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Cytotoxic *ent*-kaurane diterpenoids from *Isodon sinuolata*

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ABSTRACT

Five (**1**–**5**) *ent*-kaurane diterpenoids and 17 other known ones, were isolated from the leaves and stems of *Isodon sinuolata*. Their structures were determined on the basis of spectroscopic methods including 1D and 2D NMR spectroscopic analysis. All compounds were evaluated for cytotoxicity against a small panel of cell lines. Some compounds exhibited significant cytotoxicity.

1. Introduction

The *Isodon* genus (also named *Rabdosia*) is a cosmopolitan and important genus of the Labiatae family and includes about 150 species (Sun et al., 2006). This genus has attracted considerable attention as a prolific source of new natural products with diverse structures and biological properties. Since 1976, more than 50 *Isodon* species in China have been investigated systematically by our group and over 500 new diterpenes, including kauranes, abietanes, labdanes, pimaranes, isopimaranes, gibberellanes, clerodanes, and atisanes, have been isolated and characterized (Sun et al., 2001; Huang et al., 2006, 2007). Among them, eriocalyxin B, a typical *ent*-kaurane diterpenoid, was found to effectively induce apoptosis of murine leukemia cells through modulation of the NF-κB, acute myeloid leukemia AML1–ETO, and mitogen-activated protein kinase (MAPK) pathways, and may be a potential apoptosis inducer for treatment of murine leukemia (Wang et al., 2007).

Isodon sinuolata C.Y. Wu et H.W. Li, a kind of herb, is mainly distributed in southwest of China, especially in Shangrila and Tibet regions. This species has never been phytochemically investigated before. In our ongoing research for bioactive constituents from *Isodon* genus plants, we have investigated the aerial parts of *I. sinuolata*, collected in Shangrila region, Yunnan, PR China, which led to isolation of 22 compounds, including five new diterpenoids (**1**–**5**) and 17 known ones (**6**–**22**). In the present paper, we report the isolation, structure elucidation, and cytotoxic properties of these new compounds.

2. Results and discussion

Phytochemical study of the ethyl acetate extract of *I. sinuolata* led to isolation of five new *ent*-kaurane diterpenoids, sinuolatins A–E (**1**–**5**), together with 17 known substances, including maoecrystal A (**6**) (Hou et al., 2000), maoecrystal G (**7**) (Xiang et al., 2004), longikaurin E (**8**) (Fujita et al., 1981), lasiodonin (**9**) (Yang et al., 2007), nodosin (**10**) (Zhao et al., 2004), oreskaurin C (**11**) (Xiang et al., 2004), rubescensin Q (**12**) (Han et al., 2005), enmenin (**13**) (Shen et al., 2005), trichorabdal A (**14**) (Node et al., 1982), adenolin A (**15**) (Zhang et al., 1992), oreskaurin B (**16**) (Xiang et al., 2004), isodonal (**17**) (Yang et al., 2007), adenolin B (**18**) (Zhang et al., 1992), wikstroemioidin B (**19**) (Wu et al., 1993), wikstroemioidin C (**20**) (Wu et al., 1993), macrocalyxin J (**21**) (Shi et al., 2007), and effusanin E (**22**) (Fujita et al., 1980b) (Fig. 1). The structures of the known compounds were identified by comparison of their spectroscopic data with those reported in the literature.

Compound **1** was obtained as colorless needles. The molecular formula $C_{21}H_{32}O_7$ was determined from quasi-molecular ion peak at *m/z* 419.2045 $[M+Na]^+$ in its HRESIMS, indicating 6 degrees of unsaturation. On the basis of the characteristic signals of three methines (δ_C 62.4, 55.3, 29.3 due to C-5, 9, and 13), three quaternary carbons (δ_C 60.9, 43.0, 33.3 assignable to C-8, 10 and 4), two methyls (δ_C 34.1, 22.1 attributable to C-18, and 19), an oxygenated methylene (δ_C 66.0, assigned as C-20), and a hemiketal quaternary

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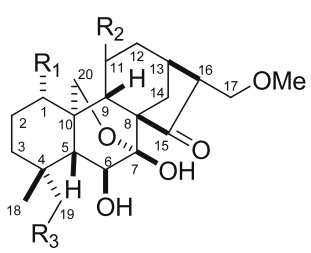

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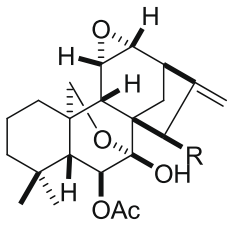
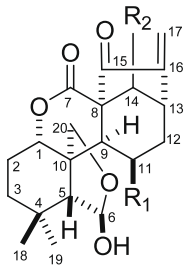
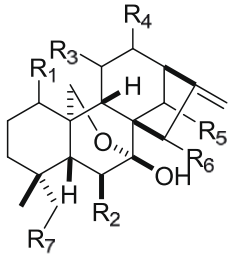
1	OH	α-OH	H
15	H	α-OAc	OAc
23	OH	β-OH	H



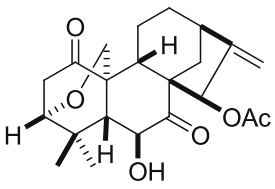
	R
4	OAc
16	OH



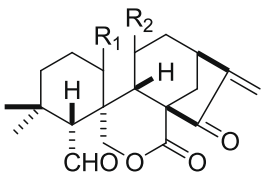
	R_1	R_2
5	OAc	H
10	OH	H
21	H	α-OH



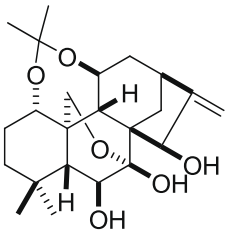
	R_1	R_2	R_3	R_4	R_5	R_6	R_7
2	H	β-OH	H	α-OH	β-OH	C=O	OAc
3	H	β-OAc	H	β-OH	H	β-OH	H
7	H	β-OAc	H	H	H	β-OH	H
8	H	β-OH	H	α-OAc	H	C=O	H
9	α-OH	β-OH	β-OH	H	H	β-OAc	H
11	H	β-OH	H	β-OH	H	β-OH	H
12	H	β-OAc	H	H	β-OH	β-OH	H
13	β-OH	β-OAc	H	H	H	β-OAc	H
18	α-OH	β-OH	α-OH	H	H	C=O	OAc
20	H	β-OH	H	α-OH	H	C=O	OAc
22	α-OH	β-OH	α-OH	H	H	C=O	H
24	H	β-OH	H	H	β-OH	C=O	OAc



6



	R_1	R_2
14	H	α-OH
17	α-OAc	β-OH



19

Fig. 1. The structures of compounds **1**–**24**.

carbon (δ_C 96.0, C-7), we assume that **1** should be a 7,20-epoxy-*ent*-kaurane diterpenoid, similar to lushanrubescensin F (**23**) (Han et al., 2003). Comparison of the NMR spectroscopic data between these two compounds confirmed the above deduction, and indicated that **1** was identical to **23**, except for an hydroxyl substituent on C-11. This was supported by the shift of C-9 (δ_Δ +3.0) and C-11 (δ_Δ −3.4) in **1** relative to **23**. Correlations from H-11 to C-9, C-8 and C-13 in the HMBC spectrum further confirmed the hydroxyl position on C-11 in **1**. The relative configuration of hydroxyl on C-11 was determined on the basis of the ROESY spectrum. Correlations between H-11β and H-9β, and between H-1β and H-9β in **1** confirmed that the hydroxyl groups on C-1 and C-11 are both

α-orientated, respectively. Therefore, **1** is established to be *ent*-1β,6α,7α,11β-tetrahydroxy-17-methoxy-7β,20-epoxy-kauran-15-one.

Compound **2** was isolated as a white powder that gave an $[M+Na]^+$ ion peak at *m*/*z* 445.1840 in the HRESIMS, consistent with a molecular formula of $C_{22}H_{30}O_8$ (calcd. 445.1838), requiring 8 degrees of unsaturation. The IR spectrum of **2** showed characteristic absorption bands at 3392, 1708, and 1641 cm^{-1} corresponding to hydroxyl and α,β-unsaturated ketone groups, respectively. The 1D NMR spectra of **2** (Table 1) showed a tertiary methyl, seven methylenes (including an exomethylene), six methines (three of which were oxygenated), six quaternary carbons (including one carbonyl carbon), and an acetoxyl. In addition, the partial structure of an exomethylene conjugated with a carbonyl group on a five-membered ring was established from analysis of its NMR spectra [δ_C at 210.3 (s), 151.1 (s), 117.5 (t); δ_H at 6.12 (br s), 5.38 (br s)]. Analysis of these data, along with the structure characteristics of diterpenoids isolated from the genus *Isodon* previously, indicated that **2** is an *ent*-kaur-16-en-15-one diterpene. In comparison with longikaurin B (**24**) (Fujita et al., 1980a), the ^{13}C NMR signal of C-12 in **2** was shifted downfield [from δ_C 30.6 to 75.1]. HMBC correlations from H-12 to C-9, C-14, and C-16, in conjunction with the molecular formula, indicated the presence of a hydroxyl group at C-12. A ROESY correlation between H-11β and H-12β showed that the hydroxyl at C-12 is α-orientated. Thus **2** is elucidated as *ent*-6α,7α,12β,14α-tetrahydroxy-19-acetoxy-7β,20-epoxy-aur-16-en-15-one.

Compound **3** was isolated as colorless needles, and yielded a pseudo molecular ion peak in the positive HRESIMS spectrum at *m*/*z* 415.2090 $[M+Na]^+$, indicative of the molecular formula $C_{22}H_{32}O_6$ and 7 degrees of unsaturation. In its NMR spectra (^1H and ^{13}C NMR), a double bond (δ_C 109.8, 157.0), a hemiketal quaternary carbon (δ_C 95.7, C-7), an oxygenated methylene (δ_C 65.9, C-20), two methyls (δ_H 0.86, 1.09; δ_C 32.3, 22.2), four oxygenated methines, and an acetoxyl group (δ_C 21.4, 169.2) were observed. These signals were consistent with the presence of a tetrasubstituted 7, 20-epoxy-*ent*-kaurane diterpenoids related to maoecrystall G (**7**). HMBC correlations indicated the presence of a hydroxyl group at C-12 (from H-12 to C-9 and C-16), and C-15 (from H-15 to C-14 and C-16), while ROESY correlations established the β configurations of the C-12 and C-15 hydroxyl groups. Thus *ent*-7α,12α,15α-trihydroxy-6α-acetoxy-7β,20-epoxy-kaur-16-ene is assigned to **3**.

Compound **4**, white amorphous powder, whose molecular formula was determined as $C_{24}H_{32}O_7$ by the HRESIMS (*m*/*z* 455.2051 $[M+Na]^+$, calcd. 455.2045). According to ^1H–^1H COSY, HSQC, and HMBC experiments, overlapping proton signals at 3.13 ppm and carbon resonances at 50.0 and 52.8 ppm were characteristic of an 11, 12-epoxide. Comparison of the spectroscopic NMR data with those of oreskaurin B (**16**) suggested that the only difference between compounds **4** and **16** was that the hydroxyl at C-15 in **16** was esterified in **4**, which was confirmed by the HMBC correlation from H-15 (δ_H 5.81, 1H, s) to OAc (δ_C 170.2). The relative configuration of all substituents of **4** is assigned on the basis of ROESY correlations. Thus compound **4** is deduced as *ent*-7α-hydroxy-6α,15α-diacetoxy-7β,20-epoxy-11α,12α-epoxy-kaur-16-ene.

Compound **5**, isolated as colorless needles, has a molecular formula $C_{22}H_{28}O_8$, from its HREISMS, implying 9 degrees of unsaturation. Examination of the ^1H, ^{13}C, and DEPT NMR spectra showed the presence of a partial structure of a carbonyl group conjugated with an exomethylene, one lactonic carbonyl group, two singlet methyls, five methylenes (one of which was oxygenated), six methines (including three oxygenated ones), and three quaternary carbons (Table 1). These data indicated a 6,7-seco-1,7-olide-*ent*-kauranoid skeleton. The ^1H and ^{13}C NMR spectra of **5** were very similar to those of nodosin (**10**), and differed only in the appearance of an additional acetate signal (δ_H 2.13, and δ_C 21.7, 169.1) in **5**. From COSY spin system (H-9/H-11/H_2-12/H-13/H_2-14), and HMBC correlations [from H-11 (δ_H 5.59, 1H, dd, J = 4.6, 4.6 Hz) to OAc (δ_C 169.1)], the acetate group was placed on C-11 (δ_C 69.7). The ROESY correlations of H-1 to H-3β, H-14β, and H-11 to H-9α indicated the stereochemistry of H-1β and H-11α. Thus **5** is determined to be *ent*-11α-acetoxy-6,7-seco-kaur-16-en-15-one-1α,7-olide.

Compounds **1**–**22** were all screened for their cytotoxicity against NB4 (acute promyelocytic leukemia), A549 (lung cancer), SHSY5Y (neuroblastoma), PC-3 (prostate cancer), MCF-7 (breast cancer) cell lines (Table 2), using taxol as positive control. Compounds **1**, **2**, **5**, **6**, **10**, **12**–**14**, **16**–**18**, and **20**–**22** exhibited significant cytotoxicity against one or more cell lines. The new compounds **2**

Table 1
^{13}C NMR spectroscopic data for compounds **1**–**5**.

	1[a,c]	**2**[a,c]	**3**[b,c]	**4**[b,d]	**5**[a,c]
1	73.1 *d*	31.1 *t*	41.1 *t*	29.7 *t*	77.9 *d*
2	29.1 *t*	18.6 *t*	18.8 *t*	18.3 *t*	23.0 *t*
3	39.2 *t*	36.2 *t*	30.9 *t*	40.8 *t*	37.3 *t*
4	33.4 *s*	37.7 *s*	33.8 *s*	33.8 *s*	30.9 *s*
5	62.4 *d*	61.5 *d*	55.6 *d*	57.6 *d*	54.4 *d*
6	75.0 *d*	74.2 *d*	75.5 *d*	75.0 *d*	101.6 *d*
7	96.0 *s*	96.5 *s*	95.7 *s*	95.9 *s*	170.7 *s*
8	60.9 *s*	59.8 *s*	52.3 *s*	50.0 *s*	54.9 *s*
9	55.3 *d*	47.0 *d*	38.3 *s*	42.7 *d*	46.9 *d*
10	43.0 *s*	37.5 *s*	36.3 *s*	37.4 *s*	48.7 *s*
11	66.7 *d*	26.8 *t*	27.4 *t*	50.0 *d*	69.7 *d*
12	29.4 *t*	75.1 *d*	75.0 *d*	52.8 *d*	37.6 *t*
13	29.3 *d*	51.4 *d*	48.4 *d*	37.7 *d*	34.2 *d*
14	29.8 *t*	68.9 *d*	75.3 *t*	26.1 *t*	33.9 *t*
15	225.0 *s*	210.3 *s*	76.0 *d*	73.4 *d*	199.4 *s*
16	58.4 *d*	151.1 *s*	157.0 *s*	150.1 *s*	148.4 *s*
17	69.3 *t*	117.5 *t*	109.8 *t*	110.5 *t*	119.5 *t*
18	34.1 *q*	28.4 *q*	22.2 *q*	29.7 *q*	33.2 *q*
19	22.1 *q*	70.7 *t*	32.3 *q*	21.4 *q*	23.2 *q*
20	66.0 *t*	67.1 *t*	65.9 *t*	69.7 *t*	74.0 *t*
OAc	–	170.2/20.7	169.2/21.4	170.2/21.1	169.1/21.7
	–	–	–	173.9/21.9	–
OMe	58.7	–	–	–	–

Chemical shifts (δ) are expressed in ppm with reference to the center peak of the most downfield signal of C_5D_5N (δ 149.9 ppm) and $CDCl_3$ (77.0 ppm).
[a] Data were recorded in C_5D_5N.
[b] Data were recorded in $CDCl_3$.
[c] At 500 MHz.
[d] At 400 MHz.

Table 2
Cytotoxicity data for isolates from *I. sinuolata* in selected human cell lines.[a,b]

Compounds	NB4	A549	SHSY5Y	PC-3	MCF-7
1	1.7	>10	2.7	>10	4.3
2	3.6	>10	8.1	>10	>10
5	>10	>10	6.5	>10	>10
6	6.5	>10	>10	>10	>10
10	3.5	>10	>10	>10	>10
12	7.7	>10	>10	>10	>10
13	9.1	>10	>10	>10	>10
14	2.7	7.7	8.8	2.9	6.3
16	8.8	>10	>10	>10	>10
17	1.1	>10	>10	8.2	>10
18	>10	>10	>10	>10	>10
20	7.4	>10	>10	>10	>10
21	5.4	5.3	6.6	>10	>10
22	4	>10	7.7	>10	>10
Taxol	0.03	0.02	0.2	0.2	0.1

[a] Results are expressed as IC_{50} values in μM, and data were obtained from triplicate experiments.
[b] Compounds **3**, **4**, **7**–**9**, **11**, **15**, and **19** were not listed in the table which showed noncytotoxicity towards above cell lines.

and **5** showed cytotoxicity against the SHSY5Y cell lines with IC_{50} values of 8.1 μM and 2.7 μM, respectively, and compound **2** also exhibited cytotoxicity against the NB4 cell lines with IC_{50} values of 3.6 μM. The above results suggest that the carbonyl conjugated with an exomethylene group is the active center.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Mass spectra were performed on a VG Autospec-3000 spectrometer under 70 eV. Column chromatography (CC) was performed with silica gel (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, PR China). Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm × 25 cm column. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC–ODS (K) column. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

3.2. Plant material

The leaves and stems of *I. sinuolata* were collected in Shangrila County, Yunnan Province, PR China, in August 2006. Voucher specimens (KIB20060814) have been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, and were identified by Prof. Xi-Wen Li.

3.3. Extraction and isolation

The air-dried and powdered leaves of *I. sinuolata* (5.5 kg) were extracted with Me_2CO (3 × 10 L, each 2 days) at room temperature and filtered. The filtrate was evaporated to give a residue, which was suspended in H_2O (4 L) and then extracted with petroleum ether (b.p. 60–90 °C, 3 × 2 L), EtOAc (4 × 2.5 L), and *n*-BuOH (2 × 2 L), successively. The EtOAc extract (302 g) was decolorized on MCI gel, eluted with MeOH–H_2O (9:1) to yield a yellowish gum (248 g). The gum was subjected to silica gel CC (100–200 mesh, 1.5 kg), eluted with $CHCl_3$–Me_2CO (1:0–0:1 gradient system) to obtain fractions A–E. After repeated silica gel CC (petroleum ether–Me_2CO, 20:1–5:1 gradient system), Fraction A (27 g) afforded compounds **4** (3 mg), **7** (21 mg), **16** (200 mg), and **17** (250 mg). Fraction B (60 g) was also applied to a silica gel eluted with petroleum ether–Me_2CO (15:1–3:1) to provide sub-fractions B1–B3. Sub-fraction B1 (28 g) was further separated by RP-18 (MeOH–H_2O, 60–90% gradient system) and normal silica gel (petroleum ether–Me_2CO, 8:1) to yield compounds **8** (6 mg), **12** (5 mg), **13** (310 mg), and **22** (23 mg). Sub-fraction B2 (12 g) was purified over silica gel ($CHCl_3$–MeOH, 20:1), and then by preparative HPLC with MeOH–H_2O (55:45) to afford compounds **3** (5 mg), **18** (12 mg), and **20** (7 mg), respectively. Sub-fraction B3 (5.3 g) provided **2** (6 mg) after being purified over silica gel developing with $CHCl_3$–2-PrOH (30:1), followed by semi-preparative HPLC (MeOH–H_2O, 45–55% gradient system). Compound **10** (200 mg) was crystallized from fraction C (32 g), and the rest was further applied to a silica gel column, eluted with a gradient system (petroleum ether–Me_2CO, 9:1–2:1) to yield sub-fractions C1–C3. Compounds **11** (13 mg) and **21** (8 mg) were obtained from sub-fraction C1 (7.5 g) by repeated silica gel CC (petroleum ether–Me_2CO, 9:1–4:1 gradient system), and then by preparative HPLC (CH_3CN:H_2O, 30:70). Sub-fraction C2 (9.0 g) was purified over RP-18 (MeOH–H_2O, 30–50% gradient system), and semi-preparative HPLC (MeOH–H_2O 45:55) to afford compounds **1** (12 mg) and **15** (7 mg). Sub-fraction C3 (8.0 g) was separated by silica gel CC eluted with $CHCl_3$–MeOH (15:1) to yield compounds **6** (4 mg) and **9** (8 mg). Fraction D (43.0 g) was subjected to silica gel CC with a gradient elution (petroleum ether–Me_2CO, 4:1–1:1) to obtain sub-fractions D1 (14 g) and D2 (20 g). Compound **5** (11 mg) was crystallized from sub-fraction D1. Compounds **14** (30 mg) and **19** (13 mg) were isolated from sub-fraction D2 by silica gel CC (petroleum ether–Me_2CO, 2:1) and then followed by preparative HPLC (MeOH–H_2O, 40–60%).

3.3.1. *ent*-1β,6α,7α,11β-Tetrahydroxy-17-methoxy-7β,20-epoxy-kauran-15-one (**1**)

Colorless needles (MeOH); $[\alpha]_D^{27.6}$ – 68.8 (c 0.08, pyridine); UV (MeOH) λ_{max} (log ε): 208 (2.85) nm; IR (KBr) ν_{max} 3308, 2932, 1716, 1455, 1392, 1194, 1163, 1088, 1063 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz): δ 6.36 (1H, d, J = 15.0 Hz, OH-6β), 5.17 (1H, d, J = 9.50 Hz, H-20a), 4.36 (1H, d, J = 9.50 Hz, H-20b), 4.55 (1H, br s, H-11α), 4.22 (1H, dd, J = 5.2, 5.2 Hz, H-6α), 3.89 (1H, m, H-1β), 3.76 (1H, d, J = 10.0, 4.0 Hz, H-17a), 3.65 (1H, d, J = 10.0, 4.0 Hz, H-17b), 2.87 (1H, br s, H-13α), 2.32 (1H, m, H-14α), 1.85 (1H, overlapped, H-14β and H-12β), 2.30 (1H, dd, J = 12.8, 5.0 Hz, H-12α), 1.83 (1H, overlapped, H-12β and H_2-14β), 1.63 (1H, m, H-2α), 1.56 (1H, m, H-2β), 1.39 (1H, m, H-3α), 1.33 (1H, m, H-3β), 1.48 (1H, d, J = 4.8 Hz, H-5β), 1.58 (1H, d, J = 3.5 Hz, H-9β), 1.23 (3H, s, Me-18), 1.08 (3H, s, Me-19), 3.23 (3H, s, –OMe); for ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic assignments, see Table 1; positive ESIMS: m/z 419 $[M+Na]^+$; positive HRESIMS $[M+Na]^+$ m/z 419.2045 (calcd. for $C_{21}H_{32}O_7Na$ $[M+Na]^+$, 419.2045).

3.3.2. *ent*-6α,7α,12β,14α-Tetrahydroxy-19-acetoxy-7β,20-epoxy-kaur-16-en-15-one (**2**)

A white powder; $[\alpha]_D^{27.0}$ – 60.8 (c 0.07, pyridine); UV (MeOH) λ_{max} (log ε): 238 (3.29) nm; IR (KBr) ν_{max} 3392, 2930, 1707, 1641, 1461, 1396, 1378, 1247, 1058 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) 6.12 (1H, s, H-17a), 5.38 (1H, br s, H-17b), 5.27 (1H, d, J = 8.7 Hz, H-19a), 4.25 (1H, d, J = 8.7 Hz, H-19b), 4.53 (1H, overlapped, H-14α and H-6α), 4.51 (1H, overlapped, H-6α, H-14α, and H_2-20), 4.47 (2H, overlapped, H_2-20 and H-6α), 3.91 (1H, d, J = 4.5 Hz, H-12β), 3.71 (1H, d, J = 11.8 Hz, H-11α), 2.55 (1H, m, H-11β), 3.34 (1H, d, J = 3.9 Hz, H-9β), 1.67 (1H, d, J = 7.7 Hz, H-5β), 1.36 (2H, overlapped, H_2-2 and H-1α), 1.46 (3H, s, H_3-18), 2.23 (1H, m, H-1β), 1.38 (1H, overlapped, H-1α and H_2-2), 1.93 (1H, dd, J = 2.6, 11.2 Hz, H-3β), 1.09 (1H, dd, J = 2.6, 11.2 Hz, H-3α), 1.49 (1H, overlapped, H-13α and Me-18); for ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic assignments, see Table 1; positive ESIMS: m/z 445 $[M+Na]^+$; positive HRESIMS $[M+Na]^+$ m/z 445.1840 (calcd. for $C_{22}H_{30}O_8Na$ $[M+Na]^+$, 443.1840).

3.3.3. *ent*-7α,12α,15α-Trihydroxy-6α-acetoxy-7β,20-epoxy-kaur-16-ene (**3**)

Colorless needles (MeOH); $[\alpha]_D^{27.2}$ – 20.84 (c 0.15, pyridine); UV (MeOH) λ_{max} (log ε): 205 (3.44) nm; IR (KBr) ν_{max} 3529, 3281, 2927, 1750, 1370, 1230, 1209, 1054, 1025 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 5.62 (1H, s, H-17a), 5.32 (1H, s, H-17b), 5.73 (1H, d, J = 6.3 Hz, H-6α), 5.04 (1H, d, J = 2.3 Hz, H-12α), 4.26 (1H, d, J = 7.7 Hz, H-15α), 4.11 (2H, br s, H-20), 3.02 (1H, d, J = 4.4 Hz, H-13α), 2.02 (1H, m, H-11α), 1.81 (1H, m, H-11β), 2.27 (1H, overlapped, H-14β and H-9β), 2.42 (1H, d, J = 12.3 Hz, H-14α), 2.24 (1H, overlapped, H-9β and H-14β), 1.62 (1H, d, J = 6.2 Hz, H-5β), 1.35 (1H, overlapped, H-3β and H-1α), 0.97 (1H, m, H-3α), 1.28

(1H, overlapped, H-1α, H-3α, and H_2-2), 1.09 (1H, overlapped, H-1β and H_3-18), 1.09 (3H, s, Me-18), 0.86 (3H, s, Me-19); for ^{13}C NMR (C_5D_5N, 100 MHz) spectroscopic assignments, see Table 1; positive ESIMS: *m/z* 415 $[M+Na]^+$; positive HRESIMS $[M+Na]^+$ *m/z* 415.2090 (calcd. for $C_{22}H_{32}O_6Na$ $[M+Na]^+$, 415.2096).

3.3.4. ent-7α-Hydroxy-6α,15α-diacetoxy-7β,20-epoxy-11α,12α-epoxy-kaur-16-ene (**4**)

White amorphous powder; $[\alpha]_D^{27.7}$ – 170.5 (c 0.09, pyridine); UV (MeOH) λ_{max} (log ε): 204 (4.03) nm; IR (KBr) ν_{max} 3436, 2930, 1737, 1660, 1431, 1372, 1250, 1052, 1014 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 5.13 (1H, d, *J* = 1.7 Hz, H-17a), 5.01 (1H, d, *J* = 1.7 Hz, H-17b), 5.81 (1H, s, H-15α), 4.12 (1H, dd, *J* = 9.6, 1.7 Hz, H-20a), 4.02 (1H, dd, *J* = 9.6, 1.7 Hz, H-20b), 5.04 (1H, d, *J* = 5.3 Hz, H-6α), 1.35 (1H, d, *J* = 5.2 Hz, H-5β), 3.13 (1H, overlapped, H-11β and H-12β), 3.13 (1H, overlapped, H-12β and H-11β), 2.93 (1H, br s, H-13α), 2.28 (1H, br s, H-9β), 2.28 (1H, m, H-14α), 1.76 (1H, dd, *J* = 4.8, 12.6 Hz, H-14β), 1.57 (1H, m, H-3α), 1.35 (1H, m, H-3β), 1.86 (1H, m, H-1β), 1.36 (1H, m, H-1α), 1.11 (3H, s, Me-19), 0.81 (3H, s, Me-18); for ^{13}C NMR (C_5D_5N, 100 MHz) spectroscopic assignments, see Table 1; positive ESIMS: *m/z* 455 $[M+Na]^+$; positive HRESIMS $[M+Na]^+$ *m/z* 455.2051 (calcd. for $C_{24}H_{32}O_7Na$ $[M+Na]^+$, 455.2051).

3.3.5. ent-11α-Acetoxy-6,7-seco-kaur-16-en-15-one-1α,7-olide (**5**)

Colorless needles (MeOH); $[\alpha]_D^{27.5}$ – 162.2 (c 0.22, pyridine); UV (MeOH) λ_{max} (log ε): 230 (3.57) nm; IR (KBr) ν_{max} 3420, 2946, 2894, 1743, 1701, 1637, 1455, 1376, 1234, 1106, 1010 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 6.09 (1H, br s, H-17a), 5.54 (1H, br s, H-17b), 5.34 (1H, s, H-6α), 4.93 (1H, dd, *J* = 5.8, 11.8 Hz, H-1β), 4.01 (1H, dd, *J* = 9.6, 1.7 Hz, H-20a), 3.97 (1H, dd, *J* = 9.6, 1.7 Hz, H-20b), 5.59 (1H, dd, *J* = 4.6, 4.6 Hz, H-11α), 3.17 (1H, dd, *J* = 4.4, 11.8 Hz, H-14β), 2.86 (1H, d, *J* = 11.8 Hz, H-14α), 2.14 (1H, overlapped, H-13β and –OAc), 1.74 (1H, overlapped, H-5β and H-2β), 2.63 (1H, d, *J* = 4.2 Hz, H-9α), 1.11 (1H, m, H-12α), 1.53 (1H, m, H-12β), 2.29 (1H, dd, *J* = 7.0, 9.2 Hz, H-3α), 1.87 (1H, overlapped, H-2α and H-3β), 1.75 (1H, overlapped, H-2β and H-5β), 1.90 (1H, overlapped, H-3β and H-2α), 0.95 (3H, s, Me-18); for ^{13}C NMR (C_5D_5N, 100 MHz) spectroscopic assignments, see Table 1; positive ESIMS: *m/z* 443 $[M+Na]^+$; positive HRESIMS $[M+Na]^+$ *m/z* 443.1701 (calcd. for $C_{22}H_{28}O_8Na$ $[M+Na]^+$, 443.1681).

3.4. Cellular proliferation assay

Colorimetric assays were performed to evaluate compound activity. The NB4 acute promyelocytic leukemia cell line, the A549 lung cancer cell line, the PC-3 prostate cancer cell line, the MCF-7 breast cancer cell line, and the SHSY5Y neuroblastoma cell line were treated with various concentrations of compounds (0, 0.01, 0.1, 1, 10, 50 μM) in 96-well culture plates for 48 h in 200 μL media and pulsed with 10 μL of 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-8; Cell Counting Kit-8; Dojindo, Kumamoto, Japan) to each well for 4 h. WST-8 is converted to WST-8-formazan upon bioreduction in the presence of an electron carrier 1-methoxy-5-methyl-phenazinium methyl sulfate that is abundant in viable cells. Absorbance readings at a wavelength of 450 nm were taken on a spectrophotometer (Multiscan MK3, Thermo Lab systems). The concentration resulting in 50% of cell-growth inhibition (IC_{50}) was calculated using the Probit program in SPSS 7.5 for windows 98 (SPSS Inc., Chicago). Taxol was used as positive control.

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