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Melosuavines A−H, Cytotoxic Bisindole Alkaloid Derivatives from *Melodinus suaveolens*

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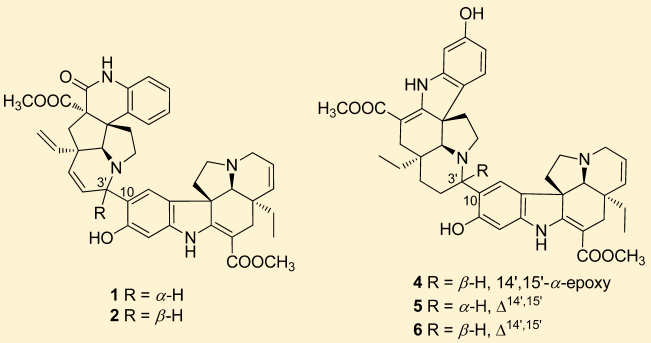
Supporting Information

ABSTRACT: Eight new bisindole alkaloids, melosuavines A−C (**1**−**3**), having an aspidosperma-scandine linkage, melosuavines D−F (**4**−**6**), possessing an aspidosperma-aspidosperma skeleton, and melosuavines G and H (**7** and **8**) of the aspidosperma-venalatonine type, tenuicausine (**9**), and melodinine J (**10**) were isolated from the twigs and leaves of *Melodinus suaveolens*. The structures of **1**−**8** were elucidated by extensive spectroscopic methods, and compounds **9** and **10** were identified by comparison with data in the literature. The relative configuration **9** was determined from the ROESY spectrum, and some NMR signals were reassigned. Compounds **1**, **2**, **4**−**6**, **8**, and **10** exhibited low micromolar cytotoxicity against one or more of five human cancer cell lines.



Bisindole alkaloids have been a topic of intensive research since the structure of the pharmacologically important bisindole alkaloid vinblastine was established.[1] To date, hundreds of bisindole alkaloids have been isolated from plants.[2] Vinblastine,[3] vincristine,[4] and vindesine[5] are well known for their antitumor activities and are currently commercial drugs in many countries.[6] In our continuous investigation on bioactive indole alkaloids from the genus *Melodinus* (Apocynaceae),[7] eight new bisindole alkaloids (**1**−**8**) and two known bisindole alkaloids, tenuicausine (**9**)[8] and melodinine J (**10**),[9] were isolated from the twigs and leaves of *M. suaveolens*.[7] The compounds were evaluated for cytotoxicity against five human cancer cell lines. Reported herein are the isolation, structural elucidation, and cytotoxicities of these compounds.

RESULTS AND DISCUSSION

Melosuavine A (**1**) was obtained as a white, amorphous powder, and the molecular formula $C_{42}H_{44}N_4O_6$ was assigned on the basis of positive HRESIMS ($[M + H]^+$ at m/z 701.3332). The UV spectrum showed absorption maxima characteristic of a β-anilinoacrylate chromophore (329, 250, 203 nm), and its IR spectrum showed absorption bands at 3431, 1744, and 1729 cm^{-1}.[10] In the ^1H NMR spectrum, three broad singlets [δ_H 9.60 (1H), 9.55 (1H), and 9.30 (1H)] were the characteristic resonances for OH and NH groups. A triplet at δ_H 0.62 (3H, t, J = 7.4 Hz, Me-18) was assigned to a methyl connected to a methylene, and two singlets (δ_H 3.63 and 3.68) were assigned to protons of two OCH_3 groups. Olefinic signals at δ_H 5.61 (1H, dd, J = 11.4, 17.4 Hz), 4.93 (1H, d, J = 17.4 Hz), and 4.86 (1H, d, J = 11.4 Hz) indicated a $-CH{=}CH_2$ moiety and were assigned to CH-19′ and CH_2-18′. In the ^{13}C NMR spectrum, 42 carbon resonances were observed. Three quaternary carbon resonances (δ_C 92.3, 167.5, 168.6) and an OCH_3 (δ_C 50.9) were assigned to a β-anilinoacrylate moiety conjugated with a methyl ester. Two quaternary carbon resonances (δ_C 168.5, 171.4) and an OCH_3 (δ_C 52.7) were readily assigned to acylamide and carboxyl methyl ester units. The NMR data (Table 1) of compound **1** were consistent with a bisindole alkaloid having aspidosperma (unit A)[11] and scandine (unit B) skeletons,[8,12] respectively (as shown in Figure 1S, Supporting Information).

The ^1H NMR spectrum indicated six aromatic protons [δ_H 7.12 (1H, s), 6.52 (1H, s), 7.46 (1H, d, J = 7.3 Hz), 7.05 (1H, t, J = 7.3 Hz), 7.18 (1H, t, J = 7.3 Hz), and 6.91 (1H, d, J = 7.3 Hz)]. According to the peak shape and coupling constants, it was deduced that two of these (δ_H 7.12 and 6.52) must be *para* and the OH at C-11 in unit A of **1**, as evidenced by HMBC correlations of δ_H 9.60 (1H, s, OH-11) with δ_C 157.9 (s, C-11), 116.9 (s, C-10), and 99.1 (d, C-12), of H-9 (δ_H 7.12, s) with δ_C 157.9 (s, C-11) and 145.2 (s, C-13), and of H-12 (δ_H 6.52, s) with δ_C 130.0 (s, C-8) and 116.9 (s, C-10). The quinoline ring in unit B of **1** was unsubstituted, as supported by peak shape and coupling constants of the other four aromatic protons. In the

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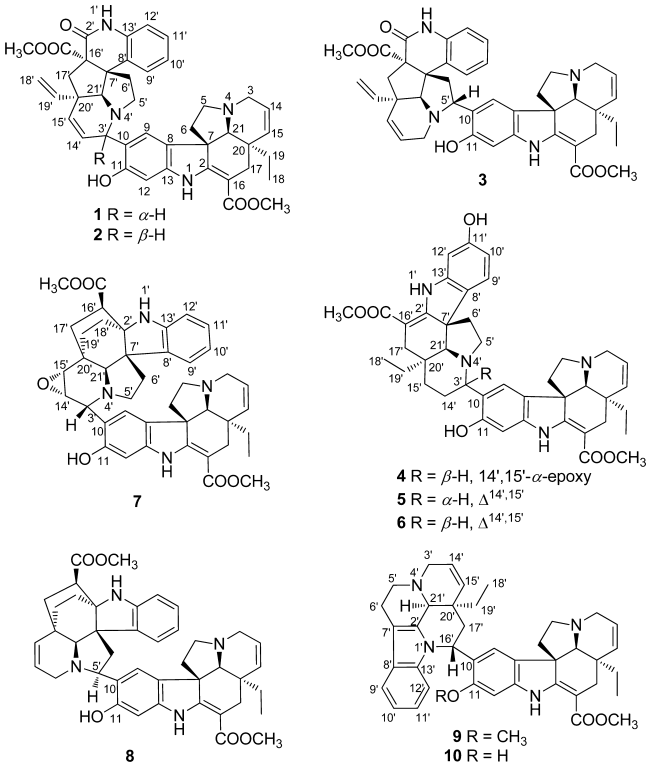

^{1}H NMR spectrum, olefinic protons at δ_H 5.77 (1H, ddd, J = 10.2, 4.8, 1.2 Hz) and 5.69 (1H, d, J = 10.2 Hz) were assigned to a double bond at C-14/15 in unit A of **1**, on the basis of HMBC correlations of H-14 (δ_H 5.77) with δ_C 50.9 (t, C-3) and 42.2 (s, C-20), and of H-15 (δ_H 5.69) with δ_C 70.6 (s, C-21), 27.4 (t, C-19), and 50.9 (t, C-3). The remaining two olefinic protons were ascribed to a double bond at C-14′/15′ in unit B of **1**, as supported by HMBC correlations of H-14′ (δ_H 5.49) with δ_C 64.0 (d, C-3′) and 49.7 (s, C-20′), and of H-15′ (δ_H 5.60) with δ_C 87.0 (s, C-21′), 141.3 (d, C-19′), and 64.0 (d, C-3′). Finally, the linkage of units A and B by C-3′/C-10 was established by the HMBC correlations of δ_H 4.32 (1H, br s, H-3′) with δ_C 116.9 (s, C-10), 122.0 (d, C-9), and 157.9 (s, C-11) (Figure 1S). In the ROESY spectrum, correlations of H-9/H-21, H-21/Me-18, H-9′/H-21′, and H-21′/H-19′ suggested that the relative configuration of unit A was the same as in tabersonine, and unit B was the same as in scandine. The ROESY correlation of H-3′/H-21′ suggested the α-orientation for H-3′ (Figure 2S). All of the NMR signals were assigned by HSQC, HMBC, and ROESY experiments, and the structure of melosuavine A (**1**) was established as shown.

The molecular formula of **2** was also revealed to be $C_{42}H_{44}N_4O_6$ by positive HRESIMS. Compounds **1** and **2** had similar physical constant data, and analysis of 1D and 2D NMR spectra (Table 1) suggested that they were isomers. The key difference was that H-3′ in **2** was β-oriented, as supported by ROESY correlations of H-21′/Me-18′, H-5′a/H-21′, and H-3′/H-5′b. Thus, compound **2** was assigned as shown, and it was named melosuavine B.

Melosuavine C (**3**) had the molecular formula $C_{42}H_{44}N_4O_6$ (by HRESIMS). The similar physical constant data of compounds **1**, **2**, and **3** (HRESIMS, UV, and IR spectra) suggested the same functional groups for them. The 1D NMR spectra data of **3** (Table 1) resembled those of **2**, except for the linkage of two units by C-5′/C-10 in **3** instead of C-3′/10 in **2**. This was supported by HMBC correlations of δ_H 4.56 (1H, t, J = 7.8 Hz, H-5′) with δ_C 116.5 (s, C-10), 123.5 (d, C-9), and 159.1 (d, C-11). ROESY correlations of H-21′/Me-18′, H-17′b/H-21′, and H-5′/H-17′a suggested the β-orientation of H-5′. Detailed analysis of 2D NMR data confirmed that the other parts of the molecule were the same as those of **2**.

The UV spectrum of melosuavine D (**4**) showed absorption maxima characteristic of a β-anilinoacrylate chromophore (330, 248, 202 nm), while the IR spectrum showed absorption bands at 3417, 3378, 1673, and 1629 cm^{-1}.[10] The molecular formula $C_{42}H_{46}N_4O_7$ was established by the positive HRESIMS ($[M + H]^+$ at m/z 719.3462). In the ^{1}H NMR spectrum, broad singlets at δ_H 9.58 (1H) and 9.59 (1H) were characteristic resonances for two NH groups. Triplets at δ_H 0.59 (3H, t, J = 7.3 Hz, Me-18) and 0.70 (3H, t, J = 7.8 Hz, Me-18′) were assigned to protons of methyl groups connected to methylenes, and singlets at δ_H 3.67 and 3.68 were assigned to protons of two methoxy groups. In the ^{13}C NMR spectrum, 42 carbon resonances were observed. Of them, six sp^2 quaternary carbon resonances (δ_C 88.5, 90.2, 166.1, 167.1, 167.2, 167.3) and two methoxy signals (δ_C 50.6, 50.6) were readily assigned to two β-anilinoacrylate moieties conjugated with two methyl ester units. According to the 1D NMR spectra, compound **4** was identified as a bisindole alkaloid with two aspidosperma-type units (A and B) similar to tabersonine (Figure 3S, Supporting Information).[11]

In unit A of **4**, the ^{1}H NMR spectrum displayed singlet aromatic protons at δ_H 7.45 and 6.66, which were ascribed to the *ortho*-disubstituted indole moiety, and one OH was substituted at C-11, as evidenced by HMBC correlations of aromatic protons at δ_H 7.45 (s, H-9) with δ_C 155.1 (s, C-11) and 143.1 (s, C-13), and of δ_H 6.66 (s, H-12) with δ_C 127.7 (s, C-8) and 116.0 (s, C-10). In addition, olefinic signals at δ_H 5.72 (dd, J = 9.9, 4.3 Hz) and 5.68 (d, J = 9.9 Hz) were ascribed to double-bond protons at C-14/15 and supported by HMBC correlations of δ_H 5.72 (1H, H-14) with δ_C 50.1 (t, C-3), 132.6 (d, C-15), and 40.8 (s, C-20), and of δ_H 5.68 (1H, H-15) with δ_C 50.1 (t, C-3), 26.4 (t, C-19), and 69.7 (d, C-21). In unit B, three aromatic protons at δ_H 6.54 (1H, d, J = 7.9 Hz, H-9′), 6.07 (1H, dd, J = 7.9, 2.0 Hz, H-10′), and 6.51 (1H, d, J = 2.0 Hz, H-12′) suggested that one OH was at C-11′, which was supported by coupling constants among H-9′, H-10′, and H-11′, and by HMBC correlations of H-9′ with δ_C 157.5 (s, C-11′) and 144.7 (s, C-13′). Three sp^3 carbon signals at δ_C 49.3 (d, C-3′), 56.6 (d, C-14′), and 56.9 (d, C-15′) allowed the connection of a heteroatom to them, and the ^{1}H−^{1}H COSY correlations of δ_H 4.98 (1H, d, J = 4.8 Hz, H-3′)/3.64 (1H, t, J = 4.5 Hz, H-14′)/3.20 (1H, d, J = 4.5 Hz, H-15′) revealed the connection of C-3′/C-14′/C-15′ (Figure 3S). HMBC correlations of H-3′ with δ_C 116.0 (s, C-10), 121.6 (d, C-9), and 155.1 (s, C-11) suggested the linkage of the two units at C-3′/C-10 (Figure 3S). The above data indicated that compound **4** was similar to melodinine K[9] except that C-11 and C-11′ in **4** were substituted by OH groups, and an epoxy was formed between C-14′ and C-15′ in **4**, as supported by HMBC correlations of H-14′ with δ_C 49.3 (d, C-3′), and 56.9 (d, C-15′), and of H-15′ with δ_C 49.3 (d, C-3′), 24.9 (t, C-19′), and 63.1 (d, C-21′). Thus, the gross structure of **4** was assigned.

The relative configuration of **4** was elucidated by ROESY correlations, as shown in a computer-generated 3D drawing (Figure 4S). ROESY correlations of H-21′/Me-18′, H-21′/H-9′, and H-21′/H-5′a suggested the α-orientation of H-21′ and H-5′a; then another proton δ_H 2.75 (1H, t, J = 15.8 Hz, H-5′b) at C-5′ was assigned as β-oriented. Furthermore, H-3′, H-14′, and H-15′ were β-oriented and the epoxy ring at C-14′ and C-15′ was α-oriented, according to ROESY correlations of H-3′/H-5′b and H-3′/H-14′/H-15′ (Figure 4S). ROESY correlations of H-9/H-21, H-21/Me-18, H-9′/H-21′, and H-21′/Me-18′ further indicated that the configurations of two units were identical to that of tabersonine. Hence, the structure of melosuavine D (**4**) was established as shown.

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds 1−3[α] (δ in ppm and J in Hz)

	1		2		3	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	9.30, s		9.17, s			
2		167.5 qC		167.6 qC		166.8 qC
3a	3.19, d (16.2)	50.9 CH_2	3.07, d (15.6)	51.1 CH_2	3.34, m	51.1 CH_2
3b	3.42, ddd (1.2, 4.8, 16.2)		3.36, ddd (1.2, 4.5, 15.6)		3.49, m	
5a	2.75, m	51.2 CH_2	2.49, m	51.0 CH_2	2.95, m	51.8 CH_2
5b	3.00, t (7.2)		2.88, t (7.2)		3.13, m	
6a	1.71, dd (4.2, 11.4)	45.7 CH_2	1.45, dd (4.2, 11.4)	45.6 CH_2	1.88, m	45.3 CH_2
6b	1.96, m		1.83, m		2.04, m	
7		55.6 qC		55.5 qC		57.7 qC
8		130.0 qC		128.7 qC		129.1 qC
9	7.12, s	122.0 CH	6.78, s	122.1 CH	7.15, s	123.5 CH
10		116.9 qC		116.7 qC		116.5 qC
11		157.9 qC		159.4 qC		159.1 qC
12	6.52, s	99.1 CH	6.35, s	99.2 CH	6.57, s	100.4 CH
13		145.2 qC		144.8 qC		145.9 qC
14	5.77, ddd (1.2, 4.8, 10.2)	126.0 CH	5.72, ddd (1.2, 4.8, 9.6)	125.9 CH	5.78, overlap	124.7 CH
15	5.69, d (10.2)	133.5 CH	5.65, d (9.6)	133.4 CH	5.78, overlap	134.3 CH
16		92.3 qC		92.0 qC		92.6 qC
17a	2.45, d (15.0)	29.2 CH_2	2.38, d (15.0)	29.0 CH_2	2.36, d (15.6)	29.3 CH_2
17b	2.51, d (15.0)		2.46, d (15.0)		2.63, d (15.6)	
18	0.62, t (7.4)	7.7 CH_3	0.59, t (7.2)	7.6 CH_3	0.62, t (7.2)	7.8 CH_3
19a	0.83, m	27.4 CH_2	0.76, m	27.3 CH_2	0.81, m	28.3 CH_2
19b	0.99, m		0.94, m		1.02, m	
20		42.2 qC		42.3 qC		42.2 qC
21	2.65, s	70.6 CH	2.49, s	70.6 CH	2.98, s	72.1 CH
CO_2Me	3.68, s	50.9 CH_3	3.64, s	50.9 CH_3	3.76, s	51.1 CH_3
CO_2Me		168.6 qC		168.6 qC		169.8 qC
11-OH	9.60, s		10.75, s			
1′	9.55, s		9.64, s			
2′		168.5 qC		167.2 qC		169.3 qC
3′a	4.32, br s	64.0 CH	4.64, d (4.8)	56.5 CH	2.17, m	45.5 CH_2
3′b					2.78, m	
5′a	2.91, m	51.4 CH_2	2.24, m	52.1 CH_2	4.56, t (7.8)	67.6 CH
5′b	3.15, d (7.8)		3.50, m			
6′a	1.98, m	43.8 CH_2	1.94, m	35.3 CH_2	3.22, m	43.6 CH_2
6′b	2.30, dd (6.0, 13.8)		2.73, m		3.37, m	
7′		57.8 qC		59.1 qC		58.0 qC
8′		128.9 qC		129.2 qC		129.1 qC
9′	7.46, d (7.3)	128.7 CH	7.47, d (7.2)	126.5 CH	7.82, d (7.2)	126.9 CH
10′	7.05, t (7.3)	124.0 CH	7.07, t (7.2)	123.9 CH	7.02, t (7.2)	125.0 CH
11′	7.18, t (7.3)	128.5 CH	7.19, t (7.2)	128.5 CH	7.20, t (7.2)	129.1 CH
12′	6.91, d (7.3)	116.0 CH	6.94, d (7.2)	116.0 CH	6.90, d (7.2)	117.0 CH
13′		136.0 qC		136.6 qC		136.9 qC
14′	5.49, d (10.8)	128.9 CH	6.14, dd (5.4, 10.2)	122.6 CH	5.65, dd (4.8, 9.6)	119.5 CH
15′	5.60, d (10.8)	131.2 CH	6.12, d (10.2)	133.7 CH	5.91, d (9.6)	132.8 CH
16′		66.0 qC		62.9 qC		64.2 qC
17′a	2.95, d (13.8)	45.4 CH_2	2.41, d (14.4)	44.8 CH_2	2.54, d (13.8)	45.4 CH_2
17′b	3.05, d (13.8)		2.97, d (14.4)		2.97, d (13.8)	
18′a	4.86, d (11.4)	116.0 CH_2	4.68, d (10.8)	114.1 CH_2	4.96, d (10.8)	115.0 CH_2
18′b	4.93, d (17.4)		4.72, d (17.4)		5.04, d (17.4)	
19′a	5.61, dd (11.4, 17.4)	141.3 CH	5.59, dd (10.8, 17.4)	144.2 CH	5.79, overlap	144.9 CH
19′b						
20′		49.7 qC		43.8 qC		45.1 qC
21′	3.10, s	87.0 CH	3.18, s	78.3 CH	3.73, s	82.1 CH
CO_2Me'	3.63, s	52.7 CH_3	3.52, s	52.9 CH_3	3.52, s	53.4 CH_3
CO_2Me'		171.4 qC		170.5 qC		171.5 qC

[α]Compounds **1** and **2** were measured in acetone-d_6, **3** in CD_3OD.

Melosuavine E (**5**) had the molecular formula $C_{42}H_{46}N_4O_6$. The UV spectrum showed absorption maxima characteristic of a β-anilinoacrylate chromophore (329, 250, and 203 nm).[10] The 1D NMR data of **5** (Table 2) were similar to those of **4** except that a double bond appeared at C-14′/C-15′ in **5** instead of the epoxy ring in **4**, which was assumed by the chemical shifts of

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Table 2. ^{1}H and ^{13}C NMR Data of Compounds 4–6[α] (δ in ppm and J in Hz)

	4		5		6	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	9.58, s		9.27, s		9.25, s	
2		166.1 qC		166.6 qC		167.7 qC
3a	2.98, m	50.1 CH_2	3.18, d (16.0)	50.0 CH_2	3.08, m	51.1 CH_2
3b	3.39, dd (9.9, 16.6)		3.43, dd (4.0, 16.0)		3.37, dd (4.2, 16.2)	
5a	2.54, m	50.2 CH_2	2.74, m	50.5 CH_2	2.55, m	51.2 CH_2
5b	2.95, m		3.01, t (7.5)		2.89, t (7.2)	
6a	1.68, m	45.2 CH_2	1.72, dd (4.0, 11.5)	44.8 CH_2	1.60, dd (4.2, 11.4)	45.8 CH_2
6b	1.95, m		1.95, overlap		1.93, m	
7		54.5 qC		54.8 qC		55.6 qC
8		127.7 qC		129.0 qC		128.9 qC
9	7.45, s	121.6 CH	7.10, s	121.2 CH	7.06, s	122.4 CH
10		116.0 qC		116.5 qC		117.7 qC
11		155.1 qC		157.1 qC		158.5 qC
12	6.66, s	98.0 CH	6.48, s	98.2 CH	6.64, s	99.1CH
13		143.1 qC		144.3 qC		144.7 qC
14	5.72, dd (4.3, 9.9)	125.1 CH	5.78, dd (2.5, 9.5)	125.0 CH	5.74, dd (3.6, 10.2)	125.9 CH
15	5.68, d (9.9)	132.6 CH	5.69, d (9.5)	132.7 CH	5.68, d (10.2)	133.4 CH
16		90.2 qC		91.4 qC		92.2 qC
17a	2.36, d (8.5)	28.4 CH_2	2.44, d (15.0)	28.3 CH_2	2.42, d (14.4)	29.1 CH_2
17b	2.46, overlap		2.51, d (15.0)		2.50, d (14.4)	
18	0.59, t (7.3)	7.2 CH_3	0.64, t (7.5)	6.8 CH_3	0.64, t (7.5)	7.6 CH_3
19a	0.77, m	26.4 CH_2	0.84, m	26.6 CH_2	0.81, m	27.3 CH_2
19b	0.94, m		1.00, m		1.01, m	
20		40.8 qC		41.3 qC		42.3 qC
21	2.48, overlap	69.7 CH	2.66, s	70.0 CH	2.57, s	70.6 CH
CO_2Me	3.67, s	50.6 CH_3	3.68, s	50.1 CH_3	3.67, s	50.9 CH_3
CO_2Me		167.1 qC		167.8 qC		168.5 qC
1′	9.59, s		9.30, s		9.35, s	
2′		167.3 qC		166.7 qC		165.9 qC
3′	4.98, d (4.8)	49.3 CH	4.46, br s	62.0 CH	4.85, d (4.8)	55.5 CH
5′a	2.44, overlap	46.8 CH_2	2.70, m	47.8 CH_2	3.13, t (6.8)	51.2 CH_2
5′b	2.75, t (15.8)		2.94, m		3.13, t (6.8)	
6′a	1.42, m	43.7 CH_2	1.77, dd (4.0, 11.5)	44.8 CH_2	2.05, m	43.6 CH_2
6′b	1.71, m		1.95, overlap		2.14, m	
7′		54.7 qC		54.4 qC		56.0 qC
8′		128.1 qC		128.1 qC		130.2 qC
9′	6.54, d (7.9)	120.8 CH	7.20, d (8.0)	121.5 CH	7.07, d (8.1)	123.2 CH
10′	6.09, dd (2.0, 7.9)	106.2 CH	6.35, dd (2.1, 8.0)	106.8 CH	6.31, dd (2.1, 8.1)	108.0 CH
11′		157.5 qC		157.9 qC		158.3 qC
12′	6.53, d (2.0)	98.5 CH	6.62, d (2.1)	98.2 CH	6.54, d (2.1)	98.5 CH
13′		144.7 qC		144.8 qC		145.8 qC
14′	3.64, t (4.5)	56.6 CH	5.50, d (10.0)	129.2 CH	6.10, dd (4.8, 10.2)	125.5 CH
15′	3.20, d (4.5)	56.9 CH	5.76, dd (2.0, 10.0)	132.3 CH	6.01, d (10.2)	134.7 CH
16′		88.5 qC		91.4 qC		90.8 qC
17′a	2.32, d (8.5)	23.4 CH_2	2.33, d (15.5)	28.2 CH_2	2.26, d (15.0)	22.8 CH_2
17′b	2.53, m		2.69, d (15.5)		2.66, d (15.0)	
18′	0.70, t (7.8)	7.1 CH_3	0.66, t (7.5)	6.8 CH_3	0.64, t (7.5)	8.9 CH_3
19′a	0.80, m	24.9 CH_2	0.94, m	26.5 CH_2	1.02, m	30.8 CH_2
19′b	1.01, m		1.09, m		1.02, m	
20′		41.0 qC		41.2 qC		38.4 qC
21′	3.08, s	63.1 CH	2.96, s	69.9 CH	3.06, s	65.1 CH
CO_2Me'	3.68, s	50.6 CH_3	3.71, s	50.2 CH_3	3.68, s	50.9 CH_3
CO_2Me'		167.2 qC		167.6 qC		168.6 qC

[α]Compound **4** was measured in DMSO, compounds **5** and **6** in acetone-d_6.

C-14′/C-15′ in **5**, and then supported by HMBC correlations of δ_H 4.46 (1H, br s, H-3′) with δ_C 129.2 (d, C-14′) and 132.3 (d, C-15′), and of δ_H 2.96 (1H, s, H-21′) with δ_C 132.3 (d, C-15′). ROESY correlations of H-3′/H-21′/Me-18′ indicated the α-orientation of H-3′, and correlations of H-9/H-21, H-21/Me-18, H-9′/H-21′, and H-21′/Me-18′ suggested that the other configurations of **5** were the same as those of **4**. Hence, the structure of compound **5** was determined to be as shown.

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Table 3. ^{1}H and ^{13}C NMR Data of Compounds 7−9 in Acetone-d_6 (δ in ppm and J in Hz)

	7		8		9	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	9.27, s		9.23, s		9.49, s	
2		167.9 qC		167.8 qC		167.9 qC
3a	3.19, d (15.5)	51.1 CH_2	3.17, d (10.2)	51.1 CH_2	2.80, d (15.6)	51.1 CH_2
3b	3.45, m		3.41, m		3.25, dd (6.0, 15.6)	
5a	2.78, overlap	51.3 CH_2	2.75, m	51.3 CH_2	2.51, m	51.3 CH_2
5b	2.99, m		2.97, t (7.2)		2.87, m	
6a	1.75, m	45.9 CH_2	1.70, overlap	45.8 CH_2	1.71, dd (3.6, 10.8)	45.6 CH_2
6b	1.97, m		1.94, m		1.92, overlap	
7		55.7 qC		55.7 qC		55.5 qC
8		129.6 qC		129.1 qC		130.9 qC
9	7.22, s	121.8 CH	6.93, s	122.7 CH	7.17, s	121.1 CH
10		117.9 qC		117.7 qC		122.1 qC
11		159.1 qC		158.9 qC		157.4 qC
12	6.54, s	99.5 CH	6.49, s	99.8 CH	7.00, s	95.3 CH
13		144.8 qC		144.9 qC		145.1 qC
14	5.77, dd (4.0, 9.5)	126.0 CH	5.74, ddd (1.2, 4.8, 9.6)	125.9 CH	5.59, overlap	126.0 CH
15	5.69, d (9.5)	133.6 CH	5.68, d (9.6)	133.5 CH	5.54, d (10.2)	133.6 CH
16		92.0 qC		91.9 qC		93.1 qC
17a	2.45, d (15.0)	29.3 CH_2	2.41, d (14.4)	29.2 CH_2	2.41, d (15.0)	29.3 CH_2
17b	2.51, d (15.0)		2.48, d (14.4)		2.44, d (15.0)	
18	0.64, t (7.3)	7.7 CH_3	0.58, t (7.2)	7.6 CH_3	0.47, t (7.2)	7.9 CH_3
19a	0.84, m	27.6 CH_2	0.75, m	27.4 CH_2	0.70, m	27.1 CH_2
19b	1.02, m		0.93, m		0.91, m	
20		42.3 qC		42.2 qC		42.2 qC
21	2.67, s	70.9 CH	2.58, s	70.9 CH	2.30, s	69.6 CH
CO_2Me	3.68, s	51.0 CH_3	3.66, s	50.9 CH_3	3.70, s	51.1 CH_3
CO_2Me		168.7 qC		168.7 qC		168.7 qC
O*Me*					3.91, s	56.4 CH_3
1′	4.92, s		4.82, s			
2′		68.2 qC		66.5 qC		136.0 qC
3′a	4.65, br s	61.8 CH	3.38, d (4.8)	45.3 CH_2	2.84, m	44.8 CH_2
3″b			3.65, m		3.01, m	
5′a	2.93, m	52.4 CH_2	4.74, t (8.4)	70.5 CH	3.29, m	50.1 CH_2
5′b	3.12, m		3.50, m		3.36, dd (7.8, 13.8)	
6′a	1.09, dd (4.2, 12.2)	33.9 CH_2	1.44, m	44.3 CH_2	2.49, d (6.6)	17.5 CH_2
6′b	2.78, overlap		3.46, dd (8.4, 15.0)		3.16, m	
7′		59.7 qC		57.6 qC		105.7 qC
8′		138.5 qC		139.7 qC		130.0 qC
9′	7.02, d (7.5)	129.1 CH	7.30, d (7.2)	122.7 CH	7.32, d (7.8)	118.2 CH
10′	6.68, t (7.5)	119.3 CH	6.67, t (7.2)	119.8 CH	6.85, t (7.8)	119.6 CH
11′	6.97, t (7.5)	128.2 CH	6.92, t (7.2)	127.8 CH	6.64, t (7.8)	120.6 CH
12′	6.66, d (7.5)	111.3 CH	6.59, d (7.2)	111.0 CH	6.20, d (7.8)	112.8 CH
13′		151.0 qC		151.0 qC		137.7 qC
14′	3.46, m	56.4 CH	5.72, overlap	124.6 CH	5.59, overlap	128.0 CH
15′	2.97, m	56.8 CH	5.72, overlap	132.2 CH	5.59, overlap	128.2 CH
16′	3.08, m	43.9 CH	3.08, t (9.6)	44.4 CH	5.19, d (10.8)	50.4 CH
17′a	1.82, overlap	29.0 CH_2	1.43, m	33.6 CH_2	1.94, m	43.8 CH_2
17′b	2.36, t (15.0)		1.93, m		2.21, dd (3.0, 13.8)	
18′a	1.41, d (10.7)	32.2 CH_2	1.45, m	32.8 CH_2	0.96, t (7.8)	9.0 CH_3
18′b	1.82, overlap		1.69, overlap			
19′a	1.68, m	30.4 CH_2	1.84, dd (10.2, 10.8)	35.1 CH_2	1.91, overlap	35.1 CH_2
19′b	1.75, m		2.52, m			
20′		35.8 qC		32.3 qC		38.3 qC
21′	3.42, s	62.9 CH	3.46, s	67.1 CH	4.07, s	58.8 CH
CO_2Me'	3.69, s	52.1 CH_3	3.69, s	52.2 CH_3		
CO_2Me′		174.7 qC		175.5 qC		

The other closely related compound, **6**, with a lower R_f value on silica TLC than **5**, showed similar physical data in the HRESIMS, UV, and IR spectra, indicating the presence of the same molecular formula and functional groups as in **5**. Detailed

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analysis of 1D and 2D NMR spectral data (Table 2) suggested that **6** was a bisindole alkaloid with a planar structure the same as that of **5**. The coupling constant (d, J = 4.8 Hz) of H-3′ in **6** was the same as that of **4**, not that of **5**, suggesting that H-3′ in **6** was β-oriented.[13] Other parts of the structure were identical to those of **5** by detailed analysis of its 2D NMR spectra.

Melosuavine G (**7**) had the molecular formula $C_{42}H_{46}N_4O_6$. The ^{1}H NMR spectrum indicated NH protons at δ_H 9.27 (1H, s, N1-H) and 4.92 (1H, s, N1′-H), one methyl group at δ_H 0.64 (Me-18) attached to methylene, two OCH_3 groups (δ_H 3.68 and 3.69), and two olefinic protons at δ_H 5.77 (1H, dd, J = 9.5, 4.0 Hz, H-14) and 5.69 (1H, d, J = 9.5 Hz, H-15). The ^{13}C NMR spectrum displayed 42 carbon resonances ascribable to three methyls, 10 methylenes, 14 methines, and 15 quaternary carbons (Table 3). It showed characteristic chemical shifts for a β-anilinoacrylate moiety conjugated with a methyl ester unit (δ_C 92.0, 167.9, 168.7, 51.0).[11] The 1D NMR spectra of compound **7** revealed a bisindole alkaloid, possessing aspidosperma (unit A)[11] and venalstonidine (unit B) units,[12] similar to scandomelidine.[14] Six aromatic protons [δ_H 7.22 (1H, s), 6.54 (1H, s), 7.02 (1H, d, J = 7.5 Hz), 6.68 (1H, t, J = 7.5 Hz), 6.97 (1H, t, J = 7.5 Hz), and 6.66 (1H, d, J = 7.5 Hz)] in the ^{1}H NMR spectrum suggested that one indole ring was *ortho*-disubstituted and another one was unsubstituted. An OH was placed at C-11, as evidenced by the chemical shift of C-11 (δ_C 159.1, s), and HMBC correlations of H-9 (δ_H 7.22, s) with δ_C 159.1 (s, C-11) and 144.8 (s, C-13), and of H-12 (δ_H 6.54, s) with δ_C 129.6 (s, C-8) and 117.9 (s, C-10). An epoxy ring was formed between C-14′ and C-15′, which was supported by the upfield chemical shift of C-14′ (δ_C 56.4, d) and C-15′ (δ_C 56.8, d), and by HMBC correlations of H-14′ with δ_C 61.8 (d, C-3′) and 35.8 (s, C-20′), and of H-15′ with δ_C 61.8 (d, C-3′), 30.4 (t, C-19′), and 62.9 (d, C-21′) (Figure 5S). Linkage of units A and B by C-3′/C-10 was established by the HMBC correlations of δ_H 4.65 (1H, br s, H-3′) with δ_C 117.9 (s, C-10), 121.8 (d, C-9), and 159.1 (s, C-11). ROESY correlations of H-21′/Me-18′ and H-21′/H-5′a positioned H-21′, Me-18′, and H-5′a at the same side, α-oriented. The ROESY correlations of H-5′b/H-3′ and H-3′/H-14′/H-15′ suggested that H-3′, H-14′, and H-15′ were β-oriented, while the epoxy was α-oriented (Figure 6S). Detailed analysis of 2D NMR spectral data (HSQC, HMBC, ROESY) indicated that the other parts of **7** were the same as those of scandomelidine. Compound **7** was established, therefore, as shown.

Melosuavine H (**8**) had the molecular formula $C_{42}H_{46}N_4O_5$, and its ^{1}H and ^{13}C NMR spectra (Table 3) were similar to those of **7** except for the olefinic carbons [δ_C 124.6 (d) and 132.2 (d)] in **8** instead of epoxy carbons [δ_C 56.4 (d) and 56.8 (d)] in **7** and linkage of the two units by C-5′/C-10 in **8** instead of C-3′/C-10 in **7**. The double bond was placed at C-14′/15′, on the basis of HMBC correlations of δ_H 5.72 (1H, H-15′) with δ_C 45.3 (t, C-3′), 67.1 (d, C-21′), and 35.1 (t, C-19′). HMBC correlations of δ_H 4.74 (1H, t, J = 8.4 Hz, H-5′a) with δ_C 117.7 (s, C-10), 122.7 (s, C-9), and 158.9 (s, C-11) supported the linkage of C-5′/C-10. ROESY correlations of H-5′/H-21′ and H-21′/Me-18′ indicated the α-orientation of H-5′. Other parts of the structure of **8** were identical to those of **7** by detailed analysis of 2D NMR spectral data.

Compound **9** gave NMR spectra similar to those of melodinine J (**10**),[9] except for an OCH_3 in **9** instead of an OH in **10**, which was in agreement with its molecular formula. The methoxy group was connected to δ_C 157.4 (s, C-11) by the HMBC correlations. In the ROESY spectrum, correlations of H-21′/Me-18′/H-17′b and H-17′a/H-16′ suggested that H-21′ and the ethyl group were located on the same side, while H-16′ and 17′a were on another side of the molecule. The coupling constant (br d, J = 10.8 Hz) for H-16′ suggested its β-orientation.[9,15] Other parts of the structure were identical to those of melodinine J by detailed analysis of 2D NMR spectral data. Thus, the structure of **9** was established as shown. The ^{1}H and ^{13}C NMR data of **9** were similar to those of tenuicausine,[8] whose relative configuration is undetermined, as its NMR spectral data were assigned incorrectly in the literature.[8] Thus, we presented structure **9** for tenuicausine and reassigned its NMR spectral data in Table 3.

All compounds were evaluated for their cytotoxicity against five human cancer cell lines using the MTT method reported previously.[16] Compounds **1**, **2**, **4**–**6**, **8**, and **10** showed low micromolar cytotoxicities (Table 4). Compounds **3**, **7**, and **9** were inactive (IC_{50} values of >40 μM). It is noteworthy that bisindole alkaloids **1**, **2**, **6**, and **8** exhibited stronger inhibitory effects against one or more of the five human cancer cell lines with lower IC_{50} values than those of cisplatin.

Table 4. Cytotoxicity of Compounds 1–10 (IC_{50}, μM)

entry	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	2.6	5.9	11.0	11.6	9.8
2	2.8	3.1	8.9	6.1	2.9
3	17.6	29.0	>40	>40	>40
4	5.5	13.8	9.7	11.6	14.4
5	4.8	12.6	12.4	10.7	12.5
6	3.5	3.6	7.5	6.6	4.0
7	>40	>40	>40	>40	>40
8	1.0	3.1	3.4	9.5	3.8
9	>40	>40	>40	>40	>40
10	3.6	10.0	14.3	9.5	9.7
cisplatin	1.1	15.3	11.1	18.4	18.6

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 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. HRESIMS was 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. suaveolens* was collected in 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. Air-dried and powdered twigs and leaves of *M. suaveolens* (16 kg) were extracted with 90% MeOH (24 h × 4). The extract was partitioned between EtOAc and 0.5% HCl solution. The acidic water solution, adjusted to pH 9–10 with 10% ammonia solution, was first extracted with EtOAc and then extracted with *n*-butanol to give an alkaloidal extract (33 g). The extract was subjected to silica gel CC ($CHCl_3$–MeOH, 1:0, 20:1, 15:1, 10:1, 8:1, 6:1, 4:1, 2:1) to afford fractions I–VI. Fraction II (4.5 g) was subjected to MPLC with RP-18 CC (MeOH–H_2O, 6:4–10:0), then followed by silica gel CC ($CHCl_3$–Me_2CO, 8:1–6:1),

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to yield **4** (32 mg) and a mixture. The mixture was chromatographed on silica gel CC (petroleum ether–Me_2CO, 1:1–0:1) and then purified by Sephadex LH-20 CC (MeOH) to afford **7** (5 mg). Fraction III (6.8 g) was subjected to MPLC with RP-18 CC (MeOH–H_2O, 5:5–10:0) to give subfractions III-a and III-b. Fraction III-a was subjected to silica gel CC ($CHCl_3$–MeOH, 15:1, 10:1) to yield **5** (24 mg). Fraction III-b was subjected to Sephadex LH-20 CC (MeOH), then MPLC with RP-18 CC (MeOH–H_2O, 50:50, 55:45), to give **6** (60 mg). Fraction IV (2.3 g) was separated by silica gel CC ($CHCl_3$–Me_2CO, 6:1, 4:1, 2:1), then by RP-18 CC, eluted with MeOH–H_2O (50:50, 55:45), to afford **3** (17 mg) and a mixture. The latter was purified by Sephadex LH-20 CC (MeOH) to give **8** (15 mg). Fraction V (3.5 g) was separated by RP-18 CC, eluted with MeOH–H_2O (4:6–10:0), and then by silica gel CC ($CHCl_3$–MeOH, 10:1, 8:1) to afford **2** (21 mg) and a mixture. Separation of the mixture by RP-18 CC (MeOH–H_2O, 45:55) yielded **1** (26 mg). Fraction VI (7.2 g) was subjected to MPLC with RP-18 CC (MeOH–H_2O, 3:7–8:2) to give subfractions VI-a and VI-b. Fraction VI-a was separated by silica gel CC ($CHCl_3$–MeOH, 10:1), then further by Sephadex LH-20 CC (MeOH), to yield **9** (20 mg). Fraction VI-b was subjected to Sephadex LH-20 CC (MeOH), then RP-18 CC (CH_3OH–H_2O, 55:45, 60:40), to give **10** (68 mg).

*Melosuavine A (**1**):* white powder; mp 132–134 °C; $[\alpha]^{24}_D$ +87.5 (*c* 0.099, MeOH); UV (MeOH) λ_{max} (log ε) 329 (4.69), 250 (4.55), 203 (4.72) nm; IR (KBr) ν_{max} 3431, 2954, 2927, 1744, 1729, 1677, 1619, 1484, 1437, 1264, 1232, 1150, 1106, 923, 754, 584 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (Me_2CO-d_6), see Table 1; positive ion HRESIMS *m*/*z* 701.3332 (calcd for $C_{42}H_{45}N_4O_6$ $[M + H]^+$, 701.3339).

*Melosuavine B (**2**):* white powder; mp 92–95 °C; $[\alpha]^{24}_D$ −242.7 (*c* 0.103, MeOH); UV (MeOH) λ_{max} (log ε) 330 (4.23), 254 (4.29), 207 (4.64) nm; IR (KBr) ν_{max} 3433, 2954, 2921, 1743, 1678, 1618, 1483, 1437, 1262, 1233, 1151, 1104, 920, 751, 585 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (Me_2CO-d_6), see Table 1; positive ion HRESIMS *m*/*z* 701.3324 (calcd for $C_{42}H_{45}N_4O_6$ $[M + H]^+$, 701.3339).

*Melosuavine C (**3**):* white powder; mp 140–142 °C; $[\alpha]^{25}_D$ +143.7 (*c* 0.106 MeOH); UV (MeOH) λ_{max} (log ε) 330 (4.22), 254 (4.29), 208 (4.66) nm; IR (KBr) ν_{max} 3432, 2953, 2923, 1742, 1678, 1620, 1483, 1438, 1264, 1234, 1150, 1107, 919, 755, 574 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (CD_3OD), see Table 1; positive ion HRESIMS *m*/*z* 701.3346 (calcd for $C_{42}H_{45}N_4O_6$ $[M + H]^+$, 701.3339).

*Melosuavine D (**4**):* white powder; mp 149–150 °C; $[\alpha]^{25}_D$ −545.2 (*c* 0.179 MeOH); UV (MeOH) λ_{max} (log ε) 330 (4.53), 248 (4.34), 224 (4.31), 202 (4.52) nm; IR (KBr) ν_{max} 3417, 3378, 2976, 2783, 1673, 1653, 1629, 1601, 1440, 1274, 1213, 1160, 1101, 1045, 840, 609 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data (DMSO), see Table 2; positive ion HRESIMS *m*/*z* 719.3462 (calcd for $C_{42}H_{47}N_4O_7$ $[M + H]^+$, 719.3444).

*Melosuavine E (**5**):* red powder; mp 98–99 °C; $[\alpha]^{24}_D$ −247.0 (*c* 0.112, MeOH); UV (MeOH) λ_{max} (log ε) 329 (4.69), 250 (4.55), 203 (4.72) nm; IR (KBr) ν_{max} 3414, 3385, 2961, 2785, 1677, 1615, 1482, 1437, 1263, 1210, 1149, 1104, 1041, 837, 602 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data (Me_2CO-d_6), see Table 2; positive ion HRESIMS *m*/*z* 703.3511 (calcd for $C_{42}H_{47}N_4O_6$ $[M + H]^+$, 703.3495).

*Melosuavine F (**6**):* red powder; mp 93–95 °C; $[\alpha]^{24}_D$ −532.4 (*c* 0.211, MeOH); UV (MeOH) λ_{max} (log ε) 329 (4.69), 250 (4.55), 203 (4.72) nm; IR (KBr) ν_{max} 3415, 3387, 2959, 2784, 1677, 1615, 1480, 1437, 1262, 1210, 1151, 1102, 1038, 837, 563 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data (Me_2CO-d_6), see Table 2; positive ion HRESIMS *m*/*z* 703.3495 (calcd for $C_{42}H_{47}N_4O_6$ $[M + H]^+$, 703.3495).

*Melosuavine G (**7**):* colorless syrup; $[\alpha]^{24}_D$ −142.3 (*c* 0.112, MeOH); UV (MeOH) λ_{max} (log ε) 331 (4.01), 248 (4.07), 207 (4.39) nm; IR (KBr) ν_{max} 3440, 3426, 2954, 2923, 2854, 1727, 1675, 1614, 1480 1436, 1264, 1231, 1152, 1102, 746 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (Me_2CO-d_6), see Table 3; positive ion HRESIMS *m*/*z* 703.3512 (calcd for $C_{42}H_{47}N_4O_6$ $[M + H]^+$, 703.3495).

*Melosuavine H (**8**):* white powder; mp 93–95 °C; $[\alpha]^{24}_D$ −34.0 (*c* 0.101 MeOH); UV (MeOH) λ_{max} (log ε) 331 (4.29), 245 (4.34), 204 (4.71) nm; IR (KBr) ν_{max} 3439, 3425, 2950, 2923, 2869, 1731, 1675, 1615, 1481, 1438, 1266, 1210, 1151, 1106, 748, 593 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (Me_2CO-d_6), see Table 3; positive ion HRESIMS *m*/*z* 687.3527 (calcd for $C_{42}H_{47}N_4O_5$ $[M + H]^+$, 687.3546).

*Tenuicausine (**9**):* blue powder; mp 100–102 °C; $[\alpha]^{25}_D$ −4.8 (*c* 0.103 MeOH); UV (MeOH) λ_{max} (log ε) 331 (4.18), 229 (4.50), 202 (4.52) nm; IR (KBr) ν_{max} 3437, 2952, 2921, 2846, 1738, 1705, 1678, 1594, 1488, 1434, 1355, 1246, 1230, 1154, 1034, 912, 754, 569 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (Me_2CO-d_6), see Table 3; positive ion HRESIMS *m*/*z* 643.3663 (calcd for $C_{41}H_{47}N_4O_3$ $[M + H]^+$, 643.3648).

Cytotoxicity Assay. 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 cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method in 96-well microplates.[16] 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 0.064, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin (Sigma, USA) as a positive control. After compound treatment, cell viability was detected, and the cell growth curve was graphed. The IC_{50} value was calculated by Reed and Muench's method.[17]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures 1S–6S and the 1D, 2D NMR and MS spectra of melosuavines A–H (**1**–**8**), tenuicausine (**9**), and melodinine J (**10**). These materials are available free of charge via Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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