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Bioactive Guaianolides from Siyekucai (*Ixeris chinensis*)

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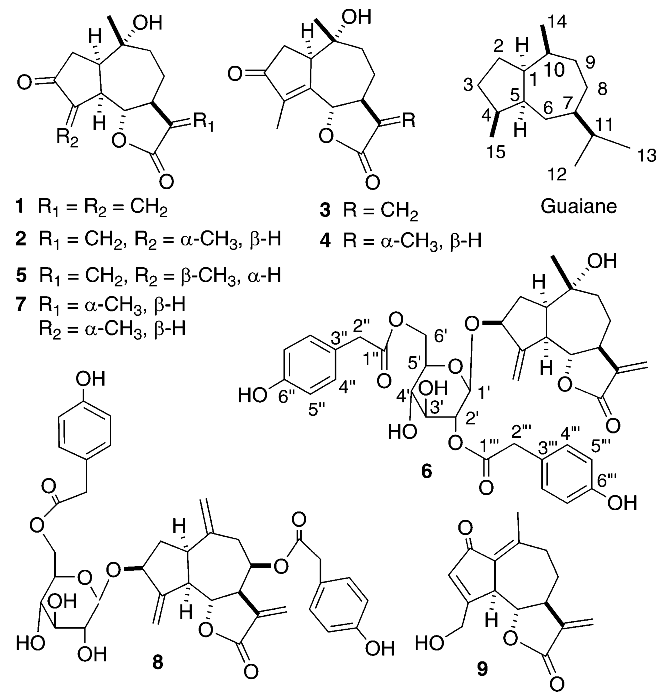
Two new guaianolides, named chinensiolides D (**5**) and E (**6**), were isolated from *Ixeris chinensis* Nakai, and their structures were determined to be 10α-hydroxy-3-oxoguaia-11(13)-eno-12,6α-lactone (**5**) and 10α-hydroxy-3*β*-*O*-[2,6-di(*p*-hydroxyphenylacetyl)-*β*-glucopylanosyl]guaia-4(15),11(13)-dieno-12,6α-lactone (**6**). The first isolation of (11*S*)-10α-hydroxy-3-oxoguaia-4-eno-12,6α-lactone (**4**) from natural sources and its characterization are also reported. Chinenciolide E (**6**) showed significant growth inhibitory activity toward VA-13 malignant lung tumor cells (IC_{50} = 0.72 μM).

Ixeris chinensis Nakai (Compositae) is a perennial plant that is found in various places in China and used as a folk medicine for the treatment of bronchitis, pneumonia, pharyngitis, dysentery, and poisonous indigestion on the basis of its antifebrile, antidotal, and analgesic effects.[1] The known constituents of this plant include several triterpenes.[2] Studies on some other species of this genus revealed the presence of sesquiterpene lactones such as guaianolides, eudesmanolides, germacranolides, and their glycosides.[3−10] Because of our interest in the biological activity of sesquiterpene lactones related to the medicinal effects of *I. chinensis*, we previously isolated three guaianolides (**1**−**3**) from this plant.[11] Lee et al. and Khalil et al. also reported glycosides of guaianolides from this plant.[12] The diverse biological activity reported for guaianolides[13−22] prompted us to undertake a further investigation of the guaianolides in this plant.

In this paper, we report the isolation of two additional new natural guaianolides, which we have named chinensiolides D (**5**) and E (**6**), and the first isolation of a known compound, (11*S*)-10α-hydroxy-3-oxoguaia-4-eno-12,6α-lactone (**4**), from natural sources. Also isolated were three known compounds, (11*S*)-10α-hydroxy-3-oxo-4*βH*-guaiano-12,6α-lactone (**7**),[23] ixerochinoside (**8**),[12b] and 8-deoxylactucin (**9**).[24] We also report their potential as immunomodulators because of their influence on induction of ICAM-1 (intercellular adhesion molecule-1) and on the basis of the reported antiinflammatory activity of this plant and their cytotoxic activity against A 549 lung carcinoma, WI-38 lung fibroblast, VA-13 lung malignant tumor, and HepG2 human liver tumor cells.

Results and Discussion

The methanolic extract of the fresh whole plant of *I. chinensis* was defatted by extraction with hexane. The MeOH layer was concentrated, diluted with H_2O, and extracted with ethyl acetate. The ethyl acetate extract was subjected to separation using flash chromatography followed by HPLC on a silica gel column to give compounds **4**−**9**.



Compound **4** had the composition $C_{15}H_{20}O_4$, on the basis of HREIMS. The IR spectrum showed the presence of hydroxyl (3612 cm^{-1}), α,*β*-unsaturated carbonyl (1706 cm^{-1}), and *γ*-lactone (1784 cm^{-1}) groups. The presence of a cyclopentenone moiety in **4** was supported by the UV spectrum [λ_{max} 237.5 nm (log ϵ 4.03)]. The ^{13}C NMR spectrum displayed 15 carbon resonances (see Experimental Section). Lactone and ketone carbonyl signals were observed at δ 177.2 and 207.7, respectively. Two signals for carbons bearing oxygen were observed at δ 81.5 (d) and 74.4 (s). It was clear that the remaining protonated carbon resonances were due to three methyl carbons, three methylene carbons, and three methine carbons. The ^1H NMR spectrum showed one singlet methyl, one doublet methyl, one broad singlet methyl connected to an olefin carbon, and one oxymethine proton. The $^1H-^1H$ correlations [H-1 and H-2α,*β*; H-1 and H-6; H-1 and H-15; H-6 and H-7; H-6 and H-15; H-7 and H-8*β*; H-7 and H-11; H-8α,*β* and H-9α,*β*; H-11 and H-13] were determined by analysis of the $^1H-^1H$ COSY spectrum. The HMBC correlation of the signal due to the tertiary

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carbon bearing a hydroxyl group at δ 74.4 (s) with those of H-1, H-2α,β, H-8α,β, H-9α,β, and H-14 placed the hydroxyl group at C-10. The correlation of the carbonyl carbon signal at δ 207.7 with those of H-2α,β and H-15 indicated that the ketone carbonyl group was at C-3. The HMBC correlation of a nonprotonated olefinic carbon at δ 143.0 (C-4) with H-2β, H-6, and H-15 and that at δ 161.3 (C-5) with H-1, H-2α,β, H-6, H-7, and H-15 indicated the nature of the five-membered A ring of **4**. The multiple-bond ^{1}H$-$^{13}C correlations of three carbon atoms of the γ-lactone ring [C-12 (δ 177.2) with H-11and H-13; C-11 with H-7, H-8α, and H-13; C-6 with H-7 and H-8α,β] were determined by HMBC experiment. This result as well as the ^{1}H$-$^{1}H COSY correlation of H-6 with H-7 and H-15 allowed unambiguous connection of the α-methyl-γ-lactone moiety to C-6 and C-7. Hence, **4** possesses the guaianolide structure of 10-hydroxy-3-oxoguaia-4-eno-12,6-lactone. The coupling constant of H-6 ($J_{6,7}$ = 12.0 Hz) indicated the existence of a *trans*-fused γ-lactone. The orientations of H-6, H-11, and C-10 Me (H-14) were determined to be β and the orientations of H-1 and H-7 were determined to be α by the NOESYcorrelation (H-6 with H-14 and H-11) as well as by the coupling constant between H-6 and H-7. Thus, compound **4** was determined to be (11*S*)-10α-hydroxy-3-oxoguaia-4-eno-12,6α-lactone. Although this is the first isolation of **4** from natural sources, the structure is the same as that of isophotosantonic lactone.[25a,b]

Chinensiolide D (**5**) had the composition $C_{15}H_{20}O_4$, which was determined by HREIMS spectra. The IR spectrum of **5** showed the existence of a hydroxyl group (3620 cm^{-1}), a five-membered ring carbonyl (1744 cm^{-1}), and an α,β-unsaturated γ-lactone (1764 cm^{-1}). The ^{13}C NMR spectrum displayed 15 carbon resonances. Lactone and ketone carbonyl signals were located at δ 170.0 and 217.2, respectively. Two signals for carbons bearing oxygen were observed at δ 81.3 (d) and 74.0 (s). Judging from the DEPT and HMQC spectra, it was clear that the remaining protonated carbon resonances were due to two methyl carbons, four methylene carbons including an *exo*-olefin, and four methine carbons. The ^{1}H NMR spectrum showed one singlet methyl, one doublet methyl, one oxymethine proton, and two olefinic protons of an α-methylene-γ-lactone moiety. The eight carbon connections of C-1 to C-2, C-1 to C-5, and C-4$-$C-9 were determined by the ^{1}H$-$^{1}H COSY spectrum. The HMBC correlation of the signal due to the tertiary carbon bearing a hydroxyl group at δ 74.0 (s) with those of H-2β, H-8α, H-9α,β, and H-14 indicated that the hydroxyl group was at C-10. The correlation of the carbonyl carbon signal at δ 217.2 with those of H-2α, H-4, and H-5 placed the ketone carbonyl group at C-3. Multiple-bond ^{1}H$-$^{13}C correlations of carbon atoms of the γ-lactone ring [C-12 (δ 170.0) with H-13a,b; C-7 with 8α,β, H9α,β, and H-13a,b; C-6 with H-1, H-4, H-5, and H-8α] were determined by HMBC experiment. These spectroscopic analyses indicated that compound **5** possesses the guaianolide structure of 10-hydroxy-3-oxoguaia-11(13)-eno-12,6-lactone. The coupling constant of H-6 ($J_{6,7}$ = 12.0 Hz) indicated the existence of a *trans*-fused γ-lactone. The stereochemistries of H-6 and C-10 Me (H-14) were determined to be β and the correlations of H-1, H-5, and H-7 determined them to be α-oriented by the NOESY correlations [H-6 and H-14; H-1 and H-5; H-5 and H-7] as well as by the coupling constants of H-6 with H-5 and H-7. The C-4 Me was determined to be β-oriented by the NOESY correlations [H-1 with H-4 and H-5; H-15 (C-4 Me) with H-6] as well as by the *cis*-vicinal coupling between H-4 and H-5 ($J_{4,5}$ = 7.5 Hz). Thus, compound **5** was determined to be 10α-hydroxy-3-oxoguaia-11(13)-eno-12,6α-lactone.

Chinensiolide E (**6**) had the composition $C_{37}H_{42}O_{13}$ (HRESIMS). The IR spectrum of **6** indicated the existence of hydroxyl (3440 cm^{-1}), α,β-unsaturated γ-lactone and ester carbonyl (1744 cm^{-1}), and *exo*-methylene (1618 cm^{-1}) groups. The ^{13}C NMR spectrum displayed 33 carbon resonances (see Experimental Section). A lactone carbonyl (δ 170.3) and two ester carbonyl signals (δ 172.1 and 171.5) were evident. Signals for carbons bearing oxygen were observed (δ 79.4, 81.9, and 73.3) in addition to six oxygenated carbon signals of a hexose sugar. The remaining protonated carbon resonances were due to seven methylene carbons including two *exo*-olefins, three methine carbons, and two *p*-hydroxyphenyl groups. The ^{1}H NMR spectrum showed two *exo*-olefin methylenes including the α-methylene-γ-lactone moiety, two oxymethine protons, two *p*-hydroxyphenylacetyl moieties, and those of a hexose sugar. The eight carbon connections of C-1$-$C-3 and C-5$-$C-9 were defined by the ^{1}H$-$^{1}H COSY spectrum. HMBC correlation of nonprotonated olefin carbons [δ 150.2 with H-2α, H-3, H-5, H-6, and H-15; δ 142.8 with H-8β and H-13a,b] placed *exo*-olefins at C-4 and C-11, respectively. The tertiary hydroxyl group at C-10 and the γ-lactone moiety at C-6 and C-7 of **6** were evident from the HMBC experiment. Attachment of a sugar at C-3 and two *p*-hydroxyphenylacetoxyl groups at C-2 and C-6 of the sugar portion were also determined by HMBC experiment. The sugar portion of **6** was assigned on the basis of ^{13}C and ^{1}H NMR data and was supported by the NOESY correlation of H-1′ with H-3′ and H-5′, and H-2′ with H-4′ of the sugar portion. The β-glucosyl bond at C-3β of the guaiane skeleton was deduced by results of the NOE between H-1′ of the glucose moiety and H-3 of the guaiane skeleton. These analyses indicated that compound **6** possesses the guaianolide structure of 10α-hydroxy-3β-*O*-[2,6-di(*p*-hydroxyphenylacetyl)-β-glucopylanosyl]guaia-4(15),11(13)-dieno-12,6-lactone. NOESY correlations [H-1 and H-3; H-1 and H-5; H-5 and H-7; H-6 and H-14] indicated the stereochemistry of this compound as shown in structure **6**, and the coupling constants of H-1, H-3, H-5, and H-6 are in good agreement with this conclusion.

Table 1. Effect of Sesquiterpene Lactones **1**, **2**, **3**, **4**, **6**, **8**, and **9** on Induction of ICAM-1 and Cell Viability

	1	2	3	4	6	8	9
			IC$_{50}$ (μM)[a]				
ICAM-1[b]							
IL-1α	10.9	16.4	20.9[c]	>100	>100	>100	16.1
TNF-α	11.4	16.8	NT	>100	>100	>100	20.1
			IC$_{50}$ (μM)[d]				
cell viability by MTT assay[e]	64.9[f]	>100	>100	>100	>100	>100	>100

[a] IC$_{50}$ was calculated using the following equation. Expression of ICAM-1 (% of control) = [(absorbance with sample and IL-1α/TNF-α treatment $-$ absorbance without IL-1α/TNF-α treatment)/(absorbance with IL-1α/TNF-α treatment $-$ absorbance without IL-1α/TNF-α treatment)] $\times$ 100. [b] A549 cells (2 $\times$ 10^4 cells/well) were pretreated with various concentrations of the compounds for 1 h and then incubated in the presence of IL-1α or TNF-α for 6 h. Absorbance at 415 nm was assayed after treatment of the cells with primary and secondary antibodies and addition of the enzyme substrate as described in the Experimental Section. The experiments were carried out in triplicate. [c] The experiment of **3** was carried out at a different time from that of other compounds. IC$_{50}$ value of **3** was corrected on the basis of that of compound **2**. [d] IC$_{50}$ was determined using the following equation. Cell viability (%) = [(experimental absorbance $-$ background absorbance)/(control absorbance $-$ background absorbance)] $\times$ 100. [e]A549 cells were incubated with serial dilutions of the compounds for 24 h. Cell viability (%) was measured by MTT assay. The experiments were carried out in triplicate cultures. [f] IC$_{50}$ value of **1** against A549 lung carcinoma cells.

Compound **7** was identical with a substance previously isolated from *Picris altissima* by Kisiel.[23a] Compound **7** has also been prepared as an intermediate in the synthesis of plagiochiline N from santonin.[23b] Compound **8** was identical with ixerochinoside that was isolated from *I. chinensis*.[12b] Compound **9** was identical with 8-deoxylactucin.[24a]

Expression of excess amount of the intercellular adhesion molecule-1 (ICAM-1) on the surface of endothelial cells of a blood vessel plays an important role in the progress of inflammatory reaction.[20−22] The inhibitory effects of **1**, **2**, **3**, **4**, **6**, **8**, and **9** on the induction of ICAM-1 were evaluated in the presence of IL-1α and TNF-α using human A549 cells (lung carcinoma) (Table 1).

Table 2. Cytotoxicity of Compounds against WI-38, VA-13, and HepG2 Cells

compd	IC_{50} (μM)		
	WI-38	VA-13	HepG2
2	25	27	22
3	24	22	23
4	25	130	230
6	23	0.72	140
7	32	160	230
9	2.7	8.5	25
Taxol	0.04	0.005	8.1
ADM	0.7	0.4	1.3

Compounds **1**, **2**, **3**, and **9**, each having an α-methylene-γ-lactone moiety, inhibited the induction of ICAM-1 significantly or moderately. Although **1** showed cytotoxicity to A549 cells, the IC_{50} values were much higher (ca. 6-fold) than those of inhibition of ICAM-1. It is interesting that glycosyl guaianolides containing an α-methylene-γ-lactone moiety (**6** and **8**) and a guaianolide containing an α-methyl-γ-lactone moiety (**4**) did not inhibit induction of ICAM-1 and also did not exhibit cytotoxicity toward A549 cells at concentrations below 100 μM. Compounds **1**, **2**, and **9** showed inhibitory activity on the induction of ICAM-1 induced by IL-1α and TNF-α at nearly the same levels. The results suggest that these compounds block the common signaling pathway of NF-κB activation downstream of IκB kinase activation, *de novo* RNA/protein synthesis of ICAM-1, or its intracellular transport to the plasma membrane.

The cytotoxic activity of compounds **2**−**4**, **6**, **7**, and **9** against WI-38, VA-13, and HepG2 cell lines is listed in Table 2. Chinensiolide E (**6**) exhibited the strongest cytotoxicity against VA-13 cells, with an IC_{50} value of 0.72 μM, and the IC_{50} value of **6** toward WI-38 was 32-fold higher than that toward VA-13. 8-Deoxylactucin (**9**) showed significant cytotoxicity against VA-13 cells with an IC_{50} value of 8.5 μM but inhibited the growth of WI-38 cells at 3-fold lower concentration. Chinenciolides B (**2**) and C (**3**), possessing an α-methylene-γ-lactone moiety, showed moderate cytotoxicity against WI-38, VA-13, and HepG2 cells at nearly identical concentrations. Chinensiolide A (**1**) showed weak inhibitory activity toward A549 cells (lung carcinoma).

Experimental Section

General Experimental Procedures. All melting points are uncorrected, and $[\alpha]_D$ values were measured using a Horiba Sepa-200 polarimeter. UV spectra were measured in MeOH using a Nihonbunko V-550 UV/vis spectrophotometer. ^{1}H NMR spectra were recorded at 500 MHz in $CDCl_3$, and ^{13}C NMR spectra were recorded at 125 MHz in $CDCl_3$. ^{1}H NMR assignments were determined by ^{1}H−^{1}H COSY experiments. ^{13}C NMR assignments were determined by DEPT, HMBC, and HMQC experiments. HREIMS and HRESIMS were recorded on a JEOL-JMS 700TZ instrument. Si gel (160−200 mesh) was employed for column chromatography and Si gel (230−400 mesh) for flash column chromatography. To describe HPLC conditions, we designate column, solvent, flow rate (mL/min), and retention time (t_R (min)). The isolated yields of compounds were calculated on the basis of the weight of fresh plant material.

Plant Material. The whole plant of *Ixeris chinensis* Nakai was collected in Qiqihar City, Heilongjiang Province, China, on July 17, 2000. The plant was identified by Dr. Takashi Kurosawa, Department of Biology, Faculty of Science, Tohoku University. A voucher specimen (2000-7-20) was deposited at the Laboratory of Natural Products, Department of Pharmacy Engineering, Qiqihar University.

Extraction and Isolation. The fresh whole plant (2091 g) was crushed using a mixer and extracted with MeOH (5 L) by dipping for 3 days. The MeOH extract was concentrated to 2.0 L and extracted with hexane (2 × 1000 mL). A saturated aqueous solution of NaCl (1.5 L) was added to the MeOH layer, and the aqueous solution was extracted with EtOAc (4 × 1000 mL). The EtOAc extracts were dried (Na_2SO_4) and concentrated to give an oily material (20.9 g), which was separated into six fractions by column chromatography [7.0 cm i.d. column packed with silica gel (628 g), EtOAc−hexane (1:1, 1.5 L, F1, F2), EtOAc (2 L, F3, F4), EtOAc−MeOH (4:1, 1 L, F5), MeOH (2.5 L, F6]. Fraction 3 (4.286 g) was further separated into four fractions using column chromatography [5.0 cm i.d. column packed with silica gel (215 g), EtOAc−hexane (2:3, 1.2 L, F3-1), EtOAc−hexane (3:1, 1 L, F3-2), EtOAc (1 L, F3-3), MeOH (1 L, F3-4]. Fraction 3-3 (0.408 g) was further separated by HPLC [Inertsil PREP-SIL (GL-Science), 25 × 1 cm i.d. stainless column, EtOAc−hexane (9:1), 6 mL/min] into four fractions. The second peak (t_R 7.61 min) gave **9** (26.6 mg, 0.00127%), the third peak (t_R 9.34 min) gave **2** (44.5 mg, 0.00213%), and the fourth peak (t_R 10.16 min) gave **7** (11.4 mg, 0.00055%). F4 (1.322 g) was separated into two fractions with column chromatography [3.0 cm i.d. column packed with silica gel (66 g), EtOAc−hexane (3:1, 1.2 L, F4-1), EtOAc (500 mL, F4-1), MeOH (1 L, F4-2]. Fraction 4-1 (0.965 g) was further separated by HPLC [Inertsil PREP-SIL(GL-Science), 25 × 2 cm i.d. stainless column, EtOAc−hexane (9:1), 9 mL/min] into seven fractions. The third peak (t_R 15.23 min), the fourth peak (t_R 18.55 min), the fifth peak (t_R 19.74 min), and the sixth peak (t_R 20.78 min) gave **6** (64.5 mg, 0.00308%), **8** (9.2 mg, 0.00044%), **4** (14.2 mg, 0.00068%), and **3** (59.8 mg, 0.00286%), respectively. The second peak (t_R 14.1 min) gave a crude solid compound (152.3 mg), which was recrystallized from MeOH to give **2** (131.7 mg, 0.00630%). The mother liquor (20.1 mg) was further purified by HPLC [Shodex C18, 25 × 1 cm i.d. stainless column, H_2O−MeOH (2:1), 4 mL/min] into four fractions. The first peak (t_R 15.2 min), the second peak (t_R 16.79 min), the third peak (t_R 18.07 min), and the fourth peak (t_R 19.67 min) gave compounds **7** (6.1 mg, total 0.00084%), **5** (3.2 mg, 0.00015%), **2** (7.8 mg, 0.00879%), and **1** (2.2 mg, 0.00011%), respectively.

Compound 4: colorless prisms (EtOAc); mp 160.5−162 °C; $[\alpha]^{20}_D$ +79.2 (*c* 1.08, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 237.5 (4.03) nm; IR ($CHCl_3$) ν_{max} 3612, 1784, 1706 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.82 (1H, br d, J = 12.0 Hz, H-6), 3.24 (1H, m, H-1), 2.61 (1H, br dd, J = 19.5, 2.7 Hz, H-2β), 2.55 (1H, dd, J = 19.5, 6.1 Hz, H-2α), 2.33 (1H, dq, J = 12.0, 6.8 Hz, H-11), 2.14 (1H, dd, J = 12.0, 12.0 Hz, H-7), 2.10 (1H, m, H-8α), 2.08 (1H, m, H-9β), 1.90 (3H, br s, H-15), 1.81 (1H, m, H-9α), 1.45 (1H, m, H-8β), 1.31 (3H, d, J = 6.8 Hz, H-13), 0.97 (3H, s, H-14); ^{13}C NMR ($CDCl_3$ 125 MHz) δ 207.7 (C, C-3), 172.2 (C, C-12), 161.3 (C, C-5), 143.0 (C, C-4), 81.5 (CH, C-6), 74.4 (C, C-10), 50.5 (CH, C-1), 48.4 (CH, C-7), 45.3 (CH_2, C-9), 41.4 (CH, C-11), 37.1 (CH_2, C-2), 25.8 (CH_2, C-8), 21.3 (CH_3, C-14), 12.5 (CH_3, C-13), 9.4 (CH_3, C-15); HREIMS *m/z* 264.1389 (calcd for $C_{15}H_{20}O_4$ 264.1362). [Isophotosantonic lactone: (EtOAc−light petroleum): mp 165−167 °C; $[\alpha]_D$ +129 (*c* 1.34, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 239 (4.11) nm.][20a]

Chinensiolide D (5): colorless needles (EtOAc); 169−172 °C; $[\alpha]^{20}_D$ +33.4 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 211.0 (3.83) nm; IR ($CHCl_3$) ν_{max} 3620, 1764, 1744 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.18 (1H, d, J = 3.4 Hz, H-13a), 5.45 (1H, d, J = 3.4 Hz, H-13b), 3.98 (1H, dd, J = 12.0, 9.3 Hz, H-6), 3.41 (1H, m, H-7), 2.89 (1H, ddd, J = 9.3, 7.5, 7.4 Hz, H-5), 2.64 (1H, dq, J = 7.5, 7.5 Hz, H-4), 2.57 (1H, ddd, J = 11.2, 8.8, 7.4 Hz, H-1), 2.44 (1 H, dd, J = 19.0, 8.8 Hz, H-2α), 2.28 (1H, m, H-8α), 2.18 (1 H, dd, J = 19.0, 11.2 Hz, H-2β), 1.88 (1H, ddd, J = 16.5, 8.0, 8.0 Hz, H-9β), 1.78 (1H, ddd, J = 16.5, 8.0, 4.7 Hz, H-9α), 1.52 (1H, m, H-8β), 1.28 (3H, s, H-14), 1.28 (3H, d, J = 7.5 Hz, H-15); ^{13}C NMR ($CDCl_3$ 125 MHz) δ 217.2 (C, C-3), 169.9 (C, C-12), 140.0 (C, C-11), 119.6 (CH_2, C-13), 81.3 (CH, C-6), 74.0 (C, C-10), 50.0 (CH, C-4), 48.3 (CH, C-1), 47.1 (CH, C-5), 44.0 (CH, C-7), 39.0 (CH_2, C-2), 33.0 (CH_2, C-9), 32.0 (CH_3, C-14), 24.4 (CH_2, C-8), 11.6 (CH_3, C-15); HRESIMS *m/z* 264.1364 (calcd for $C_{15}H_{20}O_4$ 264.1362).

Chinensiolide E (6): microcrystals (acetone); mp 113.5−117 °C; $[\alpha]^{20}_D$ −11.9 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ϵ) 277.5 (2.85), 212.5 (3.58) nm; IR (KBr) ν_{max} 3440, 1744, 1618 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 7.36 (2H, d, J = 8.0 Hz, H-4″), 7.32 (2H, d, J = 8.0 Hz, H-4‴), 7.12 (2H, d, J = 8.0 Hz, H-5″), 7.11 (2H, d, J = 8.0 Hz, H-5‴), 6.19 (1H, d, J = 3.2 Hz, H-13a), 5.64 (1H, dd, J = 10.0, 8.5 Hz, H-2′), 5.50 (1H, s, H-15a), 5.47 (1H, s, H-15b), 5.35 (1H, d, J = 3.2 Hz, H-13b), 5.13 (1H, d, J = 8.5 Hz, H-1′), 5.01 (1H, dd, J = 10.2, 6.0 Hz, H-6′a), 4.75 (1H, dd, J = 8.0, 6.5 Hz, H-3), 4.68 (1H, br d, J = 10.2 Hz, H-6′b), 4.36 (1H, dd, J = 10.2, 10.2 Hz, H-6), 4.23 (1H, dd, J = 10.0, 8.0 Hz, H-3′), 4.02 (1H, dd, J = 8.0, 8.5 Hz, H-4′), 4.00 (1H, br dd, J = 8.5, 6.0 Hz, H-5′), 3.84 (2H, d, J = 9.2 Hz, H-2‴), 3.70 (2H, d, J = 4.0 Hz, H-2″), 3.44 (1H, m, H-7), 2.99 (1H, br dd, J = 8.0, 10.2 Hz, H-5), 2.45 (1H, dd, J = 8.0, 8.0 Hz, H-1), 2.41 (1H, dd, J = 17.5, 8.0 Hz, H-2α), 2.25 (1H, m, H-8α), 1.99 (1H, ddd, $J = 17.5, 8.0, 6.5$ Hz, H-2β), 1.90 (1H, m, H-9β), 1.69 (1H, m, H-9α), 1.36 (1H, m, H-8β), 1.27 (3H, s, H-14); ^{13}C NMR (C_5D_5N, 125 MHz) δ 172.1 (C, C-1″), 171.5 (C, C-1‴), 170.3 (C, C-12), 158.0 (C, C-6‴), 157.9 (C, C-6″), 150.2 (C, C-4), 142.8 (C, C-11), 131.3 (CH, C-4‴), 131.0 (CH, C-4″), 125.2 (C, C-3‴), 125.2 (C, C-3″), 118.6 (CH_2, C-13), 116.3 (CH, C-5‴), 116.2 (CH, C-5″), 113.2 (CH_2, C-15), 100.0 (CH, C-1′), 81.9 (CH, C-6), 79.4 (CH, C-3), 75.9 (CH, C-3′), 75.3 (CH, C-2′), 75.1 (CH, C-5′), 73.3 (C, C-10), 71.8 (CH, C-4′), 64.7 (CH_2, C-6′), 51.0 (CH, C-5), 50.5 (CH, C-1), 44.5 (CH, C-7), 40.6 (CH_2, C-2‴), 40.5 (CH_2, C-2″), 35.3 (CH_2, C-2), 34.8 (CH_2, C-9), 30.5 (CH_3 C-14), 25.0 (CH_2, C-8); HREIMS m/z 717.2518 (calcd for $C_{37}H_{40}O_{13}Na$ 717.2518).

Inhibitory Activity on Induction of ICAM-1. Experimental details were described previously.[20,26,27]

Growth Inhibitory Activity to WI-38, VA-13, and HepG2 Cells. Experimental details were described previously.[27]

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