its ROESY data (Figure 1). The β-orientations of H-7 and H-12 are indicated by their associations with H-5β and H-9β, respectively, while the association between H-14 and H_3-20α indicates the α-orientation of H-14. Similarly, the association between H_2-18 and H-6β demonstrates the β-orientation of the C-18 hydroxymethyl group.

A single crystal of **1** was obtained from MeOH and analyzed by X-ray crystallography. The final refinement of the Cu Kα data resulted in a Flack parameter of 0.0(2)[28] and a Hooft parameter of 0.10(10) for 1487 Bijvoet pairs, enabling the absolute configuration of **1** to be assigned unambiguously as (4*S*, 5*S*, 7*R*, 8*S*, 9*S*, 10*R*, 12*S*, 13*R*, 14*R*) (Figure 2). The structure of compound **1** was thus defined as 12α,14β,18-trihydroxy-7α-acetoxy-*ent*-kaur-16-en-15-one.

Wikstroemioidin F (**2**) has the same molecular formula as **1** and yields similar NMR data (Tables 1 and 2). The ^{1}H−^{1}H COSY correlations of H-5/H_2-6/H-7 and H-9/H_2-11/H-12/H-

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Table 1. ^{1}H NMR Spectroscopic Data for Wikstroemioidins E–J (1–6) (δ in ppm, J in Hz)

position	1[a]	2[b]	3[b]	4[a]	5[b]	6[b]
1a	1.65, overlap	1.68, overlap	1.63, m	1.75, overlap	1.89, overlap	2.04, m
1b	0.63, m	0.67, m	0.61, m	0.68, m	0.93, overlap	1.25, m
2a	1.66, overlap	1.68, overlap	1.33, m	1.72, overlap	1.75, overlap	1.93, m
2b	1.40, overlap	1.41, m	0.82, m	1.42, overlap	1.44, m	1.80, m
3a	1.65, overlap	1.78, overlap	1.39, m	1.80, overlap	1.78, overlap	3.37, d (11.2)
3b	1.40, overlap	1.36, m	1.25, m	1.40, overlap	1.38, m	
5	1.75, overlap	1.79, overlap	1.57, overlap	1.81, overlap	1.92, overlap	1.13, d (12.0)
6a	2.37, dd (11.4, 4.1)	2.50, br q	2.02, overlap	2.34, dd (11.4, 4.5)	2.49, m	2.33, dd (12.0, 4.0)
6b	1.85, overlap	2.27, br d (11.5)	1.79, overlap	1.87, overlap	2.19, br q	2.20, br q
7	6.07, dd, (11.4, 4.1)	4.92, dd (11.5, 3.7)	5.86, dd (11.7, 4.0)	4.81, dd (11.4, 4.5)	4.67, br d (11.4)	5.02, overlap
9	1.65, overlap	1.75, overlap	1.69, s	1.62, overlap	2.40, s	2.11, br s
11a	1.88, overlap	1.95, overlap	1.89, overlap	1.94, m	2.42, overlap	4.44, s
11b	1.68, overlap	1.70, overlap	1.72, m	1.77, overlap	1.96, overlap	
12	4.35, br s	4.40, br s	4.35, br s	4.23, br s	4.43, br s	4.75, s
13	3.62, br s	3.49, d (3.5)	3.62, d (2.9)	3.33, overlap	3.34, overlap	3.78, br s
14a	5.77, s	7.05, s	5.71, br s	3.01, d (11.8)	5.72, s	5.97, s
14b				2.56, dd (11.8, 4.5)		
15					6.07, s	
17a	6.25, s	6.22, s	6.25, s	6.05, s	5.68, s	6.40, s
17b	5.38, s	5.39, s	5.38, s	5.20, s	5.30, s	5.51, s
18a	3.60, d (10.2)	3.65, d (10.5)	4.03, d (11.0)	3.68, d (10.5)	3.69, d (10.5)	1.18, s
18b	3.39, d (10.2)	3.30, d (10.5)	3.66, d (11.0)	3.34, overlap	3.32, overlap	
19	0.86, s	0.90, s	0.74, s	0.88, s	0.91, s	1.07, s
20	1.62, s	1.96, s	1.56, s	1.70, s	1.78, s	1.68, s
AcO-7	1.87, s		1.84, s			
AcO-14		2.01, s				
AcO-18			2.14, s			

[a]Recorded at 400 MHz in pyridine-d_5. [b]Recorded at 500 MHz in pyridine-d_5.

Table 2. ^{13}C NMR Spectroscopic Data for Wikstroemioidins E–J (1–6) (δ in ppm)

position	1[a]	2[b]	3[b]	4[a]	5[b]	6[a]
1	39.3, CH_2	39.0, CH_2	39.2, CH_2	39.4, CH_2	40.6, CH_2	38.1, CH_2
2	18.2, CH_2	18.4, CH_2	18.0, CH_2	18.2, CH_2	18.6, CH_2	28.0, CH_2
3	35.6, CH_2	35.6, CH_2	35.8, CH_2	35.8, CH_2	35.9, CH_2	77.7, CH
4	38.9, C	38.0, C	36.6, C	38.5, C	38.0, C	39.4, C
5	47.0, CH	46.9, CH	47.2, CH	45.8, CH	46.6, CH	52.5, CH
6	25.6, CH_2	28.7, CH_2	25.3, CH_2	29.2, CH_2	30.8, CH_2	29.9, CH_2
7	76.2, CH	72.9, CH	76.0, CH	70.7, CH	76.0, CH	75.0, CH
8	62.2, C	63.0, C	62.1, C	58.8, C	54.7, C	59.9, C
9	57.1, CH	58.5, CH	57.1, CH	54.9, CH	50.3, CH	67.4, CH
10	38.1, C	39.2, C	39.1, C	38.0, C	38.3, C	38.8, C
11	26.5, CH_2	26.4, CH_2	26.6, CH_2	27.1, CH_2	26.3, CH_2	71.2, CH
12	72.6, CH	73.2, CH	72.8, CH	71.4, CH	74.3, CH	79.2, CH
13	55.5, CH	53.5, CH	55.5, CH	46.8, CH	59.0, CH	54.7, CH
14	70.1, CH	72.4, CH	69.9, CH	22.2, CH_2	73.0, CH	71.7, CH
15	207.3, C	208.0, C	207.0, C	210.9, C	74.2, CH	208.2, C
16	146.9, C	146.3, C	146.8, C	148.7, C	157.3, C	147.7, C
17	117.6, CH_2	116.7, CH_2	117.7, CH_2	114.4, CH_2	107.4, CH_2	115.9, CH_2
18	71.4, CH_2	71.3, CH_2	72.8, CH_2	71.3, CH_2	71.5, CH_2	28.7, CH_3
19	17.8, CH_3	18.1, CH_3	17.5, CH_3	18.0, CH_3	18.2, CH_3	16.4, CH_3
20	17.0, CH_3	16.8, CH_3	16.9, CH_3	16.9, CH_3	17.4, CH_3	17.5, CH_3
AcO-7	21.1, 169.2		21.0, 169.4			
AcO-14		21.9, 171.3				
AcO-18			20.7, 171.0			

[a]Recorded at 100 MHz in pyridine-d_5. [b]Recorded at 125 MHz in pyridine-d_5.

13/H-14, along with the HMBC correlations of H_2-6, H-7, H_2-18, H-19, and H_3-20 with C-5 (δ_C 46.9) and of H_3-20 (δ_H 1.96) with C-1, C-5, C-9, and C-10 suggest that **2** is also a C-20-nonoxygenated-*ent*-kauranoid. Further analysis of its 2D NMR data and comparison with those of **1** indicate that the two compounds are C-7 and C-14 regioisomers. The presence of

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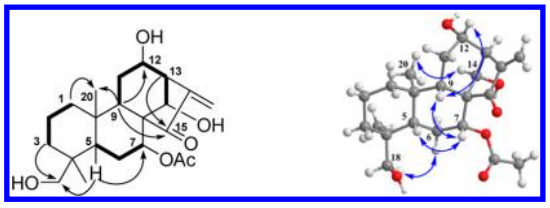

Figure 1. $^{1}H-^{1}H$ COSY (bold), selected HMBC (arrow), and key ROESY correlations of **1**.

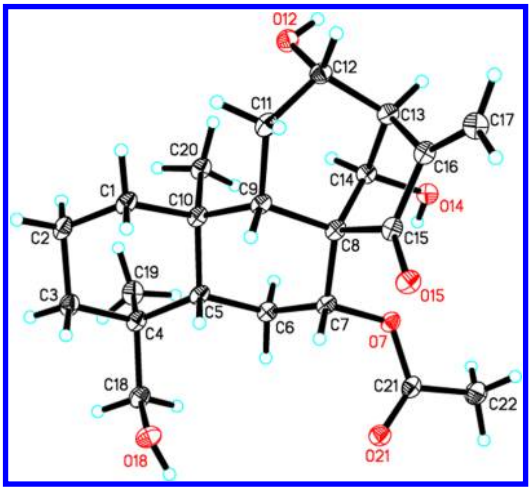

Figure 2. ORTEP drawing of compound **1**.

three hydroxy groups at C-7, C-12, and C-18 of **2** is confirmed by the observation of HMBC correlations from H-7 (δ_H 4.92) to C-5, C-6, C-8, and C-15, from H-12 (δ_H 4.40) to C-9, C-13, and C-14, and from H_2-18 (δ_H 3.65 and 3.30) to C-3, C-5, and C-19. The acetoxy group is assumed to be located at C-14, causing deshielding of H-14 from δ_H 5.77 in **1** to δ_H 7.05 in **2**. This assumption was confirmed by the HMBC correlations of H-14 with C-9, C-13, C-15, C-16, and an acetyl carbonyl. The ROESY spectrum of **2** indicates that the relative configurations of the stereogenic centers in **2** are identical to those of **1**. Compound **2** was thus identified as 7*α*,12*α*,18-trihydroxy-14*β*-acetoxy-*ent*-kaur-16-en-15-one.

Wikstroemioidin G (**3**) was isolated as a white, amorphous powder, and its molecular formula was established to be $C_{24}H_{34}O_7$ by HREIMS and ^{13}C NMR data. Comparisons of the ^{1}H and ^{13}C NMR data of **3** with those of **1** (Tables 1 and 2) indicate that both compounds have identical skeletons and substitution patterns, differing only in that **3** has an acetoxy group at C-18 rather than the hydroxy group in **1**. This conclusion is verified by the HMBC correlations of H_2-18 (δ_H 4.03 and 3.66) with C-3, C-5, C-19, and an acetyl carbonyl. The ROESY spectrum of **3** indicates that the relative configurations of its stereogenic carbons are identical to those of **1**. Compound **3** was thus identified as 12*α*,14*β*-dihydroxy-7*α*,18-diacetoxy-*ent*-kaur-16-en-15-one.

The molecular formula of wikstroemioidin H (**4**) was determined to be $C_{20}H_{30}O_4$ by HREIMS and ^{13}C NMR data. A comparison of the ^{1}H and ^{13}C NMR spectroscopic data for compounds **4** and **2** indicates that they have identical A- and B-rings but different C-rings. The ^{13}C NMR spectrum of compound **4** suggests that the C-14 oxymethine carbon of **2** is reduced to a methylene group in **4**, causing shielding of C-13 from δ_C 53.5 in **2** to δ_C 46.8 in **4**. The HMBC correlations of H_2-14 (δ_H 3.01 and 2.56) with C-7, C-9, C-15, and C-16 confirm this assumption. The correlations observed in the ROESY spectrum of **4** indicate that the orientations of the substituents at C-7 and C-12 in **4** are the same as in **2**. Therefore, the structure of compound **4** was defined as 7*α*,12*α*,18-trihydroxy-*ent*-kaur-16-en-15-one.

The molecular formula of wikstroemioidin I (**5**) was determined to be $C_{20}H_{32}O_5$ by HREIMS and ^{13}C NMR data, indicating five indices of hydrogen deficiency. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **5** are similar to those of the known compound rabdokunmin C.[22] The only significant difference between the spectra of the two compounds is that the C-15 carbonyl resonance in rabdokunmin C is replaced by an oxymethine resonance (δ_C 74.2) in **5**. Such an assignment is confirmed by the HMBC correlations of H-15 (δ_H 6.07) with C-7, C-9, and C-16. Moreover, whereas the chemical shift of C-9 of rabdokunmin C occurs at δ_C 57.3, it is shielded to δ_C 50.3 in **5**. This is presumably due to a *γ*-steric compression effect between HO-15*β* and H-9*β*, which suggests that H-15 is *α*-oriented. The structure of compound **5** was thus assigned as 7*α*,12*α*,14*β*,15*β*,18-pentahydroxy-*ent*-kaur-16-ene.

Wikstroemioidin J (**6**) was obtained as a white, amorphous powder whose molecular formula was determined to be $C_{20}H_{30}O_6$ by HREIMS and ^{13}C NMR data. The ^{1}H and ^{13}C NMR data of **6** are similar to those of known compound **28**[21] except that the C-3 and C-12 methylene resonances in **28** are replaced by oxymethine resonances in **6**. The HMBC correlations (Figure 3) of H_3-18 (δ_H 1.18) and H_3-19 (δ_H 1.07) with C-3 (δ_C 77.7) and of H-12 (δ_H 4.75) with C-9 and C-13 confirm this conclusion. The ROESY correlations (Figure 3) from H-3 to H-1*β* and H-5*β* and from H-12 to H-9*β* in **6** indicate that the C-3 and C-12 hydroxy groups in **6** are *α*-oriented. Therefore, the structure of compound **6** was assigned as 3*α*,7*α*,11*β*,12*α*,14*β*-pentahydroxy-*ent*-kaur-16-en-15-one.

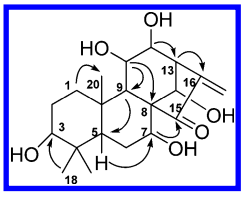

Figure 3. Selected HMBC (arrow) correlations of compound **6**.

Wikstroemioidin K (**7**), colorless needles from MeOH, has a molecular formula of $C_{20}H_{30}O_6$ based on HREIMS and ^{13}C NMR data. Its 1D and 2D NMR data indicate that **7** is an *ent*-kauranoid. Carbon-3, C-7, C-12, C-14, and C-18 all carry hydroxy groups based on the HMBC correlations of H_2-18 (δ_H 4.20 and 3.71) with C-3 (δ_C 72.8), C-4, C-5, and C-19, of H-7 and H-14 with C-8 (δ_C 61.6), and of H-12 (δ_H 4.37) with C-9, C-11, and C-14. A ROESY experiment revealed that H-3, H-7, and H-12 in **7** are *β*-oriented, while H-14 is *α*-oriented based on the correlations of H-3/H-1*β*, H-7/H-9*β*, and H-14/H-6*α* and H_3-20*α*. Therefore, the structure of **7** was defined as 3*α*,7*α*,12*α*,14*β*,18-pentahydroxy-*ent*-kaur-16-en-15-one.

Wikstroemioidin L (**8**) was identified as 15*β*,18-dihydroxy-*ent*-kaur-16-en-3-one by comparing its spectroscopic data to those of the known compound *ent*-3-oxo-16-kaurene-15*β*,18-diol.[27] Both compounds have similar NMR data, differing only with respect to the configuration of C-15. The resonance corresponding to C-9 in the ^{13}C NMR spectrum of **8** is shielded (Δ 8.8 ppm) relative to the equivalent resonance of the reference compound due to a *γ*-steric compression effect

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Table 3. ^{1}H NMR Spectroscopic Data for Wikstroemioidins K–P (7–12) (δ in ppm, J in Hz)

position	7[b]	8[b]	9[a]	10[b]	11[b]	12[b]
1a	1.69, overlap	1.95, overlap	1.78, overlap	1.79, m	1.87, m	1.88, m
1b	0.82, m	1.57, overlap	0.89, m	0.75, overlap	1.06, overlap	1.25, overlap
2a	1.94, m	2.66, m	2.00, m	1.57, m	1.70, m	1.52, m
2b	1.84, overlap	2.55, m	1.87, m	1.28, m	1.32, m	1.32, m
3a	4.14, overlap		4.19, overlap	1.26, m	1.28, m	1.19, m
3b				1.00, m	1.09, overlap	0.97, m
5	1.84, overlap	2.46, dd (12.2, 1.8)	1.70, overlap	1.10, br d (12.2)	1.00, s	2.41, s
6a	2.49, dd (12.2, 3.6)	1.55, overlap	1.78, overlap	2.09, m	4.73, br s	
6b	2.18, br q	1.44, overlap	1.50, m	1.86, m		
7a	5.09, overlap	2.04, overlap	2.43, m	5.66, d (4.1)	2.78, dd (14.4, 2.5)	3.27, dd (16.5, 13.7)
7b		1.36, m	1.44, m		2.22, dd (14.4, 2.5)	
9	1.66, overlap	1.99, s	1.56, d (9.6)	2.24, d (9.6)	2.16, s	2.61, s
11a	1.84, overlap	2.07, overlap	1.92, overlap	2.36, m	4.33, br s	4.31, d (4.2)
11b	1.72, overlap	1.48, m	1.75, overlap	1.92, overlap		
12a	4.37, br s	1.61, m	4.19, overlap	4.40, br s	2.41, br s	2.43, m
12b		1.55, overlap				2.26, m
13	3.63, br s	2.71, br s	3.24, t (3.9)	3.34, d (3.9)	3.33, br s	3.33, br s
14a	5.91, s	1.95, overlap	3.17, d (11.8)	5.34, d (6.2)	5.36, s	4.75, s
14b		1.07, overlap	1.30, dd (11.8, 4.7)			
15		4.08, br s		5.50, s		
17a	6.31, s	5.48, s	6.11, s	5.64, s	6.23, s	6.29, s
17b	5.39, s	5.12, s	5.27, s	5.29, s	5.36, s	5.44, s
18a	4.20, overlap	3.96, d (10.3)	4.22, overlap	0.80, s	0.97, s	1.00, s
18b	3.71, d (10.4)	3.64, d (10.3)	3.72, d (10.4)			
19	1.07, s	1.08, s	1.06, s	0.78, s	1.44, s	1.35, s
20	1.73, s	1.04, s	1.65, s	1.61, s	1.63, s	1.02, s
AcO-7				1.98, s		

[a]Recorded at 600 MHz in pyridine-d_5. [b]Recorded at 500 MHz in pyridine-d_5.

Table 4. ^{13}C NMR Spectroscopic Data for Wikstroemioidins K–P (7–12) (δ in ppm)

position	7[b]	8[b]	9[c]	10[c]	11[a]	12[a]
1	38.2, CH_2	38.2, CH_2	38.6, CH_2	40.8, CH_2	42.1, CH_2	39.4, CH_2
2	27.6, CH_2	36.6, CH_2	27.8, CH_2	19.2, CH_2	19.1, CH_2	18.6, CH_2
3	72.8, CH	217.2, C	73.4, CH	42.2, CH_2	43.7, CH_2	42.3, CH_2
4	42.9, C	52.7, C	43.4, C	33.7, C	34.3, C	32.5, C
5	45.5, CH	46.9, CH	47.7, CH	53.6, CH	56.4, CH	64.9, CH
6	29.7, CH_2	21.6, CH_2	19.0, CH_2	26.9, CH_2	66.0, CH	212.6, C
7	74.7, CH	38.3, CH_2	34.2, CH_2	78.4, CH	35.4, CH_2	42.9, CH_2
8	61.6, C	46.1, C	52.7, C	55.5, C	55.8, C	61.7, C
9	57.1, CH	45.4, CH	55.3, CH	50.8, CH	66.8, CH	65.8, CH
10	38.6, C	38.1, C	38.9, C	38.8, C	39.1, C	45.5, C
11	26.8, CH_2	18.7, CH_2	27.7, CH_2	26.5, CH_2	65.1, CH	64.5, CH
12	72.5, CH	33.7, CH_2	71.1, CH	74.5, CH	41.8, CH_2	41.4, CH_2
13	55.8, CH	40.7, CH	47.2, CH	58.5, CH	46.6, CH	46.1, CH
14	71.3, CH	36.6, CH_2	30.9, CH_2	74.1, CH	74.8, CH	72.9, CH
15	209.1, C	82.1, CH	211.5, C	71.8, CH	209.8, C	205.8, C
16	147.9, C	159.5, C	148.7, C	156.9, C	149.6, C	149.1, C
17	116.8, CH_2	104.5, CH_2	115.3, CH_2	108.1, CH_2	112.6, CH_2	114.7, CH_2
18	67.4, CH_2	68.5, CH_2	67.9, CH_2	33.8, CH_3	34.0, CH_3	32.6, CH_3
19	13.0, CH_3	17.9, CH_3	13.3, CH_3	22.1, CH_3	23.8, CH_3	22.0, CH_3
20	17.1, CH_3	17.7, CH_3	17.1, CH_3	17.1, CH_3	19.6, CH_3	19.1, CH_3
AcO-7				21.7, 170.6		

[a]Recorded at 100 MHz in pyridine-d_5. [b]Recorded at 125 MHz in pyridine-d_5. [c]Recorded at 150 MHz in pyridine-d_5.

between HO-15β and H-9β. This implies that H-15 is α-oriented in **8**. A ROESY correlation between H-15 and H-13α confirms that the C-15 hydroxy group of **8** is β-oriented. The structure of compound **8** was thus assigned as 15β,18-dihydroxy-*ent*-kaur-16-en-3-one.

The HREIMS, ^{13}C NMR (Table 4), and DEPT data for wikstroemioidin M (**9**) indicated a molecular formula of $C_{20}H_{30}O_4$. The ^{1}H and ^{13}C NMR data of **9** are similar to those of **7** (Tables 3 and 4) except that the C-7 and C-14 oxymethine resonances in **7** are replaced by methylene resonances in **9**.

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This is consistent with the observed HMBC correlations of H_2-7 (δ_H 2.43 and 1.44) with C-5, C-6, C-8, and C-14 (δ_C 30.9) and of H_2-14 (δ_H 3.17 and 1.30) with C-8, C-9, C-12, C-13, and C-15. Further analysis of its 2D NMR data facilitated definition of the structure of **9** as 3*α*,12*α*,18-trihydroxy-*ent*-kaur-16-en-15-one.

The molecular formula of wikstroemioidin N (**10**) was determined to be $C_{22}H_{34}O_5$ by HREIMS and ^{13}C NMR data. Its NMR spectra indicate that it is an *ent*-kaurane diterpenoid with an acetoxy group, an olefinic quaternary carbon, an olefinic methylene carbon, seven methine carbons (four of which are oxygenated), and five methylene, three quaternary, and three methyl carbons. The HMBC correlations of H-5 and H-6 with C-7 (δ_C 78.4) and of H-7 (δ_H 5.66) with the acetyl carbonyl show that the acetoxy group is located at C-7. In addition, the HMBC correlations of H-12 (δ_H 4.40) with C-9 and C-14, of H-14 (δ_H 5.34) with C-9 and C-13, and of H-15 (δ_H 5.50) with C-14 and C-16 indicate that C-12, C-14, and C-15 are substituted with a hydroxy group. The relative configuration of **10** was established by a ROESY experiment and by analyzing its proton coupling constants. The ROESY spectrum shows that H-7 correlates with H-5*β* and H-9*β*, while H-15 correlates with H-13*α*, indicating that the C-7 acetoxy group is *α*-oriented and the C-15 hydroxy group is *β*-oriented. The structure of compound **10** was thus defined as 12*α*,14*β*,15*β*-trihydroxy-7*α*-acetoxy-*ent*-kaur-16-ene.

Wikstroemioidin O (**11**) was obtained as a white, amorphous powder with a molecular formula of $C_{20}H_{30}O_4$ based on HREIMS and ^{13}C NMR data. Its spectroscopic data indicate that its structure is similar to that of known compound **28**[21] but for the location of the hydroxy group at C-6 in **11**. This assignment was verified by the $^1H-^1H$ COSY correlations between H-5, H-6, and H_2-7 as well as the HMBC correlations from H-6 (δ_H 4.73) to C-4, C-5, and C-8. The relative configurations of HO-6*α*, HO-11*β*, and HO-14*β* were determined from the ROESY correlations of H-6 with H_3-18*β*, of H-11 with H-1*α* and H_3-20*α*, and of H-14 with H_3-20*α*. Therefore, the structure of compound **11** was assigned as 6*α*,11*β*,14*β*-trihydroxy-*ent*-kaur-16-en-15-one.

The HREIMS and ^{13}C NMR data for wikstroemioidin P (**12**) indicated a molecular formula of $C_{20}H_{28}O_4$. A comparison of the NMR data for **12** and **11** (Tables 3 and 4) revealed that they only differ in that **12** has a carbonyl instead of an oxymethine group at C-6. This is demonstrated by the presence of HMBC correlations from H_2-7 and H-5 to C-6 (δ_C 212.6) in the spectra of **12**. The C-6 carbonyl group of compound **12** causes deshielding of H-5 in its ^1H NMR spectrum relative to the corresponding resonance in the spectrum of **11** (from δ_H 1.00 to δ_H 2.41) and also causes shielding of the resonance of H_3-20, from δ_H 1.63 to δ_H 1.02. In addition, correlations observed in the ROESY spectrum indicate that the orientations of the substituents in **12** are the same as in **11**. The structure of compound **12** was thus defined as 11*β*,14*β*-dihydroxy-*ent*-kaur-16-ene-6,15-dione.

The HREIMS and ^{13}C NMR data for wikstroemioidin Q (**13**) indicated a molecular formula of $C_{20}H_{28}O_4$. The NMR data of **13** closely resemble those for the known compound **30**.[22] However, the resonance for the C-18 hydroxymethyl group in the ^{13}C NMR spectum of **30** is replaced by a formyl carbonyl resonance in the spectrum of **13**. The formyl proton (δ_H 9.26) exhibits HMBC correlations with C-3, C-4, and C-19, confirming its location at C-18. The ROESY correlations of H-12/H-9*β* and H-14/H_3-20*α* indicate that HO-12 is *α*-oriented while HO-14 is *β*-oriented. Therefore, the structure of **13** was assigned as 12*α*,14*β*-dihydroxy-*ent*-kaur-16-en-18-al-15-one.

The molecular formula of wikstroemioidin R (**14**) was determined to be $C_{20}H_{32}O_3$ by HREIMS and ^{13}C NMR data. Its NMR data indicated that C-12, C-15, and C-18 are all substituted with hydroxy groups. The configuration of HO-12*α* was determined by analyzing proton coupling constants, while that of HO-15*β* was deduced from the shielded C-9 (Δ 7.0 ppm) resonance in the ^{13}C NMR spectrum of **14** relative to the equivalent resonance in the ^{13}C NMR spectrum of compound **9**. This shift was attributed to a *γ*-steric compression effect between HO-15*β* and H-9*β*. Compound **14** was thus identified as 12*α*,15*β*,18-trihydroxy-*ent*-kaur-16-ene.

Wikstroemioidin S (**15**) was obtained as a white, amorphous powder with a molecular formula of $C_{20}H_{30}O_4$ based on HREIMS and ^{13}C NMR data. Its NMR spectra indicate that it is an *ent*-kauranoid with three oxygenated carbons and a carbonyl group. The HMBC correlations of H-5, H-6, and H-15 with C-7 (δ_C 211.1), of H-14 (δ_H 4.91) with C-13, C-15, and C-16, of H-15 (δ_H 6.33) with C-7, C-8, C-9, and C-16, and of H_2-18 (δ_H 3.53 and 3.27) with C-3, C-5, and C-19 indicate that the carbonyl group is located at C-7 and that C-14, C-15, and C-18 are all substituted with hydroxy groups. In addition, its ROESY data suggest that H-14 and C-18 are *α*- and *β*-oriented, respectively, based on the correlation of H-14 with H_3-20*α* and H_2-18 with H-5*β*. Therefore, the structure of compound **15** was assigned as 14*β*,15*β*,18-trihydroxy-*ent*-kaur-16-en-7-one.

Wikstroemioidin T (**16**), colorless needles from MeOH, has a molecular formula of $C_{20}H_{30}O_6$ by HREIMS ($[M]^+$ 366.2068, calcd 366.2042) and ^{13}C NMR data, indicating six indices of hydrogen deficiency. Analysis of its 1D and 2D NMR data suggests that it is an *ent*-kauranoid with an exocyclic double bond, a carbonyl carbon, six methine carbons (of which three are oxygenated), seven methylene carbons (two of which are oxygenated), three quaternary carbons, and a methyl carbon. Carbon-7, C-12, C-14, C-18, and C-20 are all substituted with hydroxy groups, as demonstrated by the HMBC correlations from H-7 (δ_H 5.16) to C-8, C-14 (δ_C 71.8), and C-15, from H-12 (δ_H 4.35) to C-9, C-11, and C-14, and from H_2-20 (δ_H 4.63 and 4.41) to C-1, C-9, and C-10 (Figure 4). The relative

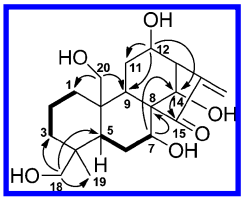

Figure 4. $^1H-^1H$ COSY (bold) and selected HMBC (arrow) correlations of **16**.

configuration of **16** was determined via its ROESY data (Figure 4): the NOEs of H-7/H-5*β* and of H-12/H-9*β* indicate that H-7 and H-12 are both *β*-oriented, while the NOEs of H-14/H-6*α* suggest that H-14 is *α*-oriented. A single-crystal X-ray diffraction analysis using the anomalous scattering of Cu K*α* radiation yielded a Flack parameter of 0.1(3) and a Hooft parameter of 0.11(7) for 1133 Bijvoet pairs, confirming the absolute configuration of **16** as (4*S*, 5*R*, 7*R*, 8*S*, 9*S*, 10*S*, 12*S*, 13*R*, 14*R*) (Figure 5). Therefore, the structure of compound **16** was defined as 7*α*,12*α*,14*β*,18,20-pentahydroxy-*ent*-kaur-16-en-15-one.

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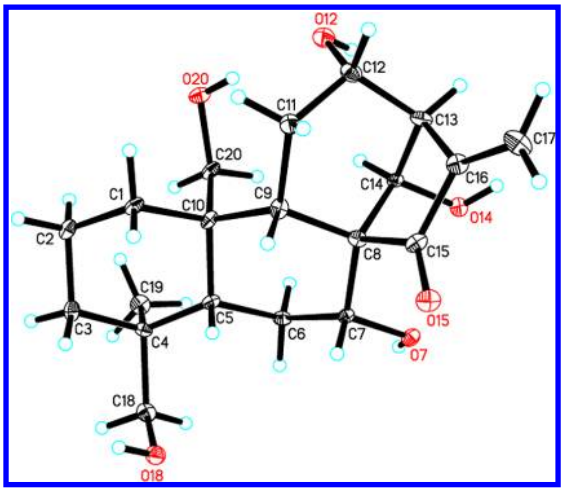

Figure 5. ORTEP drawing of compound **16**.

Wikstroemioidin U (**17**) was shown to have the molecular formula $C_{20}H_{28}O_4$ by HREIMS and ^{13}C NMR data. Its NMR data indicate that its structure is similar to that of **26**,[19] but the C-7 oxymethine group and C-20 methyl group of **26** are replaced by a methylene and a formyl group, respectively, in **17**. These replacements are verified by the HMBC correlations of H_2-7 (δ_H 2.67 and 2.15) with C-9 and C-14 and of H-20 (δ_H 10.66) with C-1 and C-10. A ROESY experiment confirmed that **17** has the same configuration as **26**. The structure of compound **17** was thus defined as 14β,18-dihydroxy-*ent*-kaur-16-en-20-al-15-one.

Wikstroemioidin V (**18**) was assigned the molecular formula $C_{20}H_{30}O_4$ based on its HREIMS and ^{13}C NMR data. Its spectroscopic data indicate that its structure resembles that of **16** (Tables 5 and 6) save that the C-12 and C-14 oxymethine groups of **16** are replaced with methylene groups in **18**. This causes shielding of C-13 from δ_C 55.9 in **16** to δ_C 38.7 in **18**. This conclusion was supported by the HMBC correlations of H_2-12 (δ_H 2.82 and 1.61) with C-13 and C-16 and of H_2-14 (δ_H 2.70) with C-8, C-9, C-13, C-15, and C-16. The relative configuration of **18** was identical to that of **16** based on the observed ROESY correlations. Therefore, the structure of compound **18** was assigned as 7α,18,20-trihydroxy-*ent*-kaur-16-en-15-one.

Compounds **1**–**35** are the main small-molecule components of the ethyl acetate extracts of *I. wikstroemioides* plants collected from the Ranwu Prefecture of Sichuan Province. All of these are either C-20-nonoxygenated-*ent*-kauranoids or C-20-oxygenated-nonepoxy-*ent*-kauranoids, both of which have different structures from the 7,20-epoxy-*ent*-kauranoids that were previously isolated from plants of this species growing in the Deqin Prefecture of Yunnan.[10] This finding confirms that the structures of the secondary metabolites produced by plants from the genus *Isodon* depend on the ecological conditions under which the plant is grown.

Previous studies on the structure–activity relationships of diterpenoids isolated from the genus *Isodon* indicated that the *exo*-methylene cyclopentanone D-ring system contributes significantly to their antitumor and anti-inflammatory activities.[29] Most of the isolates obtained from *I. wikstroemioides* in this work had D-rings containing the *exo*-methylene cyclo-

Table 5. ^1H NMR Spectroscopic Data for Wikstroemioidins Q–V (13–18) (δ in ppm, J in Hz)

position	13[a]	14[b]	15[c]	16[b]	17[a]	18[a]
1a	1.64, m	1.94, overlap	1.82, m	2.68, m	2.64, overlap	2.51, m
1b	0.64, m	0.93, overlap	0.86, m	0.55, m	0.60, m	0.63, m
2a	1.52, m	1.69, overlap	1.61, m	1.82, overlap	1.86, overlap	1.82, m
2b	1.31, m	1.43, overlap	1.42, m	1.50, m	1.49, overlap	1.48, m
3a	1.27, m	1.81, overlap	1.74, m	1.87, overlap	1.85, overlap	1.86, m
3b	1.03, overlap	1.41, overlap	1.39, m	1.40, m	1.32, m	1.42, m
5	1.52, overlap	1.68, overlap	2.11, overlap	1.97, d (12.5)	2.07, overlap	1.97, overlap
6a	1.54, overlap	1.76, m	2.78, br q	2.37, overlap	2.03, overlap	2.27, m
6b	1.04, overlap	1.53, m	2.74, overlap	2.08, br q	2.01, overlap	1.97, overlap
7a	2.51, m	2.25, m		5.16, dd (11.4, 4.4)	2.67, overlap	4.84, dd (10.8, 4.4)
7b	2.12, m	1.49, m			2.15, m	
9	1.75, overlap	2.14, d (9.4)	2.63, d (8.1)	1.73, s	1.90, overlap	1.67, d (8.6)
11a	1.83, m	2.29, m	2.07, m	2.40, overlap	1.45, overlap	2.19, dd (14.3, 5.8)
11b	1.77, m	1.91, m	1.67, m	1.77, overlap	1.43, overlap	1.56, m
12a	4.40, br s	4.21, overlap	2.03, m	4.35, br s	1.81, overlap	2.82, m
12b			1.80, m		1.55, m	1.61, m
13	3.61, d (3.4)	2.98, br s	3.10, br s	3.69, d (3.1)	3.23, br s	3.10, d (2.8)
14a	5.42, s	2.89, d (11.7)	4.91, s	6.08, s	4.41, s	2.70, m
14b		1.04, dd (11.7, 4.4)				
15		4.19, overlap	6.33, br s			
17a	6.25, s	5.50, s	5.69, s	6.31, s	6.23, s	6.04, s
17b	5.34, s	5.14, s	5.29, s	5.39, s	5.29, s	5.18, s
18a	9.26, s	3.66, d (10.4)	3.53, d (10.6)	3.63, d (10.5)	3.68, d (10.6)	3.67, d (10.5)
18b		3.35, d (10.4)	3.27, d (10.6)	3.28, d (10.5)	3.33, d (10.6)	3.33, d (10.5)
19	0.98, s	0.90, s	0.82, s	0.91, s	0.72, s	0.93, s
20a	1.49, s	1.70, s	1.27, s	4.63, d (12.4)	10.66, s	4.45, d (11.5)
20b				4.41, d (12.4)		4.36, d (11.5)

[a]Recorded at 500 MHz in pyridine-d_5. [b]Recorded at 400 MHz in pyridine-d_5. [c]Recorded at 600 MHz in pyridine-d_5.

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Table 6. ^{13}C NMR Spectroscopic Data for Wikstroemioidins Q–V (13–18) (δ in ppm)

position	13[a]	14[a]	15[c]	16[a]	17[b]	18[b]
1	38.7, CH_2	40.5, CH_2	40.9, CH_2	34.5, CH_2	34.2, CH_2	34.8, CH_2
2	17.3, CH_2	18.5, CH_2	18.8, CH_2	18.3, CH_2	19.2, CH_2	18.5, CH_2
3	32.2, CH_2	36.1, CH_2	36.2, CH_2	35.5, CH_2	35.6, CH_2	35.8, CH_2
4	50.0, C	38.2, C	38.9, C	37.8, C	38.7, C	37.8, C
5	47.5, CH	48.6, CH	48.5, CH	46.6, CH	48.7, CH	46.4, CH
6	21.6, CH_2	20.3, CH_2	38.7, CH_2	29.3, CH_2	17.8, CH_2	29.1, CH_2
7	25.7, CH_2	39.0, CH_2	211.1, C	74.8, CH	26.2, CH_2	71.0, CH
8	59.3, C	46.0, C	67.1, C	61.7, C	58.8, C	59.0, C
9	56.7, CH	48.3, CH	51.1, CH	53.9, CH	54.6, CH	53.5, CH
10	37.8, C	37.9, C	39.6, C	44.0, C	54.2, C	43.2, C
11	26.9, CH_2	26.7, CH_2	18.1, CH_2	25.3, CH_2	18.3, CH_2	19.5, CH_2
12	73.3, CH	72.3, CH	34.5, CH_2	73.5, CH	30.4, CH_2	32.7, CH_2
13	55.6, CH	48.9, CH	50.2, CH	55.9, CH	47.2, CH	38.7, CH
14	68.4, CH	30.9, CH_2	76.8, CH	71.8, CH	75.5, CH	29.4, CH_2
15	210.6, C	83.0, CH	73.1, CH	208.8, C	208.7, C	210.5, C
16	147.3, C	157.4, C	159.0, C	147.3, C	149.2, C	151.7, C
17	116.6, CH_2	105.2, CH_2	107.4, CH_2	117.1, CH_2	116.0, CH_2	112.5, CH_2
18	206.4, CH	71.6, CH_2	71.0, CH_2	71.2, CH_2	70.4, CH_2	71.5, CH_2
19	14.0, CH_3	18.1, CH_3	17.8, CH_3	19.4, CH_3	17.1, CH_3	18.9, CH_3
20	16.6, CH_3	17.1, CH_3	18.0, CH_3	61.2, CH_2	207.7, CH	60.7, CH_2

[a]Recorded at 100 MHz in pyridine-d_5. [b]Recorded at 125 MHz in pyridine-d_5. [c]Recorded at 150 MHz in pyridine-d_5.

pentanone motif. Therefore, all of the isolates except **10** and **15** (which could not be tested due to sample limitations) were evaluated for their cytotoxicity against the HL-60 (acute leukemia), SMMC-7721 (hepatic cancer), A-549 (lung cancer), MCF-7 (breast cancer), and SW-480 (colon cancer) human tumor cell lines using the MTS method,[30] with cisplatin and paclitaxel as positive controls. Compounds **3**, **4**, **9**, **11**–**13**, **23**, **25**–**28**, and **33** exhibited significant cytotoxic activity, with IC_{50} values ranging from 0.4 to 5.1 μM for all five cell lines, while compounds **1**, **2**, **18**, **24**, **29**, **30**, and **32** showed moderate cytotoxic potency (Table 7). These results suggested that the α,β-unsaturated carbonyl function is a structural requirement for cytotoxicity in these compounds.[31] It is worth mentioning that compound **25**, whose absolute configuration was determined in an X-ray diffraction experiment using Cu Kα radiation, as shown in Figure 6, which has three OH groups on its B- and C-rings, exhibited significant inhibitory activity against all of the tested cancer cell lines, with IC_{50} values ranging from 0.4 to 1.2 μM. Compounds **3** and **27** have an OAc group at C-18 and also exhibited significant inhibitory activities against all of the tested cancer cell lines. Compounds **1** and **2**, which are major chemical constituents of *I. wikstroemioides* and have an OH group at C-18, exhibited lower activity against the five cancer cell lines and selective antiproliferative activity against the SW-480 cancer cell line, with IC_{50} values of 6.9 and 8.6 μM, respectively.

Inflammation is a complicated physiological phenomenon that occurs in response to injury, infection, and stress. Cytokines are released by activated macrophages and are known to be important in pro-inflammatory responses, partly because they stimulate nitric oxide production. Nitric oxide (NO) is a product of inflammation that is synthesized by inducible NO synthase (iNOS) isoforms and plays a central role in immune and inflammatory responses to diverse pathogens. [32] Because inflammation is closely related to cancer, and cytotoxic diterpenoid isolates from the genus *Isodon* often exhibit inhibitory activity against NO production,[4b] the 21 isolates obtained in this study were tested for their capacity to inhibit NO production in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. Strong inhibitory activity was observed for compounds **3**, **4**, **9**, **11**–**13**, **18**, **23**–**30**, **32**, and **33** (Table 8).

Table 7. Cytotoxic Activities of Diterpenoids from *I. wikstroemioides* against Tumor Cell Lines[a]

compound	HL-60	SMMC-7721	A-549	MCF-7	SW-480
1	>10	>10	>10	>10	6.9
2	>10	>10	>10	>10	8.6
3	2.9	3.5	2.7	1.6	0.9
4	3.6	4.0	4.4	4.9	1.4
9	4.1	4.1	3.7	4.8	2.7
11	4.8	5.1	5.1	3.7	2.3
12	3.1	0.8	1.0	1.6	1.0
13	3.2	1.4	0.9	1.7	1.0
18	>10	7.1	>10	7.3	4.0
23	3.6	4.0	3.3	2.8	2.0
24	8.8	5.8	>10	>10	4.7
25	0.5	1.2	0.8	0.4	0.6
26	3.0	2.7	2.1	1.6	1.3
27	2.0	1.4	3.2	1.7	0.9
28	3.3	2.1	2.3	1.5	1.1
29	8.8	5.8	>10	>10	4.7
30	8.8	4.0	>10	>10	4.0
32	3.7	4.0	>10	3.7	2.1
33	2.9	3.4	4.4	3.0	2.4
DDP[b]	1.7	4.5	7.6	15.7	15.0
paclitaxel[b]	<0.008	<0.008	<0.008	<0.008	<0.008

[a]Results are expressed as IC_{50} values in μM. Cell lines: HL-60, acute leukemia; SMMC-7721, hepatic cancer; A-549, lung cancer; MCF-7, breast cancer; SW-480, colon cancer. Compounds **5**–**8**, **14**, **16**, **17**, **19**–**22**, **31**, **34**, and **35** were inactive (IC_{50} > 10 μM) for all cell lines. [b]DDP (cisplatin) and paclitaxel were used as positive controls.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on an XRC-1 apparatus and are uncorrected. Optical rotations were measured in MeOH with Horiba SEPA-300 and

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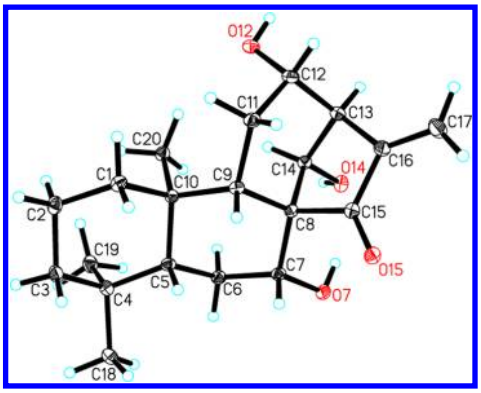

Figure 6. ORTEP drawing of compound **25**.

Table 8. Inhibitory Effects of the Diterpenoids from *I. wikstroemioides* on LPS-Activated NO Production in RAW264.7 Cells[a]

compound	IC_{50} (μM)	compound	IC_{50} (μM)
1	16.8	**23**	3.3
2	16.5	**24**	7.2
3	5.0	**25**	0.8
4	3.5	**26**	1.0
9	4.7	**27**	1.9
11	2.5	**28**	1.0
12	1.1	**29**	7.2
13	1.9	**30**	4.8
14	23.9	**32**	2.7
17	9.9	**33**	1.9
18	2.8	MG-132	0.1

[a]Each value represents the mean ± SEM (n = 3).

JASCO P-1020 polarimeters. UV spectra were recorded using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained on a Tenor 27 FT-IR spectrometer using KBr pellets. NMR spectra were recorded on Bruker AM-400, DRX-500, and DRX-600 spectrometers using TMS as the internal standard. All chemical shifts (δ) are expressed in ppm relative to the solvent signals. HREIMS was performed on an API QSTAR TOF spectrometer. X-ray crystallographic data were collected on a Bruker APEX DUO diffractometer equipped with an APEX II CCD using Cu Kα radiation. Column chromatography (CC) was performed with silica gel (100−200 mesh and 200−300 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40−63 μm, Merck, Darmstadt, Germany), and MCI gel (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Thin-layer chromatography was performed on precoated TLC plates (200−250 μm thickness, silica gel 60 F_{254}, Qingdao Marine Chemical, Inc.), and spots were visualized by UV light (254 nm) or by spraying heated silica gel plates with 10% H_2SO_4 in EtOH. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC-ODS (K) column. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18} (9.4 mm × 25 cm) column.

Plant Material. The aerial parts of *I. wikstroemioides* were collected in the Ranwu District of Sichuan Province, People's Republic of China, in July 2011 and identified by Prof. Xi-Wen Li at the Kunming Institute of Botany. A voucher specimen (KIB 20110939) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The aerial parts of *I. wikstroemioides* (7.5 kg) were extracted with 70% aqueous acetone (14 L) three times (for three days each time) at room temperature and filtered. The filtrate was concentrated under reduced pressure to a volume of 5 L and then partitioned between EtOAc and H_2O. The EtOAc-soluble portion (380 g) was subjected to silica gel CC (2 kg, 100−200 mesh), eluting with $CHCl_3$/acetone (1:0−0:1 gradient system). Seven fractions were obtained from the silica gel column and individually decolorized on MCI gel, after which they were eluted with 90:10 MeOH/H_2O to yield fractions A−G.

Fraction C ($CHCl_3$/acetone, 8:2; 19 g), which was a brown gum, was subjected to RP-18 column chromatography (MeOH/H_2O, 27:73 to 60:40 gradient) to provide three fractions, C1−C3. Fraction C2 (15 g) was separated into five subfractions (C2-1−C2-5) using RP-18 CC (MeOH/H_2O, 25:75 to 40:60 gradient). C2-5 (80 mg) was subjected to semipreparative HPLC (CH_3CN/H_2O, 32:68) to obtain **11** (4 mg), **13** (5 mg), and **14** (6 mg). C2-4 (12 g) was subjected to RP-18 CC (MeOH/H_2O, 25:75 to 40:60 gradient) and then semipreparative HPLC (CH_3CN/H_2O, 28:72) to yield **32** (200 mg) and **17** (13 mg). Fraction C3 (2 g) was separated by preparative HPLC (CH_3CN/H_2O, 34:66) to afford 17 fractions (C3-1−C3-17). C3-14 (65 mg) was submitted to semipreparative HPLC (MeOH/H_2O, 72:28) to yield **8** (30 mg). Compounds **19** (7 mg), **20** (16 mg), **22** (27 mg), **23** (75 mg), and **21** (10 mg) were isolated from fraction C3-17 (210 mg) by semipreparative HPLC (MeOH/H_2O, 60:40). C3-13 (1 g) was subjected to repeated preparative HPLC (MeOH/H_2O, 65:35) to afford **3** (4 mg), **10** (2 mg), **25** (70 mg), **26** (500 mg), and **27** (53 mg). C3-8 (50 mg) was subjected to semipreparative HPLC (CH_3CN/H_2O, 35:65) to yield **28** (6 mg).

Fraction D ($CHCl_3$/acetone, 7:3; 50 g) was subjected to silica gel CC (1 kg, 200−300 mesh) and eluted with $CHCl_3$/MeOH (80:1) to afford seven fractions (D1−D7). D1 (800 mg) was submitted to preparative HPLC (CH_3CN/H_2O, 34:66) and then semipreparative HPLC (CH_3CN/H_2O, 32:68) to yield **12** (12 mg), **15** (1 mg), **29** (8 mg), and **24** (8 mg). D4 (20 g) was applied to a silica gel column (200 g, 200−300 mesh) and eluted with $CHCl_3$/MeOH (80:1) to afford six fractions (D4-1−D4-6). D4-4 (14 g) was separated by preparative HPLC (CH_3CN/H_2O, 30:70) to yield **1** (4 g) and **2** (5 g) and then semipreparative HPLC (CH_3CN/H_2O, 28:72) to yield **33** (8 mg), **34** (24 mg), and **18** (3 mg). In the same way, **5** (3 mg) and **9** (2 mg) were isolated from fraction D4-5, and **4** (20 mg) was isolated from D4-6.

Fraction E ($CHCl_3$/acetone, 6:4; 3 g) was separated by RP-18 CC (MeOH/H_2O, 13:87 to 40:60 gradient) into fractions E1−E5. Compounds **16** (8 mg), **30** (2 mg), **31** (17 mg), and **35** (4 mg) were isolated from E2 (150 mg) by semipreparative HPLC (CH_3CN/H_2O, 16:84).

Fraction F ($CHCl_3$/acetone, 5:5; 12 g) was separated by RP-18 CC (MeOH/H_2O, 13:87 to 40:60 gradient) and then semipreparative HPLC (CH_3CN/H_2O, 18:82) to afford **6** (10 mg) and **7** (9 mg).

*Wikstroemioidin E (**1**):* colorless needles (MeOH); mp 147−148 °C; $[\alpha]^{27}_D$ −67 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 233 (3.87), 200 (3.73) nm; IR (KBr) ν_{max} 3466, 2928, 1719, 1646, 1264, 1036 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESIMS m/z 415 $[M + Na]^+$ (100); positive-ion HREIMS $[M]^+$ m/z 392.2183 (calcd for $C_{22}H_{32}O_6$, 392.2199).

*Wikstroemioidin F (**2**):* colorless needles (MeOH); mp 135−136 °C; $[\alpha]^{25}_D$ −50 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 233 (3.78), 201 (3.80) nm; IR (KBr) ν_{max} 3427, 2927, 1728, 1648, 1249, 1038 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESIMS m/z 415 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 392.2202 (calcd for $C_{22}H_{32}O_6$, 392.2199).

*Wikstroemioidin G (**3**):* white, amorphous powder; $[\alpha]^{26}_D$ −53 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.75) nm; IR (KBr) ν_{max} 3427, 2931, 1728, 1647, 1244, 1038 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESIMS m/z 457 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 434.2395 (calcd for $C_{24}H_{34}O_7$, 434.2305).

*Wikstroemioidin H (**4**):* white, amorphous powder; $[\alpha]^{24}_D$ −108 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 232 (3.74) nm; IR (KBr) ν_{max} 3427, 2926, 1717, 1642, 1267, 1019 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESIMS m/z 357 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 334.2143 (calcd for $C_{20}H_{30}O_4$, 334.2144).

*Wikstroemioidin I (**5**):* white, amorphous powder; $[\alpha]^{25}_D$ −36 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.79) nm; IR (KBr) ν_{max} 3416, 2936, 1459, 1438, 1088, 1018 cm^{-1}; ^1H and ^{13}C NMR data, see

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Tables 1 and 2; positive-ion ESIMS m/z 375 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 352.2258 (calcd for $C_{20}H_{32}O_5$, 352.2250).

*Wikstroemioidin J (**6**):* white, amorphous powder; $[\alpha]^{25}_D$ −62 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 241 (3.74), 200 (3.64) nm; IR (KBr) ν_{max} 3396, 2931, 1720, 1649, 1232, 1023 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESIMS m/z 389 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 366.2041 (calcd for $C_{20}H_{30}O_6$, 366.2042).

*Wikstroemioidin K (**7**):* colorless needles (MeOH); mp 162−163 °C; $[\alpha]^{25}_D$ −69 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 232 (3.73), 201 (3.67) nm; IR (KBr) ν_{max} 3417, 2931, 1726, 1647, 1254, 1041 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; positive-ion ESIMS m/z 451 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 366.2043 (calcd for $C_{20}H_{30}O_6$, 366.2042).

*Wikstroemioidin L (**8**):* white, amorphous powder; $[\alpha]^{24}_D$ −90 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 253 (2.70), 203 (3.80) nm; IR (KBr) ν_{max} 3384, 2934, 1700, 1659, 1278, 1045 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; positive-ion ESIMS m/z 341 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 318.2198 (calcd for $C_{20}H_{30}O_3$, 318.2195).

*Wikstroemioidin M (**9**):* white, amorphous powder; $[\alpha]^{25}_D$ −97 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 232 (3.71) nm; IR (KBr) ν_{max} 3427, 2933, 1719, 1641, 1265, 1042 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; positive-ion ESIMS m/z 357 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 334.2153 (calcd for $C_{20}H_{30}O_4$, 334.2144).

*Wikstroemioidin N (**10**):* white, amorphous powder; $[\alpha]^{26}_D$ −20 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.80) nm; IR (KBr) ν_{max} 3441, 2925, 1630 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; positive-ion ESIMS m/z 401 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 378.2413 (calcd for $C_{22}H_{34}O_5$, 378.2406).

*Wikstroemioidin O (**11**):* white, amorphous powder; $[\alpha]^{26}_D$ −110 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 236 (3.74) nm; IR (KBr) ν_{max} 3441, 2927, 1721, 1649, 1268, 1064 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; positive-ion ESIMS m/z 357 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 334.2151 (calcd for $C_{20}H_{30}O_4$, 334.2144).

*Wikstroemioidin P (**12**):* white, amorphous powder; $[\alpha]^{26}_D$ −128 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 237 (3.76), 195 (3.51) nm; IR (KBr) ν_{max} 3431, 2924, 1728, 1692, 1259, 1071 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; positive-ion ESIMS m/z 355 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 332.1989 (calcd for $C_{20}H_{28}O_4$, 332.1988).

*Wikstroemioidin Q (**13**):* white, amorphous powder; $[\alpha]^{26}_D$ −94 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.78) nm; IR (KBr) ν_{max} 3449, 2935, 1715, 1638, 1260, 1059 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 5 and 6; positive-ion ESIMS m/z 355 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 332.1988 (calcd for $C_{20}H_{28}O_4$, 332.1988).

*Wikstroemioidin R (**14**):* white, amorphous powder; $[\alpha]^{26}_D$ −36 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 253 (3.16), 203 (3.72) nm; IR (KBr) ν_{max} 3427, 2925, 1634, 1046 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 5 and 6; positive-ion ESIMS m/z 343 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 320.2354 (calcd for $C_{20}H_{32}O_3$, 320.2351).

*Wikstroemioidin S (**15**):* white, amorphous powder; $[\alpha]^{27}_D$ −36 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.01) nm; IR (KBr) ν_{max} 3440, 2924, 1670, 1632, 1068 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 5 and 6; positive-ion ESIMS m/z 357 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 334.2133 (calcd for $C_{20}H_{30}O_4$, 334.2144).

*Wikstroemioidin T (**16**):* colorless needles (MeOH); mp 258−259 °C; $[\alpha]^{26}_D$ −68 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.77), 201 (3.82) nm; IR (KBr) ν_{max} 3420, 2936, 1728, 1649, 1093, 1027 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 5 and 6; positive-ion ESIMS m/z 389 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 366.2068 (calcd for $C_{20}H_{30}O_6$, 366.2042).

*Wikstroemioidin U (**17**):* white, amorphous powder; $[\alpha]^{26}_D$ −85 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.71), 211 (3.71) nm; IR (KBr) ν_{max} 3433, 2937, 1717, 1644, 1240, 1040 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 5 and 6; positive-ion ESIMS m/z 355 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 332.1989 (calcd for $C_{20}H_{28}O_4$, 332.1988).

*Wikstroemioidin V (**18**):* white, amorphous powder; $[\alpha]^{26}_D$ −175 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 234 (3.91), 199 (3.64) nm; IR (KBr) ν_{max} 3425, 2926, 1713, 1641, 1270, 1042 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 5 and 6; positive-ion ESIMS m/z 357 $[M + Na]^+$; positive-ion HREIMS $[M]^+$ m/z 334.2136 (calcd for $C_{20}H_{30}O_4$, 334.2144).

X-ray Crystal Structure Analysis. The intensity data for wikstroemioidin E (**1**), wikstroemioidin T (**16**), and isoscoparin L (**25**) were collected at 100 K on a Bruker APEX DUO diffractometer equipped with an APEX II CCD using Cu Kα radiation. Cell refinement and data reduction were performed with Bruker SAINT. The structures were solved by direct methods using SHELXS-97,[33] expanded using difference Founier techniques, and refined by the program and full-matrix least-squares calculations. The non-hydrogen atoms were refined anisotropically, and hydrogen atoms were fixed at calculated positions. Crystallographic data (excluding structure factor tables) for the reported structures have been deposited with the Cambridge Crystallographic Data Center (CCDC) as supplementary publications no. CCDC 975166 for **1**, CCDC 975167 for **16**, and CCDC 975168 for **25**. Copies of the data can be obtained free of charge from the CCDC, 12 Union Road, Cambridge CB 1EZ, UK [fax: Int. +44(0) (1223) 336 033); e-mail: deposit@ccdc.cam.ac.uk].

*Crystallographic data for wikstroemioidin E (**1**):* $C_{22}H_{32}O_6{\cdot}H_2O$, M_w = 410.49, orthorhombic, a = 6.25210(10) Å, b = 16.7938(4) Å, c = 20.7157(5) Å, $\alpha = \beta = \gamma$ = 90°, V = 2175.08(8) Å^3, T = 100(2) K, space group $P212121$, Z = 4, μ(Cu Kα) = 0.759 mm^{-1}, 10 178 reflections measured, 3801 independent reflections (R_{int} = 0.0468). The final R_1 values were 0.0527 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1372 ($I > 2\sigma(I)$). The final R_1 values were 0.0536 (all data). The final $wR(F^2)$ values were 0.1380 (all data). The goodness of fit on F^2 was 1.026. Flack parameter = 0.0(2). The Hooft parameter is 0.10(10) for 1487 Bijvoet pairs.

*Crystallographic data for wikstroemioidin T (**16**):* $C_{20}H_{30}O_6$, M = 366.44, monoclinic, a = 6.58330(10) Å, b = 18.2138(3) Å, c = 7.99620(10) Å, α = 90.00°, β = 111.6750(10)°, γ = 90.00°, V = 891.01(2) Å^3, T = 100(2) K, space group $P21$, Z = 2, μ(Cu Kα) = 0.817 mm^{-1}, 6001 reflections measured, 2708 independent reflections (R_{int} = 0.0392). The final R_1 values were 0.0698 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.2039 ($I > 2\sigma(I)$). The final R_1 values were 0.0706 (all data). The final $wR(F^2)$ values were 0.2086 (all data). The goodness of fit on F^2 was 1.097. Flack parameter = 0.1(3). The Hooft parameter is 0.11(7) for 1133 Bijvoet pairs.

*Crystallographic data for isoscoparin L (**25**):* $C_{20}H_{30}O_4$, M = 334.44, orthorhombic, a = 6.23380(10) Å, b = 15.2634(3) Å, c = 18.2796(4) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 1739.29(6) Å^3, T = 100(2) K, space group $P212121$, Z = 4, μ(Cu Kα) = 0.698 mm^{-1}, 12 250 reflections measured, 3018 independent reflections (R_{int} = 0.0435). The final R_1 values were 0.0419 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1068 ($I > 2\sigma(I)$). The final R_1 values were 0.0423 (all data). The final $wR(F^2)$ values were 0.1074 (all data). The goodness of fit on F^2 was 1.158. Flack parameter = 0.15(18). The Hooft parameter is 0.15(7) for 1250 Bijvoet pairs.

Cytotoxicity Assays. HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human tumor cell lines were used in the cytotoxic assay. These cell lines were obtained from ATCC (Manassas, VA, USA). Cells were cultured in RMPI-1640 or DMEM medium (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. The cytotoxicity assay was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) (Sigma, St. Louis, MO, USA) assay.[30] Briefly, cells were seeded into each well of a 96-well cell culture plate. After 12 h of incubation at 37 °C, the test compound (40 μM) was added. After incubating for 48 h, cells were subjected to the MTS assay. Compounds with a growth inhibition rate of 50% were further evaluated at concentrations of 0.064, 0.32, 1.6, 8, and 40 μM in triplicate, with cisplatin and paclitaxel (Sigma) as positive controls. The IC_{50} value of each compound was calculated with Reed and Muench's method.[34]

Nitric Oxide Production in RAW264.7 Macrophages. RAW264.7 cells were seeded in 96-well cell culture plates (2×10^5 cells/well). The cells were treated with serial dilutions of the compounds with a maximum concentration of 25 μM, followed by

stimulation with LPS (1 μg/mL) for 18 h. NO production in the supernatant was assessed by Griess reagents. The absorbance at 550 nm was measured with a 2104 Envision multilabel plate reader (Perkin-Elmer Life Sciences, Inc., Boston, Ma, USA). MG-132 was used as a positive control.[35] The viability of RAW264.7 cells was evaluated by the MTT assay simultaneously to exclude the interference of the cytotoxicity of the test compounds. The absorbance was read at 595 nm.

■ ASSOCIATED CONTENT

Supporting Information

Supplemetary data associated with this article (^{1}H, ^{13}C NMR, DEPT, HSQC, HMBC, COSY, NOESY, HREIMS, IR, and UV spectra of compounds **1**, **6**, and **16**; ^{1}H, ^{13}C NMR, DEPT, and HREIMS spectra of compounds **2**−**5**, **7**−**15**, **17**, and **18**; X-ray data of compounds **1**, **16**, and **25**) are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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