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Melodinines A−G, Monoterpenoid Indole Alkaloids from *Melodinus henryi*

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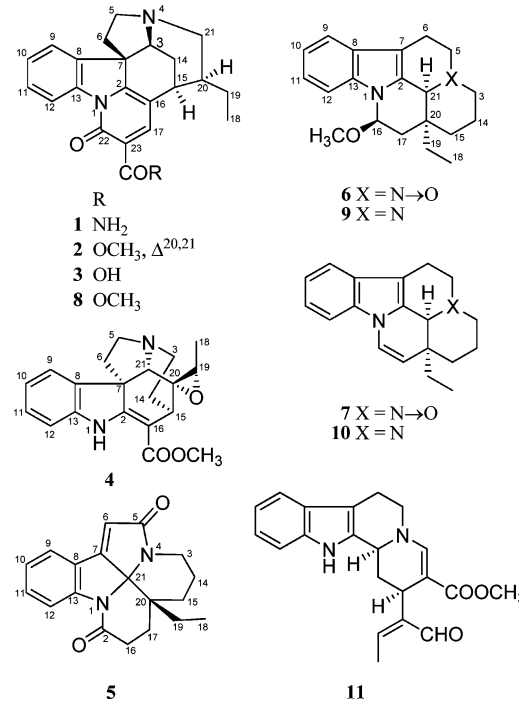
Nineteen monoterpenoid indole alkaloids including seven new ones, melodinines A−G (**1**−**7**), were isolated from *Melodinus henryi*. The structures of the new compounds were elucidated using spectroscopic methods, and the structure of compound **4** was confirmed by single-crystal X-ray diffraction analysis. The known compounds were identified by comparing their spectroscopic data with those reported in the literature. All of the compounds were evaluated for cytotoxic activity against five human cancer cell lines, and compound **11** exhibited cytotoxicity against HL-60, SMMC-7721, A-549, and SK-BR-3 cells with IC_{50} values of 2.0, 16.8, 25.9, and 24.7 μM, respectively.

Plants of the family Apocynaceae have been proven to be good sources of monoterpenoid indole alkaloids, which originate from the condensation of tryptophan with secologanin.[1] Our previous studies reported isolation of (19,20)-*E*/*Z*-alstoscholarine,[2] scholarisines A−G,[3] and alstoyunines A−H[4] from the genus *Alstonia*. Pharmacological investigations on these alkaloids demonstrated promising anti-inflammatory and cytotoxic activities.[4] As part of our search for novel and bioactive alkaloids we investigated the chemical constituents of *Melodinus henryi* Craib. (Apocynaceae), a cane used for treating meningitis and fractures in China, Thailand, and Burma.[5] This led to the isolation of seven new monoterpenoid indole alkaloids, melodinines A−G (**1**−**7**), together with 12 known compounds. The structures of the new compounds were elucidated by means of spectroscopic methods, and the structure of **4** was confirmed by single-crystal X-ray diffraction analysis. Compounds **1**−**3** possessed 21 skeletal carbons arranged compactly in six rings, and compound **5** was a diazaspiroindole alkaloid, a structural type seldom reported previously. The known compounds were *Leuconotis* alkaloid 376 (**8**),[6] *O*-methylepivincanol (**9**),[7] (−)-eburnamenine (**10**),[8] vallesiachotamine (**11**),[9] decarbomethoxydihydrogambirtannine,[10] eburenine,[11] rhazinilam,[12] epivincanol,[13] 19-(*R*)-methoxytubotaiwine,[13] stemmadenine,[14] stemmadenine-*N*-oxide,[15] and isositsirikine.[16] Structures of these known compounds were identified by comparison with data reported in the literature. All of the compounds were evaluated for cytotoxicity against five human cancer cell lines: breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 cells.



Results and Discussion

Melodinine A (**1**) was isolated as colorless needles. The molecular formula was established as $C_{22}H_{23}N_3O_2$ by HRESIMS ($[M + H]^+$ at *m*/*z* 362.1867), which indicated 13 degrees of unsaturation. IR absorption bands at 1668 and 1666 cm^{-1} revealed the existence of C═O groups. The ^1H and ^{13}C NMR spectra indicated an *ortho*-disubstituted phenyl ring (Table 1). In addition to the phenyl ring, **1** possessed four olefinic carbons, two amide groups, three methines, five methylenes, and one methyl (Table 1). These data were closely related to that of *Leuconotis* alkaloid 376 (**8**),[6] except for an acylamide group in **1** instead of the methyl ester group in **8**. In the ^1H NMR spectrum, two doublets at δ_H 5.80 (1H, d, J = 3.6 Hz, N-Hb) and 9.47 (1H, d, J = 3.6 Hz, N-Ha) were assigned to protons of a NH_2 group, as evidenced by $^1H-^1H$ COSY and ROESY correlations between them and by HMBC correlations of N-Hb with δ_C 165.7 (s, C═O) and 120.2 (s, C-23) (Figure 1). The downfield chemical shift of N-Ha might be caused by the hydrogen bond formed between N-Ha and the acylamide group at C-23 (Figure 1). Detailed analysis of 2D NMR data and comparison of 1D NMR data with those of **8** established the planar structure of **1**. The relative configurations of chiral carbons were established by a ROESY experiment. The ROESY correlation of H-9/H-3 determined the relative configuration of C-3 and C-7, which subsequently allowed the relative configuration of H-15 to be α-oriented. ROESY correlation of H-15 with H-20 suggested the α-orientation of H-20. Thus, the structure and the relative configuration of melodinine A were established as shown in structure **1**.

Melodinine B (**2**) possessed the molecular formula $C_{23}H_{22}N_2O_3$, as deduced from HRESIMS ($[M + H]^+$ at *m*/*z* 375.1072). The ^1H and ^{13}C NMR data (Table 1) were very similar to those of **8** except for two olefinic carbons [δ_C 122.8 (s) and 129.9 (d)] in **2** instead of two sp^3 carbons in **8**. Two olefinic carbons were assigned to a double bond at C-20/21 on the basis of HMBC correlations of δ_H

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Table 1. ^{1}H and ^{13}C NMR Data of **1**−**3** ($CDCl_3$, δ in ppm and J in Hz)

	1		**2**		**3**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		161.1		158.7		162.0
3	4.08 br s	62.2	4.18 d (2.0)	60.0	4.40 br s	62.1
5a	3.12 m	54.5	3.27 m		3.40 m	
5b	2.91 m		3.17 m	53.6	3.03 m	54.2
6a	2.89 m	44.9	2.26 overlap	46.1	2.89 m	44.6
6b	1.99 m		1.83 dd (12.0, 4.4)		2.13 dd (14.0, 7.0)	
7		55.6		56.7		55.6
8		140.0		139.4		139.5
9	7.36 d (8.0)	120.2	7.43 d (8.0)	120.3	7.48 d (8.0)	120.5
10	7.27 t (8.0)	126.9	7.28 t (8.0)	126.7	7.37 t (8.0)	128.0
11	7.34 t (8.0)	128.1	7.37 t (8.0)	128.5	7.44 t (8.0)	128.7
12	8.48 d (8.0)	117.4	8.63 d (8.0)	118.2	8.47 d (8.0)	117.9
13		140.6		140.8		139.7
14a	2.17 dd (12.8, 3.2)	31.4	2.26 overlap	30.9	2.36 br d (13.5)	30.9
14b	1.34 br d (12.8)		1.32 br d (12.8)		1.45 dd (13.5, 3.0)	
15	2.90 m	36.3	3.04 d (2.0)	33.7	2.98 d (3.0)	36.1
16		115.7		118.7		117.4
17	8.27 s	145.4	8.11 s	143.9	8.26 s	146.4
18	1.02 t (7.6)	11.5	1.04 t (7.2)	12.8	1.07 t (7.5)	11.4
19a	1.48 m	26.5	2.09 m	27.5	1.48 m	26.3
19b	1.22 m		2.11 m		1.30 m	
20	1.88 m	38.7		122.8	2.06 overlap	38.3
21a	2.99 dd (10.8, 3.6)	51.4	5.53 s	129.9	3.26 br d (9.2)	51.2
21b	1.92 m				2.06 overlap	
22		161.6		159.4		163.3
23		120.2		119.4		117.1
CO_2NH_2		165.7				
CO_2NH_2	9.47 d (3.6)					
	5.80 d (3.6)					
CO_2CH_3				166.2		
CO_2CH_3			3.94 s	52.4		
CO_2H						165.6

5.53 (1H, s, H-21) with δ_C 60.0 (d, C-3), 53.6 (t, C-5), 33.7 (d, C-15), and 27.5 (t, C-19). The ROESY correlations were very similar to those of **1**, which indicated that the relative configurations at chiral centers were the same as in **1**. Finally, detailed analysis of 2D NMR (HSQC, HMBC, ^{1}H−^{1}H COSY, ROESY) data revealed melodinine B to have structure **2**, as shown.

Compound **3** had the molecular formula $C_{22}H_{22}N_2O_3$ as determined by HRESIMS. The 1D NMR data (Table 1) were closely related to those of **8** except for a carboxyl group (δ_C 165.6) in **3** instead of the methyl ester group in **8**, as supported by the lack of signals of the OCH_3 group in the ^{1}H and ^{13}C NMR spectra and the presence of an OH group as indicated by the IR absorption band at 3463 cm^{-1}. ROESY correlations of H-3/H-9 and H-15/H-20 suggested that the relative configuration of **3** was the same as that of **1**. Analysis of 2D NMR data (HSQC, HMBC, ROESY) established the structure of **3** to be as shown, and it was named melodinine C.

Melodinine D (**4**) was isolated as colorless needles. The molecular formula $C_{20}H_{22}N_2O_3$ was established by HRESIMS ([M + H]$^+$ at m/z 339.1709). The UV spectrum showed absorption maxima characteristic of a β-anilinoacrylate chromophore (327, 294, and 240 nm), while the IR spectrum showed absorption bands due

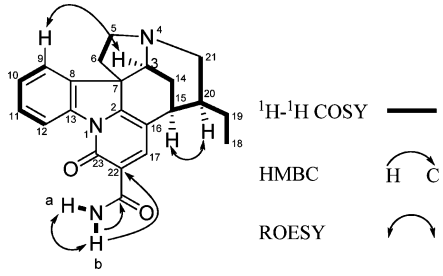

Figure 1. Key 2D NMR correlations of **1**.

to NH (3353 cm^{-1}) and conjugated ester (1673 cm^{-1}) functions.[17] The ^{1}H NMR data revealed the existence of an *ortho*-disubstituted phenyl ring, an indolic NH group, and two methyl groups (Table 2). The ^{13}C NMR and DEPT spectra displayed 20 carbon resonances, which were ascribed to two methyl, four methylene, seven methine, and seven quaternary carbons (Table 2). The signals at δ_C 168.7 and 98.7 were readily assigned to C-2 and C-16, respectively, corresponding to the acrylate double bond. The above NMR data resembled those of condylocarpine,[18] except for the oxidation of the C_{19}═C_{20} double bond in condylocarpine into an epoxy ring in **4** [δ_C 59.2 (C-19) and δ_C 63.3 (C-20)] in the ^{13}C NMR spectrum. This was supported by MS analysis and HMBC correlations of δ_H 2.81 (1H, q, J = 5.6 Hz, H-19) with Me-18 and C-20. The relative configuration of **4** was established to be the same as that of condylocarpine. The absolute configuration of C-7 in condylocarpine-type alkaloids such as condylocarpine,[18] tubotaiwine,[19] (19*S*,20*S*)-19-hydroxytubotaiwine,[20] and 19-(*R*/*S*)-methoxytubotaiwine,[13] was assigned previously to be *R*. Thus, the absolute configurations at C-7, C-15, and C-21 in **4** were assumed to be 7*S*, 15*S*, and 21*R* according to the relative configuration. The ROESY spectrum did not reveal the configuration of the epoxy ring, even though correlations of Me-18/H-21 and H-19/H-15 were observed. A single-crystal X-ray diffraction study clarified the relative configuration of **4** (Figure 2).

Melodinine E (**5**) had the molecular formula $C_{19}H_{20}N_2O_2$ as established by HRESIMS. The UV spectrum had absorption maxima at 350 and 254 nm, indicating a conjugated moiety, while the IR spectrum showed absorption bands due to lactam groups (1688 and 1639 cm^{-1}). The ^{1}H NMR spectrum showed the presence of an *ortho*-disubstituted phenyl ring with the characteristically deshielded H-12 (δ_C 8.14) due to anisotropy by the proximate lactam carbonyl (Table 2).[21] The ^{13}C NMR spectrum displayed 19 carbon signals, which were ascribed to one methyl, six methylene, five methine, and seven quaternary carbons (Table 2). Of them,

Table 2. ^{1}H and ^{13}C NMR Data of **4** and **5** ($CDCl_3$, δ in ppm and J in Hz)

	4		**5**	
position	δ_H	δ_C	δ_H	δ_C
2		168.7		173.5
3a	3.22 m	44.7	4.42 m	36.9
3b	2.68 m		3.21 m	
5a	3.11 m	53.2		176.0
5b	3.02 m			
6a	2.80 m	44.5	6.20 s	118.1
6b	1.99 m			
7		59.6		164.2
8		133.5		123.4
9	7.15 d (8.0)	120.3	7.44 d (7.5)	121.5
10	6.87 t (8.0)	121.2	7.10 t (7.5)	124.3
11	7.11 t (8.0)	127.9	7.31 t (7.5)	131.5
12	6.80 d (8.0)	109.9	8.14 d (7.5)	115.8
13		144.5		148.5
14a	2.15 m	23.8	2.00 m	16.7
14b	1.86 m		1.79 m	
15a	2.46 m	34.9	1.62 m	26.0
15b			1.05 m	
16a		98.7	3.04 m	33.1
16b			2.59 m	
17a			2.09 m	30.4
17b			1.66 m	
18	0.99 d (5.6)	14.1	0.73 t (7.4)	8.2
19a	2.81 q (5.6)	59.2	1.42 q (7.4)	34.1
19b			1.32 q (7.4)	
20		63.3		44.5
21	3.65 br d	61.4		93.5
CO_2CH_3		167.7		
CO_2CH_3	3.75 s	51.3		
NH	8.79 br s			

signals at δ_C 173.5 and 176.0 were readily assigned to two lactam carbonyls (C-2 and C-5, respectively). One sp^3 quaternary carbon signal (δ_C 93.5) was assigned to C-21 bearing the connections to both N-1 and N-4. The above NMR data showed that **5** had a structure similar to that of leuconoxine[21] except for the replacement of two sp^3 carbons of C-6 and C-7 in leuconoxine by two olefinic carbons [δ_C 118.1 (d, C-6), 164.2 (s, C-7)] in **5**, as supported by MS analysis and HMBC correlations of δ_H 6.20 (1H, s, H-6) with δ_C 176.0 (s, C-5) and 123.4 (s, C-8). Finally, detailed analysis of 1D and 2D NMR data established the structure of **5** as 6,7-dehydroleuconoxine.

Melodinine F (**6**) was isolated as colorless needles. The UV spectrum showed absorption maxima characteristic of an indole chromophore at 274 and 239 nm.[22] The HRESIMS had an [M + H]$^+$ peak at m/z 327.2081, which analyzed for $C_{20}H_{26}N_2O_2$, 16 mass units higher than that of *O*-methylepivincanol (**9**).[7] Compound **6** was readily identified as epivincanol-*N*(4)-oxide from ^{1}H and ^{13}C NMR data (Table 3), in particular the characteristic downfield shifts of the carbon resonances for C-3 (δ_C 58.0), C-5 (δ_C 69.7), and C-21 (δ_C 71.8), when compared to those of **9**.

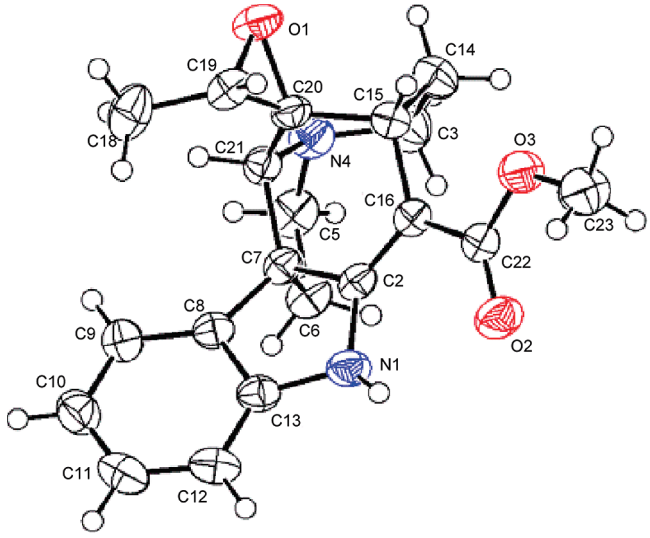

Figure 2. X-ray diffraction of **4** showing relative configuration.

Table 3. ^{1}H and ^{13}C NMR Data of **6** and **7** ($CDCl_3$, δ in ppm and J in Hz)

	6		**7**	
position	δ_H	δ_C	δ_H	δ_C
2		127.8		126.7
3a	3.32 m	58.0	3.48 m	58.5
3b	3.09 m		3.16 br d (11.5)	
5a	3.93 m	69.7	3.98 m	70.8
5b	3.82 m		3.86 m	
6a	3.04 m	19.5	3.11 m	20.1
6b	3.06 m		3.04 m	
7		105.2		106.4
8		127.1		126.5
9	7.45 d (8.0)	118.5	7.45 d (7.8)	118.8
10	7.14 t (8.0)	120.9	7.24 t (7.8)	120.7
11	7.23 t (8.0)	122.7	7.14 t (7.8)	123.1
12	7.25 d (8.0)	110.9	7.33 d (7.8)	108.9
13		136.4		134.5
14a	2.59 m	16.6	2.66 m	16.2
14b	1.39 br d (13.6)		1.46 br d (14.2)	
15a	1.93 m	25.4	1.59 br d (14.0)	30.7
15b	1.47 br d (14.0)		1.24 dd (14.0, 3.5)	
16a	5.42 d (3.4)	82.3	6.93 d (7.8)	119.9
16b				
17a	2.18 br d (15.2)	34.4	5.03 d (7.8)	117.6
17b	1.96 dd (15.2, 4.4)			
18	0.99 t (7.3)	8.3	1.07 t (7.4)	9.6
19a	2.60 q (7.3)	31.0	2.78 q (7.4)	30.2
19b	2.25 q (7.3)		1.88 q (7.4)	
20		36.5		39.1
21	4.43 s	71.8	4.78 s	69.5
OCH_3	3.50 s	55.7		

Melodinine G (**7**) was isolated as a colorless oil. The HRESIMS displayed an [M + H]$^+$ peak at m/z 295.1811 ($C_{19}H_{22}N_2O$), 16 mass units higher than that of (−)-eburnamenine (**10**).[8] Compound **7** was readily identified as (−)-eburnamenine-*N*(4)-oxide from ^{1}H and ^{13}C NMR data (Table 3), in particular the characteristic downfield shifts of the carbon resonances for C-3 (δ_C 58.55), C-5 (δ_C 70.8), and C-21 (δ_C 69.5) with respect to those of **10**.

All compounds were evaluated for their cytotoxicity against five human cancer cell lines using the MTT method as reported previously.[23] Cisplation (Sigma, USA) was used as the positive control. Compound **11** showed cytotoxicity against HL-60, SMMC-7721, A-549, and SK-BR-3 cells, with IC_{50} values of 2.0, 16.8, 26.0, and 24.7 μM, respectively, while cisplatin gave IC_{50} values of 1.1, 16.5, 23.2, and 30.0 μM. The other compounds were inactive (IC_{50} values >40 μM).

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 spectrophotometer. IR spectra were obtained with a Tenor 27 spectrophotometer using KBr pellets. 1D and 2D spectra were run on a Bruker DRX-500 spectrometer or on an AV-400 spectrometer with TMS as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Mass spectra were recorded on a VG Autospec-3000 spectrometer or 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 using Dragendorff's reagent or by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. *M. henryi* plants were collected in Mengna County, Yunnan Province, P. R. China, and identified by Mr. Jing-Yun Cui, Xishuangbanna Tropical Plant Garden. A voucher specimen (No. Cui20081128) has been deposited at the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Air-dried and powdered plant material (12 kg) was extracted with 90% EtOH (24 h × 3) to give a crude extract (950 g). The extract was partitioned between EtOAc and 5% HCl solution. The acidic water-soluble material was adjusted to pH 9−10 with 10% ammonia solution and then extracted with EtOAc to give an alkaloidal extract (77 g). The alkaloidal extract was subjected to a silica gel CC ($CHCl_3$−CH_3OH, 1:0 to 0:1) to afford fractions A−H. Fraction A (7 g) was separated by silica gel CC (petroleum ether−Me_2CO, 15:1−3:1) to afford **9** (201 mg), **10** (7 mg), decarbomethoxydihydrogambirtannine (23 mg), and eburenine (18 mg). Fraction B (8 g) was subjected to silica gel CC (petroleum ether−Me_2CO, 8:1−1:1) to yield **2** (12 mg), **5** (23 mg), **11** (31 mg), rhazinilam (112 mg), and 19-(*R*)-methoxytubotaiwine (41 mg). Fraction C (10 g) was chromatographed on a silica gel column (petroleum ether−Me_2CO, 3:1−1:1) to yield **1** (24 mg), **4** (21 mg), and a mixture (800 mg). The mixture was further separated by chromatography on RP-18 (MeOH−H_2O, 6:4) to yield **8** (48 mg) and epivincanol (120 mg). Fraction D (3 g) was separated by silica gel CC ($CHCl_3$−MeOH, 15:1) and then by RP-18 (MeOH−H_2O, 4:6) to yield stemmadenine (700 mg) and isositsirikine (45 mg). Fraction E (7 g) was subjected to silica gel CC ($CHCl_3$−MeOH, 10:1) to afford **3** (18 mg), **6** (38 mg), and a mixture. The mixture was separated further on RP-18 (CH_3OH−H_2O, 4:6) and Sephadex LH-20 (MeOH) columns to yield **7** (13 mg) and stemmadenine-*N*-oxide (40 mg).

Melodinine A (1): colorless needles (Me_2CO); mp 228−229 °C; $[\alpha]_D^{20}$ −887.2 (*c* 0.18, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 368 (3.73), 241 (3.78) nm; IR (KBr) ν_{max} 3339, 2924, 1668, 1666, 1540, 1458, 747 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see Table 1; positive ion HRESIMS *m/z* 362.1867 (calcd for $C_{22}H_{24}N_3O_2$ $[M + H]^+$, 362.1868).

Melodinine B (2): colorless needles (Me_2CO); mp 176−177 °C; $[\alpha]_D^{20}$ −665.1 (*c* 0.20, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 378 (3.73), 241 (3.79) nm; IR (KBr) ν_{max} 2956, 2951, 1738, 1700, 1659, 1544, 749 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see Table 1; positive ion HRESIMS *m/z* 375.1702 (calcd for $C_{23}H_{23}N_2O_3$ $[M + H]^+$, 375.1708).

Melodinine C (3): white powder; $[\alpha]_D^{20}$ −502.7 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 363 (3.76), 283, (3.21), 205 (4.39) nm; IR (KBr) ν_{max} 3463, 2930, 1657, 1589, 1459, 749 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see Table 1; positive ion HRESIMS *m/z* 363.1718 (calcd for $C_{22}H_{23}N_2O_3$ $[M + H]^+$, 363.1708).

Melodinine D (4): colorless needles (Me_2CO); mp 172−173 °C; $[\alpha]_D^{20}$ +816.8 (*c* 0.17, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 327 (3.71), 294 (3.60), 240 (3.65), 204 (3.37), 193 (3.37) nm; IR (KBr) ν_{max} 3353, 2925, 1673, 1596, 1435, 1236, 1160, 756 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see Table 2; positive ion HRESIMS *m/z* 339.1709 (calcd for $C_{20}H_{23}N_2O_3$ $[M + H]^+$, 339.1708).

Melodinine E (5): colorless oil; $[\alpha]_D^{20}$ +304.0 (*c* 0.19, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 350 (3.47), 254 (4.09) nm; IR (KBr) ν_{max} 3439, 2924, 1688, 1639, 1459, 1376, 1020, 759 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see Table 2; positive ion HRESIMS *m/z* 309.1597 (calcd for $C_{19}H_{21}N_2O_2$ $[M + H]^+$, 309.1603).

Melodinine F (6): colorless needles (Me_2CO); mp 187−189 °C; $[\alpha]_D^{20}$ +16.6 (*c* 0.23, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 274 (3.66), 239 (3.68) nm; IR (KBr) ν_{max} 3423, 2924, 1459, 1074, 747 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see Table 3; positive ion HRESIMS *m/z* 327.2081 (calcd for $C_{20}H_{27}N_2O_2$ $[M + H]^+$, 327.2072).

Melodinine G (7): colorless oil; $[\alpha]_D^{20}$ +147.9 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 312 (3.62), 303 (3.66), 257 (4.20), 219 (4.23), 204 (4.29) nm; IR (KBr) ν_{max} 3424, 2929, 1644, 1462, 1440, 1253, 743 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data ($CDCl_3$), see Table 3; positive ion HRESIMS *m/z* 295.1811 (calcd for $C_{19}H_{23}N_2O$ $[M + H]^+$, 295.1810).

Crystallographic Data of Melodinine D (4): $C_{20}H_{22}N_2O_3 \cdot H_2O$, MW = 356.41; monoclinic, space group $P2_1$; a = 6.9521(9) Å, b = 14.5835(19) Å, c = 17.382(2) Å, α = 90.00°, β = 90.00(10)°, γ = 90.00°, V = 1762.3(14) $Å^3$, Z = 4, d = 1.343 g/cm^3. A colorless crystal of dimensions 0.24 × 0.18 × 0.12 mm was used for measurement with SHELXL-97 on a graphite monochromator, Mo Kα radiation. The total number of reflections measured was 3993, of which 3109 were observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0505, wR_2 = 0.1529. The crystal structure of **4** was solved by the direct method SHLXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of **4** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 743265). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Cytotoxicity Assay. Five human cancer cell lines, breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 cells, were used in the cytotoxic assay. Cells were cultured in RPMI-1640 or in 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-diphenyl tetrazolium bromide) method in 96-well microplates.[23] 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 addition of test compounds, while suspended cells were seeded just before drug addition with initial density of 1 × 10^5 cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin (Sigma, USA) as positive control. 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.[24]

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Supporting Information Available: 1D and 2D NMR and MS spectra of melodinines A−H (**1**−**7**), ^1H and ^{13}C NMR spectra of known compounds, and X-ray crystallographic data (CIF file) of **4**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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