ent-Kaurane and Cembrane Diterpenoids from *Isodon sculponeatus* and Their Cytotoxicity

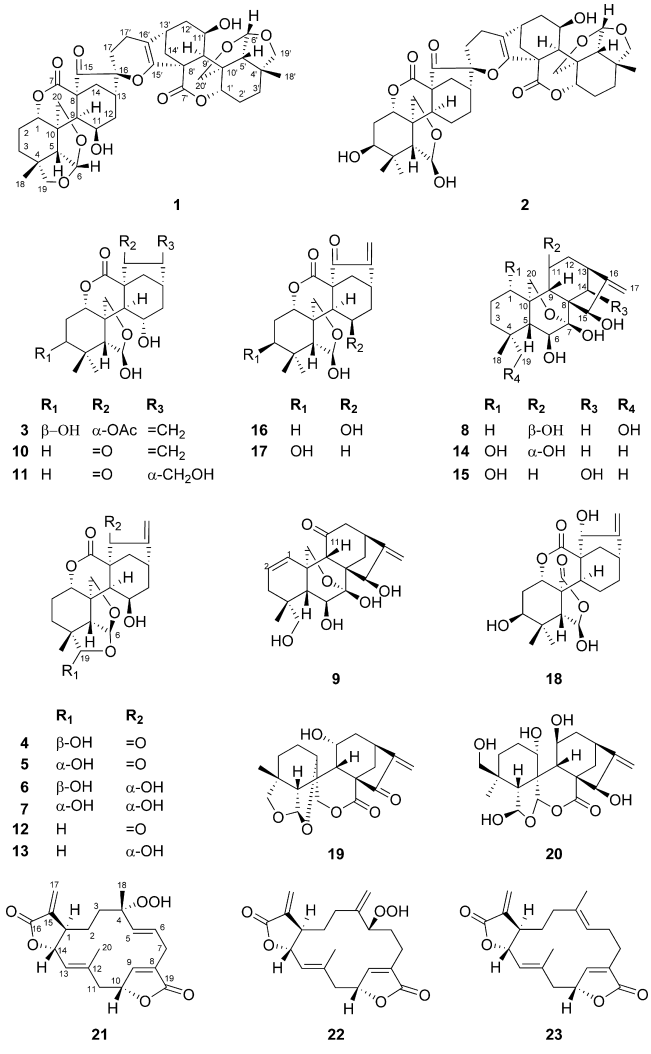
Li-Mei Li,[†,‡] Guo-You Li,[§] Jian-Xin Pu,[†] Wei-Lie Xiao,[†] Li-Sheng Ding,[§] and Han-Dong Sun*[,†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China, Scientific Research Center, Chengdu Medical College, Chengdu 610083, People's Republic of China, and Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, People's Republic of China

Received July 16, 2009

Nine new *ent*-kauranoids, sculponins D−L (**1**−**9**), and 14 known diterpenoids (**10**−**23**) were isolated from the aerial parts of *Isodon sculponeatus*. The structures of the new diterpenoids were determined by detailed interpretation of their 1D and 2D NMR spectra and HRESIMS. The isolated compounds were evaluated for their cytotoxic activities against a small panel of human cancer cell lines.

Isodon sculponeatus (Vaniot) Kudo (Lamiaceae) is mainly distributed in southern China.[1] Its stems and leaves have long been used in traditional Chinese medicine for the treatment of diarrhea.[2] Previous phytochemical study of *I. sculponeatus* indigenous to Xichang Prefecture of Sichuan Province led to the isolation of three *ent*-kauranoids that possessed multicyclic skeletons formed via the oxygen atoms.[3] Continued investigation on this plant has now furnished an additional 23 diterpenoids, including nine new *ent*-kauranoids (sculponins D−L, **1**−**9**), 11 known *ent*-kauranoids, epinodosin (**10**),[4] maoyecrystal D (**11**),[5] sculponeatin A (**12**),[6] sculponeatin B (**13**),[6] nervosanin B (**14**),[7] enmenol (**15**),[8] nodosin (**16**),[9,10] enmein (**17**),[4] ememogin (**18**),[11] sculponeatin C (**19**),[6] and sculponeatin D (**20**),[12] and three known cembrane diterpenoids, 4α-hydroperoxy-5-enovatodiolide (**21**),[13] 4-methylene-5β-hydroperoxyovatodiolide (**22**),[13] and ovatodiolide (**23**).[14] Although these cembrane diterpenoids have been isolated from *Anisomeles indica* (Lamiaceae),[13] they are reported from the genus *Isodon* for the first time. All of the diterpenoids were evaluated for cytotoxicity against K562, A549, and HepG2 human cell lines, and some of them showed moderate activity. This paper describes the characterization and cytotoxicity of these diterpenoids.



Results and Discussion

Compound **1** was isolated as a white powder. Its molecular formula was determined as $C_{40}H_{48}O_{12}$ by HRESIMS, corresponding to 17 degrees of unsaturation. In the ^{13}C NMR spectrum, most of the 40 carbon signals appeared in pairs, due to the asymmetric skeleton of the diterpene dimer (Table 1). Analysis of ^{1}H and ^{13}C NMR, DEPT, and HSQC spectra revealed that the two subunits of **1** (Figure 1, **1a** and **1b**), comprising rings A−D with their associated substituents, were identical to those of sculponeatin A (**12**).[6] The α,β-unsaturated carbonyl group [δ 200.9 (s, C-15), δ 150.8 (s, C-16), and δ 118.1 (t, C-17)] of **12** was replaced by a carbonyl carbon [δ 208.9 (s, C-15)], an oxygenated quaternary carbon [δ 85.5 (s, C-16)], an olefinic bond [δ 156.3 (s, C-15′), δ 117.9 (s, C-16′)], and two methylenes [δ 17.8 (t, C-17) and δ 24.1 (t, C-17′)] in **1a** and **1b**. These key changes of signals suggested that subunits **1a** and **1b** were linked by a dihydropyran ring. This conclusion was confirmed by the related HMBC correlations (Figure 1). Considering the seriously overlapped signals, key correlations were not easily observed in the ROESY spectrum. However, the ^{13}C NMR data of C-13, C-15, C-16, C-17, and C-15′ of compound **1** were almost identical to those of lushanrubescensin J,[15] whose structure was confirmed by X-ray diffraction. Therefore, the configuration at C-16 in compound **1** was deduced to be *S*. The upfield shift of C-12 (Δ −8.3 ppm) caused by the γ-steric compression effect between H-17β and H-12α (Figure 2) further supported the above conclusion. Thus, the structure of compound **1** was determined to be as shown, and it was named sculponin D.

The molecular formula of sculponin E (**2**) was determined to be $C_{40}H_{50}O_{12}$ by HRESIMS in combination with ^{13}C NMR. The 1D NMR data indicated that it was also an asymmetric *ent*-kauranoid dimer. By comparison with the ^{13}C NMR data of compounds **1** and **17**, the substructures **2a** and **2b** corresponded to compounds

* Corresponding author. Tel: 86-871-5223251. Fax: 86-871-5216343. E-mail: hdsun@mail.kib.ac.cn.
† Kunming Institute of Botany.
‡ Chengdu Medical College.
§ Chengdu Institute of Biology.

10.1021/np900406c CCC: $40.75
Published on Web 08/21/2009

Table 1. ^{1}H and ^{13}C NMR Data of Compound **1** and ^{13}C NMR Data of Compound **12** (in C_5D_5N, δ in ppm, J in Hz)

	1a			**1b**		**12**
no.	δ_H	δ_C	no.	δ_H	δ_C	δ_C
1	5.57 (dd, 11.8, 4.8)	78.8 CH	1′	5.51 (t, 8.4)	78.6 CH	78.8 CH
2	1.77−1.98 (2H, overlap)	23.4 CH_2	2′	1.77−1.98 (2H, overlap)	23.5 CH_2	23.4 CH_2
3	1.60−1.66 (2H, m)	29.5 CH_2	3′	1.55−1.57 (2H, m)	29.6 CH_2	29.4 CH_2
4		41.8 C	4′		41.5 C	41.7 C
5	2.92 (d, 5.0)	54.4 CH	5′	2.89 (d, 5.0)	53.3 CH	54.1 CH
6	6.10 (d, 5.0)	111.6 CH	6′	6.00 (d, 5.0)	111.6 CH	111.6 CH
7		170.9 C	7′		172.8 C	171.5 C
8		57.2 C	8′		52.6 C	56.0 C
9	2.16−2.22 (overlap)	47.8 CH	9′	2.16−2.22 (overlap)	44.1 CH	46.1 CH
10		50.6 C	10′		49.8 C	50.5 C
11	4.57−4.60 (overlap)	64.6 CH	11′	4.57−4.60 (overlap)	67.2 CH	65.4 CH
12	2.33−2.39 (m)	32.6 CH_2	12′	2.10 (dd, 14.4, 7.6)	34.7 CH_2	40.9 CH_2
	1.93−1.98 (overlap)			1.86−1.90 (overlap)		
13	2.76 (dd, 9.8, 3.5)	35.9 CH	13′	2.57 (dd, 7.2, 4.0)	36.2 CH	35.2 CH
14	3.84−3.88 (overlap)	32.8 CH_2	14′	3.58 (d, 11.6)	38.3 CH_2	34.1 CH_2
	2.90 (dd, 10.8, 5.0)			2.16−2.22 (overlap)		
15		208.9 C	15′		156.3 C	200.9 C
16		85.5 C	16′		117.9 C	150.8 C
17	2.26−2.33 (m)	17.8 CH_2	17′	1.77−2.01 (2H, overlap)	24.1 CH_2	118.1 CH_2
	1.77−1.83 (overlap)					
18	1.03 (3H, s)	30.6 CH_3	18′	1.03 (3H, s)	30.6 CH_3	30.7 CH_3
19	4.02 (d, 8.4)	77.2 CH_2	19′	3.94 (d, 8.8)	77.4 CH_2	77.2 CH_2
	3.45 (d, 8.4)			3.43 (d, 8.8)		
20	4.29 (d, 9.2)	72.9 CH_2	20′	4.00 (d, 8.0)	73.7 CH_2	72.8 CH_2
	4.15 (d, 9.2)			3.84−3.88 (overlap)		

17 and **12**, respectively. NMR assignments were established by analysis of 2D NMR data, especially HMBC correlations (Figure 3). Just as for compound **1**, the configuration at C-16 in **2** was assigned to be *S*, as indicated by the upfield shift of C-12 (Δ −11.8 ppm) due to the γ-steric compression effect between H-17β and H-12α.

The HRESIMS spectrum of compound **3** exhibited a molecular ion peak at *m*/*z* 445.1843 ([M + Na]$^+$), suggesting a molecular formula of $C_{22}H_{30}O_8$, with 8 degrees of unsaturation. Its ^{1}H and ^{13}C NMR data indicated a skeleton of 6,7-*seco*-1,7-olide-*ent*-kauranoid, similar to epinodosin (**10**).[4] The most notable difference was that the α,β-unsaturated carbonyl group (δ_C 200.7) in **10** was replaced by an oxygenated methine in **3**. Moreover, compound **3** has one more oxygenated methine, an acetyl group, and one less methylene than compound **10**. In its HMBC spectrum, the proton at δ 5.52 (dd, J = 11.8, 6.0 Hz, H-1) correlated to C-3 (δ 75.0, d), C-7 (δ 174.2, s), C-10 (δ 51.0, s), and C-20 (δ 73.6, t), the proton at δ 3.82 (br s, H-3) correlated to C-4 (δ 36.5, s), C-5 (δ 50.7, d), and C-18 (δ 28.6, q), and the proton at δ 6.70 (s, H-15) correlated to OAc (δ 170.1, s), suggesting that the OH and the OAc groups were located at C-3 and C-15, respectively. The β-orientation of OH-3 was deduced from the proton coupling constants between H-3α and H_2-2. The α-orientation of OAc-15 was determined by the upfield shift of C-9 (Δ −5.7 ppm) because of the γ-steric compression effect between OAc-15α and H-9α. ROESY correlations between H-3α and H-19, and H-15β and H-14β, confirmed the above deduction. Consequently, compound **3** was identified as

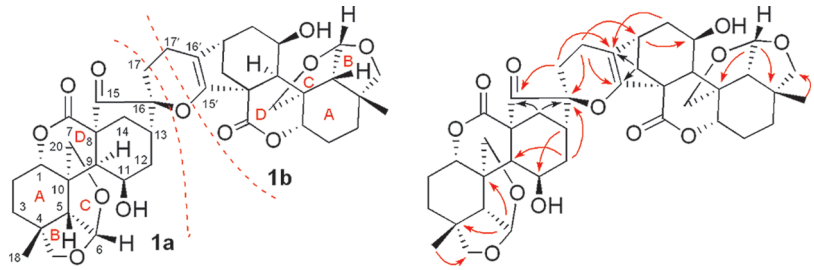


Figure 1. Key HMBC correlations of **1**.

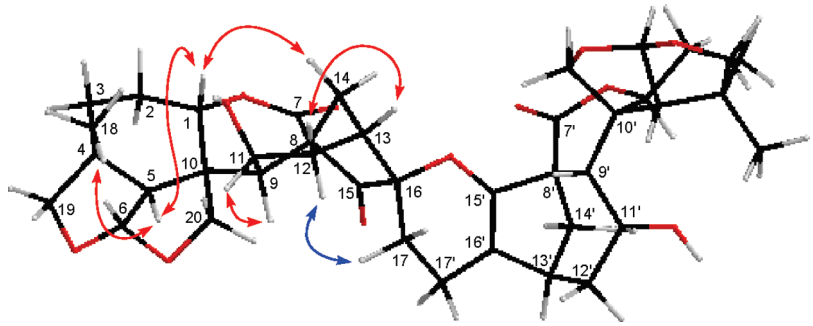


Figure 2. Key ROESY correlations (red ↔) and γ-steric compression effects (blue ↔) of **1**.

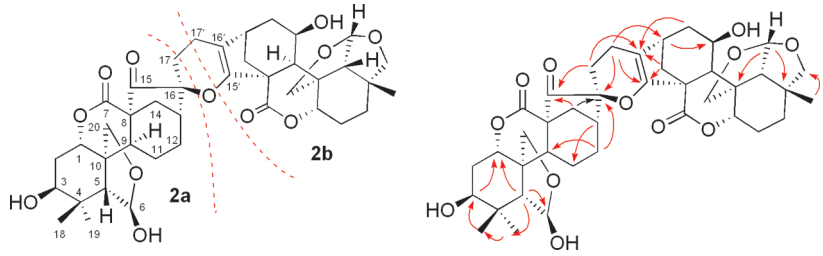


Figure 3. Key HMBC correlations of **2**.

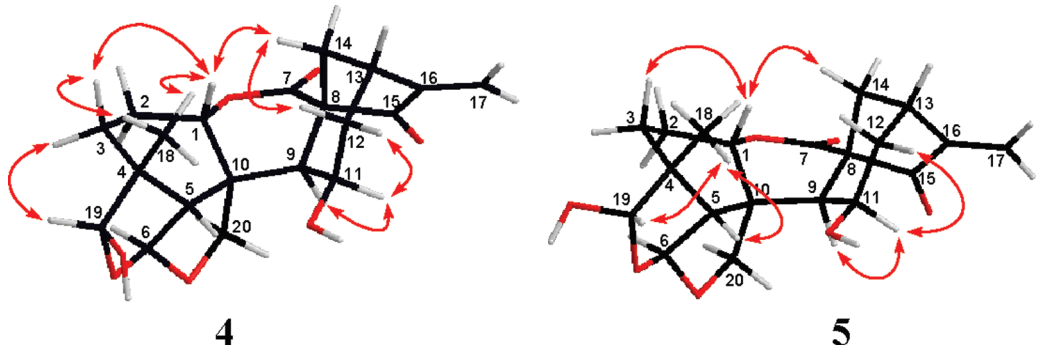


Figure 4. Key ROESY correlations of **4** and **5**.

3β,6β,11α-trihydroxy-15α-acetoxy-6,20-epoxy-6,7-*seco*-*ent*-kaur-16-en-1β,7-olide.

Compounds **4** and **5** were isolated as a pair of epimers both having the molecular formula $C_{20}H_{24}O_7$ from their HRESIMS. Their ^{13}C NMR signals were similar to those of sculponeatin A (**12**),[6] except that the C-19 methylene group (δ 77.2) of **12** was oxygenated to a hemiacetal group in **4** and **5** (δ 101.2, 106.4, respectively). Compounds **4** and **5** have one more OH group (at C-19) than compound **12**, which was supported by HMBC correlations of H-5, H-6, and H-18 with C-19. In the ROESY spectra of compounds **4** and **5**, H-19 (δ 6.10, 5.36, respectively) correlated to H-3α and H-18, verifying the respective β- and α-orientations of OH-19 in compounds **4** and **5** (Figure 4). Thus, compounds **4** and **5** were elucidated as 11β,19β-dihydroxy-6,19;6,20-diepoxy-6,7-*seco*-*ent*-kaur-16-en-15-one-1β,7-olide and 11β,19α-dihydroxy-6,19;6,20-diepoxy-6,7-*seco*-*ent*-kaur-16-en-15-one-1β,7-olide, respectively.

Compounds **6** and **7** were also obtained as a pair of epimers (both $C_{20}H_{26}O_7$ by HRESIMS), possessing one less unsaturation degree than **4** and **5**. Their ^{13}C NMR data closely resembled those of compounds **4** and **5**, except for an oxygenated methine group (δ_C 80.0) in **6** and **7** instead of the carbonyl group in **4** and **5**. The OH-15 was α-oriented, as shown by the significant upfield shift of C-9 (Δ -6.2 ppm) due to the γ-steric compression effect between OH-15 and H-9α. Therefore, compounds **6** and **7** were determined to be 11β,15α,19β-trihydroxy-6,19;6,20-diepoxy-6,7-*seco*-*ent*-kaur-16-en-1β,7-olide and 11β,15α,19α-trihydroxy-6,19;6,20-diepoxy-6,7-*seco*-*ent*-kaur-16-en-1β,7-olide, respectively.

The molecular formula of compound **8** was determined to be $C_{20}H_{30}O_6$ (HRESMS), indicating six degrees of unsaturation. The following typical NMR signals, including one hemiketal quaternary carbon (δ_C 98.0, C-7) and one oxygenated methylene carbon [δ_C 69.9, C-20, δ_H 5.47 and 4.35 (each 1H, d, J = 8.2 Hz), H-20], were characteristic of a 7α,20-epoxy-*ent*-kauranoid similar to maoecrystal K.[16] The only difference between them was that the β-OH was shifted from C-3 to C-11, which was confirmed by HMBC correlations between H-11 and C-9, C-8, and C-13. The relative configuration of all substituents was established unambiguously by a ROESY experiment. Accordingly, the structure of **8** was assigned as 6β,7β,11β,15β,19-pentahydroxy-7α,20-epoxy-*ent*-kaur-16-ene.

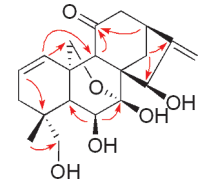


Figure 5. Key HMBC correlations of **9**.

The 1D NMR spectra of compound **9** indicated that it was also a 7α,20-epoxy-*ent*-kauranoid, substituted by four OH, one terminal double bond, one 1,2-disubstituted double bond, and one carbonyl group. In the HMBC spectrum, δ_H 6.81 (H-1) correlated to C-9 and C-20, δ_H 5.79$-$5.66 (H-2) correlated to C-4 and C-10, and H-9, H-12, and H-13 correlated to the carbonyl carbon (δ_C 210.7), which proved that the 1,2-disubstituted double bond was located between C-1 and C-2, and the carbonyl carbon was placed at C-11 (Figure 5). Analysis of the ROESY spectrum revealed that **9** had the same relative configuration as **8**. Compound **9** was determined to be 6β,7β,15β,19-tetrahydroxy-7α,20-epoxy-*ent*-kaur-1,16-dien-11-one.

All of the isolated *ent*-kaurane diterpenoids were C-20 oxygenated. Both monomeric (**3**$-$**20**) and dimeric *ent*-kauranes (**1** and **2**) were obtained from this species. The two dimeric *ent*-kauranes may be formed from their corresponding monomers by Diels$-$Alder reactions. The monomeric compounds consisted of 7,20-epoxy-*ent*-kauranes (**8**, **9**, **14**, and **15**) and 6,7-*seco*-*ent*-kauranes (**3**$-$**7**, **10**$-$**13**, and **16**$-$**20**). The 6,7-*seco*-*ent*-kauranes can be regarded as products of oxidative cleavage of the C_6-C_7 bond of 7,20-epoxy-*ent*-kaurane precursors. This group was further divided into 1,7-lactone (**3**$-$**7**, **10**$-$**13**, and **16**$-$**18**) and 7,20-lactone (**19** and **20**) types. From a biosynthetic point of view, if no substituent is present at C-1, oxidative cleavage would lead to the 7,20-lactone type, but if an α-orientated OH is present at C-1, relactonization could occur to produce the 1,7-lactone type. However, compound **20** is a particular case.

All of the isolates (**1**$-$**23**) were evaluated for cytotoxic activity against the K562, A549, and HepG2 human cell lines. Results are expressed as IC_{50} values (concentration required to inhibit cell growth by 50%) in μM, and data were obtained from triplicate experiments. Cisplatin was used as a positive control. As sum-

Table 2. ^{1}H and ^{13}C NMR Data of Compound **2** and ^{13}C NMR Data of Compound **17** (in C_5D_5N, *J* in Hz)

	2a			**2b**		**17**
no.	δ_H	δ_C	no.	δ_H	δ_C	δ_C
1	5.44−5.52 (overlap)	74.0 CH	1′	5.44−5.52 (overlap)	78.6 CH	75.1 CH
2	2.31−2.38 (m) 2.18−2.29 (overlap)	30.9 CH_2	2′	2.19−2.26 (overlap) 1.72−1.83 (overlap)	23.5 CH_2	31.0 CH_2
3	3.78 (overlap)	75.1 CH	3′	1.55−1.57 (2H, m)	29.6 CH_2	74.1 CH
4		36.0 C	4′		41.6 C	36.0 C
5	2.72−2.74 (overlap)	51.4 CH	5′	2.95 (d, 4.8)	53.3 CH	51.2 CH
6	5.90 (br s)	102.5 CH	6′	6.12 (d, 4.8)	111.7 CH	102.5 CH
7		171.6 C	7′		172.8 C	172.2 C
8		58.0 C	8′		52.5 C	57.1 C
9	2.97−3.02 (m)	47.9 CH	9′	2.14−2.16 (overlap)	44.1 CH	46.6 CH
10		50.1 C	10′		49.7 C	50.2 C
11	2.18−2.29 (overlap) 1.74−1.94 (overlap)	19.3 CH_2	11′	4.62 (br s)	67.3 CH	19.9 CH_2
12	1.74−1.94 (overlap) 1.45−1.50 (m)	21.0 CH_2	12′	2.06 (dd, 14.5, 7.4) 1.74−1.78 (m)	34.7 CH_2	32.8 CH_2
13	2.41 (br d, 9.2)	36.1 CH	13′	2.50−2.55 (m)	36.2 CH	35.4 CH
14	2.72−2.74 (2H, overlap)	31.9 CH_2	14′	2.14−2.17 (overlap) 3.55 (d, 9.2)	38.3 CH_2	29.7 CH_2
15		208.6 C	15′		156.3 C	200.9 C
16		85.7 C	16′		117.8 C	151.5 C
17	2.18−2.29 (2H, overlap)	17.7 CH_2	17′	1.74−1.94 (2H, overlap)	24.0 CH_2	117.4 CH_2
18	1.32 (3H, s)	28.4 CH_3	18′	1.05 (3H, s)	30.7 CH_3	28.4 CH_3
19	1.02 (3H, s)	23.3 CH_3	19′	3.95 (d, 8.4) 3.46 (d, 8.4)	77.4 CH_2	23.3 CH_3
20	4.54 (d, 9.0) 4.36 (d, 9.0)	74.5 CH_2	20′	3.97 (d, 8.5) 3.79 (d, 8.5)	73.7 CH_2	74.5 CH_2

Table 3. ^{13}C NMR Data of Compounds **3**−**9** (in C_5D_5N, δ in ppm)

no.	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	74.3 CH	78.9 CH	78.8 CH	78.6 CH	78.5 CH	31.5 CH_2	128.4 CH
2	31.3 CH_2	23.5 CH_2	25.8 CH_2	23.7 CH_2	26.0 CH_2	19.1 CH_2	128.7 CH
3	75.0 CH	29.7 CH_2	30.5 CH_2	29.9 CH_2	30.8 CH_2	36.1 CH_2	36.9 CH_2
4	36.5 C	43.6 C	43.8 C	43.7 C	43.8 C	37.3 C	37.5 C
5	50.7 CH	53.8 CH	54.0 CH	53.9 CH	54.1 CH	58.8 CH	54.7 CH
6	102.7 CH	106.7 CH	111.4 CH	106.8 CH	111.6 CH	73.9 CH	72.7 CH
7	174.2 C	171.5 C	171.8 C	175.8 C	176.1 C	98.0 C	97.1 C
8	52.7 C	56.1 C	56.1 C	52.4 C	52.4 C	52.2 C	55.7 C
9	48.3 CH	46.2 CH	46.2 CH	40.0 CH	40.0 CH	47.3 CH	56.0 CH
10	51.0 C	50.3 C	50.4 C	50.2 C	50.2 C	39.1 C	39.0 C
11	62.6 CH	65.6 CH	65.6 CH	65.3 CH	65.3 CH	65.1 CH	210.7 C
12	45.0 CH_2	41.1 CH_2	41.0 CH_2	45.3 CH_2	45.3 CH_2	45.3 CH_2	53.1 CH_2
13	37.6 CH	35.3 CH	35.3 CH	37.6 CH	37.6 CH	37.4 CH	35.9 CH
14	34.3 CH_2	34.2 CH_2	34.1 CH_2	34.2 CH_2	34.1 CH_2	27.1 CH_2	31.3 CH_2
15	78.8 CH	200.9 C	200.8 C	80.0 CH	80.0 CH	75.9 CH	76.4 CH
16	153.2 C	151.0 C	150.9 C	159.9 C	159.6 C	162.8 C	158.5 C
17	110.1 CH_2	117.8 CH_2	118.0 CH_2	108.4 CH_2	108.3 CH_2	106.7 CH_2	110.0 CH_2
18	28.6 CH_3	24.9 CH_3	31.8 CH_3	25.1 CH_3	32.0 CH_3	28.8 CH_3	25.9 CH_3
19	23.5 CH_3	101.2 CH	106.4 CH	101.2 CH	106.5 CH	64.6 CH_2	65.4 CH_2
20	73.6 CH_2	72.0 CH_2	70.5 CH_2	71.8 CH_2	70.2 CH_2	69.9 CH_2	68.0 CH_2
OAc	170.1 C 20.8 CH_3						

Table 4. Cytotoxic Activity (IC_{50}) of Compounds **1**, **2**, **10**, **12**, **16**, **17**, **19**, and **21**−**23**

compound	K562	A549	HepG2
1	1.50	1.00	1.12
2	2.00	1.85	1.62
10	0.56	2.10	1.62
12	1.62	1.90	1.77
16	0.54	>10	1.92
17	1.62	>10	2.02
19	0.78	2.73	0.68
21	1.20	1.55	0.90
22	4.30	4.18	3.11
23	1.25	1.96	1.40
cisplatin	1.14	3.84	1.27

marized in Table 4, compounds **1**, **2**, **10**, **12**, **19**, and **21**−**23** were cytotoxic to all cell lines, while **16** and **17** showed cytotoxicity only to K562 and HepG2 cell lines. Compounds **3**−**9**, **11**, **13**−**15**, **18**, and **20** were noncytotoxic, with IC_{50} values > 10 μM for all cell lines.

Experimental Section

General Experimental Procedures. Melting points were obtained on an XRC-1 micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were carried out on a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy. NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Electrospray ionization (ESIMS), high-resolution electrospray ionization (HRESIMS), and fast atom bombardment (FABMS) mass spectra were acquired on an API QSTAR time-of-flight mass spectrometer and a VG Autospec-3000 mass spectrometer, respectively. Analytical and semipreparative HPLC was performed on an Agilent 1100 apparatus equipped with a diode-array detector and a Zorbax SB-C18 (Agilent, 4.6 mm × 250 mm, 1 mL/min; 9.4 mm × 250 mm, 3 mL/min, respectively) column. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatographic apparatus with a Shimadzu PRC-ODS (K) column (34 mm × 15 cm). Column chromatography (CC) was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Factory, Qingdao, People's Republic of China), silica gel H (60 μm,

Qingdao Marine Chemical Factory), Lichroprep RP-18 gel (40−63 μm, Merck, Darmstadt, Germany), and MCI gel (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). All solvents including petroleum ether (60−90 °C) were distilled prior to use.

Plant Material. The aerial parts of *I. sculponeatus* were collected in Xichang Prefecture of Sichuan Province, People's Republic of China, in August 2005. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (KIB 050810305) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Cell Cultures. Chronic myelogenous leukemia (K562), lung cancer (A549), and hepatocellular carcinoma (HepG2) human cell lines were obtained from the Shanghai Cell Bank, Chinese Academy of Sciences. The cells were maintained in RPMI1640 medium with hormone-free 15% heat-inactivated FBS (fetal bovine serum). In each case, 2 mM glutamine, 100 U/mL penicillin G, and 100 μg/mL streptomycin were added.

Cytotoxicity Assay. K562 cells were seeded into 96-well plates at an initial density of 5×10^4 cells/mL, and A549 and HepG2 cells were seeded at 4×10^4 cells/mL. After incubation with the indicated concentrations of compounds (10^2−10^{-2} μM) for 48 h, cell viability was assayed at 450 and 650 nm with the Cell Counting Kit-8 (CCK-8; Dojindo, Gaithersburg, MD).[17] Pure compounds were tested against the K562 cell line using the established protocols.[18] Testing against A549 and HepG2 cell lines was carried out according to the established protocols.[19]

Extraction and Isolation. The air-dried powdered aerial parts of *I. sculponeatus* (1.5 kg) were extracted at room temperature with acetone and filtered. The filtrate was evaporated in vacuo to afford a residue, which was partitioned by a liquid−liquid extraction between EtOAc and H_2O (1500 mL of each). The EtOAc extract (45.4 g) was decolorized using MCI gel, eluted with 90% MeOH−H_2O, to yield a yellowish gum (39.3 g). The gum was separated on a silica gel column, eluted with a $CHCl_3$−Me_2CO step gradient (1:0 to 0:1), to obtain nine fractions, A−I. This separation yielded **12** (2.1 g) (fraction B), **19** (1.2 g) (fraction C), **10** (0.9 g) (fraction D), **17** (1.4 g) (fraction E), and **13** (1.6 g) (fraction F). Fraction A (0.21 g) was chromatographed over silica gel eluted with petroleum ether−Me_2CO (5:1), followed by semipreparative HPLC (35% MeOH−H_2O), to give compounds **21** (8.2 mg), **22** (5.1 mg), and **23** (98.5 mg). Part of the mother liquid of compound **10** (0.13 g) was purified by preparative HPLC using 40% MeOH−H_2O, affording **10** (85.2 mg) and **16** (32.6 mg). Fraction G (5.8 g) was fractionated over silica gel eluted with petroleum ether−Me_2CO (5:1 to 1:2) to afford five subfractions, G1−G5. Subfraction G2 (0.54 g) was further chromatographed over RP-18 with 60% MeOH−H_2O eluent to give compound **3** (30.7 mg). Subfraction G3 was purified by semipreparative HPLC eluting with 20% MeCN−H_2O to yield compounds **4** and **5** (5.6 mg) and **6** and **7** (3.1 mg). Subfraction G4 was applied to Sephadex LH-20 eluting with MeOH followed by semipreparative HPLC eluting with 30% MeOH−H_2O to afford compounds **1** (15.3 mg), **2** (5.0 mg), **14** (5.2 mg), and **15** (2.3 mg), respectively. Compounds **18** (12.5 mg) and **20** (15.1 mg) were isolated from subfraction G5 using an RP-18 column and isocratic elution (50% MeOH−H_2O). Fraction H (2.4 g) was subjected to silica gel CC, eluted with a $CHCl_3$−MeOH step gradient (10:1 to 5:1), to afford **10** (0.5 g) and subfraction H1 (0.14 g), respectively. Subfraction H1 was further purified by preparative HPLC eluting with 15% MeCN−H_2O to provide compounds **8** (22.0 mg), **9** (4.2 mg), and **11** (14.5 mg), respectively.

Sculponin D (1): white, amorphous powder; $[\alpha]^{27}_D$ −146.5 (*c* 0.58, C_5H_5N); UV (MeOH) λ_{max} (log ε) 203 (3.82) nm; IR (KBr) ν_{max} 3442, 2950, 2930, 1769, 1723, 1710, 1630, 1455, 1364, 1341, 1282, 1267, 1224, 1189, 1151, 1089, 1061, 1021, 958, 943 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz), see Table 1; positive FABMS *m/z* 721 $[M + H]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 743.3049 (calcd for $C_{40}H_{48}O_{12}Na$ $[M + Na]^+$, 743.3043).

Sculponin E (2): white, amorphous powder; $[\alpha]^{27}_D$ −78.1 (*c* 0.70, C_5H_5N); UV (MeOH) λ_{max} (log ε) 204 (3.80) nm; IR (KBr) ν_{max} 3442, 2937, 2887, 1770, 1722, 1634, 1457, 1376, 1360, 1341, 1324, 1279, 1248, 1225, 1183, 1147, 1091, 1068, 1022, 967, 937, 886 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz), see Table 2; positive FABMS *m/z* 723 $[M + H]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 745.3189 (calcd for $C_{40}H_{50}O_{12}Na$ $[M + Na]^+$, 745.3199).

Sculponin F (3): white, amorphous powder; $[\alpha]^{13}D$ −94.5 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.66) nm; IR (KBr) ν_{max} 3429, 2949, 2894, 1743, 1640, 1459, 1376, 1342, 1303, 1234, 1189, 1167, 1140, 1106, 1062, 1010, 983, 971, 904, 815, 742, 645, 614, 565 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 6.70 (1H, s, H-15β), 5.87 (1H, d, J = 3.6 Hz, H-6α), 5.52 (1H, dd, J = 11.8, 6.0 Hz, H-1β), 5.14 (1H, br s, H-17a), 5.12 (1H, br s, H-17b), 4.62 (1H, m, H-11β), 4.31 (1H, d, J = 9.0 Hz, H-20a), 4.16 (1H, d, J = 9.0 Hz, H-20b), 3.82 (1H, br s, H-3α), 3.66 (1H, br s, H-5β), 3.43 (1H, d, J = 9.6 Hz, H-9α), 2.72−2.80 (1H, m, H-12β), 2.61−2.64 (1H, m, H-13β), 2.36−2.42 (1H, m, H-2β), 2.23 (1H, t, J = 12.4 Hz, H-2α), 2.06 (3H, s, OAc), 2.00 (1H, d, J = 12.0 Hz, H-14β), 1.85 (1H, dd, J = 13.2, 6.2 Hz, H-12α), 1.64 (1H, dd, J = 12.0, 5.0 Hz, H-14α), 1.30 (3H, s, Me-18), 1.07 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 3; positive ESIMS *m/z* 445 $[M + Na]^+$, 867 $[2\ M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 445.1843 (calcd for $C_{22}H_{30}O_8Na$ $[M + Na]^+$, 445.1838).

Sculponin G (4): white, amorphous powder; $[\alpha]^{26}_D$ −113.3 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.77) nm; IR (KBr) ν_{max} 3440, 2962, 2930, 2905, 2887, 1739, 1697, 1648, 1459, 1441, 1365, 1343, 1325, 1299, 1284, 1270, 1189, 1096, 1074, 1043, 1015, 993, 964, 952, 906, 883, 850, 772, 577 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) δ 6.18 (1H, d, J = 5.5 Hz, H-6β), 6.10 (1H, br s, H-19α), 6.02 (1H, br s, H-17a), 5.65−5.72 (1H, overlap, H-1β), 5.36 (1H, br s, H-17b), 4.55−4.59 (1H, overlap, H-11α), 4.27 (1H, d, J = 9.5 Hz, H-20a), 4.19 (1H, d, J = 9.5 Hz, H-20b), 3.60−3.67 (1H, overlap, H-14β), 3.10−3.13 (2H, overlap, H-5β, 13β), 2.44−2.50 (1H, m, H-12β), 2.23 (1H, d, J = 3.5 Hz, H-9α), 2.14−2.24 (1H, overlap, H-14α), 2.03−2.11 (1H, overlap, H-3α), 1.85−1.96 (2H, m, H_2-2), 1.70 (1H, dd, J = 15.0, 4.0 Hz, H-12α), 1.53−1.66 (1H, overlap, H-3β), 1.29 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 125 MHz), see Table 3; positive ESIMS: *m/z* 399 $[M + Na]^+$, 775 $[2\ M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 399.1419 (calcd for $C_{20}H_{24}O_7Na$ $[M + Na]^+$, 399.1419).

Sculponin H (5): white, amorphous powder; $[\alpha]^{26}_D$ −113.3 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.77) nm; IR (KBr) ν_{max} 3440, 2962, 2930, 2905, 2887, 1739, 1697, 1648, 1459, 1441, 1365, 1343, 1325, 1299, 1284, 1270, 1189, 1096, 1074, 1043, 1015, 993, 964, 952, 906, 883, 850, 772, 577 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) δ 6.30 (1H, d, J = 5.5 Hz, H-6β), 6.02 (1H, br s, H-17a), 5.65−5.72 (1H, overlap, H-1β), 5.36 (1H, br s, H-17b), 5.35 (1H, overlap, H-19β), 5.20 (1H, d, J = 8.5 Hz, H-20a), 4.55−4.59 (1H, overlap, H-11α), 4.20 (1H, d, J = 8.5 Hz, H-20b), 3.60−3.67 (1H, overlap, H-14β), 3.10−3.13 (1H, overlap, H-13β), 3.02 (1H, d, J = 5.5 Hz, H-5β), 2.93−3.00 (1H, m, H-2α), 2.44−2.50 (1H, m, H-12β), 2.32 (1H, d, J = 3.5 Hz, H-9α), 2.14−2.24 (2H, overlap, H-3α, 14α), 2.03−2.11 (1H, overlap, H-2β), 1.70 (1H, dd, J = 15.0, 4.0 Hz, H-12α), 1.53−1.66 (1H, overlap, H-3β), 1.07 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 125 MHz), see Table 3; positive ESIMS *m/z* 399 $[M + Na]^+$, 775 $[2\ M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 399.1419 (calcd for $C_{20}H_{24}O_7Na$ $[M + Na]^+$, 399.1419).

Sculponin I (6): white, amorphous powder; $[\alpha]^{26}_D$ −81.6 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.82) nm; IR (KBr) ν_{max} 3420, 2968, 2934, 2895, 1727, 1656, 1631, 1467, 1384, 1354, 1280, 1233, 1190, 1166, 1101, 1083, 1070, 1058, 1020, 1008, 963, 938, 912, 890, 754, 561 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) δ 6.17 (1H, d, J = 5.5 Hz, H-6β), 6.11 (1H, br s, H-19α), 5.92−5.99 (1H, overlap, H-1β), 5.71 (1H, br s, H-15β), 5.53 (1H, br s, H-17a), 5.23 (1H, br s, H-17b), 4.53−4.57 (1H, overlap, H-11α), 4.52 (1H, d, J = 9.0 Hz, H-20a), 4.22 (1H, d, J = 9.0 Hz, H-20b), 3.14−3.22 (2H, overlap, H-5β, 14β), 2.93−3.01 (1H, overlap, H-13β), 2.81 (1H, d, J = 3.5 Hz, H-9α), 2.43−2.49 (1H, overlap, H-12β), 2.10−2.15 (1H, m, H-2α), 2.03−2.10 (1H, m, H-3α), 1.92−1.97 (1H, overlap, H-12α), 1.86−1.93 (1H, overlap, H-14α), 1.85−1.91 (1H, overlap, H-2β), 1.62−1.72 (1H, m, H-3β), 1.32 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 125 MHz), see Table 3; positive ESIMS *m/z* 401 $[M + Na]^+$, 779 $[2\ M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 401.1575 (calcd for $C_{20}H_{26}O_7Na$ $[M + Na]^+$, 401.1576).

Sculponin J (7): white, amorphous powder; $[\alpha]^{26}_D$ −81.6 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.82) nm; IR (KBr) ν_{max} 3420, 2968, 2934, 2895, 1727, 1656, 1631, 1467, 1384, 1354, 1280, 1233, 1190, 1166, 1101, 1083, 1070, 1058, 1020, 1008, 963, 938, 912, 890, 754, 561 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) δ 6.30 (1H, d, J = 5.5 Hz, H-6β), 5.92−5.99 (1H, overlap, H-1β), 5.75 (1H, br s, H-15β), 5.52 (1H, br s, H-17a), 5.38 (1H, br s, H-19β), 5.23 (1H, br s, H-17b), 5.14 (1H, d, J = 8.0 Hz, H-20a), 4.53−4.57 (1H, overlap, H-11α), 4.51 (1H, d, J = 8.0 Hz, H-20b), 3.14−3.22 (2H, overlap, H-5β, 14β), 2.95−3.03 (1H, m, H-2α), 2.93−3.01 (1H, overlap, H-13β), 2.91 (1H, overlap, H-9α), 2.43−2.49 (1H, overlap, H-12β), 2.19−2.22 (1H, m, H-3α), 1.92−1.97 (2H, overlap, H-2β, 12α), 1.86−1.93 (1H, overlap,

H-14α), 1.59−1.62 (1H, m, H-3β), 1.10 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 125 MHz), see Table 3; positive ESIMS *m/z* 401 $[M + Na]^+$, 779 $[2\ M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 401.1575 (calcd for $C_{20}H_{26}O_7Na$ $[M + Na]^+$, 401.1576).

Sculponin K (8): colorless needles; mp 190−192 °C; $[\alpha]^{13}_D$ −36.8 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.79) nm; IR (KBr) ν_{max} 3417, 2925, 2898, 2870, 1659, 1632, 1450, 1432, 1377, 1339, 1322, 1279, 1255, 1222, 1177, 1160, 1141, 1091, 1048, 1007, 985, 973, 945, 896, 888, 676, 573, 513 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 8.10 (1H, d, J = 4.4 Hz, OH-6β), 6.83 (1H, br s, OH-15β), 5.80 (1H, d, J = 3.0 Hz, OH-11β), 5.47 (1H, d, J = 8.2 Hz, H-20a), 5.46 (1H, br s, H-17a), 5.28 (1H, br s, H-15α), 5.20 (1H, br s, H-17b), 4.71 (1H, br s, H-6α), 4.51 (1H, br s, H-11α), 4.42 (1H, d, J = 10.6 Hz, H-19a), 4.35 (1H, d, J = 8.2 Hz, H-20b), 4.14 (1H, d, J = 10.6 Hz, H-19b), 3.24 (1H, d, J = 11.4 Hz, H-14α), 2.89 (1H, br s, H-13α), 2.55 (1H, dd, J = 14.6, 9.4 Hz, H-12β), 2.39 (1H, br s, H-9β), 2.33−2.38 (2H, overlap, H-1α, 3α), 2.18 (1H, dd, J = 11.4, 4.5 Hz, H-14β), 2.02 (1H, d, J = 6.5 Hz, H-5β), 1.97 (1H, dd, J = 14.6, 5.1 Hz, H-12α), 1.60−1.65 (1H, m, H-2α), 1.63 (3H, s, Me-18), 1.42−1.50 (2H, overlap, H-1β, 2β), 1.12 (1H, t, J = 12.0 Hz, H-3β); ^{13}C NMR (C_5D_5N, 125 MHz), see Table 3; positive ESIMS *m/z* 389 $[M + Na]^+$, 755 $[2\ M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 389.1929 (calcd for $C_{20}H_{30}O_6Na$ $[M + Na]^+$, 389.1940).

Sculponin L (9): white, amorphous powder; $[\alpha]^{13}_D$ −73.8 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.83) nm; IR (KBr) ν_{max} 3441, 2962, 2900, 1713, 1639, 1415, 1384, 1328, 1267, 1241, 1176, 1122, 1079, 1062, 1052, 1031, 975, 913, 885, 728, 706, 657 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 8.44 (1H, d, J = 4.4 Hz, OH-6β), 6.94 (1H, br s, OH-15β), 6.81 (1H, dd, J = 10.4, 2.0 Hz, H-1), 5.66−5.79 (1H, m, H-2), 5.65 (1H, br s, H-17a), 5.29 (1H, br s, H-17b), 5.20 (1H, br s, H-15α), 5.02 (1H, br s, H-6α), 4.56 (1H, d, J = 8.8 Hz, H-20a), 4.31 (1H, d, J = 8.8 Hz, H-20b), 4.14 (1H, br d, J = 10.2 Hz, H-19a), 3.99 (1H, br d, J = 10.2 Hz, H-19b), 3.27 (1H, s, H-9β), 2.84−2.87 (1H, m, H-13α), 2.69 (1H, dd, J = 16.4, 7.6 Hz, H-12α), 2.49 (1H, br d, J = 12.5 Hz, H-14β), 2.41 (1H, d, J = 16.4 Hz, H-12β), 2.26−2.34 (2H, overlap, H-3β, 5β), 1.93 (1H, br d, J = 17.6 Hz, H-3α), 1.88 (1H, d, J = 12.5 Hz, H-14α), 1.58 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 3; positive ESIMS *m/z* 385 $[M + Na]^+$, 747 $[2\ M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 385.1633 (calcd for $C_{20}H_{26}O_6Na$ $[M + Na]^+$, 385.1627).

Acknowledgment. Financial support of this research project was provided by the National Natural Science Foundation of China (Nos. 30772637 and 30830119), the NSFC-Joint Foundation of Yunnan Province (No. U0832602), the Major State Basic Research Development Program of China (No. 2009CB522300), the Natural Science Foundation of Yunnan Province (No. 2008CD162), and the Key Project of Knowledge Innovation Project of CAS (KSCX2-YW-R-25).

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1–9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP900406C