Cytotoxic 3,20-Epoxy-*ent*-Kaurane Diterpenoids from *Isodon eriocalyx* var. *laxiflora*

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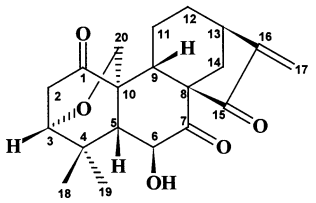
Four new *ent*-kaurane diterpenoids, laxiflorins J−M (**1**−**4**), along with maoecrystal A (**5**) and maoecrystal P (**6**), were isolated from the leaves of *Isodon eriocalyx* var. *laxiflora*. Their structures were determined by spectroscopic analyses. All the compounds were assayed for their cytotoxic effects on human tumor K562, A549, and T24 cell lines. Compounds **1** and **6** showed significant inhibitory effects on human tumor K562 and T24 cells, with IC_{50} values less than 0.5 μg/mL. Compound **3** also demonstrated cytotoxic activities against all three types of human tumor cells, with IC_{50} values in the range of 1−25 μg/mL.

Isodon eriocalyx (Dunn) Hara var. *laxiflora* C. Y. Wu et H. W. Li (Labiatae), a perennial shrub native to Yunnan province,[1] is a member of the genus *Isodon*, which is notable for being abundant in *ent*-kaurane diterpenoids.[2,3] Since eriocalyxin B, which was isolated from an ethyl alcohol extract of the leaves of *I. eriocalyx* as one of the major constituents in 1982,[4] has been successfully developed into a drug and put into use for sore throat and inflammation in China in 1996, much attention has been focused on *I. eriocalyx* as a natural drug resource. Many phytochemical studies have been reported on *I. eriocalys* and its variation, from which about 40 new *ent*-kaurane diterpenoids have been isolated.[4−14] Our searching for bioactive diterpenoids from *Isodon eriocalyx* var. *laxiflora* led to the isolation of four new diterpenoids, laxiflorin J−M (**1**−**4**), and two known ones, maoecrystal A (**5**)[6] and maoecrystal P (**6**).[11] All the compounds possess the same skeleton of 3,20-epoxy-*ent*-kaurane diterpenoids. Compounds **1** and **6** displayed prominent inhibitory effects on two human tumor K562 and T24 cells, with IC_{50} values less than 0.5 μg/mL. Maoecrystal P (**6**) was particularly active, with an IC_{50} value against T24 lower than 0.06 μg/mL. Compound **3** also showed obvious inhibitory activities toward all three lines of human tumor cells, with IC_{50} values in the range of 1−25 μg/mL. We report herein the isolation and structure elucidation of **1**−**4** and the cytotoxic activities of the isolates.



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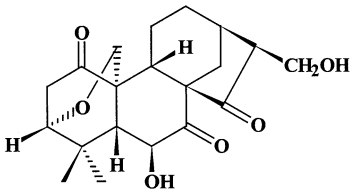

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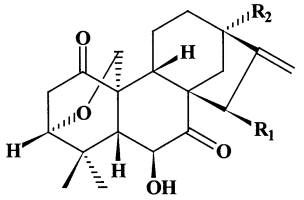
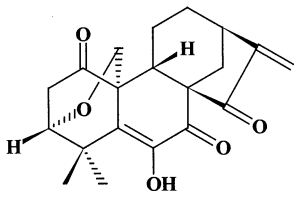


3 R_1 = OH, R_2 = H
4 R_1 = OAc, R_2 = OH
5 R_1 = OAc, R_2 = H



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Results and Discussion

Compound **1**, obtained as colorless crystals from Me_2CO, has molecular formula $C_{20}H_{24}O_5$, as evidenced by HREIMS. The ^{1}H NMR spectrum (Table 1) exhibits two singlets at δ_H 6.05 (1H, s) and 5.21 (1H, s), attributable to an *exo*-cyclic methylene, a pair of AB doublets at δ_H 4.83 (1H, d, J = 9.6 Hz) and 4.13 (1H, d, J = 9.6 Hz) assignable to an oxygenated methylene, two signals at δ_H 3.74 (1H, br s) and 4.86 (1H, br d, J = 11.8 Hz) assignable to two oxygen-bearing methines, respectively, and two sharp signals at δ_H 1.14 (3H, s) and 1.68 (3H, s) due to two tertiary methyl groups. The ^{13}C NMR and DEPT spectra (Table 2) indicated the presences of two methyls, four methylenes, one oxygen-bearing methylene, three methines, two oxygenated methines, three skeleton quaternary carbons, two carbonyl carbons, and an α,β-unsaturated moiety of a ketone carbon conjugated with an *exo*-methylene [δ_C 201.2 (s), 148.7 (s), and 116.0 (t)]. Considering the structures of diterpenoids isolated thus far from the genus *Isodon*, all the spectral evidence suggested the basic skeleton of an *ent*-kaur-16-en-15-one,[11,12] which was further supported by the UV absorption at 233 nm.[6] The negative optical rotation value ($[\alpha]_D^{23.9}$ −167.93°) of **1**, being very

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Table 1. ^{1}H NMR Data for Compounds **1**−**4**[a,b] (δ in ppm with reference to the signal of C_5D_5N, J in Hz)

proton	**1**	**2**	**3**	**4**
2	2.79 (1H, d, 9.2)	2.77 (1H, d, 19.2)	2.76 (2H, d, 19.0)	2.84 (1H, d, 19.0)
	2.70 (1H, brd, 9.2)	2.70 (1H, brd, 19.2)	2.72 (2H, brd, 19.0)	2.78 (1H, brd, 19.0)
3β	3.74 (1H, brs)	3.75 (1H, brs)	3.75 (1H, brs)	3.78 (1H, brs)
5β	1.90 (1H, d, 11.8)	1.90 (1H, overlap)	1.76 (1H, overlap)	1.93 (1H, overlap)
6α	4.86 (1H, brd, 11.8)	4.91 (1H, brd, 12.1)	4.94 (1H, brd, 11.7)	5.06 (1H, brd, 11.8)
9β	3.02 (1H, brd, 8.1)	2.97 (1H, overlap)	3.57 (1H, d, 8.1)	3.30 (1H, d, 8.0)
11α	1.54 (1H, m)	1.58 (1H, m)	1.50 (1H, overlap)	1.80 (brd, 14.4)
11β	1.75 (1H, m)	1.76 (1H, m)	2.42 (1H, m)	2.26 (1H, m)
12α	1.47−1.42 (2H, m)	1.42 (1H, m)	1.39 (1H, overlap)	1.89 (2H, overlap)
12β		1.90 (1H, overlap)	1.53 (1H, m)	
13α	2.88 (1H, brs)	2.79 (1H, brs)	2.68 (1H, brs)	
14α	2.10 (1H, d, 11.9)	2.18 (1H, d, 12.0)	1.79 (1H, overlap)	2.31 (1H, d, 11.7)
14β	1.81 (1H, brd, 11.9)	1.90 (1H, overlap)	1.46 (1H, overlap)	1.91 (1H, overlap)
15α			5.41 (1H, brs)	6.79 (1H, brs)
16α		2.95 (1H, m)		
17	6.05 (1H, s)	4.43 (1H, dd, 4.6, 11.1)	5.52 (1H, s)	5.60 (1H, s)
	5.21 (1H, s)	3.94 (1H, dd, 9.1, 11.1)	5.11 (1H, s)	5.45 (1H, s)
18	1.14 (3H, s)	1.14 (3H, s)	1.11 (3H, s)	1.67 (3H, s)
19	1.68 (3H, s)	1.70 (3H, s)	1.70 (3H, s)	1.71 (3H, s)
20	4.83 (1H, d, 9.6)	4.85 (1H, d, 9.4)	4.85 (1H, d, 9.7)	4.96 (1H, d, 9.6)
	4.13 (1H, d, 9.6)	4.11 (1H, d, 9.4)	4.20 (1H, d, 9.7)	4.27 (1H, d, 9.6)
6β-OH	6.70 (1H, brs)		6.08 (1H, brs)	
15β-OH			7.36 (1H, brs)	
OAc				2.04 (3H, s)

[a] Recorded at 125 MHz. [b] Measured in C_5D_5N.

Table 2. ^{13}C NMR Data for Compounds **1**−**6** (δ in ppm with reference to the signal of solvent)

carbon	**1**[b,c]	**2**[b,c]	**3**[b,c]	**4**[b,c]	**5**[a,d]	**6**[a,d]
1	208.3 s	208.4 s	209.1 s	209.3 s	208.2 s	204.6 s
2	42.2 t	42.2 t	42.2 t	42.3 t	41.7 t	41.7 t
3	77.5 d	77.6 d	77.5 d	77.6 d	77.0 d	77.8 d
4	38.1 s	38.1 s	38.1 s	38.3 s	37.6 s	40.8 s
5	50.5 d	50.8 d	51.8 d	51.7 d	51.9 d	133.1 s
6	72.3 d	72.5 d	72.1 d	72.2 d	71.0 d	144.6 s
7	207.4 s	207.8 s	212.0 s	209.7 s	209.5 s	192.6 s
8	60.4 s	61.6 s	57.2 s	56.0 s	55.8 s	58.6 s
9	39.2 d	39.7 d	33.6 d	34.6 d	34.7 d	31.9 d
10	52.5 s	52.4 s	52.0 s	51.9 s	51.4 s	54.6 s
11	21.0 t	21.0 t	21.2 t	24.3 t	20.4 t	19.4 t
12	31.5 t	24.7 t	33.3 t	40.8 t	32.5 t	31.0 t
13	37.3 d	32.5 d	40.0 d	77.3 s	39.6 d	37.8 d
14	37.3 t	37.8 t	36.3 t	43.7 t	35.8 t	38.4 t
15	201.2 s	213.4 s	74.6 d	73.5 d	73.9 d	202.3 s
16	148.7 s	57.4 d	156.5 s	155.1 s	149.8 s	147.7 s
17	116.0 t	58.0 t	106.3 t	108.3 t	108.7 t	117.3 t
18	29.5 q	29.5 q	29.5 q	29.6 q	29.2 q	23.3 q
19	23.1 q	23.1 q	23.3 q	23.2 q	22.9 q	22.0 q
20	61.6 t	61.9 t	62.6 t	62.5 t	62.0 t	66.8 t
OAc				170.6 s	170.0 s	
				20.9 q	20.9 q	

[a] Recorded at 125 MHz. [b] Recorded at 100 MHz. [c] Measured in C_5D_5N. [d] Measured in $CDCl_3$.

similar with that of *ent*-kaurane diterpenoids such as maoecrystal P[11] and eriocalyxin C[12] (both were isolated from a closely related species, *I. eriocalyx*), indicated that **1** belongs to *ent*-kauranoids instead of kauranoids.[18] The characteristic ^{13}C NMR signals at δ_C 77.5 (d), which showed HMBC interactions with Me-18 and Me-19, and δ_C 61.6 (t), which showed HMBC cross-peaks with H-5 and H-9, together with the corresponding ^{1}H NMR signals at δ_H 3.74 (1H, br s), 4.83 (1H, d, J = 9.6 Hz), and 4.13 (1H, d, J = 9.6 Hz), suggested that compound **1** possesses an epoxy unit between C-3 and C-20, as found and proven by X-ray diffraction in neorabdosin (the first 3,20-epoxy-*ent*-kauranoid discovered from *Isodon nervosa*).[15] This was further supported by the HMBC spectrum, in which ^{1}H−^{13}C long-range correlations between H-3 and C-20 and between H_2-20 and C-3 were observed. In the ^{1}H−^{1}H COSY spectrum, the coupling of the proton signal at δ_H 4.86 (1H, d, J = 11.8 Hz) with the signal at δ_H 1.90 (1H, d, J = 11.8 Hz), which was assigned to H-5 by its correlations in the HMBC spectrum with two methyls, indicated that a hydroxy group was attached to C-6. H-6 was assigned with the α-orientation on the basis of its coupling constant $J_{5\beta,6\alpha}$ = 11.8 Hz, as well as the ROESY correlation between H-6 and Me-19. The correlations of the signal at δ_C 208.3 (s) with H_2-20, H-3, H-5, and H-9 in the HMBC spectrum suggested that one carbonyl group could be assigned to position C-1. The other carbonyl group was deduced to be located at C-7 by the long-range couplings of the signal at δ_C 207.4 (s) with H-5, H-6, H-9, and H-14. Placement of H-3 in the β-orientation was based on observation of cross-peaks of H-3/CH_3-18 and H-3/CH_3-19 in the ROESY spectrum. The distorted boat form of the A-ring would result in H-3β existing between the space of CH_3-18 and CH_3-19, thus causing H-3 to show NOE correlations not only with CH_3-18 but also with CH_3-19. Thus, compound **1** was characterized as 6β-hydroxy-3α,20-epoxy-*ent*-kaur-16-en-1,7,15-trione and named laxiflorin J.

Compound **2** has a molecular formula $C_{20}H_{26}O_6$, as determined by HREIMS. The ^{13}C NMR and DEPT spectra (Table 2) revealed the presence of two methyls, four methylenes, two oxygenated methylenes, four methines, two oxygen-bearing methines, three skeletal quaternary carbons, and three carbonyl carbons. Comparison of the ^{1}H and ^{13}C NMR data of **2** with those of **1** suggested that compound **2** is very similar to **1** except for the D-ring. In comparing their ^{13}C NMR spectra, the signals for the α,β-unsaturated moiety of a ketone carbon conjugated with an *exo*-methylene in **1** (δ_C 201.2, 148.7, 116.0) are replaced by signals at δ_C 213.4 (s), 57.4 (d), and 58.4 (t) in **2**, suggesting that the double bond of the α,β-unsaturated moiety is reduced and the conjugated system is absent. This was further supported by comparison of the ^{1}H NMR spectra (Table 1), which show the absence of the two sharp singlets for an *exo*-methylene in **1** and the presence of signals at δ_H 2.95 (1H, m), 4.43 (1H, dd, J = 4.6, 11.1 Hz), and 3.94 (1H, dd, J = 9.1, 11.1 Hz) in **2**. That both C-12 (δ_C 24.7) and the oxygenated C-17 (δ_C 58.0) appeared relatively upfield due to a γ-steric compression effect between H-12β and 16-CH_2OH suggested β-orientation of the hydroxym-

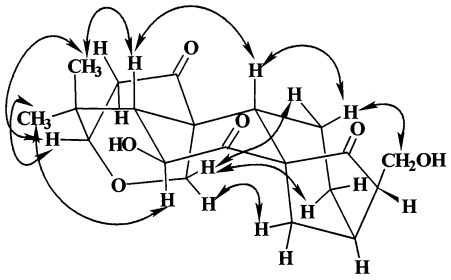

Figure 1. Key correlations of compound **2** in the ROESY spectrum.

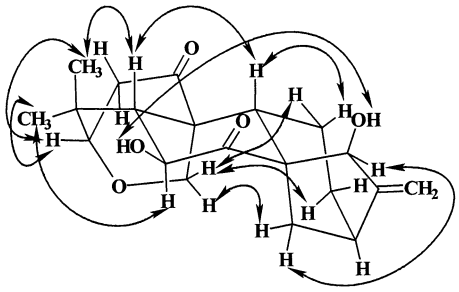

Figure 2. Key correlations of compound **3** in the ROESY spectrum.

ethylene at C-16.[10] This was supported by a NOE correlation between H_2-17 and H-11β in the ROESY spectrum of **2** (Figure 1). Thus, **2** was concluded to be 16(*S*)-6β,17-dihydroxy-3α,20-epoxy-*ent*-kaur-1,7,15-trione and named laxiflorin K.

Compound **3**, obtained as colorless needles, has a molecular formula of $C_{20}H_{26}O_5$ as determined by HREIMS, as well as ^{1}H (Table 1) and ^{13}C NMR spectra (Table 2). The ^{1}H and ^{13}C NMR and DEPT spectra of **3** display signals for two tertiary methyls, four methylenes, one oxygen-bearing methylene, three methines, three oxygenated methines, three skeletal quaternary carbons, two carbonyl carbons, one olefinic quaternary carbon, and one *exo*-methylene. The signals at δ_C 156.5 (s) and 106.3 (t) in the ^{13}C NMR spectrum, which are typical of an isolated double bond, suggested the absence of an α,β-unsaturated ketone moiety in the D-ring. Together with the presence of a proton signal at δ_H 5.41 (1H, br s) and a corresponding carbon signal at δ_C 74.6 (d) in the HMQC spectrum, it seemed evident that the C-15 ketone group of **1** and **2** was reduced to a hydoxyl group. Comparing the NMR data of **3** with those of **5**[6] revealed that the two compounds are very similar except for the absence of an acetyl group at C-15 in **3**. The analysis of the ROESY spectrum of **3** revealed that the stereochemistry of **3** is the same as that of **5**. In the ROESY spectrum (Figure 2), 15-OH showed a NOE correlation with 6β-OH, while H-15 displayed an NOE correlation with H-14β, indicating that 15-OH was in the β-orientation. This was also supported by the relative upfield shift of the C-9 signal in **3** (δ_C 33.6, compared with that of **1** and **2**), which was caused by the γ-steric compression effect between H-9β and 15-OH.[12] Thus, compound **3** was established as 6β,15β-dihydoxy-3α,20-epoxy-*ent*-kaur-16-en-1,7-dione and named laxilorin L.

Compound **4** has a molecular formula of $C_{22}H_{28}O_7$, determined by HREIMS and ^{1}H (Table 1) and ^{13}C NMR spectra (Table 2). The EIMS spectrum exhibited a fragment ion peak as a base peak at *m*/*z* 344 that was attributed to the loss of an acetic acid $[M - 60]^+$ from the molecular ion peak at *m*/*z* 404. On the basis of NMR data, compound **4** contains two tertiary methyls, four methylenes, one oxymethylene, two methines, three oxygenated methines, three skeletal quaternary carbons, one oxygen-bearing quaternary carbon, two carbonyl carbons, one olefinic quaternary carbon, an *exo*-methylene carbon, and an acetoxy group [δ_C 170.6 (s), 20.9 (q) and δ_H 2.04 (3H, s)]. Normally, in the upfield region of their ^{13}C NMR (DEPT) spectra, *ent*-kauranoids show three methine signals for C-5, 9, and 13. The existence of an oxygenated quaternary carbon [δ_C 77.3 (s)] and the lack of one methine could be easily observed in the ^{13}C NMR (DEPT) spectra of **4**. The comparison of the spectral data with those of **5** revealed that compound **4** closely resembles **5** except for the existence of one additional hydroxy group. The noticeable downfield shifts of the signals for C-12 [δ_C 40.8 (t)] and C-14 [δ_C 43.7 (t)] in the ^{13}C NMR, along with the disappearance of the broad singlet at ca. δ_H 2.80 ascribable to H-13 in the ^{1}H NMR spectrum, disclosed that the additional hydroxyl is attached to C-13. The configurations of other chiral centers in **4** were established to be consistent with those of **5** via a ROESY experiment. Full assignments of all protons and carbons were achieved by ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. Therefore, compound **4** was identified as 15β-acetoxy-6β,13α-dihydroxy-3α,20-epoxy-*ent*-kaur-16-en-1,7-dione and named laxiflorin M.

Table 3. Cytotoxic Activities of Compounds **1**−**6**

test substance	MW	IC_{50} (μg/mL) K562	A549	T24
1	344	0.473	49.054	0.314
2	362	11.874	ND[a]	1753.166
3	346	1.126	24.659	5.162
4	404	218.685	ND[a]	ND[a]
5	388	ND[a]	ND[a]	ND[a]
6	342	0.132	69.494	0.051
cis-platin		2.018	11.940	1.155

[a] ND = not determined.

The cytotoxic activities of all the compounds were tested against human tumor K562, A549, and T24 cells. Their IC_{50} (50% inhibitory concentration) values are shown in Table 3, in which *cis*-platin was included as a positive reference substance. The IC_{50} values (0.473/K562 and 0.314/T24 for compound **1**; 0.132/K562 and 0.0512/T24 for compound **6**) demonstrated significant cytotoxicities of compounds **1** and **6** against human tumor K562 and T24 cell lines. Compound **3** also showed strong inhibitory activities toward all three kinds of human tumor cells, with IC_{50} values of 1.126/K562, 24.659/A549, and 5.162/T24 μg/mL. Compound **2** exhibited only moderate cytotoxicity against K562 cells (IC_{50} 11.874 μg/mL). Compounds **4** and **5** were inactive.

From the above, it is plausible to suggest that the D-ring is an important function for cytotoxic activity against human tumor cells in 3α,20-epoxy-*ent*-kaurane diterpenoids, since the A-, B-, and C-rings of the skeletons of compounds **1**−**5** are all the same. The fact that compound **1** shows the most significant inhibitory activity among compounds **1**−**5** suggests that there might exist a relationship between the D-ring α,β-unsaturated ketone and cytotoxicity. That compound **6** displays stronger activity against human tumor cells than **1** could be explained by the existence of an additional double bond between C-5 and C-6 in **6**, leading to the formation of a second α,β-unsaturated ketone moiety in the B-ring.

Experimental Section

General Experimental Procedures. Melting points were obtained on an XRC-1 apparatus and are uncorrected. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were taken on a Shimadzu double-beam 210A spectrophotometer. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets. MS

spectra were recorded on a VG Auto Spec-3000 spectrometer. 1D and 2D NMR spectra were run on Bruker AM-400 and DRX-500 instruments. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Column chromatography was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Inc., China), silica gel H (10−40 μ, Qingdao Marine Chemical Inc., China), Lichroprep Rp_{18} gel (40−63 μm, Merck, Darmstadt, Germany), and MCI gel (70−150 μ, Mitsubishi Chemical Corp., Tokyo, Japan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The leaves of *I. eriocalyx* (Dunn) Hara var. *laxiflora* C. Y. Wu et H. W. Li were collected in Xishuangbanna prefecture, Yunnan Province, People's Republic of China, in November 1999. The plant material was identified by Prof. Zhong-Wen Lin at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, the Chinese Academy of Sciences. A voucher specimen (KIB-99-9-10 Lin) was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and Isolation. The dried and powdered leaves (25 kg) were extracted with 70% aqueous Me_2CO (60 L × 3) at room temperature and filtered. The filtrate was concentrated and partitioned with EtOAc. The EtOAc part was evaporated in vacuo to give 1000 g of a residue, which was subjected to silica gel column chromatography (9 × 200 cm, 3000 g) using $CHCl_3/Me_2CO$ (1:0, 9:1, 8:2, 7:3, and 0:1) as eluent. According to differences in composition detected by silica gel TLC, six crude fractions were obtained. Compound **5** (11.6 g) was recrystallized from fraction 1 in Me_2CO. The remaining fraction 1 (280 g) was repeatedly subjected to silica gel column chromatography developing with $CHCl_3/Me_2CO$ (9:1) and followed by recrystallization in Me_2CO to provide compound **6** (100 mg). Fraction 2 (200 g) was chromatographed by MPLC over silica gel (800 g) developing with petroleum ether/Me_2CO (4:1, 3:1, 2:1, 1:1) to give four subfractions, the third of which was further purified by MPLC over silica gel (100 g) eluting with $CHCl_3/Me_2CO$ (9:1, 8:2) to yield compound **1** (53 mg). Fraction 3 (80 g) was further purified by repeated column chromatography over silica gel column chromatography (200 g) [petroleum ether/Me_2CO (3:1, 2:1, 1:1)], MCI-gel CHP-20P (100 g) column chromatography [aqueous MeOH 50%:60%:100%], and silica gel column chromatography (100 g) [*n*-hexane/isopropyl alcohol (9:1, 8:2)] to afford compound **3** (23 mg). Fraction 4 (50 g) was rechromatographed separately on silica gel column chromatography (200 g) [*n*-hexane/isopropyl alcohol (9:1, 8:2)], MCI-gel CHP-20P (100 g) column chromatography [aqueous MeOH 50%:60%:100%], and Sephadex LH-20 (100) [aqueous MeOH 50%:60%:100%] to give compounds **2** (12 mg) and **4** (48 mg).

Laxiflorin J (1): colorless crystal from Me_2CO; mp 180.5−182.5 °C; $[\alpha]_D^{23.9}$ −167.93° (*c* 0.40, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 233 (4.21) nm; IR (KBr) ν_{max} 3430, 2937, 1744, 1701, 1644, 1453, 1411, 1385, 1315, 1289, 1256, 1205, 1130, 1105, 1064, 938, 906, 891, 861, 836, 779, 759, 684, 524, 556 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) spectral data, see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 2; EIMS *m*/*z* 344 $[M]^+$ (100), 326 (7), 316 (12), 302 (10), 288 (16), 273 (13), 255 (16), 245 (30), 231 (32), 213 (60), 203 (26), 185 (22), 173 (20), 159 (18), 145 (21), 131 (21), 129 (21), 115 (23), 105 (41), 91 (67), 79 (52), 69 (38), 67 (37), 56 (64); HREIMS *m*/*z* 344.1612 (calcd for $C_{20}H_{24}O_5$, 344.1624).

Laxiflorin K (2): white powder; $[\alpha]_D^{11.7}$ −65.17° (*c* 0.29, MeOH); UV (MeOH) λmax (log ϵ) 204 (3.68) nm; IR (KBr) ν_{max} 3434, 2940, 1728, 1664, 1459, 1366, 1326, 1214, 1057, 958, 929, 859, 785, 754, 534 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) spectral data, see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 2; EIMS *m*/*z* 362 $[M]^+$ (15), 344 (20), 334 (5), 316 (12), 299 (4), 288 (50), 274 (16), 259 (6), 245 (12), 231 (13), 217 (13), 203 (16), 187 (10), 175 (14), 149 (14), 135 (11), 121 (12), 105 (18), 91 (32), 84 (100), 79 (30), 67 (25), 56 (88); HREIMS *m*/*z* 362.1708 (calcd for $C_{20}H_{26}O_6$, 362.1729).

Laxiflorin L (3): colorless needles from Me_2CO; mp 302.5−304.0 °C; $[\alpha]_D^{15.1}$ −131.84° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (3.67) nm; IR (KBr) ν_{max} 3391, 2942, 2927, 2897, 2879, 1662, 1492, 1461, 1398, 1376, 1342, 1277, 1213, 1192, 1168, 1128, 1104, 1077, 1050, 1016, 991, 962, 947, 891, 855 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) spectral data, see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 2; EIMS *m*/*z* 346 $[M]^+$ (100), 328 (50), 318 (61), 300 (66), 288 (40), 269 (15), 257 (26), 243 (29), 231 (59), 229 (64), 215 (65), 201 (26), 187 (36), 173 (25), 159 (18), 145 (19), 129 (10), 115 (10), 105 (13), 91 (19), 79 (17), 69 (15), 55 (26); HREIMS *m*/*z* 346.1793 (calcd $C_{20}H_{26}O_5$, 346.1780).

Laxiflorin M (4): colorless needles from Me_2CO; mp 179.5−180.5 °C; $[\alpha]_D^{15.2}$ −56.00° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.67) nm; IR (KBr) ν_{max} 3663, 3442, 2952, 2887, 1723, 1457, 1374, 1332, 1252, 1202, 1155, 1127, 1062, 1041, 944, 913, 892, 858, 762, 727, 682, 526 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) spectral data, see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 2; EIMS *m*/*z* 404 $[M]^+$ (15), 386 (3), 376 (5), 361 (34), 344 (100), 326 (29), 316 (34), 301 (15), 287 (19), 273 (13), 255 (18), 245 (19), 231 (27), 211 (29), 203 (17), 187 (19), 167 (39), 149 (77), 135 (91), 121 (41), 105 (40), 91 (62), 79 (54), 69 (60), 55 (98); HREIMS *m*/*z* 404.1815 (calcd for $C_{22}H_{28}O_7$, 404.1835).

Tests of Cytotoxicity against Human Tumor K562 Cells. An improved MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay[16] (referred to as MTT method[17]) was performed in 96-well plates. The assay is based on reduction of MTT by the mitochondrial dehydrogenase of viable cells to yield a blue formazan product that can be measured spectrophotometrically. In the experiment, the negative controls were isochoric normal saline, 1% DMSO, or 0.1% DMSO; positive control was *cis*-platin at concentrations of 0.1, 1, and 10 μg/mL. K562 cells at a log phase of their growth cycle (5 × 10^4 cell/mL) were added to each well (90 μL/well), then treated in four replicates at various concentrations of the drugs (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} g/L) with six vacant reference wells set in one plate (100 μL cultured media in each well) and incubated for 48 h at 37 °C in a humidified atmosphere of 5% CO_2. After 48 h, 10 μL of MTT solution (5 mg/mL) was added to each well, which was incubated for another 4 h, after which a three-system solution of 10% SDS/5% isobutanol/0.012 mL/L HCl (w/v/v) was added to each well (100 μL/well). Twelve hours later at room temperature, the OD of each well was recorded on an ELISA reader (Bioteck EL-340) at two wavelengths (570 and 630 nm).

Tests of Cytotoxicity against Human Tumor A549 and T24 Cells. Exponentially growing A549 or T24 cells were dissociated with 0.25% trypsin and 0.02% $EDTANa_2$ in D-Hanks buffer and made into a single-cell suspension of density (4−5) × 10^4/mL by RPMI-1640 containing 10% heat-inactived bovine serum. Some of the cell suspension (90 μL) was inoculated into each well of a 96-well microplate. Cultures were preincubated for 24 h in 5% CO_2 atmosphere and 100% humidity incubator, then 10 μL controlled or test solution was added to each well of a 96-well microplate (each concentration was tested in quadruplex wells). The cells were incubated for an additional 72 h, then fixed by the addition of 50 μL of cold 50% trichloroacetic acid (TCA) at 4 °C for 1 h. After fixation, cultures were washed five times with tap water. After plates were air-dried, TCA-fixed cells of each well were stained with a 0.4% 50 μL SRB (sulforhodamin B) solution in 1% acetic for 30 min. At the end of the staining period, SRB was removed, cultures were rinsed four times with 1% acetic acid to remove unbound dye and air-dried until no standing moisture was visible, and 10 mM unbuffered Tris solution of 150 μL was added to each well. Plates were agitated for 5 min on a gyratory shaker, and OD was read on a plate reader at a wavelength of 570 nm.

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