Sabiperones A—F, New Diterpenoids from *Juniperus sabina*

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Six new diterpenoids, sabiperones A—F (1—6) have been isolated from the aerial part of *Juniperus sabina*. Their structures were elucidated by spectroscopic methods including 2D NMR techniques. Sabiperone F showed moderate cell growth inhibitory activities against five human cancer cell lines.

Key words diterpenoid; *Juniperus sabina*; sabiperone; cell growth inhibitory activity

Juniperus sabina (Cupressaceae) has been used in Kazakh traditional folk medicine for the treatment of various diseases.[1)] We have conducted phytochemical investigation of *J. sabina* growing in the Altay Mountain, and isolated two new lignans, sabinaperins A and B.[2)] In our continuous investigation, six new diterpenoids, sabiperones A—F (**1**—**6**) have been isolated from *J. sabina* together with three known diterpenoids, juniperolide (**7**),[3)] 3β,7*α*-dihydroxy-abieta-8,11,13-triene (**8**),[4)] and labd-*E*-13-ene-8,15-diol (**9**).[5)] In this paper we describe the isolation and structure elucidation of sabiperones A—F (**1**—**6**) as well as their *in vitro* cell growth inhibitory activities evaluation of all isolated diterpenoids against five human cell lines.

Results and Discussion

The *n*-hexane soluble layer prepared from the aerial parts of *J. sabina* was subjected to a silica gel column chromatography. The cytotoxic fractions rechromatographed by columns over silica gel and octadecyl silica (ODS), were further separated by an ODS HPLC column to afford sabiperones A (**1**)—F (**6**) together with known related diterpenoids, juniperolide (**7**), 7*α*-dihydroxy-abieta-8,11,13-triene (**8**), and labd-*E*-13-ene-8,15-diol (**9**).

Sabiperone A (**1**), colorless solid, showed molecular formula, $C_{20}H_{28}O_3$, which was established by high resolution electron spray ionization time of flight mass spectrum (HR-ESI-TOF-MS) [*m*/*z* 339.1939 $(M+Na)^+$]. The IR absorption bands indicated the presence of a hydroxyl (3510 cm^{-1}) and a *γ*-lactone (1730 cm^{-1}) groups. The ^{1}H-NMR spectrum exhibited the characteristic signals of a poly-oxygenated abietane-type diterpenoid[3)] such as two singlet methyls at δ_H 1.32 and 0.85, an isopropyl group at δ_H 1.14 (d), 1.11 (d), and 2.47 (sept.), a *γ*-proton of a *γ*-lactone at δ_H 4.99, and two olefinic protons at δ_H 5.82 and 5.97. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra revealed the presence of a lactone moiety at δ_C 182.3 and 74.0, four sp^2 carbons at δ_C 118.9, 141.4, 149.4, and 124.7, and an oxymethine at δ_C 65.7.

Four partial structures **a** (from C-1 to C-2 and C-3), **b** (from C-5 to C-7), **c** (from C-9 to C-11 and C-12), and **d** (C-15 to C-17) were deduced from ^{1}H–^{1}H correlation spectroscopy (COSY) analysis of **1** in $CDCl_3$ (Fig. 1). The heteronuclear

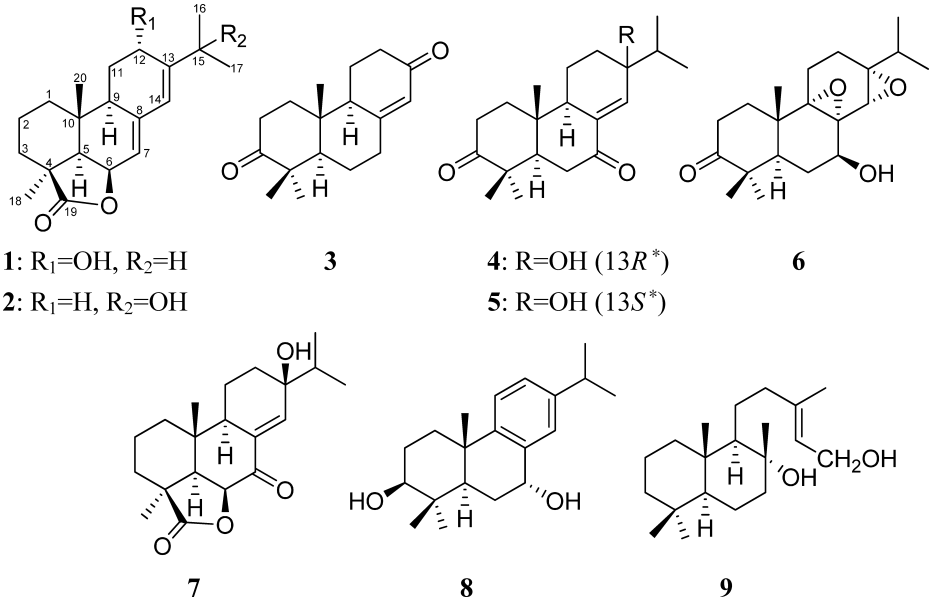

Chart 1

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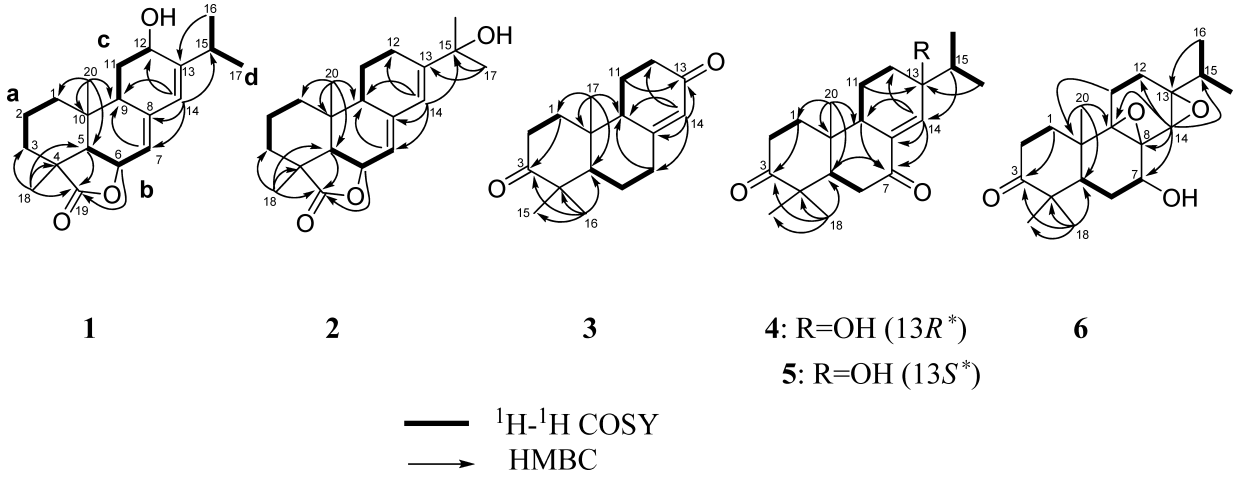

Fig. 1. Selected 2D-NMR Correlations for Sabiperones A—F (**1**—**6**)

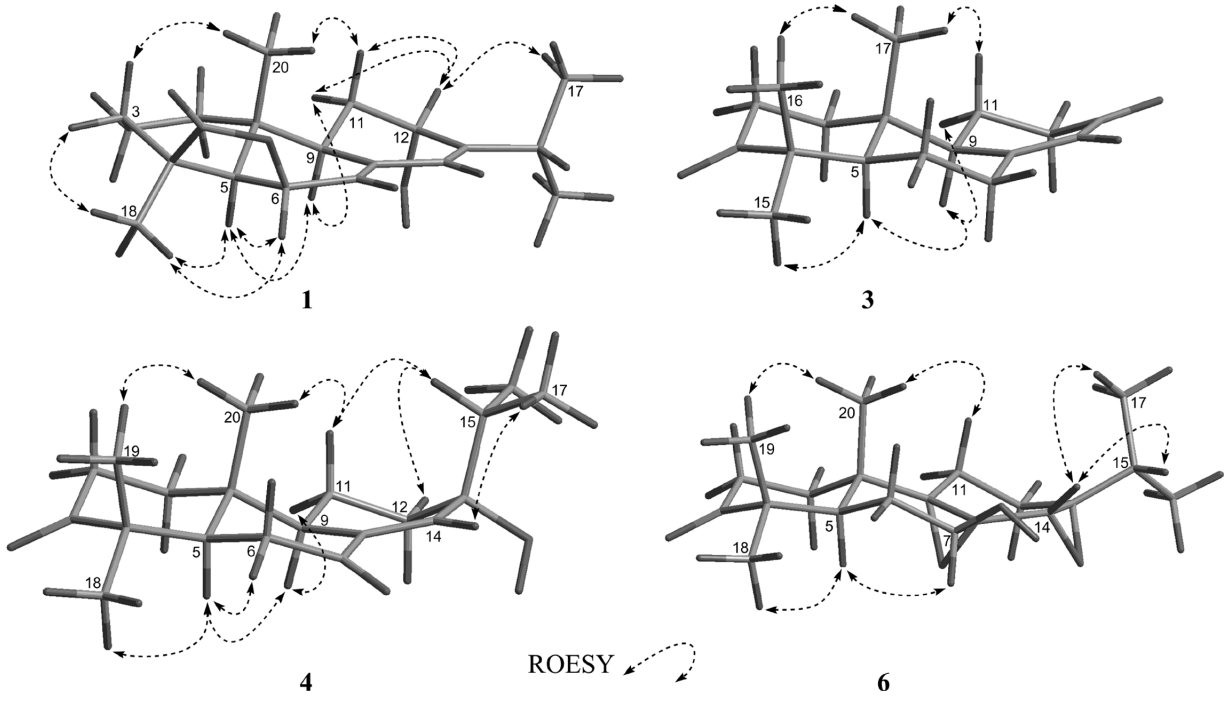

Fig. 2. Selected ROESY Correlations for Sabiperones A, C, D, and F (**1**, **3**, **4**, and **6**)

multiple bond connectivity (HMBC) correlations of H_3-20 to C-1, C-5, C-9, and C-10, H_3-18 to C-3, C-4, C-5, and C-19, H-5 to C-19, and H-7 to C-9 gave rise to the connectivity of partial structures **a**, **b**, and **c** through C-4, C-8, and C-10. Connection between partial structures **c** and **d** could be assigned by HMBC correlations of H-14 to C-7, C-8, C-9, C-12 (δ_C 65.7), and C-15, and H_3-16 to C-13, which also indicated the presence of a secondary alcohol at C-12.

The relative configuration of **1** was assigned by rotating frame Overhauser enhancement spectroscopy (ROESY) correlations as shown in computer-generated 3D drawing (Fig. 2). The ROESY correlations of H_3-18/H-3a, H-5, and H-6, H-5/H-9, and H_3-20/H-3b and H-11b indicated that C-18, H-5, H-6, and H-9 were α-oriented and C-20 was β-oriented. The correlations of H-12/H_2-11 and H_3-17 suggested the presence of an α-oriented hydroxyl group at C-12. Therefore, **1** was assigned to be an abietane derivative as shown in Chart 1.

The HR-ESI-TOF-MS of sabiperone B (**2**) gave *m/z* 339.1939 $(M+Na)^+$ in accordance with the molecular formula $C_{20}H_{28}O_3$. The IR absorptions at 3510 and 1730 cm^{-1} indicated the presence of a hydroxyl and a γ-lactone ring as the same in **1**. The ^{13}C-NMR signals (Table 2) of **2** were very similar to those of **1** except for the downfield shift on C-15 (δ_C 33.2 in **1** to 72.9 in **2**) and upfield shift on C-12 (δ_C 65.7 in **1** to 25.4 in **2**). The major differences in their ^{1}H-NMR spectra (Table 1) are the resonances of the two methyls of H-16 (δ_H 1.14, d in **1** to 1.35, s in **2**) and H-17 (δ_H 1.11, d in **1** to 1.36, s in **2**). These differences suggested the presence of a hydroxyl group on C-15 in

Table 1. ^{1}H-NMR Data for Sabiperones A—F (**1**—**6**) in $CDCl_3$ (δ_H, J in Hz)

	1	2	3	4	5	6
1a	1.31 m	1.27 m	1.59 (ddd, 14.0, 4.4, 4.4)	1.57 m	1.58 m	1.88 m
1b	1.59 m	1.64 (ddd, 13.1, 6.5, 6.5)	2.07 m	2.06 (ddd, 13.4, 5.3, 3.5)	2.16 (brd, 14.4)	1.94 m
2a	1.57 m	1.56 m	2.35 (dt, 15.0, 4.0)	2.35 (ddd, 14.4, 3.8, 3.5)	2.35 (brd, 14.8)	2.53 (2H, m)
2b	1.73 m	1.73 m	2.68 (td, 15.0, 5.7)	2.76 (ddd, 14.4, 14.2, 5.3)	2.77 (ddd, 14.8, 4.4, 5.2)	
3a	1.48 m	1.48 (ddd, 14.1, 6.8, 6.8)				
3b	2.18 m	2.18 (ddd, 14.1, 8.5, 5.2)				
4						
5	1.83 (d, 5.0)	1.76 (d, 4.9)	1.63 (dd, 12.6, 2.8)	1.93 (dd, 13.2, 5.5)	1.93 (dd, 11.2, 5.2)	1.95 (dd, 10.2, 2.7)
6a	4.99 (td, 5.0, 1.9)	4.97 (t, 4.9)	1.68 (ddd, 12.6, 4.5, 4.5)	2.50 (dd, 18.4, 13.2)	2.51 m	1.59 m
6b			1.73 m	2.55 (dd, 18.4, 5.5)	2.54 m	1.62 m
7a	5.82 (brs)	5.76 (brs)	2.34 m			4.12 (d, 5.2)
7b			2.63 (brdd, 15.7, 3.0)			
9	2.36 (brd, 13.3)	1.90 (brd, 13.3)	2.14 (dd, 9.6, 7.7)	2.14 m	2.01 m	
11a	1.90 (dt, 13.3, 3.3)	1.84 (dq, 13.3, 4.2)	2.07 m	1.78 m	1.73 m	1.86 m
11b	1.45 (td, 13.3, 3.3)	1.33 (qd, 13.3, 4.2)	1.81 (dddd, 13.9, 13.8, 9.6, 4.5)	1.46 m	1.56 m	1.63 m
12a	4.34 (brs)	2.10 (brt, 17.1)	2.25 (ddd, 16.0, 13.9, 5.5)	1.42 m	1.50 m	1.61 m
12b		2.36 (dt, 17.1, 3.2)	2.43 (ddd, 16.0, 4.4, 3.8)	2.18 m	1.76 m	1.88 m
13						
14	5.97 s	6.20 (d, 2.2)	5.94 brs	6.81 s	6.80 s	3.15 s
15	2.47 (sept., 6.9)		1.14 s	1.80 m	1.80 m	1.68 (sept., 7.0)
16	1.14 (d, 6.9)	1.35 s	1.10 s	0.96 (d, 6.8)	0.86 (d, 6.8)	0.95 (d, 7.0)
17	1.11 (d, 6.9)	1.36 s	1.04 s	1.00 (d, 6.8)	0.97 (d, 6.8)	0.99 (d, 7.0)
18	1.32 s	1.30 s		1.07 s	1.07 s	1.08 s
19				1.12 s	1.13 s	1.01 s
20	0.85 s	0.87 s		1.07 s	1.11 s	1.07 s

2 instead of that on C-12 in **1**. The position of the hydroxyl group was further confirmed by the HMBC correlations of H-14 and H_3-17 to C-15 (Fig. 1). Based on the ROESY correlations, the relative configurations of C-4, C-5, C-6, C-9, and C-10 of **2** was concluded to be the same as **1**. Therefore, **2** was elucidated to be an isomer of **1**.

Sabiperone C (**3**) was obtained as colorless solid, possessing a molecular formula, $C_{17}H_{24}O_2$ on the basis of HR-ESI-TOF-MS [m/z 261.1867 $(M+H)^+$]. The IR absorptions at 1706 and 1670 cm^{-1} can be attributed to a ketone and an α,β-unsaturated ketone groups, respectively. The ^{1}H- and ^{13}C-NMR spectra (Tables 1, 2, respectively) revealed the presence of three methyls, six sp^3 methylenes, two sp^3 methines, one sp^2 methine, two sp^3 quaternary carbons, and one sp^2 quaternary carbon, in addition to two carbonyl carbons (δ_C 215.6, 199.5).

The ^{1}H–^{1}H COSY spectrum (Fig. 1) revealed the connectivities of C-1 to C-2, C-5 to C-6 and C-7, and C-9 to C-11 and C-12. In the HMBC spectrum (Fig. 1), the locations of two ketone groups at C-3 and C-13 were deduced from the HMBC correlations of H-1 to C-3, and H_3-16 to C-3, C-4, C-5, and C-15 as well as those of H-11 to C-13, and H-14 to C-7, C-8, C-9, C-12, and C-13, respectively.

The relative configuration of **3** was assigned on the basis of ROESY correlations (Fig. 2). The intense ROESY correlations of H_3-17/H_3-16 and H-11b, and H-5/H-9 and H_3-15 were indicative of the α-orientation of H-5 and H-9 and β-orientation of C-17.

The molecular formula of sabiperone D (**4**) was determined to be $C_{20}H_{30}O_3$ by the HR-ESI-TOF-MS [m/z 341.2327 $(M+Na)^+$]. The IR spectrum suggested the presence of a hydroxyl (3400 cm^{-1}) and two carbonyls (1707, 1685 cm^{-1}) in the structure. The ^{1}H-NMR spectrum (Table 1) showed three methyl groups at δ_H 1.07 (s), 1.12 (s), and 1.07 (s), an isopropyl group at δ_H 0.96 (d), 1.00 (d), and 1.80 (m), and a proton on a trisubstituted double bond at δ_H 6.81 (s). The ^{13}C-NMR signals at δ_C 214.7 and 199.4 showed the characteristic resonances of

Table 2. ^{13}C-NMR Data for Sabiperones A—F (**1**—**6**) in $CDCl_3$ (δ_C)

	1	2	3	4	5	6
1	32.9	33.4	37.6	37.2	37.3	32.0
2	17.9	18.0	34.5	34.4	34.4	33.8
3	28.2	28.3	215.6	214.7	215.0	215.8
4	42.5	42.6	47.8	47.6	47.5	46.4
5	51.9	51.8	54.6	51.3	50.7	39.3
6	74.0	74.3	22.53	37.6	37.7	29.0
7	118.9	117.7	35.4	199.4	199.4	65.9
8	141.4	142.4	163.6	136.0	137.6	62.4
9	40.6	47.5	50.7	49.8	50.6	68.2
10	32.4	32.9	38.6	37.9	37.9	36.7
11	30.3	22.6	20.9	21.2	18.9	21.3
12	65.7	25.4	36.7	32.8	29.5	23.2
13	149.4	150.6	199.5	72.6	72.0	62.5
14	124.7	121.6	126.6	142.0	141.0	55.9
15	33.2	72.9	25.8	36.3	37.9	33.3
16	21.9	28.7	22.45	17.1	17.4	18.1
17	21.5	28.7	15.1	16.8	16.2	17.9
18	24.4	24.5		24.6	24.5	27.0
19	182.3	182.6		21.5	21.6	21.0
20	18.2	17.9		13.7	14.0	16.4

two carbonyl moiety, and that at δ_C 72.6 was indicative of the presence of a tertiary hydroxyl group. The connections of the three partial structures of C-1 to C-2, C-5 to C-6, and C-9 to C-11 and C-12, which were observed from the ^{1}H–^{1}H COSY were deduced by analysis of the HMBC spectrum (Fig. 1). The HMBC correlations of H_3-20/C-1, C-5, C-9, and C-10, H-1/C-3, H_3-18/C-3, C-4, C-5, and C-19, H-5/C-7, H-11/C-13, H-14/C-7, C-8, C-9, and C-12, H-15/C-14, and H_3-17/C-13 suggested that **4** was an abietane derivative. In addition, comparison of the structure of **4** to that of juniperolide (**7**) revealed that both of them have the same skeleton. The differences are limited to the presence of a carbonyl group (δ_C 214.7) at C-3 and lack of a γ-butyrolactone ring.

The relative configuration of **4** was confirmed by ROESY correlations (Fig. 2) in which the correlations of H_3-20/H_3-19, H-5/H_3-18 and H-9 indicated that H-5 and H-9 were α-oriented and C-20 was β-oriented. The ROESY correlations of H_3-20/H-11b and H-15/H-11b and H-12b suggested the hydroxyl group at C-13 to be α-oriented in **4**.

Sabiperone E (**5**) has a molecular formula $C_{20}H_{30}O_3$ based on the HR-ESI-TOF-MS [*m/z* 341.2327, $(M+Na)^+$]. The IR absorptions at 3400, 1710, and 1680 cm^{-1} suggested the presence of a hydroxyl and two carbonyls. Analysis of the ^{1}H-, ^{13}C-, and 2D-NMR of **5** gave the same planar structure as **4**, suggesting that **5** should be a stereoisomer of **4**.

Further analysis of ROESY spectral data also suggested that **5** had the same configuration as in **4** for both A and B rings. Thus, relation between **4** and **5** should be different at C-13 configuration of C ring which was also found between 7-oxo-13α-hydroxyabiet-8(14)-en-18-oic acid and its steroisomer.[6—8] The hydroxyl group at C-13 was confirmed to be β-oriented, since the ROESY correlation of H_3-16/H-12a (H-12α) was observed as major difference.

Sabiperone F (**6**) was found to possess the molecular formula $C_{20}H_{30}O_4$ from the HR-ESI-TOF-MS at *m/z* 357.2031 [M+Na]. The presence of a carbonyl and a hydroxyl group were inferred by the absorptions observed in the IR spectrum of **6** appearing at 1690 and 3550 cm^{-1}. The ^{13}C-NMR (Table 2) and DEPT spectra revealed the presence of a secondary hydroxyl (δ_C 65.9) and two epoxy groups (δ_C 68.2, 62.4, 62.5, 55.9). The location of the secondary hydroxyl at C-7 was established by COSY correlations of H-6/H-5 and H-7, and an HMBC correlation (Fig. 1) of H-14 to C-7. The HMBC correlations of H-14 to C-8, C-9, C-12, and C-15, H-20 to C-9, and H_3-16 to C-13 indicated that two epoxy group should be assigned between C-8 and C-9 and between C-13 and C-14, respectively.

Confirmation of structure and α configuration of epoxides of **6** were established by close comparison of ^{13}C-NMR spectra of **6** to those of a known 7β-hydroxy-8α,9α,13α,14α-diepoxy-abietan-18-oic acid,[9] which showed a general similarity except for the presence of a carbonyl at C-3 (δ_C 215.8) and a methyl at C-18 (δ_C 27.0) of **6** instead of a methylene and a carboxyl group, respectively. In addition, the upfield shifts of C-1 (δ_C 32.0) and C-5 (δ_C 39.3) of **6** due to γ-gauche effect caused by the α-oriented epoxides at 8, 9, 13, and 14 were in agreement with similar abietane diterpens which are highly oxidized at C ring.[9—11] The relative configuration of C-5, C-7, and C-10 were assigned by ROESY correlations as shown in computer-generated 3D drawing (Fig. 2). ROESY correlations of H_3-20/H_3-19 and H-5/H-7 and H_3-18 suggested that H-5 and H-7 were α-oriented, and C-20 was β-oriented.

Sabiperone F (**6**) and 3β,7α-dihydroxy-abieta-8,11,13-triene (**8**) showed moderate cell growth inhibitory activities against HL-60, MCF7, A549, HepG2, and HCT116 cells (IC_{50} for **6**: 6.37 μM for HL-60, 25.62 μM for MCF7, and 7.59 μM for HCT116). As for A549 and HepG2 cells, treatment with compound **6** at 12.5 μM resulted in cell growth plateau at 27% and 51%, respectively. Meanwhile, treatment with 50.0 μM of compound **8**, showed inhibition of MCF7 and HCT116 cells growth to 37% and 39%, respectively, whereas others did not show substantial inhibitory activity (IC_{50} >50 μM).

Experimental

General Experimental Procedures Optical rotations were measured on a JASCO DIP-1000 automatic digital polarimeter. Circular dichroism (CD) specta were measured on a JASCO J-820 spectropolarimeter, and IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. ^{1}H- and 2D-NMR spectra were recorded on a JEOL ECA600 and Bruker AV 600 spectrometers, and chemical shifts were referenced to the residual solvent peaks (δ_H 7.26 and δ_C 77.0 for $CDCl_3$). Standard pulse sequences were employed for the 2D-NMR experiments. High-resolution ESI-MS were obtained on a LTQ Orbitrap XL (Thermo Scientific). HPLC was performed on a CAPCELL PAK C_{18} MG-II, 5 μm (φ 10×250 mm).

Plant Material The aerial part of *J. sabina* was collected in Altay mountain (Xinjiang region, PRC) in 2009. The botanical identification was made by pharmacist Bahargul Konirhan, Institute of Medicine Inspection Department of Altay City, Xinjiang, China.

Extraction and Purification The aerial parts of *J. sabina* (2 kg) were extracted with 70% EtOH, and 100 g of the extract was partitioned with *n*-hexane, $CHCl_3$, *n*-BuOH, and H_2O. The *n*-hexane layer was subjected to a silica gel column chromatography (elution, *n*-hexane/EtOAc 1 : 0 to 0 : 1) to obtain 15 main fractions. The cytotoxic fraction 11 (1.3586 g), which was eluted by *n*-hexane/EtOAc (1 : 1), was rechromatographed by a column over silica gel (toluene/EtOAc 1 : 0 to 1 : 1). The fraction (58.8 mg) eluted by toluene/EtOAc (8 : 2) was further separated by an ODS column with H_2O/MeOH (60 : 40) to obtain fraction 1a (20.9 mg) which was further separated by an ODS HPLC column (MeOH/H_2O) to give sabiperones A (**1**, 1.2 mg) and B (**2**, 2.8 mg). The fraction (77.0 mg) which was eluted by toluene/EtOAc (7 : 3) was chromatographed by an ODS column MeOH/H_2O (30 : 70—80 : 20) to give fractions 1b and 2b. Further purification of fraction 1b (23.2 mg) ODS HPLC (MeOH/H_2O) gave sabiperone C (**3**, 1.1 mg), sabiperone D (**4**, 0.5 mg), and sabiperone E (**5**, 0.3 mg), while further separation of fraction 2b (11.3 mg) gave sabiperone F (**6**, 0.70 mg), juniperolide (**7**, 1.3 mg), and 7α-dihydroxy-abieta-8,11,13-triene (**8**, 0.6 mg). Labd-*E*-13-ene-8,15-diol (**9**, 9.4 mg) was obtained by separation of cytotoxic fraction 12 (50.5 mg) using a silica gel column eluted with toluene/EtOAc (8 : 2).

Sabiperone A (**1**): Colorless solid, $[\alpha]_D^{32}$ +17 (c=0.3, MeOH); HR-ESI-TOF-MS *m/z* 339.1939 (M+Na; Calcd for $C_{20}H_{28}O_3Na$, 339.1936); IR (KBr) cm^{-1}: 3510 and 1730; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) spectroscopic data, see Tables 1 and 2; UV λ_{max} (MeOH) nm (ε): 246 (15900); CD (MeOH) $[\theta]_{245}$ −48767.

Sabiperone B (**2**): Colorless solid, $[\alpha]_D^{29}$ +14 (c=0.6, MeOH); HR-ESI-TOF-MS *m/z* 339.1939 (M+Na; Calcd for $C_{20}H_{28}O_3Na$, 339.1936); IR (KBr) cm^{-1}: 3510, 1730; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) spectroscopic data, see Tables 1 and 2; UV λ_{max} (MeOH) nm (ε): 240 (17800); CD (MeOH) $[\theta]_{243}$ −61732.

Sabiperone C (**3**): Colorless solid, $[\alpha]_D^{30}$ −7 (c=0.6, MeOH); HR-ESI-TOF-MS *m/z* 261.1867 (M+H; Calcd for $C_{17}H_{25}O_2$, 261.1855); IR (KBr) cm^{-1}: 1706 and 1670; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) spectroscopic data, see Tables 1 and 2; UV λ_{max} (MeOH) nm (ε): 245 (20610); CD (MeOH) $[\theta]_{317}$ −5923, and $[\theta]_{237}$ +36022.

Sabiperone D (**4**): Oil, $[\alpha]_D^{30}$ −16 (c=0.1, MeOH); HR-ESI-TOF-MS *m/z* 341.2327 (M+Na; Calcd for $C_{20}H_{30}O_3Na$, 341.2277); IR (KBr) cm^{-1}: 3400, 1707, 1685; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) spectroscopic data, see Tables 1 and 2; UV λ_{max} (MeOH) nm (ε): 200 (6900), 245 (6980); CD (MeOH) $[\theta]_{206}$ +16178, $[\theta]_{255}$ −7840, and $[\theta]_{326}$ −2540.

Sabiperone E (**5**): Oil, HR-ESI-TOF-MS *m/z* 341.2327 (M+Na; Calcd for $C_{20}H_{30}O_3Na$, 341.2277); IR (KBr) cm^{-1}: 3400, 1707, 1685; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) spectroscopic data, see Tables 1 and 2; UV λ_{max} (MeOH) nm (ε): 200 (10900) and 243 (9300); CD (MeOH) $[\theta]_{210}$ +12125, $[\theta]_{245}$ +8480, and $[\theta]_{333}$ −2205.

Sabiperone F (**6**): Oil, $[\alpha]_D^{32}$ +30 (c=0.4, MeOH); HR-ESI-TOF-MS *m/z* 357.2031 (M+Na; Calcd for $C_{20}H_{30}O_4Na$, 357.2042); IR (KBr) cm^{-1}: 3550, 1690; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) spectroscopic data, see Tables 1 and 2; UV λ_{max} (MeOH) nm (ε): 202 (4900), 288 (600); CD (MeOH) $[\theta]_{210}$ +1161 and $[\theta]_{289}$ −429.

Cytotoxicity HL-60, human promyelocytic leukemia cells were maintained in RPMI-1640 medium; A549, human lung adenocarcinoma; MCF7, human breast adenocarcinoma; HepG2, human hepatocellular carcinoma; and HCT116, human colorectal adenocarcinoma cells were maintained in Dulbecco's modified Eagle's medium (DMEM) medium. Both growth medium were supplemented with 10% fetal calf serum and 1% penicillin–streptomycin. The cells (5×10^3 cells/well) were cultured in Nunc disposable 96-well plates containing 90 μL of growth medium per well and were incubated at 37°C in a humidified incubator of 5% CO_2. 10 μL of serially diluted samples (50 μM, 25 μM, 12.5 μM, and 6.25 μM) were added to the cultures at 24 h of incubation. After 48 h of incubation with the samples, 15 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL) were added to each of the wells. The cultures were incubated for another 3 h before the cells supernatant are removed. After the removal of the cells supernatant, 50 μL of dimethyl sulfoxide (DMSO) was added to each well. The formed formazan crystal was dissolved by re-suspension by pipette. The optical density was measured using a microplate reader (Bio-Rad) at 550 nm with reference wavelength at 700 nm. In all experiment, three replicates were used. Cisplatin was used as positive control (IC_{50}: 0.87 μM for HL-60, 27.7 μM for MCF7, 27.8 μM for A549, 12.3 μM for HepG2, and 16.0 μM for HCT116).

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