Four New *ent*-Kauranoids from *Isodon rubescens* var. *lushanensis* and Data Reassignment of Dayecrystal B

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Four new 7, 20-epoxy-*ent*-kauranoids, rubluanins A—D (1—4) and eleven known ones, were isolated from *Isodon rubescens* var. *lushanensis*, and their structures were elucidated by spectroscopic analysis. Compounds 2—14 were evaluated for their cytotoxicity in several human tumor cell lines (U937, Jurkat, HL-60, K562, SGC790, and HepG2). Among the compounds, lasiokaurin (13) showed significant cytotoxic activity of 0.62 μM (IC_{50}) in U937 cells.

Key words *Isodon rubescens* var. *lushanensis*; *ent*-kauranoid; cytotoxicity

Isodon rubescens (Helsm.) Hara (Labiatae), a perennial herb of *Isodon* genus, has been used as an antitumor folk medicine for the treatment of esophageal and cardiac carcinoma in Henan Province, China.[1)] From *Isodon rubescens* var. *lushanensis*, collected in Lushan Prefecture of Henan Province, five 20-nonoxygenated and fifteen 20-oxygenated *ent*-kaurane diterpenoids were reported previously.[2—6)] Since the metabolites of some species in *Isodon* genus often exhibit biodiversity attributed to their different ecological environments,[7—11)] we reinvestigated *Isodon rubescens* var. *lushanensis* indigenous to Luanchuan Prefecture (western area of Lushan Prefecture), Henan Province, with the aim of identifying new diterpenoids with promising antitumor activity. As a result, four new 7,20-epoxy-*ent*-kauranoids (rubluanins A—D, **1**—**4**) along with eleven known diterpenoids, dayecrystal B (**5**), kamebacetal A (**6**), henryin (**7**), kamebakaurin (**8**), oridonin (**9**), isodonoiol (**10**), megathyrin A (**11**), lasiodonin (**12**), lasiokaurin (**13**), epinodosin (**14**), and 7-isopropyl-podocarpinol (**15**) were obtained. Compounds **2**—**14** were evaluated for their cytotoxicity in 6 human tumor cell lines (U937, Jurkat, HL-60, K562, SGC790, and HepG2), and lasiokaurin (**13**) showed significant cytotoxic activity of 0.62 μM (IC_{50}) in U937 cells. Herein, we report the isolation and structure elucidation of these compounds as well as the cytotoxicity evaluation results.

Results and Discussion

Rubluanin A (**1**), obtained as a white amorphous powder, showed a quasi-molecular ion peak at *m/z* 429.2239 $[M+Na]^+$ (Calcd 429.2253) in its high resolution electrospray ionization mass spectrometry (HR-ESI-MS), corresponding to the molecular formula $C_{23}H_{34}O_6$. Analyses of its ^{1}H-, ^{13}C-NMR, and distortionless enhancement by polarization transfer (DEPT)-NMR data (Tables 1, 2) provided evidence that **1** possessed one exomethylene, one acetyl group, two tertiary methyls, one methoxyl group, five methylenes, eight methines (including one acetal methine and four oxygenated methines), and three quaternary carbons (Tables 1, 2). Thirty-two protons were all attached to corresponding carbons in heteronuclear single-quantum coherence (HSQC) spectroscopy. Three structural fragments, **a** (C-1 to C-3), **b** (C-6 to C-7), and **c** (C-9 to C-13), were deduced from the ^{1}H–^{1}H correlation spectroscopy (COSY) as shown in Fig. 1. The above data indicated that **1** had a 7,20-epoxy-*ent*-kau-

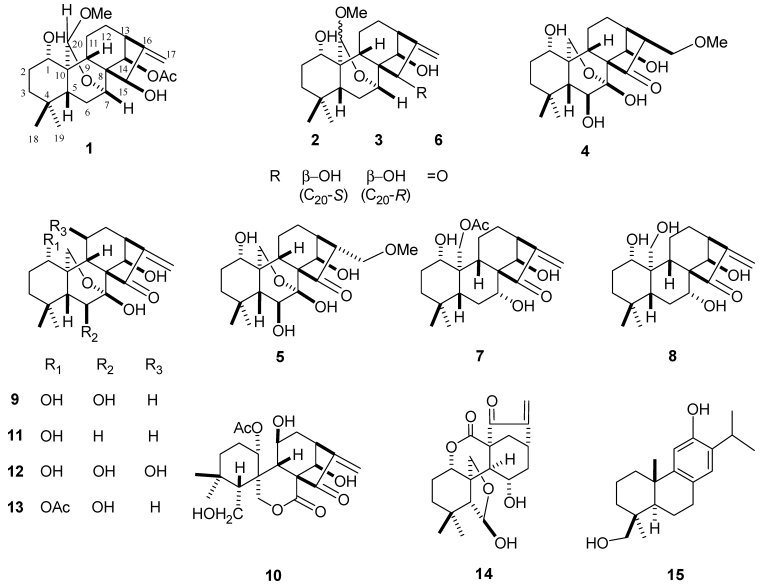

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Table 1. ^{1}H-NMR Data of Compounds **1**—**5** (Recorded at 500 MHz)

Position	**1**[a] (δ_H)	**2**[a] (δ_H)	**3**[a] (δ_H)	**4**[b] (δ_H)	**5**[b] (δ_H)
1β	3.50 (1H, dd, 12.0, 4.8 Hz)	3.50 (1H, m)	3.44 (1H, m)	3.65 (1H, m)	3.63 (1H, dd, 11.0, 5.5 Hz)
2α	1.61[c] (1H, m)	1.61[c] (1H, m)	1.62[c] (1H, m)	1.84[c] (2H, m)	1.79 (1H, m)
2β	1.79[c] (1H, m)	1.79[c] (1H, m)	1.81[c] (1H, m)	—	1.86 (1H, m)
3α	1.48 (1H, m)	1.48 (1H, m)	1.43[c] (1H, m)	1.37[c] (1H, m)	1.36[c] (2H, m)
3β	1.25 (1H, m)	1.25 (1H, m)	1.17[c] (1H, m)	1.30[c] (1H, m)	—
4	—	—	—	—	—
5β	1.35[c] (1H, m)	1.34[c] (1H, m)	1.54[c] (1H, m)	1.44 (1H, d, 6.3 Hz)	1.43 (1H, d, 7.2 Hz)
6α	1.60[c] (1H, m)	1.63[c] (1H, m)	1.83[c] (1H, m)	4.19 (1H, dd, 11.0, 6.5 Hz)	4.24 (1H, dd, 10.2, 7.4 Hz)
6β	2.08 (1H, t, 13.5, 11.5 Hz)	2.07[c] (1H, m)	1.94 (1H, m)	—	—
7	4.01 (1H, d, 3.0 Hz)	4.15 (1H, d, 2.8 Hz)	4.11 (1H, t, 2.7 Hz)	—	—
8	—	—	—	—	—
9β	1.98 (1H, m)	1.93 (1H, m)	1.81[c] (1H, m)	1.94 (1H, m)	1.94 (1H, m)
10	—	—	—	—	—
11α	2.29 (1H, m)	2.13[c] (1H, m)	1.69[c] (1H, m)	2.50 (1H, m)	2.38[c] (1H, m)
11β	1.81[c] (1H, m)	1.75[c] (1H, m)	1.58[c] (1H, m)	1.83[c] (1H, m)	2.05 (1H, m)
12α	2.42 (1H, m)	2.33 (1H, m)	2.36 (1H, m)	2.01 (1H, m)	2.38[c] (1H, m)
12β	1.34[c] (1H, m)	1.32[c] (1H, m)	1.47[c] (1H, m)	1.80[c] (1H, m)	1.60 (1H, m)
13α	2.66 (1H, d, 10.0 Hz)	2.59 (1H, d, 9.9 Hz)	2.56 (1H, d, 9.5 Hz)	2.88 (1H, t, 8.5 Hz)	2.72 (1H, d, 8.3 Hz)
14α	5.55 (1H, s)	4.53 (1H, s)	4.31 (1H, s)	5.40, (1H, s)	5.23 (1H, s)
15α	4.29 (1H, s)	4.30 (1H, s)	4.48 (1H, br s)	—	—
16	—	—	—	3.72[c] (1H, m)	2.86 (1H, t, 7.0 Hz)
17	5.14 (2H, d, 10.0 Hz)	5.18 (2H, br s)	5.20 (2H, br s)	3.83 (1H, m) 3.77 (1H, m)	3.93 (2H, d, 7.0 Hz)
18	0.81 (3H, s)	0.81 (3H, s)	0.83 (3H, s)	1.23 (3H, s)	1.28 (3H, s)
19	0.95 (3H, s)	0.96 (3H, s)	1.01 (3H, s)	1.11 (3H, s)	1.10 (3H, s)
20	5.29 (1H, s)	5.30 (1H, s)	5.10 (1H, s)	4.75 (1H, AB, 10.1 Hz) 4.39 (1H, AB, 10.1 Hz)	4.74 (1H, AB, 10.0 Hz) 4.35 (1H, AB, 10.0 Hz)
15-OAc	2.02 (3H, s)	—	—	—	—
20-OMe	3.44 (3H, s)	3.45 (3H, s)	3.40 (3H, s)	—	—
17-OMe	—	—	—	3.26 (3H, s)	3.24 (3H, s)
1-OH	—	4.35 (1H, d, 7.3 Hz)	—	5.96 (1H, br s)	5.98 (1H, br s)
6-OH	—	—	—	6.55 (1H, d, 11.0 Hz)	6.64 (1H, d, 10.5 Hz)
14-OH	—	—	—	7.75 (1H, br s)	7.14 (1H, s)

a) Determined in $CDCl_3$. *b*) Determined in C_5D_5N. *c*) Overlapping signals.

Table 2. ^{13}C-NMR Data of Compounds **1**—**5**

Position	**1**[a] (δ_C)	**2**[b] (δ_C)	**3**[a] (δ_C)	**4**[a] (δ_C)	**5**[b] (δ_C)
1	76.1 d	76.3 d	75.4 d	72.8 d	73.3 d
2	30.1 t	30.2 t	31.0 t	30.5 t	30.5 t
3	38.8 t	39.0 t	39.5 t	39.3 t	39.5 t
4	33.7 s	33.7 s	32.7 s	34.1 s	34.0 s
5	48.2 d	48.3 d	47.0 d	61.2 d	60.1 d
6	26.1 t	26.3 t	26.2 t	74.6 d	75.0 d
7	69.3 d	69.5 d	68.8 d	98.1 s	98.2 s
8	51.2 s	52.2 s	53.6 s	62.9 s	63.7 s
9	44.0 d	44.0 d	48.0 d	53.4 d	53.4 d
10	42.7 s	42.8 s	43.0 s	41.5 s	41.7 s
11	21.0 t	21.1 t	18.1 t	19.4 t	20.3 t
12	33.2 t	33.0 t	33.1 t	20.6 t	30.8 t
13	42.6 d	44.9 d	43.6 d	37.9 d	38.9 d
14	76.5 d	73.3 d	73.3 d	74.3 d	75.7 d
15	74.7 d	74.3 d	73.3 d	222.5 s	221.7 s
16	161.3 s	162.1 s	161.3 s	52.1 d	57.9 d
17	109.1 t	109.3 t	109.8 t	68.9 t	74.9 t
18	32.0 q	32.0 q	32.8 q	33.1 q	33.5 q
19	20.5 q	20.6 q	21.4 q	21.9 q	22.4 q
20	101.8 d	102.3 d	99.2 d	64.1 t	63.9 t
15-OAc	170.4 s	—	—	—	—
	21.3 q	—	—	—	—
20-OMe	55.0 q	55.1 q	54.5 q	—	—
17-OMe	—	—	—	58.7 q	58.3 q

a) Recorded at 100 MHz. *b*) Recorded at 125 MHz.

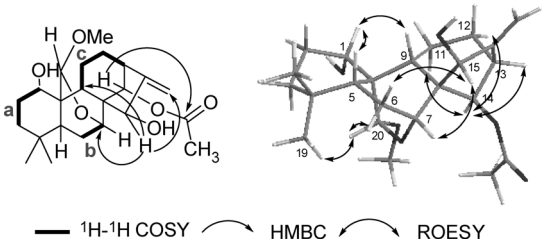

Fig. 1. Key HMBC (from H to C) and ROESY Correlations of **1**

rane skeleton, partially similar to kamebacetal A (**6**).[12] The conjugated carbonyl group (C-15) of **6** was reduced to a secondary hydroxyl in **1**, and this assumption was confirmed by the heteronuclear multiple bond coherence (HMBC) correlations from H-15 (δ_H 4.29) to C-7 (δ_C 69.3), C-9 (δ_C 44.0), C-16 (δ_C 161.3), and C-17 (δ_C 109.1) as shown in Fig. 1. HMBC correlations from H-14 (δ_H 5.55) to a carbonyl carbon (δ_C 170.4) indicated that the hydroxyl group at C-14 of **6** was replaced by an OAc group in **1**. The relative configuration of **1** was revealed by analysis of the rotating frame Overhauser effect spectroscopy (ROESY), in which the correlations of H-1 with H-5β and H-9β; H-14 with H-11α, H-12α, and H-13α; and H-15 with H-6β, H-7β, and H-17 were clearly observed, suggesting that the substituents at C-1, C-14, and C-15 possess α-, β-, and β-orientations, respectively

(Fig. 1). Since compound **1** is an *ent*-kauranoid on biogenetic grounds,[6] the configuration at C-20 was assigned as *S** from the ROESY correlations between H-20 and H-19, H-6α.[13] Consequently, compound **1** was determined to be (20*S**)-14β-acetoxy-1α,15β-dihydroxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-ene.

Rubluanin B (**2**), a white amorphous powder, was shown to possess the molecular formula $C_{21}H_{32}O_5$ from the quasi-molecular ion peak observed at *m*/*z* 387.2154, $[M+Na]^+$ (Calcd 387.2147) in its HR-ESI-MS. Analyses of the ^{1}H-, ^{13}C-, and DEPT-NMR spectra suggested that it was also a 7,20-epoxy-*ent*-kauranoid, bearing a structure similar to kamebacetal A (**6**). One secondary hydroxyl group was assigned to C-15 in **2** as the HMBC spectrum showed correlations arising from H_2-17, H-14α, H-13α and H-9β to C-15. ROESY correlations between H-1 and H-5β, H-9β; H-14 and H-11α, H-13α; H-15 and H-6β, H-7β, H-17 revealed the relative configuration of the substituents at C-1, C-14, and C-15 possess α-, β-, and β-orientations, respectively. Compound **2** also displayed ROESY correlations from H-20 to H-19, H-6α, and correlations from H-14α to H-OMe (at C-20). These correlations reflected that the configuration at C-20 was *S**. Therefore, rubluanin B was assigned to be (20*S**)-1α, 14β, 15β-trihydroxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-ene.

Rubluanin C (**3**), an epimer to **2**, exhibited a quasi-molecular ion peak at *m*/*z* 387.2141 $[M+Na]^+$ (Calcd 387.2147) in its HR-ESI-MS, in agreement with the molecular formula $C_{21}H_{32}O_5$. The only difference between them was the configuration at C-20. The ROESY spectrum of **3** showed correlations from H-20 to H-11α, H-14α, and H_3-OMe (at C-20). Therefore the configuration at C-20 of **3** was determined as *R**. Thus, the structure of compound **3** was elucidated as (20*R**)-1α, 14β, 15β-trihydroxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-ene.

Rubluanin D (**4**), isolated as an amorphous white powder, showed a quasi-molecular ion peak at *m*/*z* 419.2055 $[M+Na]^+$ (Calcd 419.2045) in its HR-ESI-MS, consistent with the molecular formula $C_{21}H_{32}O_7$. Analyses of its ^{1}H-, ^{13}C-, and DEPT-NMR data (Tables 1, 2) suggested that **4** also had a 7,20-epoxy-*ent*-kaurane skeleton, partially similar to oridonin (**9**).[14] Two structural fragments, **a** (C-1 to C-3), and **b** (C-9 to C-14), were deduced from the ^{1}H–^{1}H COSY spectra as shown in Fig. 2. Comparison of its ^{13}C-NMR data with those of oridonin revealed that the exomethylene of oridonin disappeared in **4**, and **4** had one more additional methoxyl group. The methoxyl group was assigned to C-17 (δ_C 68.9) by the HMBC correlations arising from H_2-17 (δ_H 3.83, 3.77) to C-OMe (δ_C 58.7), C-13 (δ_C 37.9), C-15 (δ_C 222.5), and C-16 (δ_C 52.1) (Fig. 2). ROESY correlations between H-17 and H-12β, H-13α; H-16α and H-12β, H-13α indicated the configuration at C-16 was *S**. Thus compound **4** was ultimately determined to be (16*S**)-1α,6β,7β,14β-tetrahydroxy-17β-methoxy-7α,20-epoxy-*ent*-kaur-15-one.

Dayecrystal B (**5**) was obtained as an epimer to compound **4**. The only difference between them was the configuration at C-16. It gave a quasi-molecular ion peak at *m*/*z* 419.2049 $[M+Na]^+$ (Calcd 419.2045) in its HR-ESI-MS, corresponding to the molecular formula $C_{21}H_{32}O_7$. Dayecrystal B has been reported previously.[15] Here, we prsent the corrections of its data assignment. Compound **5** showed ROESY correlations between H-16β and H-12β, H-9β, H-13α. This means that the configuration at C-16 was *R**. In the HMBC spectrum, correlations from ^{1}H-signals of two methyls (δ_H 1.3, CH_3-18 and δ_H 1.1, CH_3-19) to one ^{13}C-signal (δ_C 60.1) of a methine had been found simultaneously. Thus, this methine was assigned to C-5. Then the ^{13}C-signal (δ_C 75.0) was assigned to C-6 with the help of evidence from the HSQC and ^{1}H–^{1}H COSY data. Next, in the HMBC spectrum, C-15 (δ_C 221.7) was chosen as a start point. The correlation of C-15 with H-14 (δ_H 5.23) was then found. Therefore, the chemical shifts of C-14 (δ_C 75.7) and C-1 (δ_C 73.3) could be distinguished with the help of evidence from the HSQC data. Thus compound **5** was finally established to be (16*R**)-1α,6β,7β,14β-tetrahydroxy-17α-methoxy-7α,20-epoxy-*ent*-kaur-15-one, and the correction of its data assignment is given in Tables 1 and 2.

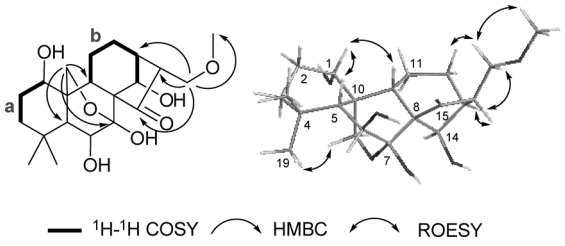

Fig. 2. Key HMBC (from H to C) and ROESY Correlations of **4**

The structures of other known compounds (**6**—**15**) were identified to be kamebacetal A (**6**),[12] henryin (**7**),[12] kamebakaurin (**8**),[16] oridonin (**9**),[14] isodonoiol (**10**),[17] megathyrin A (**11**),[18] lasiodonin (**12**),[19] lasiokaurin (**13**),[19] epinodosin (**14**),[20] and 7-isopropyl-podocarpinol (**15**),[21] by comparison of their spectroscopic data with those reported in the literature. The results were generally consistent with the constituents reported in the literature, in which fifteen 20-oxygenated *ent*-kauranoids had been isolated from *Isodon rubescens* var. *lushanensis* collected in Lushan Prefecture of Henan Province.

Since only a minute amount of compound **1** was obtained it could not be tested for cytotoxicity. Compounds **2**—**14** were tested for their inhibitory effects in the human tumor cell lines (U937, Jurkat, HL-60, K562, SGC790, and HepG2), with 10-hydroxy-camptothecine as the positive control. The results are presented in Table 3. Compounds **2** and **3** did not show bioactivity while their analogue kamebacetal A exhibited significant inhibitory effects in all of the cell lines, and this result was achieved mainly because the carbonyl groups that conjugated with the exomethylene group in kamebacetal A were replaced by secondary hydroxyl groups in compounds **2** and **3**. For compounds **4** and **5**, in their structures, the cyclopentanone group still remained, but their exomethylene groups were substituted by methoxyls, and then compounds **4** and **5** just showed weak activity in U937, Jurkat, HL-60, and K562, and they were not cytotoxic in SGC790 and HepG2. The above results further suggest that the cyclopentanone conjugated with an exomethylene group is the active center in *ent*-kauranoids.[22]

Experimental

General Procedures Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter. UV absorptions were obtained on a Shimadzu UV-2401 PC UV–VIS recording spectrophotometer. IR spectra were deter-

Table 3. Cytotoxicity Results of Compounds **2**—**14**

Compound	IC_{50} (μM) for cell lines					
	U937	Jurkat	HL-60	K562	SGC790	HepG2
2	>40	>40	>40	>40	>40	>40
3	>40	>40	>40	>40	>40	>40
4	13.57	13.65	26.69	20.41	>40	>40
5	14.11	14.18	34.15	26.67	>40	>40
6	3.23	3.58	4.32	10.91	7.14	4.00
7	0.72	0.93	1.16	4.74	3.09	4.05
8	1.11	2.09	3.66	5.59	7.12	12.62
9	3.41	3.79	15.07	16.08	20	19.15
10	16.25	25.27	>40	24.94	>40	>40
11	2.98	4.17	6.17	16.15	19.9	20.03
12	18.17	25.66	>40	>40	>40	>40
13	0.62	0.63	3.16	3.02	4.01	5.57
14	16.05	13.41	27.30	19.29	>40	>40
Control	0.105	0.03	0.036	1.6	19.80	11.90

Positive control: 10-hydroxy-camptothecine.

mined on a Bio-Rad Fts-135 spectrophotometer with KBr pellets. MS were recorded on a VG Auto spec-3000 spectrometer or Finnigan MAT 90 instrument. One-dimension and 2D-NMR spectra were run on Bruker AV-400 and DRX-500 instruments with TMS as an internal standard.

Plant Material Stems and leaves of *Isodon rubescens* (Hara) var. *lushanensis* Gao *et* Li were collected in Luanchuan Prefecture of Henan Province in June 2007 and air dried. The identity of the plant material was verified by Prof. Xi-Wen Li, and a voucher specimen is deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation The dried and powdered stems and leaves (4.0 kg) were extracted with 70% acetone and filtered. After filtration and evaporation of the solvent under vacuum, the residue was suspended in water and partitioned with EtOAc. The EtOAc extract (117 g) was applied to column chromatography over a silica gel (100—200 mesh, 800 g) column eluted with a system of $CHCl_3$–acetone (10 : 0, 9 : 1, 8 : 2, 7 : 3, 2 : 1, 1 : 1) to afford fractions A—F. Fraction B (28 g) was further chromatographed over MCI gel CHP-20 P (90% MeOH–H_2O, then 100% MeOH) to yield fraction B_1—B_3. Fraction B_1 was further subjected to silica gel eluted with petroleum ether–acetone (1 : 0→0 : 1) to afford a mixture of two diterpenoids, then semi-preparative RP18-HPLC (MeOH/H_2O, 30 : 70, 3 ml/min, detector 238 nm) was applied to yield compounds **10** (4.1 mg) and **7** (11.3 mg). Fraction C (43 g) was further chromatographed on MCI gel CHP-20 P (90% MeOH–H_2O, then 100% MeOH) to yield fraction C_1—C_4, and compound **6** (437 mg) was obtained by recrystallization in MeOH from the C_1 fraction. Fraction C_2 was further subjected to a silica gel eluted with petroleum ether–acetone (10 : 1→0 : 1) to afford a mixture of two diterpenoids, and then isolated by semi-preparative RP18-HPLC (ACN/H_2O, 35 : 65, 3 ml/min, detector 210 nm) to yield compounds **1** (2.1 mg) and **6**. Compounds **13** (7.0 mg) and **14** (4.8 mg) were obtained by recrystallization in MeOH from the C_3 fraction after repeated chromatography over silica gel and RP-18 column (30—100% MeOH). Compound **8** (5.8 mg) was obtained by semi-preparative RP18-HPLC (ACN/H_2O, 27 : 73, 3 ml/min, detector 238 nm) from the C_4 fraction after repeated chromatography. Compound **12** (3.6 mg) was also obtained by semi-preparative RP18-HPLC (MeOH/H_2O, 30 : 70, 3 ml/min, detector 238 nm) from the C_4 fraction after repeated chromatography. Fraction D (32 g) was submitted to repeated chromatography including MCI gel, silica gel to afford D_1—D_3 fractions. Compounds **2** (4.0 mg) and **3** (3.1 mg) were isolated from the D_1 fraction over a silica gel (200—300 mesh, 5 g) column eluted with a system of $CHCl_3$–MeOH (80 : 1), and then purified by Sephadex-LH 20 gel. Compounds **4** (6.2 mg) and **5** (5.4 mg) were also obtained from the D_1 fraction after repeated chromatography, and then isolated by semi-preparative RP18-HPLC (MeOH/H_2O, 45 : 55, 3 ml/min, detector 210 nm). Compound **9** (7.2 mg) was obtained by semi-preparative RP18-HPLC (MeOH/H_2O, 45 : 55, 3 ml/min, detector 238 nm) from the D_2 fraction after repeated chromatography. Compound **15** (5.7 mg) was obtained by semi-prepare RP18-HPLC (MeOH/H_2O, 50 : 50, 3 ml/min, detector 238 nm) from the D_3 fraction after repeated chromatography.

Rubluanin A (**1**): White amorphous powder, $[\alpha]_D^{26}$ +0.00° (c=0.064, MeOH). UV λ_{max} (MeOH) nm (ε): 205 (3802). ^{1}H- and ^{13}C-NMR ($CDCl_3$) data: see Tables 1, 2. HR-ESI-MS $[M+Na]^+$ m/z: 429.2239 (Calcd for $C_{23}H_{34}O_6$, 429.2253).

Rubluanin B (**2**): White amorphous powder, $[\alpha]_D^{26}$ +25.86° (c=0.116, MeOH). UV λ_{max} (MeOH) nm (ε): 205 (3090). IR (KBr) cm^{-1}: 3431, 2930, 2867, 1657, 1072, 1031. ^{1}H- and ^{13}C-NMR ($CDCl_3$) data: see Tables 1, 2. HR-ESI-MS $[M+Na]^+$ m/z: 387.2154 (Calcd for $C_{21}H_{32}O_5$, 387.2147).

Rubluanin C (**3**): White amorphous powder, $[\alpha]_D^{26}$ −3.84° (c=0.100, MeOH). UV λ_{max} (MeOH) nm (ε): 204.2 (3019). IR (KBr) cm^{-1}: 3432, 2926, 2857, 1630, 1068, 1031. ^{1}H- and ^{13}C-NMR ($CDCl_3$) data: see Tables 1, 2. HR-ESI-MS $[M+Na]^+$ m/z: 387.2154 (Calcd for $C_{21}H_{32}O_5$, 387.2147).

Rubluanin D (**4**): White amorphous powder, $[\alpha]_D^{27}$ −5.36° (c=0.280, MeOH). UV λ_{max} (MeOH) nm (ε): 204.6 (1479). IR (KBr) cm^{-1}: 3406, 2942, 1717, 1630, 1455, 1060. ^{1}H- and ^{13}C-NMR (C_5D_5N) data: see Tables 1, 2. HR-ESI-MS $[M+Na]^+$ m/z: 419.2055 (Calcd for $C_{21}H_{32}O_7$, 419.2045).

Dayecrystal B (**5**): White amorphous powder, $[\alpha]_D^{27}$ −48.80° (c=0.502, MeOH). UV λ_{max} (MeOH) nm (ε): 203.4 (1349). IR (KBr) cm^{-1}: 3397, 2948, 1716, 1641, 1453, 1060. ^{1}H- and ^{13}C-NMR (C_5D_5N) data: see Tables 1, 2. HR-ESI-MS $[M+Na]^+$ m/z: 419.2049 (Calcd for $C_{21}H_{32}O_7$, 419.2045).

Cytotoxicity Assay The following human tumor cell lines were used: U937, Jurkat, HL-60, K562, SGC790, and HepG2. All the cells were cultured in RPMI-1640 or Dulbecco's modified Eagle's medium (DMEM) (Hyclone, 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 that formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, U.S.A.).[23] Briefly, 100 μl of adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1×10^5 cells/ml in 100 μl of medium. Each tumor cell line was exposed to the tested compound at various concentrations in triplicate for 48 h, with 10-hydroxy-camptothecine (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 of 20% SDS–50% DMF after removal of 100 μl of 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 method.[24]

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