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Melodinines M−U, Cytotoxic Alkaloids from *Melodinus suaveolens*

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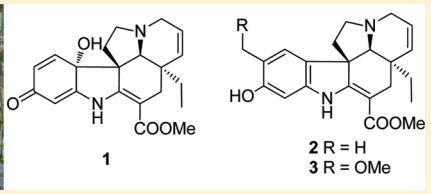
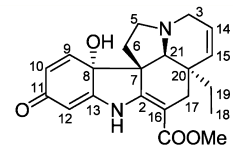
Supporting Information

ABSTRACT: Nine new alkaloids, melodinines M−U (**1**−**9**), and 11 known alkaloids were isolated from *Melodinus suaveolens*. The new structures were elucidated by extensive NMR and mass spectroscopic analyses and comparison to known compounds. All compounds were evaluated for their cytotoxicity against five human cancer cell lines. Compounds **6**, **11**, and **16** showed significant cytotoxicity.



Plants of the family Apocynaceae have been proven to be good resources of monoterpenoid indole alkaloids. These originate from condensation of tryptophan with secologanin to give strictosidine, which then elaborates to give an impressive array of structural variants.[1] Many of them, such as yohimbine,[2] reserpine,[3] and camptothecin,[4] are well known for their pharmacological significance. Previous pharmacological investigations on the crude and purified alkaloids from some *Melodinus* plants have demonstrated promising antitumor[5] and antibacterial activities.[6] Our previous studies reported the isolation and cytotoxic activities of melohenines A and B, melotenine A, and melodinines A−L from two species of *Melodinus*.[7] Continuation of our study on the genus *Melodinus* has led to the isolation of seven new monoterpenoid indole alkaloids, melodinines M−S (**1**−**7**), two new monoterpenoid quinoline alkaloids, melodinines T and U (**8**, **9**), and 11 known alkaloids from *Melodinus suaveolens* Champ. ex Benth (Apocynaceae).[8] To the best of our knowledge, compound **1** is the first *Aspidosperma*-type alkaloid possessing a dienone ring A. Compounds **2** and **3** are *Aspidosperma*-type alkaloids each bearing a methyl group at C-10, a type seldom reported previously. Structures of the new alkaloids were elucidated by spectroscopic methods, while the known alkaloids were identified as 11-hydroxytabersonine (**10**),[9] 11-methoxytabersonine (**11**),[10] tabersonine (**12**),[11] 19-(*R*)-acetoxytabersonine (**13**),[12] 11-methoxy-19-(*R*)-hydroxytabersonine (**14**),[13] lochnericine (**15**),[14] 3α-acetonyltabersonine (**16**),[15] 3-oxo-tabersonine,[16] venalstonine,[17] scandine,[18] and 10-hydroxyscandine[19] by comparison with data (1D NMR and MS) in the literature. All of the compounds were evaluated for cytotoxicity against five human cancer cell lines.



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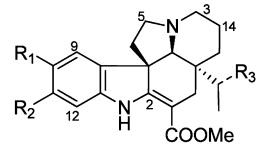
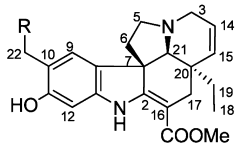
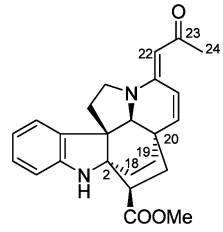


	R_1	R_2	R_3	
4	OH	H	H	$\Delta^{14,15}$
10	H	OH	H	$\Delta^{14,15}$
11	H	OMe	H	$\Delta^{14,15}$
12	H	H	H	$\Delta^{14,15}$
13	H	H	OAc	$\Delta^{14,15}$
14	H	OMe	OH	$\Delta^{14,15}$
15	H	H	H	14,15-α-epoxy



2 R = H
3 R = OMe



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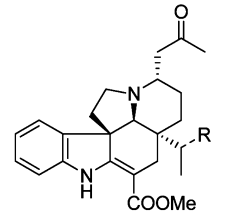
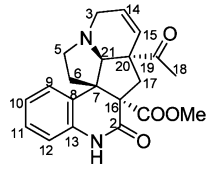


6 R = OH, $\Delta^{14,15}$
7 R = H, 14,15-α-epoxy
16 R = H, $\Delta^{14,15}$



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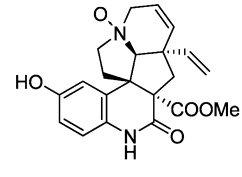

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RESULTS AND DISCUSSION

Melodinine M (**1**) was isolated as yellow needles and gave a positive reaction with Dragendorff's reagent. Its molecular formula was established as $C_{21}H_{24}N_2O_4$ by the molecular ion at m/z 369.1811 $[M + H]^+$ in the HRESIMS, indicating 11 degrees of unsaturation. The IR absorption bands at 3431, 1656, and 1610 cm^{-1} suggested the presence of a β-anilinoacrylate

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system,[20] corresponding to carbon signals characteristic of an acrylate double bond at δ_C 158.5 (C-2) and 98.6 (C-16). The ^{1}H NMR spectrum displayed two olefinic signals at δ_H 5.80 (ddd, J = 10.2, 4.8, 1.2 Hz) and 5.62 (d, J = 10.2 Hz), which were ascribed to protons of a double bond between C-14 and C-15. An indolic −NH proton was also observed at δ_H 9.32 (1H, s) (Table 1). The ^{13}C NMR and DEPT spectra of compound **1** displayed 21 carbon resonances assigned to two methyl, five methylene, six methine, and eight quaternary carbons (Table 3). These data suggested that **1** was an *Aspidosperma*-type alkaloid related to 11-hydroxytabersonine (**10**) with identical rings B−E (Figure S1, Supporting Information).[11,21] A significant difference was that the benzene ring A was oxidized to a dienone system, as deduced from three deshielded olefinic protons at δ_H 6.90 (d, J = 9.6 Hz), 6.20 (dd, J = 9.6, 1.2 Hz), and 5.56 (d, J = 1.2 Hz),[22] as well as the carbonyl carbon resonance at δ_C 186.2. The dienone system was further determined as 9(10),12(13)-dien-11-one by HMBC and ^{1}H−^{1}H COSY correlations (Figure S1, Supporting Information). In addition, C-8 was an oxygenated quaternary carbon at δ_C 74.9, on the basis of the HMBC correlations from H-6, H-10, H-12, and N-H to C-8. The relative configuration of **1** was assigned on the basis of the ROESY experiment measured in DMSO (Figure S1, Supporting Information). The ROESY correlations of OH-8/H-21 and H-21/H-19 indicated the α-orientation of OH-8, H-21, and H-19. Thus, the structure of melodinine M (**1**) was established as shown.

Table 1. ^{1}H NMR Data of 1−4[α] (δ in ppm and J in Hz)

no.	1	2	3	4
N_1-H	9.32, s	9.20, s	9.27, s	9.16, s
3a	3.00, d (15.6)	3.09, overlap	3.17, d (15.6)	3.17, d (10.2)
3b	3.38, ddd (15.6, 4.8, 1.2)	3.41, dd (15.6, 3.6)	3.41, ddd (15.6, 4.8, 1.2)	3.42, ddd (10.2, 4.8, 1.5)
5a	2.99, overlap	2.64, m	2.76, m	2.70, m
5b	2.32, m	2.94, overlap	2.98, m, overlap	3.00, t (7.8)
6a	1.67, m	1.63, dd (10.8, 3.6)	1.69, dd (11.4, 4.2)	1.73, dd (11.4, 3.6)
6b	1.74, dd (12.6, 4.8)	1.93, m	1.95, m	2.97, m, overlap
9	6.90, d (9.6)	7.12, s	7.14, s	6.74, d (2.4)
10	6.20, dd (9.6, 1.2)			
11				6.62, dd (7.8, 2.4)
12	5.56, d (1.2)	6.60, s	6.59, s	6.86, d (7.8)
14	5.80, ddd (10.2, 4.8, 1.2)	5.75, dd (10.2, 3.6)	5.77, ddd (9.9, 4.8, 1.2)	5.78, ddd (9.6, 4.8, 1.5)
15	5.62, d (10.2)	5.66, d (10.2)	5.68, d (9.9)	5.70, d (9.6)
17a	2.43, s	2.43, d (15.0)	2.44, d (14.4)	2.44, d (15.0)
17b		2.48, d (15.0)	2.50, d (14.4)	2.50, d (15.0)
18	0.79, t (7.2)	0.59, t (7.2)	0.68, t (7.5)	0.63, t (7.8)
19a	1.44, q (7.2)	0.79, m	0.83, m	0.84, m
19b		0.96, m	0.98, m	0.98, m
21	2.66, s	2.56, s	2.62, s	2.62, s
22		2.08, s	4.43, s	
OOC*Me*	3.78, s	3.66, s	3.68, s	3.67, s
O*Me*			3.33, s	

[α]Compound **1** was measured in $CDCl_3$; **2**, **3**, and **4** in acetone-d_6.

Melodinine N (**2**) had the molecular formula $C_{22}H_{26}N_2O_3$ as established by HRESIMS. The UV spectrum showed absorption maxima characteristic of a β-anilinoacrylate chromophore (333, 255, and 203 nm), while the IR spectrum showed absorption bands due to −NH (3439 cm^{-1}) and conjugated ester (1669 cm^{-1}) functions.[20] The 1D (Tables 1 and 3) and 2D NMR data of **2** were similar to those of **10** except for one more methyl (Me-22) substituted at C-10, as supported by the HMBC correlations of δ_H 2.08 (3H, s) with δ_C 123.8 (d, C-9), 120.4 (s, C-10), and 155.0 (s, C-11). ROESY correlations of H-9/H-21 and H-21/H-19 indicated that the relative configuration of **2** was also the same as that of **10**. Analysis of 2D NMR data (HSQC, HMBC, ROESY) established the structure of **2** to be as shown, and it was named melodinine N.

Melodinine O (**3**) showed almost the same NMR spectra as **2**, except for a methoxy in **3** instead of a hydrogen (Me-22) in **2**, as indicated by HMBC correlations from δ_H 3.33 (3H, s) to δ_C 71.0 (t, C-22) and from δ_H 4.43 (2H, s, H-22) to δ_C 123.0 (d, C-9), 116.9 (s, C-10), and 156.5 (s, C-11). Other parts of the structure were identical to those of **2** by detailed analysis of 2D NMR data.

Melodinine P (**4**) was isolated as a colorless oil. The UV spectrum showed absorption maxima characteristic of a β-anilinoacrylate chromophore (342, 313, and 204 nm), and the IR spectrum showed absorption bands due to −NH (3427 cm^{-1}) and conjugated ester (1669 cm^{-1}) functions. The HREIMS gave the molecular formula $C_{21}H_{24}N_2O_3$, identical to that of 11-hydroxytabersonine (**10**). The NMR data also suggested that **4** might be the same as **10**. Detailed comparison of their 1D NMR data suggested that the OH group was at C-10 in **4**, rather than at C-11 in **10**, which was further supported by the HMBC correlations of H-9 (δ_H 6.74, d, J = 2.4 Hz), H-11 (δ_H 6.62, dd, J = 7.8, 2.4 Hz), and H-12 (δ_H 6.86, d, J = 7.8 Hz) with C-10 (δ_C 152.8, s). Analysis of 2D NMR data confirmed that the other parts were the same as those of **10**. Hence, the structure of melodinine P (**4**) was determined as shown.

The molecular formula of melodinine Q (**5**) was determined to be $C_{24}H_{26}N_2O_3$ by the positive HRESIMS. The IR spectrum suggested the presence of −NH (3440 cm^{-1}), carboxyl (1718 cm^{-1}), and double-bond (1640, 1614 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra of **5** displayed 24 carbon resonances ascribed to two methyl, five methylene, nine methine, and eight quaternary carbons (Table 3). These data resembled those of venalstonine.[17] One visible difference was that **5** possessed three additional carbon signals at δ_C 31.4 (q), 195.6 (s), and 96.1 (d). In the HMBC spectrum, the correlations of δ_H 2.11 (3H, s) with δ_C 195.6 (s) and 96.1 (d) established a CH_3−CO−CH− unit among C-24, C-23, and C-22, while the HMBC correlation of δ_H 5.09 (1H, s, H-22) with δ_C 150.7 (s, C-3) suggested the unit to be connected to C-3. ROESY correlations of H-9/H-21, H-21/H-19, and H-19/H-16 indicated that the relative configuration of **5** was also the same as that of venalstonine. Detailed analysis of 2D NMR data (HSQC, HMBC, ^{1}H−^{1}H COSY, ROESY) established the structure of **5** to be as shown, and it was named melodinine Q.

Melodinine R (**6**) was obtained as a colorless oil. The molecular formula $C_{24}H_{28}N_2O_4$ was established by HREIMS, 57 Da higher than that of 19-(*R*)-hydroxytabersonine.[23] Compound **6** was readily identified as an acetonyl derivative of 19-(*R*)-hydroxytabersonine by the carbon resonances at δ_C 46.2 (t, C-22), 207.3 (s, C-23), and 30.4 (q, C-24), which was placed at C-3 on

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Table 2. ^{1}H NMR Data of 5−9$^{\alpha}$ (δ in ppm and J in Hz)

no.	5	6	7	8	9
1		9.38, s	9.34,s	7.95	
3a		4.01, dd (13.6, 6.0)	4.06, dd (13.0, 5.5)	3.23, m	3.89, m
3b					3.99, m
5a	3.37, m	3.00, m	2.78, m	3.10, dd (16.8, 9.0)	4.02, m
5b	3.59, m	3.11, dd (15.0, 6.9)	2.83, m	3.18, m	3.49, t (10.2)
6a	1.40, dd (12.8, 5.6)	1.91, m	1.63, dd (11.0, 4.0)	1.98, m	2.07, dd (14.7, 8.7)
6b	2.66, m	2.02, overlap	1.83, m	2.52, overlap	3.31, overlap
9	7.03, d (7.6)	7.35, d (7.5)	7.22, d (7.5)	7.42, d (7.8)	7.53, d (2.4)
10	6.76, t (7.6)	6.86, t (7.5)	6.84, t (7.5)	7.09, t (7.8)	
11	7.06, t (7.6)	7.13, t (7.5)	7.13, t (7.5)	7.19, t (7.8)	6.66, dd (8.4, 2.4)
12	6.72, d (7.6)	7.03, d (7.5)	7.03, d (7.5)	6.71, d (7.8)	6.72, d (8.4)
14	7.65, d (10.0)	5.94, dd (10.2, 4.8)	3.56, t (4.2)	5.92, dt (10.2, 3.6)	5.69, m
15	5.98, d (10.0)	5.81, d (10.2)	3.16, d (4.2)	5.99, d (10.2)	5.91, dd (10.5, 2.7)
16	2.92, t (9.8)				
17a	1.57, d (10.2)	2.12, d (15.0)	2.36, d (14.5)	2.51, d (13.8)	2.50, d (13.8)
17b	2.27, m	3.06, d (15.0)	2.57, d (14.5)	3.36, d (13.8)	2.97, d (13.8)
18a	1.30, t (11.6)	0.89, d (6.0)	0.72, t (7.0)	2.23, s	5.07, dd (17.4, 10.8)
18b	1.91, m				
19a	1.48, t (12.0)	3.27, q (6.0)	0.84, m		5.83, dd (17.4, 10.8)
19b	1.79, m		1.04, m		
21	3.56, s	3.21, s	2.74, s	4.18, s	3.89, s
22a	5.09, s	2.68, dd (13.6, 6.0)	2.96, m		
22b		2.88, overlap			
24	2.11, s	2.22, s	2.23, s		
OOC*Me*	3.72, s	3.66, s	3.70, s	3.57, s	3.57, s

$^{\alpha}$Compounds **5** and **8** were measured in $CDCl_3$; **6** and **7** in acetone-d_6; **9** in methanol-d_4.

Table 3. ^{13}C NMR Data of 1−9$^{\alpha}$ (δ in ppm and J in Hz)

no.	1	2	3	4	5	6	7	8	9
2	158.5 C	168.0 C	167.8 C	168.0 C	66.6 C	165.7 C	167.7 C	166.6 C	167.7 C
3	51.2 CH_2	51.1 CH_2	51.1 CH_2	51.0 CH_2	150.7 C	53.2 CH	51.0 CH	46.2 CH_2	62.7 CH_2
5	51.7 CH_2	51.2 CH_2	51.3 CH_2	51.4 CH_2	46.2 CH_2	52.0 CH_2	48.0 CH_2	52.1 CH_2	66.5 CH_2
6	36.9 CH_2	45.7 CH_2	45.8 CH_2	45.6 CH_2	34.8 CH_2	44.1 CH_2	45.0 CH_2	35.6 CH_2	34.7 CH_2
7	56.9 C	55.6 C	55.6 C	56.5 C	56.0 C	56.9 C	56.1 C	58.3 C	60.4 C
8	74.9 C	130.4 C	129.9 C	140.3 C	136.8 C	139.5 C	138.6 C	128.7 C	127.9 C
9	138.9 CH	123.8 CH	123.0 CH	110.5 CH	121.0 CH	122.8 CH	122.4 CH	126.5 CH	117.7 CH
10	132.8 CH	120.4 C	116.9 C	152.8 C	119.7 CH	121.5 CH	121.3 CH	124.1 CH	154.8 C
11	186.2 C	155.0 C	156.5 C	114.3 CH	127.8 CH	128.5 CH	128.6 CH	127.8 CH	116.2 CH
12	100.3 CH	98.8 CH	98.7 CH	110.9 CH	111.5 CH	110.3 CH	110.6 CH	115.5 CH	117.5 CH
13	164.5 C	143.6 C	145.1 C	137.1 C	148.9 C	144.7 C	144.5 C	134.7 C	129.5 C
14	125.0 CH	125.9 CH	126.1 CH	126.0 CH	122.8 CH	129.3 CH	57.2 CH	127.9 CH	117.5 CH
15	134.1 CH	133.6 CH	133.5 CH	133.5 CH	139.7 CH	130.3 CH	59.0 CH	126.7 CH	132.5 CH
16	98.6 C	92.0 C	92.3 C	91.0 C	43.1 CH	91.2 C	90.5 C	62.3 C	63.5 C
17	29.5 CH_2	29.3 CH_2	29.3 CH_2	29.4 CH_2	27.4 CH_2	30.2 CH_2	24.1 CH_2	41.5 CH_2	43.3 CH_2
18	8.2 CH_3	7.7 CH_3	7.7 CH_3	7.7 CH_3	32.4 CH_2	19.1 CH_3	7.4 CH_3	25.4 CH_3	115.5 CH_2
19	27.1 CH_2	27.4 CH_2	27.4 CH_2	27.5 CH_2	28.5 CH_2	67.5 CH	25.7 CH	207.5 C	142.3 CH
20	44.1 C	42.3 C	42.2 C	42.2 C	33.7 C	43.9 C	41.6 C	55.5 C	50.5 C
21	63.3 CH	70.6 CH	70.8 CH	70.8 CH	64.0 CH	61.8 CH	63.3 CH	74.9 CH	92.9 CH
22		30.5 CH_3	71.0 CH_2		96.1 CH	46.2 CH_2	38.0 CH_2		
23					195.6 C	207.3 C	207.7 C		
24					31.4 CH_3	30.4 CH_3	30.5 CH_3		
OOCMe	168.9 C	168.7 C	168.7 C	168.8 C	173.3 C	168.9 C	168.5 C	169.7 C	170.6 C
OOC*Me*	51.8 CH_3	50.8 CH_3	51.0 CH_3	50.9 CH_3	52.2 CH_3	50.9 CH_3	51.1 CH_3	53.1 CH_3	53.4 CH_3
O*Me*			58.0 CH_3						

$^{\alpha}$Compounds **1**, **5**, and **8** were measured in $CDCl_3$; **2**, **3**, **4**, **6**, and **7** in acetone-d_6; **9** in methanol-d_4.

the basis of HMBC correlations of δ_H 2.68 (1H, dd, J = 13.6, 6.0 Hz, H-22a) and 2.88 (1H, overlap, H-22b) with δ_C 53.2 (d, C-3), as well as the ^{1}H−^{1}H COSY cross-peak between H-22 and H-3. ROESY correlations of H-22/H-21 indicated the β-orientation of H-3. Thus, the structure of melodinine R (**6**) was determined as shown.

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Melodinine S (**7**) gave the molecular formula $C_{24}H_{28}N_2O_4$, indicating 12 degrees of unsaturation. The 1D NMR data showed that **7** had a structure similar to that of 3α-acetonyltabersonine (**16**)[15] except for the 14/15 epoxy group (δ_C 57.2, 59.0) in **7**. The suggestion was supported by the molecular formula and HMBC correlations of δ_H 3.56 (1H, t, J = 4.2 Hz, H-14) with C-3 and C-22 and of δ_H 3.16 (1H, d, J = 4.2 Hz, H-15) with C-21, C-20, C-17, and C-19. ROESY correlations of H-21/H-19, H-22/H-21, H-3/H-14, and H-14/H-15 placed the 3α-acetonyl and the 14, 15-epoxy ring on the same side of the molecule. Detailed analysis of 2D NMR data (HSQC, HMBC, ROESY) established the structure of melodinine S (**7**) to be as shown.

Melodinine T (**8**) possessed the molecular formula $C_{21}H_{22}N_2O_4$. The ^{1}H and ^{13}C NMR data (Tables 2 and 3) were very similar to those of scandine[18] except that the terminal double bond between C-19 and C-18 was oxidized to be an acetyl at δ_C 207.5 (s) and 25.4 (q), as supported by the HMBC correlations of δ_H 2.23 (3H, s, H-18) with δ_C 207.5 (s, C-19) and 55.5 (s, C-20). ROESY correlations indicated that the relative configuration of **8** was the same as that of scandine.

Melodinine U (**9**) was isolated as a white, amorphous powder ($C_{21}H_{22}N_2O_5$ by HREIMS), 16 Da higher than that of 10-hydroxyscandine.[19] Compound **9** was readily identified as 10-hydroxyscandine-*N*(4)-oxide from ^{1}H and ^{13}C NMR data, in particular the characteristic downfield shifts of the carbon resonances at δ_C 62.7, 66.5, and 92.9 for C-3, C-5, and C-21, respectively, with respect to those of 10-hydroxyscandine.[7c–7d]

All alkaloids were evaluated for their cytotoxicity against five human cancer cell lines using the MTT method reported previously.[24] Compounds **6**, **11**, and **16** exhibited stronger inhibitory effects against five human cancer cell lines with lower IC_{50} values than those of cisplatin. Compounds **2**, **3**, **7**, **12**, **13**, and **14** displayed moderate cytotoxicity against one or more of the cell lines. The other compounds were considered to be non-cytotoxic, with IC_{50} values greater than 10 μM. It is noteworthy that tabersonine derivatives with an acetonyl moiety at C-3 inhibit five human cancer cell lines significantly in comparison with tabersonine.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on an X-4 micro melting point apparatus. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrometer. IR spectra were obtained by a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. 1D and 2D NMR spectra were run on an AVANCE III-600 MHz or 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 was recorded on an API QSTAR Pulsar I 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. suaveolens* was collected from Luchun County, Yunnan Province, P. R. China, and identified by Dr. Chun-Xia Zeng, Kunming Institute of Botany. A voucher specimen (No. Zeng20091026) has been deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. An air-dried and powdered sample (16 kg) was extracted with 90% MeOH (24 h × 4). The extract was partitioned between EtOAc and a 0.5% HCl solution. The acidic water-soluble material, adjusted to pH 9–10 with 10% ammonia solution, was extracted with EtOAc to give an alkaloidal extract (42 g). The extract was subjected to a silica gel column ($CHCl_3$–Me_2CO, 1:0 to 0:1) to afford fractions I–VIII. Fraction I (4.4 g) was separated by silica gel CC (petroleum ether–EtOAc, 20:1–5:1) to afford **11** (223 mg) and **12** (1870 mg). Fraction II (3.3 g) was subjected to MPLC with RP-18 CC (MeOH–H_2O, 6:4–10:0), followed by silica gel CC (petroleum ether–Me_2CO, 15:1–5:1), to yield **15** (32 mg), **16** (37 mg), and a mixture. The mixture was chromatographed on a silica gel column (petroleum ether–EtOAc, 10:1–6:1) to afford **5** (13 mg) and **13** (63 mg). Fraction III (12.5 g) was separated by silica gel CC (petroleum ether–Me_2CO, 8:1 to 2:1), then by RP-18 CC, eluted with MeOH–H_2O (5:5–10:0), to afford venalstonine (220 mg), scandine (6560 mg), and a mixture. The latter was purified by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to give **7** (6 mg) and 3-oxo-tabersonine (23 mg). Fraction IV (2.8 g) was subjected to MPLC with RP-18 CC (MeOH–H_2O, 4:6–8:2) to give subfractions IV-a and IV-b. Subfraction IV-a was further separated by silica gel CC (petroleum ether–Me_2CO, 4:1) to yield **3** (8 mg) and **14** (28 mg). Subfraction IV-b was subjected to Sephadex LH-20 CC ($CHCl_3$–MeOH, 1:1), then silica gel CC (petroleum ether–Me_2CO, 6:1), to give **4** (7 mg) and **6** (4 mg). Fraction V (3.9 g) was separated by silica gel CC ($CHCl_3$–MeOH, 15:1) to yield **10** (1630 mg) and a mixture. Further separation of the mixture by RP-18 CC (MeOH–H_2O, 5:5) yielded **2** (5 mg). Separation of fraction VI (1.7 g) by RP-18 CC, eluted with MeOH–H_2O (3:7–8:2), and then by silica gel CC ($CHCl_3$–MeOH, 10:1) afforded **1** (62 mg) and **8** (12 mg). Fraction VII (2.5 mg) was separated by RP-18 CC (CH_3OH–H_2O, 2:8–5:5), then further by Sephadex LH-20 CC (MeOH), to yield **9** (55 mg) and 10-hydroxyscandine (432 mg).

*Melodinine M (**1**):* yellow needles (MeOH); mp 109–111 °C; $[\alpha]^{26}_D$ −66.3 (*c* 0.102, MeOH); UV (MeOH) λ_{max} (log ε) 370 (4.00), 282 (4.01), 223 (4.27), 203 (4.23) nm; IR (KBr) ν_{max} 3431, 2932, 1729, 1656, 1633, 1610, 1582, 1437, 1382, 1308, 1253, 1158, 1049, 745 cm^{-1}; ^{1}H (400 MHz) and ^{13}C NMR (150 MHz) data ($CDCl_3$), see Tables 1 and 3; positive ion HRESIMS *m*/*z* 369.1811 (calcd for $C_{21}H_{25}N_2O_4$ [M + H]$^+$, 369.1814).

*Melodinine N (**2**):* white powder; mp 87 °C; $[\alpha]^{25}_D$ −146.3 (*c* 0.231, MeOH); UV (MeOH) λ_{max} (log ε) 333 (3.94), 255 (4.02), 203 (4.22) nm; IR (KBr) ν_{max} 3439, 2958, 2925, 1669, 1487, 1438, 1264, 1155, 1102, 1058, 576 cm^{-1}; ^{1}H (400 MHz) and ^{13}C NMR (125 MHz) data (Me_2CO-d_6), see Tables 1 and 3; HREIMS *m*/*z* 366.1927 (calcd for $C_{22}H_{26}N_2O_3$ [M]$^+$, 366.1943).

*Melodinine O (**3**):* white powder; mp 95–96 °C; $[\alpha]^{25}_D$ −186.3 (*c* 0.095, MeOH); UV (MeOH) λ_{max} (log ε) 330 (4.11), 263 (4.44), 202 (4.36) nm; IR (KBr) ν_{max} 3430, 2930, 1722, 1675, 1620, 1439, 1264, 1212, 1105, 1058, 576 cm^{-1}; ^{1}H (500 MHz) and ^{13}C NMR (150 MHz) data (Me_2CO-d_6), see Tables 1 and 3; HREIMS *m*/*z* 396.2041 (calcd for $C_{23}H_{28}N_2O_4$ [M]$^+$, 396.2049).

*Melodinine P (**4**):* colorless oil; $[\alpha]^{24}_D$ −158.3 (*c* 0.164, MeOH); UV (MeOH) λ_{max} (log ε) 342 (3.87), 313 (3.98), 204 (4.23) nm; IR (KBr) ν_{max} 3427, 2960, 2926, 1669, 1613, 1468, 1439, 1382, 1273, 1189, 1111, 809, 582 cm^{-1}; ^{1}H (400 MHz) and ^{13}C NMR (150 MHz) data (Me_2CO-d_6), see Tables 1 and 3; HREIMS *m*/*z* 352.1782 (calcd for $C_{21}H_{24}N_2O_3$ [M]$^+$, 352.1787).

*Melodinine Q (**5**):* colorless needles (MeOH); mp 136–138 °C; $[\alpha]^{24}_D$ +186.8 (*c* 0.048, MeOH); UV (MeOH) λ_{max} (log ε) 362 (4.13), 239 (4.00), 206 (4.25) nm; IR (KBr) ν_{max} 3440, 2951, 2925, 1718, 1640, 1614, 1520, 1449, 1324, 1216, 1167, 961, 753 cm^{-1}; ^{1}H (400 MHz) and ^{13}C NMR (150 MHz) data ($CDCl_3$), see Tables 2 and 3; positive ion HRESIMS *m*/*z* 391.2027 (calcd for $C_{24}H_{27}N_2O_3$ [M + H]$^+$, 391.2021).

*Melodinine R (**6**):* colorless oil; $[\alpha]^{24}_D$ −42.9 (*c* 0.104, MeOH); UV (MeOH) λ_{max} (log ε) 328 (3.84), 277 (4.05), 203 (4.34) nm; IR (KBr) ν_{max} 3431, 2924, 1711, 1678, 1632, 1610, 1466, 1438, 1246, 1103, 900, 748 cm^{-1}; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data (Me_2CO-d_6), see Tables 2 and 3; HREIMS *m*/*z* 408.2050 (calcd for $C_{24}H_{28}N_2O_4$ [M]$^+$, 408.2049).

*Melodinine S (**7**):* colorless needles (Me_2CO); mp 73–75 °C; $[\alpha]^{26}_D$ −331.7 (*c* 0.100, MeOH); UV (MeOH) λ_{max} (log ε) 328 (4.33), 299 (4.18), 225 (4.17), 202 (4.23) nm; IR (KBr) ν_{max} 3440,

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3375, 2961, 2901, 1704, 1669, 1608, 1465, 1439, 1294, 1243, 1113, 757 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data (Me_2CO-d_6), see Tables 2 and 3; positive ion HRESIMS m/z 409.2122 (calcd for $C_{24}H_{29}N_2O_4$ $[M + H]^+$, 409.2127).

*Melodinine T (**8**):* white powder; mp 75−76 °C; $[\alpha]^{26}_D$ +204.0 (c 0.100, MeOH); UV (MeOH) λ_{max} (log ε) 396 (1.44), 261 (3.96), 208 (4.54) nm; IR (KBr) ν_{max} 3432, 2924, 1676, 1617, 1487, 1455, 1266, 1156, 1106, 738 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (150 MHz) data ($CDCl_3$), see Tables 2 and 3; positive ion HRESIMS m/z 367.1449 (calcd for $C_{21}H_{23}N_2O_4$ $[M + H]^+$, 367.1446).

*Melodinine U (**9**):* white powder; mp 130−132 °C; $[\alpha]^{26}_D$ +177.3 (c 0.103, MeOH); UV (MeOH) λ_{max} (log ε) 306 (3.59), 271 (3.96), 205 (4.39) nm; IR (KBr) ν_{max} 3425, 3235, 2955, 1735, 1672, 1617, 1503, 1469, 1251, 1181, 1138, 973, 732 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (MeOH), see Tables 2 and 3; positive ion HRESIMS m/z 383.1613 (calcd for $C_{21}H_{23}N_2O_5$ $[M + H]^+$, 383.1606).

Table 4. Cytotoxicity of Compounds 2−4, 6, 7, and 10−16 (IC_{50}, μM)

compd	HL-60	SMMC-7721	A-549	MCF-7	SW480
2	3.5	15.5	18.5	15.0	15.1
3	10.0	15.2	18.2	15.7	14.9
4	28.1	>40	>40	>40	>40
6	0.7	3.3	3.9	1.8	1.6
7	4.8	11.2	15.2	7.0	13.5
10	6.0	15.5	17.6	>40	>40
11	0.5	1.1	1.0	0.2	2.4
12	4.5	5.6	14.7	9.9	12.1
13	5.8	6.0	15.5	14.2	15.0
14	6.3	16.0	19.7	21.3	13.1
15	15.5	24.7	>40	>40	>40
16	0.2	0.3	0.6	0.4	0.5
cisplatin	1.3	14.6	10.7	18.4	17.7

Cytotoxicity Assays. Five human cancer cell lines, human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480 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 assays were performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates.[24] 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 at concentrations of 0.064, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin (Sigma, USA) as a positive control. After each compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method.[25]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR and MS spectra of melodinines M−U (**1**−**9**). These materials are available free of charge via the Internet at http://pubs.acs.org.

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