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

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Five new *ent*-kaurane diterpenoids, isodonhenrins A—E (1—5), together with thirteen known ones were isolated from the aerial parts of *Isodon henryi*. Their structures were identified by means of extensive spectroscopic analysis, and the absolute configurations of 1 were determined by single-crystal X-ray diffraction. Most of the diterpenoids were evaluated for their cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines. Compound 17 showed significant inhibitory effects on five cell lines, and compounds 6, 9, 10, 11, 12 and 16 exhibited selective activity.

Key words *Isodon henryi*; diterpenoid; isodonhenrin

The genus *Isodon* is a prolific source of *ent*-kauranoids which were demonstrated to exhibit antibacterial, anti-inflammatory, and anti-cancer activities.[1)] For the past 30 years, over 50 *Isodon* species from mainland of China were investigated and more than 500 diterpenoids had been isolated and characterized.[2)]

Isodon henryi (Hemsl.) Hara, a perennial herb, is distributed mainly in Hubei, Henan, Shanxi and Sichuan Provinces of China.[3)] It has been used to treat acute diarrhea and enteritis in folk medicine.[4)] Previous study on this herb collected in Qinghai Province led to separation of fourteen *ent*-kauranoids,[5)] including seven new ones. The secondary metabolites from the plants of the genus *Isodon* often differ when grown in different ecological environments. Thus, with the intent of discovering structurally unique and bioactive *ent*-kauranoids, we explored the plant indigenous to Qionglai City of Sichuan Province, which had not been studied on the secondary metabolites. As a result, five new *ent*-kaurane diterpenoids isodonhenrins A—E (**1**—**5**), together with thirteen analogues (**6**—**18**) were isolated. The current paper describes the isolation, structure elucidation, and cytotoxic evaluation of selected compounds.

Results and Discussion

The aerial parts of *I. henryi* (1.3 kg) were extracted with aqueous acetone at room temperature and then the extract was partitioned between EtOAc and H_2O. Repeated chromatography of the EtOAc soluble portions (58 g) yield 5 new (**1**—**5**) and 13 known (**6**—**18**) *ent*-kanrane diterpenoids. The known ones (**6**—**18**) which were determined by comparing the spectroscopic data with literature values were identified as glaucocalactone (**6**),[6)] 15-α-hydroxy-6,7-*seco*-1α,7:11α,6-diolide-20-al-*ent*-kaur-16-ene (**7**),[7)] sculponeatin E (**8**),[8)] ponicidin (**9**),[9)] macrocalin B (**10**),[10)] rabdoternin F (**11**),[11)] rabdoternin E (**12**),[11)] rabdoternin B (**13**),[12)] rabdoternin A (**14**),[12)] oridonin (**15**),[13)] rosthorin A (**16**),[14)] rabdocoetsin B (**17**),[15)] rabdonervosin B (**18**),[16)] respectively.

Isodonhenrin A (**1**) was obtained as colorless needle crystals from MeOH. The molecular formula was determined by positive high resolution-electrospray ionization (HR-ESI)-MS as $C_{22}H_{28}O_7$, *m*/*z* 427.1732 $[M+Na]^+$, indicating 9 degrees of unsaturation. The IR spectrum of **1** showed characteristic absorptions at 3438, 1742, 1713 and 1638 cm^{-1}, which implied the existence of hydroxy group, a lactone carbonyl group, an aldehyde group and olefinic group. In its ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra, an acetyl group, two methyl, five methylenes (including an exomethylene), eight methines (four of which were oxygenated and one was an aldehyde carbon), five quaternary carbons (including one carbonyl carbon) were observed. These data implied a skeleton of 6,7-*seco*-1,7-olide-*ent*-kauranoid, similar to glaucocalactone (**6**). The above assumption was supported by heteronuclear multiple bond connectivity (HMBC) correlations. HMBC correlations from H-6 (δ_H 5.86) to C-4, C-5 and C-11 suggested the presence of hemi acetal ring from C-6 to C-11, corresponding to the lactone moiety in **6**. HMBC correlations from H-15 (δ_H 6.66) to the OAc (δ_C 170.0) permitted the presence of exomethlene and the location of an acetyl group at C-15, respectively.

The rotating frame Overhauser enhancement spectroscopy (ROESY) corrections of H-1 to H-5β; H-6 to H-5β and Me-18; H-11 to H-5β, H-15 to H-14α revealed H-1, H-6, H-15 to be β-oriented (Fig. 1). **1** was crystallized in MeOH to afford a crystal of the orthorhombic space group $P2_12_12_1$ which was analyzed by X-ray crystallography (CCDC 838441, Fig. 2). Bearing on seven oxygen atoms in the molecular, the final refinement on Cu$K\alpha$ data resulted in a Flack

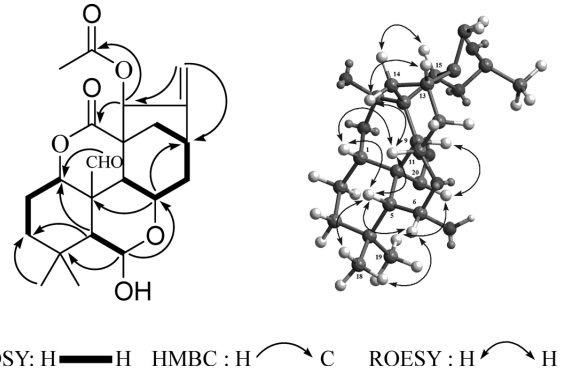

Fig. 1. ^{1}H–^{1}H COSY and Selected HMBC Correlations of **1**

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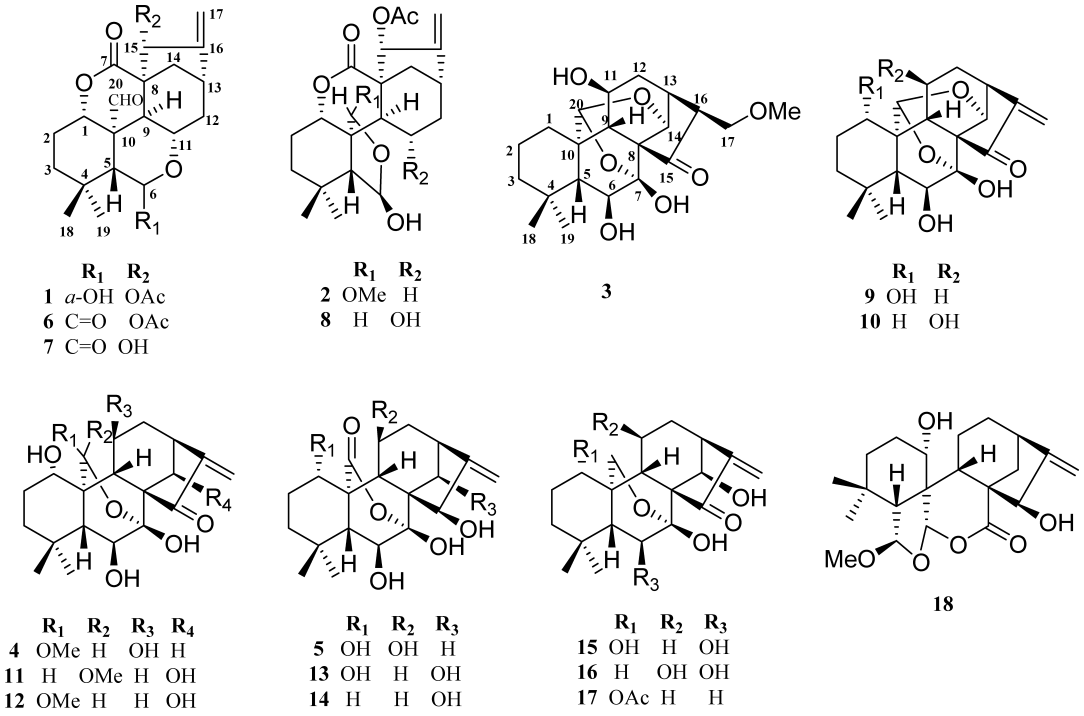

Chart 1

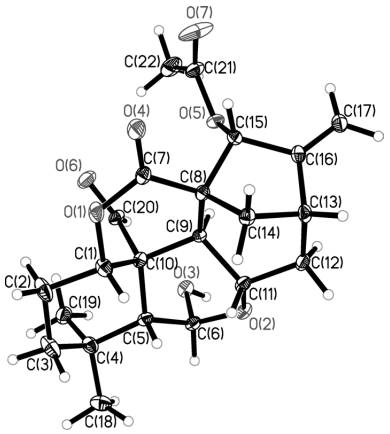

Fig. 2. X-Ray Crystal Structure of **1**

parameter of 0.2 (2),[17] allowing an unambiguous assignment of the complete absolute configuration of **1** as shown in its formula. Thus, compound **1** is elucidated as 15α-acetoxy-6α-hydroxy-20-oxo-6,11α-epoxy-6,7-*seco*-*ent*-kanr-16-en-1α,7-olide.

The molecular formula of isodonhenrin B (**2**) was $C_{23}H_{32}O_7$, with 8 degrees of unsaturation, deduced from positive HR-ESI-MS, *m*/*z* 443.2042 $[M+Na]^+$. The ^{13}C-NMR data of **2** were close to sculponeatin E (**8**),[8] except for the additional signal of a methoxy group (δ_C 57.6) and the chemical shift value of C-20. The HMBC correlations from H-20 (δ_H 5.38) to the OCH_3 and H-15 (δ_H 7.03) to OAc (δ 170.0) linked the methoxy group at C-20 and acetyl group at C-15, respectively. The ROESY correlations of H-1 to H-3β, H-15 to H-14β, H-6 to H_3-19α proved H-1, H-6, H-15 to be β, α, β-oriented, respectively. The configuration of H-20 was assigned as *S* from the ROESY correlations of H-20 to H_3-19α. Therefore, compound **2** is determined to be 20(*S*)-15α-acetoxy-6β-hydroxy-20-methoxy-6,20α-epoxy-6,7-*seco*-*ent*-kanr-16-en-1α,7-olide.

Isodonhenrin C (**3**) was isolated as amorphous powder, with molecular formula of $C_{21}H_{30}O_7$ indicated by positive HR-ESI-MS, *m*/*z* 417.1881 $[M+Na]^+$. In the IR spectrum, hydroxy and carbonyl group gave characteristic absorptions at 3438 and 1738 cm^{-1}, respectively. The ^{13}C-NMR and DEPT spectra displayed signals of three methyl (including one OCH_3), five methylenes (one oxygenated), eight methines (four oxygenated) and five quaternary carbons (two oxygenated), which were closely resembled macrocalin B (**10**).[10] The only difference was that a double bond (C-16, C-17) in **10** was changed into a methine (δ_C 55.1, C-16) and an oxygenated methylene (δ_C 70.0, C-17) in **3**. HMBC corrections from H_2-17 (δ_H 4.18, 4.51) to the carbon in OCH_3 confirmed that the OCH_3 placed at C-17. The relative configuration of **3** was the same as macrocalin B except that H-16 was α-oriented, which was supported by the ROESY correlation of H-16 and H-14α. Therefore, 16(*S*)-6β,7β,11β-trihydroxy-17-methoxy-7α,20:14α,20-diepoxy-*ent*-kaur-15-one is assigned to **3**.

Isodonhenrin D (**4**), amorphous powder, possessed a molecular formula of $C_{21}H_{30}O_7$ as derived from its HR-ESI-MS, *m*/*z* 417.1886 $[M+Na]^+$. The ^{1}H- and ^{13}C-NMR data of **4** were similar to those of rabdoternin G.[1] Three hydroxy groups were assigned at C-1, C-6, C-11 in **4** by the key correlations in HMBC from H-1 (δ_H 4.16) to C-20, C-5 and C-3; H-6 (δ_H 4.41) to C-4, C-5 and C-7; H-11 (δ_H 4.56) to C-9 and C-13. H-20 (δ_H 5.58) correlated to OCH_3 proved the location of OCH_3 at C-20. With the respective key correlations in ROESY, H-1, H-6 and H-11 were assigned to be oriented to β, α and α, respectively, same as rabdoternin G. So the only difference between **4** and raddoternin G was the configuration of C-20. The 20*R* configuration of **4** was verified by ROESY correlation of H-20 and H-11α. Hence, **4** is deter-

Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **1** and **2** (500, 125 MHz, in C_5D_5N, δ ppm, J in Hz)[a)]

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1β	4.52 (m)	77.4 d	4.71 (m)	75.3 d
2α	2.55 (m)	25.8 t	1.87 (m)	23.8 t
2β	2.00 (overlapped)			
3α	1.42 (m)	40.7 t	1.38 (overlapped)	37.6 t
3β			1.25 (m)	
4		33.5 s		31.0 s
5β	1.90 (br s)	50.0 d	2.29 (br s)	54.9 d
6α	92.3 d	5.58 (br s)	99.1 d	
6β	5.86 (br s)			
7		172.1 s		174.4 s
8		48.7 s		53.1 s
9α	3.27 (d, 11.6)	35.0 d	3.86 (dd, 6.0, 15.5)	33.2 d
10		50.6 s		50.5 s
11α		62.6 d	2.13 (overlapped)	19.8 t
11β	4.30 (dt, 8.7, 11.6)		1.36 (overlapped)	
12α	2.70 (dt, 8.7, 12.8)	39.4 t	2.10 (overlapped)	34.4 t
12β	1.70 (overlapped)		1.55 (overlapped)	
13β	2.78 (m)	37.7 d	2.58 (m)	38.1 d
14α	1.70 (overlapped)	32.3 t	2.13 (overlapped)	30.5 t
14β	2.20 (d, 12.8)		1.51 (overlapped)	
15β	6.66 (s)	80.8 d	7.03 (s)	76.5 d
16		153.7 s		156.7 s
17	5.09 (overlapped)	111.0 t	5.05 (s)	108.0 t
18	0.96 (s)	30.2 q	1.01 (s)	32.8 q
19	1.16 (s)	22.1 q	1.00 (s)	23.5 q
20	10.68 (d, 2.6)	202.8 d	5.36 (br s)	110.1 d
OAc	2.00 (s)	169.9 s	2.10 (s)	170.0 s
		20.5 q		21.3 q
OMe			3.45 (s)	57.6

a) The data of compounds **1** and **2** were assigned based on DEPT, HSQC, HMBC, COSY and ROESY experiments.

Table 2. ^{1}H- and ^{13}C-NMR Data of Compounds **3**—**5** (500, 125 MHz, in C_5D_5N, δ ppm, J in Hz)[a)]

Position	**3**		**4**		**5**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1α	1.68 (d, 13.5)	28.0 t		73.9 d		73.1 d
1β	1.05 (m)		4.16 (m)		4.10 (m)	
2α	1.44 (m)	18.9 t	2.34 (m)	29.2 t	2.21 (m)	29.6 t
2β	1.30 (overlapped)		1.81 (m)		1.95 (m)	
3α	0.97 (m)	41.3 t	1.38 (m)	41.0 t	1.25 (m)	39.3 t
3β	1.28 (overlapped)		1.50 (m)	33.2 s	1.15 (m)	
4		33.6 s				34.4 s
5β	1.51 (br s)	63.4 d	1.75 (d, 9.5)	58.6 d	1.78 (d, 5.0)	56.1 d
6α	4.20 (br s)	72.8 d	4.45 (d, 9.5)	74.3 d	4.30 (overlapped)	72.9 d
7		101.6 s		97.3 s		107.8 s
8		56.0 s		60.6 s		54.1 s
9β	3.00 (overlapped)	54.6 d	1.89 (d, 10.5)	58.9 d	2.96 (d, 9.7)	50.8 d
10		42.8 s		46.3 s		48.9 s
11α	4.31 (d, 5.2)	62.5 d	4.60 (d, 10.5)	65.0 d	4.28 (overlapped)	64.2 d
12α	2.59 (m)	28.0 t	2.83 (m)	40.2 t	2.68 (overlapped)	42.1 t
12β	2.16 (m)		1.70 (m)		1.80 (m)	
13α	3.00 (overlapped)	34.8 d	3.30 (m)	34.5 d	2.66 (overlapped)	36.6 d
14α		71.4 d	2.46 (dd, 4.3, 12.5)	26.4 t	2.15 (m)	26.4 t
14β	5.01 (br s)		2.59 (d, 12.5)		1.90 (overlapped)	
15α		209.0 s		208.8 s	5.10 (overlapped)	75.0 d
16α	3.00 (overlapped)	55.0 d		153.5 s		160.4 s
17α	4.18 (overlapped)	70.0 t	5.38 (s)	117.8 t	5.42 (s)	107.8 t
17β	4.51 (t, 9.0)		6.02 (s)		5.12 (overlapped)	
18	0.87 (s)	31.6 q	1.35 (s)	30.1 q	1.10 (s)	31.1 q
19	0.96 (s)	23.2 q	1.45 (s)	23.4 q	0.91 (s)	21.3 q
20	5.58 (s)	98.7 d	5.59 (s)	100.8 d		175.7 s
OMe	3.22 (s)	58.3 q	3.40 (s)	54.5 q		

a) The data of compounds **3**, **4** and **5** were assigned based on DEPT, HSQC, HMBC, COSY and ROESY experiments.

Table 3. IC_{50} Values (μM) of Diterpenoids from *I. henryi* for Human Tumor Cell Lines

Compound[a)]	HL-60	SMMC-7721	A-549	MCF-7	SW480
6	2.68	29.9	>40	17.0	24.9
9	7.26	15.0	>40	16.3	13.5
10	9.55	13.6	>40	21.2	15.7
11	7.50	12.8	>40	18.1	19.7
12	12.8	17.3	>40	20.6	25.0
16	16.0	31.4	32.5	24.3	35.3
17	2.14	5.02	9.86	6.09	14.1
DDP[b)]	1.97	15.2	15.48	20.3	14.7
Paclitaxel[b)]	<0.008	<0.008	1.36	<0.008	0.04

a) Other selected ones not listed in the table were inactive (IC_{50}>40 μM) for all cell lines. *b*) DDP (cisplatin) and paclitaxel were used as positive controls.

mined to be (20*R*)-1α,6β,7β,11β-tetrahydroxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-ene-15-one.

Isodonhenrin E (**5**) was isolated as amorphous powder. The molecular formula was proved by its positive HR-ESI-MS (*m*/*z* 403.1737 $[M+Na]^+$) to be $C_{20}H_{28}O_7$. Comparing the ^{13}C-NMR data of **5** with those of rabdoternin B,[12] the notable difference was at ring-D. The HMBC correlations from H-11 (δ_H 4.30) to C-9 and C-13 indicated the presence of a hydroxy group at C-11 in **5**, while the hydroxy group was at C-14 in rabdoternin B. The ROESY correlations from H-1 to H-9β established the β-configulation of H-1. The α-configulations of the H-6, H-11, H-15 were established by the correlations from H-6 to H_3-19α, H-11 to H-13α and H-14α, H-15 to H-13α and H-14β respectively. So compound **5** is deduced as 1α,6β,7β,11β,15β-pentahydroxy-*ent*-kaur-16-en-7α,20-olide.

Isolated 18 deterpenoids, except for compounds **3**, **4** and **15**, due to sample limitations, were evaluated for cytotoxic activity against five human cancer cell lines (HL-60, A549, SMMC-7721, MCF-7, SW480). The method used was the same as the description in the literature.[18] Compound **17** showed significant cytotoxicity against all five cell lines, while compounds **6**, **9**, **10**, **11**, **12** and **16** exhibited selective cytotoxicity (Table 3). Analyisis of the above bioassay results, a preliminary conclusion can be drawn that the carbonyl conjugated with an exomethylene group is the active center.

Experimental

General Procedure Petroleum ether (PE, 60—90 °C), EtOAc, $CHCl_3$, acetone, MeOH, EtOH were analytical grade and produced by Sinopharm Chemical Reagent Co., Ltd., China. Column chromatography (CC) was performed on silica gel (200—300 mesh; Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40—63 μm, Merck, Darmstadt, Germany), and Sephadex LH-20 (Pharmacia). Fractions were monitored by TLC, and spots were visualized by spraying with 10% H_2SO_4 in EtOH, followed by heating. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm×25 cm column. Melting point was obtained on an XRC-1 apparatus and was uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV data were obtained using a UV-210A spectrometer. IR spectra were obtained on a Bio-Rad FtS-135 spectrophotometer with KBr pellets. HR-ESI-MS were performed on an API QSTAR time-of flight spectrometer. NMR spectra were obtained on a Bruker DRX-500 instrument with tetramethylsilane (TMS) as an internal standard. X-ray data were collected using a Bruker APEX DUO instrument.

Plant Material The aerial part of *I. henryi* were collected in Sichuan province, China, in July 2008, and identified by Prof. Xi-Wen Li, Kunming Institute of Botany. A voucher specimen (KIB 09102010) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation The aerial parts (1.3 kg) of *I. henryi* were extracted with the 70% aqueous acetone (10 l) four times (2 d for each time) at room temperature and filtered. After the filtrate was evaporated *in vacuo* to be concentrated. Then the concentrate without acetone (0.8 l) was portioned between EtOAc and H_2O. The EtOAc soluble portion (58 g) was decolorized on MCI gel and then subjected to silica gel CC (350 g, 100—200 mesh). Five fractions were given by the silica gel column eluted with a $CHCl_3$/MeOH gradient system (100 : 1, 50 : 1, 20 : 1, 10 : 1, 5 : 1, for fraction 1 to 5, respectively). Fraction 1 was chromatographed on normal silica gel column repeatedly (eluted with petroleum ether/acetone 30 : 1 to 10 : 1 then $CHCl_3$/MeOH 80 : 1) and HPLC (eluted with 50% MeOH–H_2O) to afford isodonhenrin C (**3**, 1.5 mg), 15-α-hydroxy-6,7-*seco*-1α,7:11α,6-diolide-20-al-*ent*-kaur-16-ene (**7**, 18.2 mg), ponicidin (**9**, 3.7 mg), macrocalin B (**10**, 21.3 mg) and rabdonervosin B (**18**, 5.6 mg). Isolated by normal silica gel column (eluted with $CHCl_3$/MeOH 80 : 1 to 50 : 1), RP-18 (30 to 60% MeOH–H_2O) and semipreparative HPLC (33% acetonitrile–H_2O), isodonhenrin A (**1**, 7.1 mg), isodonhenrin D (**4**, 2.1 mg) and isodonhenrin E (**5**, 2.4 mg) was got from fraction 2, along with glaucocalactone (**6**, 6.3 mg), rabdoternin F (**11**, 4.1 mg), rabdoternin E (**12**, 4.6 mg), rabdocoetsin B (**17**, 5.2 mg) from fraction 3. Successive column chromatography by RP-18 (30 to 50% MeOH–H_2O), silica gel column (eluted with $CHCl_3$/MeOH 50 : 1 to 30 : 1) and HPLC (33% acetonitrile–H_2O, and 45% MeOH–H_2O) led to the isolation of sculponeatin E (**8**, 31.7 mg), rabdoternin B (**13**, 5.8 mg) and oridonin (**15**, 1.8 mg) from fraction 4. Isodonhenrin B (**2**, 3.1 mg), rabdoternin A (**14**, 5.6 mg) and rosthorin A (**16**, 3.4 mg) were isolated from fraction 5 by the same separation method used as fraction 4.

Isodonhenrin A (**1**): Colorless needle crystals in MeOH; mp 187—188 °C; $[\alpha]_D^{20.6}$ −193.76 (*c*=0.001, $CHCl_3$/MeOH 1 : 1); UV (MeOH) λ_{max} (log ε) 202 (3.50) nm; IR (KBr) ν_{max} cm^{-1}: 3438, 2934, 1742, 1713, 1638, 1238, 1067; HR-ESI-MS (positive): *m*/*z* 427.1732 ($[M+Na]^+$, $C_{22}H_{28}O_7$, Calcd 427.1732); ^{1}H- and ^{13}C-NMR data listed in Table 1.

X-Ray crystal structure analysis of **1**: $C_{22}H_{28}O_7$ (molecular weight (M_W)= 404.4), orthorhombic, space group, $P2_12_12_1$, Z=4, a=10.6859(2) Å, b= 12.5623(2) Å, c=14.7334(3) Å, $\alpha=\beta=\gamma$=90°, V=1977.80(6) Å. μ(Cu$K\alpha$)= 0.834 mm^{-1}, ρ_{calc}=1.358 g/cm^3; S=1.079, final R indices: R_1=0.0485, wR_2=0.1533 for 3414 observed from 8872 independent and 3387 measured reflections, (θ_{max}=69.27, $I>2\sigma(I)$ criterion and 289 parameters); maximum and minimum residues are 0.307 and −0.320 eÅ^{-3}, respectively. The Flack30 parameter value was x=0.2(2), indicating that the absolute structure has been determined correctly.

Isodonhenrin B (**2**): $C_{23}H_{32}O_7$; amorphous powder; $[\alpha]_D^{20}$ −110.9 (*c*= 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.82) nm; IR (KBr) ν_{max}: 3420, 2925, 2854, 1741, 1639, 1629 cm^{-1}; positive ESI-MS *m*/*z* (%): 863 $[2M+Na]^+$, 443 $[M+Na]^+$; positive HR-ESI-MS Found 443.2042, Calcd for $C_{23}H_{32}O_7Na$ 443.2045; ^{1}H- and ^{13}C-NMR data listed in Table 1.

Isodonhenrin C (**3**): $C_{21}H_{30}O_7$; amorphous powder; $[\alpha]_D^{20}$ −103.4 (*c*= 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.11) nm; IR (KBr) ν_{max}: 3438, 2930, 2871, 1738, 1638 cm^{-1}; positive ESI-MS *m*/*z* (%): 811 $[2M+Na]^+$, 417 $[M+Na]^+$; positive HR-ESI-MS Found 417.1881, Calcd for $C_{21}H_{30}O_7Na$ 417.1889 ; ^{1}H- and ^{13}C-NMR data listed in Table 2.

Isodonhenrin D (**4**): $C_{21}H_{30}O_7$; amorphous powder; $[\alpha]_D^{24}$ −38.5 (*c*=0.13, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.51) nm; IR (KBr) ν_{max}: 3396, 2926, 2869, 1710, 1639 cm^{-1}; positive ESI-MS *m*/*z* (%): 811 $[2M+Na]^+$, 417 $[M+Na]^+$; positive HR-ESI-MS Found 417.1886, Calcd for $C_{21}H_{30}O_7Na$ 417.1889 ; ^{1}H- and ^{13}C-NMR data listed in Table 2.

Isodonhenrin E (**5**): $C_{20}H_{28}O_7$; amorphous powder; $[\alpha]_D^{24}$ −20.4 (*c*=0.25, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.48) nm; IR (KBr) ν_{max}: 3428, 2926, 2855, 1715, 1633 cm^{-1}; positive ESI-MS *m*/*z* (%): 783 $[2M+Na]^+$, 403$[M+Na]^+$; positive HR-ESI-MS Found 403.1737, Calcd for $C_{20}H_{28}O_7Na$ 403.1732 ; ^{1}H- and ^{13}C-NMR data listed in Table 2.

Cytotoxicity Assay The following human tumor cell lines were used: HL-60 (human myeloid leukemia cell line), SMMC-7721 (human hepatocarcinoma cell line), A-549 (lung cancer cell line), MCF-7 (breast cancer cell line) and SW480 (human colon carcinoma). All the cells were cultured in RPMI-1640 or Dulbecco's modified Eagle's medium (DMEM) medium (Hyclone, Logan, UT, U.S.A.), supplemented with 10% fetal bovine serum (Hyclone, U.S.A.) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO, U.S.A.). Briefly, 100 μl adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before

drug addition, while suspended cells were seeded just before drug addition, both with initial density of 1×10^5 cells/ml in 100 μl medium. Each tumor cell line was exposed to the tested compound at various concentrations in triplicates for 48 h, with *cis*platin (Sigma, U.S.A.) as positive control. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were lysed with 100 μl 20% sodium dodecyl sulfate (SDS)–50% *N*,*N*-dimethylformamide (DMF) after removal of 100 μl medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680, U.S.A.). The IC_{50} value of each compound was calculated by the Reed and Muench's method.[18)]

Acknowledgments This work was supported financially by the NSFC-Joint Foundation of Yunnan Province (No. U0832602 to H.-D. Sun), the Major State Basic Research Development Program of China (No. 2009CB522300 and 2009CB940900), the Science and Technology Program of Yunnan Province (Nos. 2008IF010 and 2008CD162), the NSFC (No. 81172939 to J.-X. Pu), and the Major Direction Projection Foundation of CAS Intellectual Innovation Project (No. 2010KIBA05 to J.-X. Pu).

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