Cytotoxic Indole Alkaloids from *Melodinus tenuicaudatus*

Tao Feng,[†,‡] Yan Li,[†] Yuan-Yuan Wang,[†] Xiang-Hai Cai,[†] Ya-Ping Liu,[†,‡] and Xiao-Dong Luo*,[†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, People's Republic of China, and Graduate School of Chinese Academy of Sciences, Beijing, People's Republic of China

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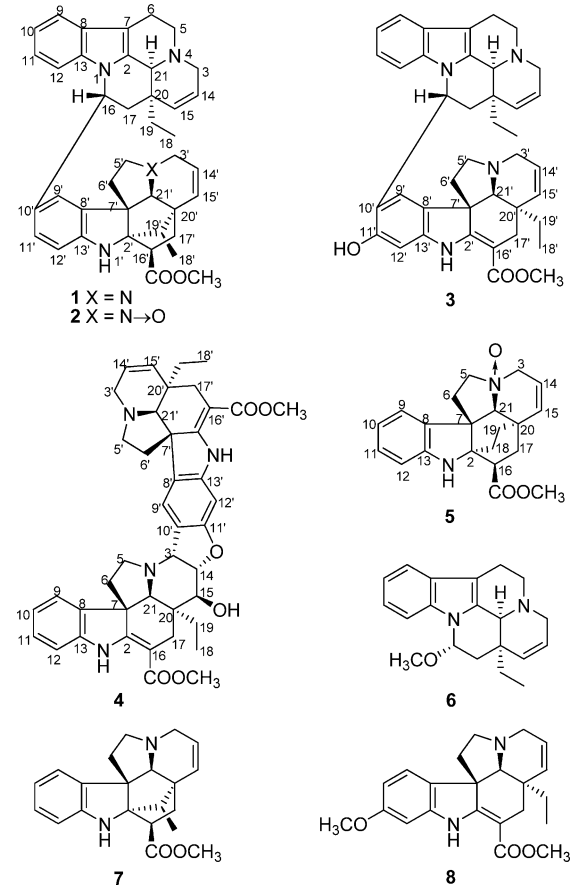
Four new bisindole alkaloids, melodinines H−K (**1**−**4**), a new monomer, melodinine L (**5**), and 11 known alkaloids were isolated from *Melodinus tenuicaudatus*. The structures of **1**−**5** were elucidated by extensive spectroscopic methods, and the known compounds were identified by comparison with data in the literature. All of the compounds were evaluated for their cytotoxicity against five human cancer cell lines. Alkaloids **1**, **3**, and **4** and the known compound 11-methoxytabersonine (**8**) exhibited inhibitory effects, with IC_{50} values comparable to those of cisplatin and vinorelbine.

Monoterpenoid indole and bisindole alkaloids have attracted the interest of many researchers due to their complicated structures and potent biological activities.[1] Many bisindole alkaloids have been isolated from plants, and most of them have been reported to display in vitro cytotoxicity against several human cancer cell lines.[2] The genus *Melodinus* is rich in monoterpenoid indole and bisindole alkaloids. A noteworthy feature of the *Melodinus* alkaloids is the preponderance of an aspidosperma unit. Previous crude alkaloid mixtures and purified alkaloids from some *Melodinus* species have demonstrated antitumor[3] and antibacterial activities.[4] Melodinines A−G and their cytotoxicity were reported earlier, from *M. henryi*.[5] Our current investigation of *Melodinus tenuicaudatus* Tsiang et P. T. Li (Apocynaceae)[6] has resulted in the isolation of four new bisindole alkaloids (**1**−**4**), named melodinines H−K, and a new monomer (**5**), named melodinine L. The new structures were elucidated by means of spectroscopic methods. Eleven known alkaloids were also isolated and identified as *O*-methyl-Δ^{14}-vincanol (**6**),[7] vindolinine (**7**),[8] 11-methoxytabersonine (**8**),[9] venalstonine,[8] 17-α-hydroxy-venalstonine,[10] 11-hydroxytabersonine,[11] 19-*R*-acetoxy-11-hydroxytabersonine,[12] 14,15-α-epoxy-11-hydroxytabersonine,[13] scandine,[14] 10-methoxyscandine,[15] and meloscandonine[16] by comparison with data in the literature. To the best of our knowledge, compounds **1** and **2** are the first examples of bisindole alkaloids containing a vindolinine unit. All of these compounds were evaluated for cytotoxicity against five human cancer cell lines.



Results and Discussion

Melodinine H (**1**) had UV absorption bands at 292, 286, 255, and 214 nm suggesting an indole chromophore,[17] and the IR absorption bands at 3381 and 1707 cm^{-1} indicated NH and ester functionalities. The molecular formula $C_{40}H_{44}N_4O_2$ was established by HRESIMS. The ^1H and ^{13}C NMR data revealed that **1** possessed three methyl, nine methylene, 16 methine, and 12 quaternary carbons (Table 1). The structure of **1** was investigated using 2D NMR spectra, including HSQC, HMBC, $^1H-^1H$ COSY, and ROESY spectra in Me_2CO-d_6. All of the data suggested that **1** was a bisindole alkaloid comprised of two units, A and B, as shown in Figure 1.

In unit A, the $^1H-^1H$ COSY spectrum revealed five partial structures (**a**, −CHCHCHCH−; **b**, $-CH_2CH_2-$; **c**, $-CH_2CHCH-$; **d**, $-CHCH_2-$; **e**, $-CH_2CH_3$) (Figure 1). Partial structure **a** was ascribed to the unsubstituted indole moiety. In the HMBC spectrum, the correlations of δ_H 4.08 (s, H-21) with δ_C 135.5 (s, C-2), 44.7 (t, C-3), 50.7 (t, C-5), and 38.3 (s, C-20) suggested the connections of partial structures **b** and **c** and C-21 with N-4 and the connections of C-21 with C-2 and C-21 with C-20. In addition, the HMBC correlations also revealed the connections of structures **c**−**e** and C-21 with C-20. A downfield signal at δ_H 4.66 (1H, dd, J = 11.6, 4.0 Hz) in the ^1H NMR spectrum, ascribed to H-16, showed HMBC correlations with δ_C 137.6 (s, C-13), which allowed the connection of C-16 (δ_C 57.6, d) with N-1. The above data suggested that unit A possessed an eburnan-type skeleton as in *O*-methyl-Δ^{14}-epivincanol (**6**).[7] In unit B, the $^1H-^1H$ COSY correlations revealed partial structures (**a′**, −CHCH−; **b′**, $-CH_2CH_2-$; **c′**, $-CH_2CHCH-$; **d′**, $-CHCH_2-$; **e′**, $-CH_2CH_3$) (Figure 1). In the ^1H NMR spectrum, a singlet at δ_H 7.11 (1H, s, H-9′) and two doublets [δ_H 7.00 (1H, d, J = 8.0 Hz, H-11′) and 6.66 (1H, d, J = 8.0 Hz, H-12′)] suggested that the phenyl ring was substituted at C-10′. A methyl doublet at δ_H 0.99 (3H, d, J = 6.8 Hz) allowed a methine to be at C-19′. Signals at δ_C 174.9 and 51.8 were assigned to carbons of a methyl ester functionality. Two characteristic quaternary carbons

* To whom correspondence should be addressed. Tel: +86-871-5223177. Fax: +86-871-5150227. E-mail: xdluo@mail.kib.ac.cn.
† Kunming Institute of Botany, Chinese Academy of Sciences.
‡ Graduate School of Chinese Academy of Sciences.

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Table 1. ^{1}H and ^{13}C NMR Data of **1** and **2** (δ in ppm and J in Hz)

	1		**2**	
entry	δ_H	δ_C	δ_H	δ_C
2		135.5, qC		135.6, qC
3a	2.80, m	44.7, CH_2	2.80 m	44.7, CH_2
3b	2.97, m		3.01 m	
5a	3.36, m	50.7, CH_2	3.32 m	50.0, CH_2
5b	3.69, m		3.34 m	
6a	2.46, dd (15.6, 6.8)	17.4, CH_2	2.47 dd (16.0, 6.0)	17.5, CH_2
6b	3.15, m		3.11 m	
7		105.2, qC		105.4, qC
8		129.9, qC		130.0, qC
9	7.31, d (8.0)	118.2, CH	7.32 d (7.5)	118.3, CH
10	6.83, t (8.0)	119.5, CH	6.84 t (7.5)	119.6, CH
11	6.70, t (8.0)	120.5, CH	6.67 t (7.5)	120.6, CH
12	6.39, d (8.0)	112.8, CH	6.47 t (7.5)	112.9, CH
13		137.6, qC		137.8, qC
14	5.55, m	127.7, CH	5.90 br, s	125.7, CH
15	5.64, d (10.4)	128.1, CH	5.56 overlap	127.8, CH
16	4.66, dd (11.6, 4.0)	57.6, CH	4.61 dd (9.8, 3.6)	57.8, CH
17a	1.94, dd (14.6, 11.6)	45.7, CH_2	1.95 overlap	45.7, CH_2
17b	2.17, dd (14.0, 4.0)		2.13 dd (13.0, 3.5)	
18	0.92, t (7.6)	8.8, CH_3	0.88 t (7.5)	8.9, CH_3
19a	1.52, dq (15.2, 7.6)	34.9, CH_2	1.51 dq (13.0, 7.5)	35.0, CH_2
19b	1.86, dq (15.2, 7.6)		1.84 dq (13.0, 7.5)	
20		38.3, qC		38.4, qC
21	4.08, s	58.7, CH	4.06 s	58.8, CH
2′		82.8, qC		82.4, qC
3a′	3.31, m	50.0, CH_2	4.31 m	69.5, CH_2
3b′	3.33, m		4.50 m	
5a′	3.24, m	58.4, CH_2	4.13 overlap	73.8, CH_2
5b′	3.26, m		4.13 overlap	
6a′	1.74, overlap	36.9, CH_2	2.29 m	34.8, CH_2
6b′	2.21, m		2.34 m	
7′		60.1, qC		59.2, qC
8′		140.4, qC		139.2, qC
9′	7.11, br s	122.3, CH	7.55 br s	124.3, CH
10′		134.8, qC		135.1, qC
11′	7.00, d (8.0)	126.6, CH	7.03 d (7.7)	127.5, CH
12′	6.66, d (8.0)	111.2, CH	6.70 d (7.7)	111.5, CH
13′		150.6, qC		150.3, qC
14′	5.67, m	129.5, CH	5.56 overlap	128.1, CH
15′	6.06, d (9.6)	131.7, CH	6.25 br, s	132.0, CH
16′	3.07, dd (12.0, 6.4)	39.8, CH	3.26 dd (12.4, 5.5)	39.6, CH
17a′	1.74, overlap	29.6, CH_2	1.95 overlap	27.9, CH_2
17b′	2.35, dd (13.6, 6.4)		2.40 m	
18′	0.99, d (6.8)	7.4, CH_3	1.00 br, s	7.1, CH_3
19′	1.98, q (6.8)	49.3, CH	2.03 m	49.2, CH
20′		46.6, qC		46.6, qC
21′	3.18, s	79.5, CH	3.78 br, s	92.7, CH
CO_2Me'		174.9, qC		174.4, qC
CO_2Me'	3.64, s	51.8, CH_3	3.69 s	52.4, CH_3

at δ_C 38.3 and 60.1 were assigned to C-20′ and C-7′, respectively, as revealed by HMBC correlations as shown in Figure 1. In addition, a quaternary carbon at δ_C 82.8 was assigned to C-2′, as supported by the HMBC correlations of δ_H 3.07 (1H, dd, J = 12.0, 6.4 Hz, H-16′) and 1.98 (1H, q, J = 6.8 Hz, H-19′) with it (Figure 1). These data suggested that unit B possessed a vindolinine skeleton as in **7**.[8] Finally, the linkage of units A and B by C-16/10′ was established by the HMBC correlations of δ_H 4.66 (1H, dd, J = 11.6, 4.0 Hz, H-16) with δ_C 134.8 (s, C-10′), 122.3 (d, C-9′), and 126.6 (d, C-11′) (Figure 1).

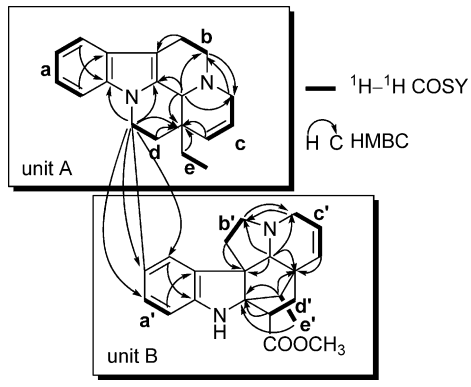

Figure 1. Selected HMBC and $^{1}H-^{1}H$ COSY correlations of **1**.

The relative configuration of **1** was elucidated by the ROESY spectrum and the $^{1}H-^{1}H$ coupling constants. The coupling constant of H-16 (dd, J = 11.6, 4.0 Hz) suggested H-16 to be β-oriented by comparison with that of *O*-methylvincanol (dd, J = 9.5, 5.7 Hz)[18] and celastromelidine (J = 10.0, 5.0 Hz).[19] The ROESY correlation of H-16/H-15 and H-21/H-19 suggested that H-21 and the ethyl were on the other side.[20] The ROESY results of H-19′/H-21′ and H-18′/H-16′ suggested that both H-19′ and H-16′ were α-oriented. Thus, the structure of melodinine H (**1**) was established as shown.

Melodinine I (**2**) had UV absorption bands at 293, 286, 259, 213, and 194 nm, and IR absorption bands at 3424 and 1709 cm^{-1} indicated the NH and ester functionalities. The HRESIMS displayed an $[M + Na]^+$ peak at m/z 651.3307, analyzed for $C_{40}H_{44}N_4O_3$, 16 mass units higher than that of **1**. Compound **2** was readily identified

Table 2. ^{1}H and ^{13}C NMR Data of **3** and **4** (δ in ppm and J in Hz)

	3		**4**	
entry	δ_H	δ_C	δ_H	δ_C
1			8.91, s	
2		135.1, qC		164.1, qC
3a	2.73, m	43.7, CH_2	4.84, d (8.0)	59.6, CH
3b	2.98, m			
5a	3.26, m	48.9, CH_2	2.90, m	45.8, CH_2
5b	3.28, m		2.97, m	
6a	2.43, overlap	16.5, CH_2	1.64, m	41.5, CH_2
6b	3.06, m		1.99, m	
7		104.4, qC		53.8, qC
8		128.5, qC		137.6, qC
9	7.27, d (8.0)	117.4, CH	6.02, d (7.3)	121.2, CH
10	6.79, t (8.0)	118.6, CH	6.39, t (7.3)	120.2, CH
11	6.57, t (8.0)	119.7, CH	6.97, t (7.3)	127.6, CH
12	6.10, d (8.0)	111.7, CH	6.69, d (7.3)	109.1, CH
13		136.4, qC		143.0, qC
14	5.56, overlap	126.8, CH	5.09, dd (8.0, 3.5)	84.8, CH
15	5.56, overlap	127.5, CH	4.14, d (3.5)	69.5, CH
16	5.04, br, d (10.6)	49.1, CH		90.8, qC
17a	1.88, dd (13.8, 10.6)	42.4, CH_2	2.40, d (15.5)	22.2, CH_2
17b	2.13, br, d (13.8)		2.76, d (15.5)	
18	0.88, t (7.5)	8.6, CH_3	0.68, t (7.0)	7.3, CH_3
19a	1.61, m	33.8, CH_2	0.85, qd (14.8, 7.0)	26.2, CH_2
19b	1.82, m		1.14, qd (14.8, 7.0)	
20		37.1, qC		44.8, qC
21	4.09, s	57.3, CH	2.58, s	65.2, CH
CO_2Me				168.7, qC
CO_2*Me*			3.77, s	50.9, CH_3
1′	9.61, s		8.99, s	
2′		166.4, qC		166.5, qC
3a′	2.82, m	49.8, CH_2	3.20, d (16.0)	50.5, CH_2
3b′	3.21, m		3.48, dd (16.0, 3.3)	
5a′	2.42, m	50.1, CH_2	2.73, m	50.8, CH_2
5b′	2.85, m		3.06, dd (15.0, 6.5)	
6a′	1.58, m	44.8, CH_2	1.78, dd (11.5, 4.5)	44.7, CH_2
6b′	1.77, m		2.07, m	
7′		54.4, qC		54.5, qC
8′		128.9, qC		130.9, qC
9′	6.87, s	119.7, CH	7.20, s	119.1, CH
10′		118.6, qC		113.3, qC
11′		153.9, qC		160.9, qC
12′	6.72, s	98.3, CH	6.33, s	92.9, CH
13′		143.7, qC		145.0, qC
14′	5.56, overlap	125.2, CH	5.80, overlap	124.7, CH
15′	5.48, d (10.0)	132.7, CH	5.80, overlap	132.5, CH
16′		90.8, qC		92.3, qC
17a′	2.29, overlap	29.1, CH_2	2.46, d (16.0)	27.5, CH_2
17b′	2.29, overlap		2.62, d (16.0)	
18′	0.36, t (7.4)	7.4, CH_3	0.80, t (7.5)	7.5, CH_3
19a′	0.53, m	26.0, CH_2	0.99, dq (15.3, 7.5)	26.9, CH_2
19b′	0.78, m		1.24, dq (15.3, 7.5)	
20′		40.8, qC		41.3, qC
21′	2.15, s	68.5, CH	2.69, s	71.3, CH
CO_2Me′		167.3, qC		168.7, qC
CO_2*Me*′	3.66, s	50.7, CH_3	3.78, s	51.0, CH_3
OH	9.67, s			

as the *N*(4)′-oxide of **1** from ^{1}H and ^{13}C NMR data (Table 1), in particular the characteristic downfield shifts of the carbon resonances at δ_C 69.5, 73.8, and 92.7 for C-3′, C-5′, and C-21′, respectively, with respect to those of **1**. Detailed analysis of 2D NMR data confirmed that the other parts of the molecule were the same as those of **1**.

Melodinine J (**3**) had IR absorption bands at 3432, 3368, and 1675 cm^{-1}, suggesting the presence of NH, OH, and ester functions. The molecular formula $C_{40}H_{44}N_4O_3$ was established by HREIMS ($[M]^+$ at *m/z* 628.3406). Preliminary analysis of ^{1}H and ^{13}C NMR data (Table 2) suggested that **3** was a bisindole alkaloid with the same structure as that of demethyhenuicausin.[21] The specific rotation, $[\alpha]_D^{20}$ +2.0 (*c* 0.10, $CHCl_3$), suggested that the configuration of **3** might be different from that of demethyhenuicausin ($[\alpha]_D^{13}$ −198.5).[21] The coupling constant ($J_{16,17a}$ = 10.6 Hz) of H-16 indicated the β-orientation of H-16,[18,19] and the key ROESY correlations of H-16/H-15 and H-21/H-19 suggested that H-21 and the ethyl group were α-oriented, with respect to that of **1**. In addition, the ROESY correlations of H-9′/H-21′ and H-21′/H-19′ suggested that both H-21′ and C-19′ were α-oriented. Thus, the structure of compound **3** was established, as shown.

The UV spectrum of melodinine K (**4**) showed absorption maxima characteristic of a β-anilinoacrylate chromophore (330, 242 nm), while the IR spectrum showed absorption bands due to NH (3432, 3377 cm^{-1}) and conjugated ester (1678 cm^{-1}) functions.[22] The molecular formula $C_{42}H_{46}N_4O_6$ was established by HRESIMS. In the ^{1}H NMR spectrum (Table 2), two broad singlets at δ_H 8.91 (1H) and 8.99 (1H) were resonances suggesting two NH groups of aspidosperma-type alkaloids. Two triplets at δ_H 0.68 (3H, t, J = 7.0 Hz, Me-18) and 0.80 (3H, t, J = 7.5 Hz, Me-18′) were assigned

Table 3. Cytotoxicity of Compounds **1**, **3**, **4**, **6**, **7**, and **8** (IC_{50}, μmol)

compd	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	1.1	3.2	4.8	2.9	1.4
3	3.0	8.5	9.1	10.0	14.8
4	0.1	3.0	5.0	2.7	5.7
6	15.9	22.1	>40	29.3	32.2
7	6.8	20.7	26.3	21.9	15.2
8	0.2	13.1	12.8	2.1	12.7
cisplatin	2.4	11.2	17.6	18.7	14.9
vinorelbine	<0.06	4.7	26.1	17.2	>40

to protons of two methyl groups, and singlets at δ_H 3.77 (3H, s, OMe) and 3.78 (3H, s, OMe′) were assigned to protons of two methoxy groups. In the ^{13}C NMR spectrum (Table 2), 42 carbon resonances were observed. Of them, six quaternary carbon resonances (δ_C 90.8, 92.3, 164.1, 166.5, 168.7, 168.7) and two methoxy signals (δ_C 50.9, 51.0) were readily assigned to two β-anilinoacrylate moieties conjugated with a methyl ester unit, respectively. According to the 1D NMR data, compound **4** was identified as a bisindole alkaloid and divided into two aspidosperma-type units similar to tabersonine.[23] Three carbon signals at δ_C 59.6 (d, C-3), 84.8 (d, C-14), and 69.5 (d, C-15) allowed the connection of a heteroatom to them, and the $^1H-^1H$ COSY correlations of δ_H 4.84 (1H, d, J = 8.0 Hz, H-3)/5.09 (1H, dd, J = 8.0, 3.5 Hz, H-14)/4.14 (1H, d, J = 3.5 Hz, H-15) revealed the connection of C-3/C-14/C-15. The HMBC correlations of H-3 with δ_C 113.3 (s, C-10′) and of H-14 with δ_C 160.9 (s, C-11′) suggested linkage of two units by bonds of C-3−C-10′ and C-15−O−C-11′. The above data suggested that compound **4** was similar to conophyllidine[24] except the aromatic carbons of C-9 to C-12 in **4** were unsubstituted, as supported by $^1H-^1H$ COSY correlations from H-9 to H-12. Detailed analysis of 2D NMR (HSQC, HMBC, and $^1H-^1H$ COSY) correlations suggested that other parts of **4** were the same as those of conophyllidine. The coupling constants of H-3, H-14, and H-15 and the ROESY correlations of H-3/H-5b, H-14/H-17a, and H-15/H-19 suggested the relative configuration of **4** was the same as that of conophyllidine. The structure of compound **4** was established, therefore, as shown.

Melodinine L (**5**) had an $[M + H]^+$ peak at *m/z* 353.1865 ($C_{19}H_{22}N_2O$) in the HRESIMS, 16 mass units higher than that of venalstonine.[7] Compound **5** was readily identified as venalstonine-*N*(4)-oxide from ^1H and ^{13}C NMR data, in particular, the characteristic downfield shifts of the carbon resonances at δ_C 65.7, 69.1, and 82.6 for C-3, C-5, and C-21, respectively, with respect to those of venalstonine. Detailed analysis of 2D NMR (HSQC, HMBC, and ROESY) data confirmed that the other parts were the same as those of venalstonine.

All compounds were evaluated for cytotoxicity against five human cancer cell lines using the MTT method as reported previously.[25] Compounds **1**, **3**, **4**, **6**, **7**, and **8** showed significant cytotoxicity, and their IC_{50} values are presented in Table 3. The other compounds were inactive (IC_{50} values of >40 μmol). It is noteworthy that bisindole alkaloids **1**, **3**, and **4** and the known compound 11-methoxytabersonine (**8**) exhibited significant inhibitory effects against five human cancer cell lines, with IC_{50} values similar to those of cisplatin and vinorelbine.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrometer. IR spectra were obtained on a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. 1D and 2D NMR spectra were run on a Bruker DRX-500 MHz spectrometer or an AV-400 MHz spectrometer with TMS as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to solvent signals. HREIMS was recorded on a Waters Auto Premier P776 spectrometer. HRESIMS were recorded on an API QSTAR Pulsar 1 spectrometer. Column chromatography (CC) was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China), RP-18 gel (20−45 μm, Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden). Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd. Qingdao), and spots were visualized by Dragendorff's reagent.

Plant Material. *M. tenuicaudatus* was collected from Mengna County, Yunnan Province, P. R. China, and identified by Mr. Jing-Yun Cui, Xishuangbanna Tropical Plant Garden. A voucher specimen (No. Cui20081129) has been deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. An air-dried and powdered sample (14 kg) was extracted with 90% EtOH (24 h × 3). The extract was partitioned between EtOAc and 0.5% HCl solution. The acidic water-soluble materials, adjusted to pH 9−10 with 10% ammonia solution, were extracted with EtOAc to give an alkaloidal extract (17 g). The extract was subjected to silica gel CC (petroleum ether−acetone, 1:0 to 0:1) to afford fractions 1−7. Fraction 1 (1.8 g) was separated by silica gel CC (petroleum ether−Me_2CO, 15:1−3:1) to afford venalstonine (130 mg), **6** (4 mg), and **8** (670 mg). Fraction 3 (2.3 g) was subjected to silica gel CC (petroleum ether−Me_2CO, 6:1−1:1) to yield 17-α-hydroxyvenalstonine (13 mg), 11-hydroxytabersonine (130 mg), and scandine (720 mg). Fraction 4 (1.1 g) was chromatographed on silica gel (petroleum ether−Me_2CO, 3:1−1:1) and further purified by RP-18 CC (MeOH−H_2O, 6:4) to yield 19-*R*-acetoxy-11-hydroxytabersonine (13 mg) and 14,15-α-epoxy-11-hydroxytabersonine (10 mg). Fraction 5 (3 g) was separated by silica gel CC ($CHCl_3$−MeOH, 15:1) to yield **3** (20 mg), **4** (16 mg), and a mixture (800 mg). Meloscandonine (200 mg) precipitated from the mixture. Further separation of the mixture by RP-18 CC (MeOH−H_2O, 5:5) led to the isolation of **7** (78 mg). Fraction 6 (1.1 g) was subjected to silica gel CC ($CHCl_3$−MeOH, 10:1) to afford **1** (5 mg) and a mixture. 10-Methoxyscandine (80 mg) precipitated from the mixture. Fraction 7 (800 mg) was separated by RP-18 CC (CH_3OH-H_2O, 3:7−5:5) and purified further by Sephadex LH-20 CC (MeOH) to yield **2** (4 mg) and **5** (11 mg).

Melodinine H (1): colorless oil; $[\alpha]_D^{20}$ −10.8 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 292 (4.07), 286 (4.07), 255 (4.22), 214 (4.91) nm; IR (KBr) ν_{max} 3381, 2933, 2873, 1707, 1613, 1489, 1455, 1205, 1096, 742 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (100 MHz) data (Me_2CO-d_6), see Table 1; positive ion HRESIMS *m/z* 613.3561 (calcd for $C_{40}H_{45}N_4O_2$ $[M + H]^+$, 613.3542).

Melodinine I (2): colorless oil; $[\alpha]_D^{20}$ −6.7 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 293 (3.87), 286 (3.87), 259 (4.00), 213 (4.86), 194 (4.21) nm; IR (KBr) ν_{max} 3424, 2920, 1709, 1639, 1487, 1454, 1164, 1058, 741 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (125 MHz) data (Me_2CO-d_6), see Table 1; positive ion HRESIMS *m/z* 651.3307 (calcd for $C_{40}H_{44}N_4O_3Na$ $[M + Na]^+$, 651.3311).

Melodinine J (3): colorless oil; $[\alpha]_D^{20}$ +2.0 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 330 (3.71), 294 (3.75), 236 (4.14), 219 (3.88), 205 (3.87), 193 (3.86) nm; IR (KBr) ν_{max} 3432, 3368, 2921, 1675, 1618, 1455, 1265, 1157, 1104, 741 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (DMSO-d_6), see Table 2; HREIMS *m/z* 628.3406 (calcd for $C_{40}H_{44}N_4O_3$ $[M]^+$, 628.3413).

Melodinine K (4): white powder; $[\alpha]_D^{20}$ −129.6 (*c* 0.24, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 330 (4.99), 242 (4.79) nm; IR (KBr) ν_{max} 3432, 3377, 2922, 1678, 1610, 1469, 1437, 1263, 1101, 745 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see Table 2; positive ion HRESIMS *m/z* 703.3481 (calcd for $C_{42}H_{47}N_4O_6$ $[M + H]^+$, 703.3495).

Melodinine L (5): colorless oil; $[\alpha]_D^{20}$ −62.3 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 289 (3.69), 241 (4.06), 224 (3.74), 220 (3.74), 213 (3.72), 205 (3.72) nm; IR (KBr) ν_{max} 3425, 2950, 1725, 1605, 1464, 1174, 752 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.21 (1H, d, J = 7.5 Hz, H-9), 7.01 (1H, t, J = 7.5 Hz, H-11), 6.77 (1H, t, J = 7.5 Hz, H-10), 6.62 (1H, d, J = 7.5 Hz, H-12), 5.67 (1H, d, J = 9.7 Hz, H-15), 5.62 (1H, m, H-14), 4.44 (2H, br s, H-3), 3.91−3.87 (2H, m, H-5), 3.78 (1H, s, H-21), 3.75 (3H, s, OMe), 2.94 (1H, m, H-16), 2.88 (1H, m, H-6b), 2.34 (1H, m, H-17b), 2.15 (1H, m, H-6a), 2.04 (1H, m, H-18b), 1.78 (1H, m, H-19b), 1.71 (1H, m, H-17a), 1.60 (1H, t, J = 12.0 Hz, H-19a), 1.30 (1H, t, J = 12.0 Hz, H-18a); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9 (C, CO_2Me), 148.5 (C, C-13), 136.4 (C, C-8), 130.0 (CH, C-15), 127.7 (CH, C-11), 126.6 (CH, C-9), 120.5 (CH, C-10), 120.1 (CH, C-14), 82.6 (CH, C-21), 69.1 (CH_2, C-5), 65.8 (C, C-2), 65.7 (CH_2, C-3), 58.7 (C, C-7), 52.3 (CH_3, CO_2Me), 43.1 (CH, C-16), 35.2 (C, C-20), 32.9 (CH_2, C-18), 32.5 (CH_2, C-17), 32.2 (CH_2,

C-6), 32.2 (CH_2, C-19); positive ion HRESIMS *m/z* 353.1865 (calcd for $C_{21}H_{25}N_2O_3$ $[M + H]^+$, 353.1866).

Cytotoxicity Assay. Five human cancer cell lines, breast cancer MCF-7, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, colon cancer SW480, and lung cancer A-549 cells, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method in 96-well microplates.[25] Briefly, 100 μL of adherent cells was 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 with an initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the test compound dissolved in DMSO at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μmol in triplicates for 48 h, with cisplatin (Sigma, USA) and vinorelbine (National Institute for the Control of Pharmaceutical and Biological Products, P. R. China) as positive controls. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method.[26]

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Supporting Information Available: 1D and 2D NMR and MS spectra of melodinines H−L (**1**−**5**). These materials are available free of charge via the Internet at http://pubs.acs.org.

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