Fitoterapia 138 (2019) 104347

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Melotenuines A-E, cytotoxic monoterpenoid indole alkaloids from *Melodinus tenuicaudatus*

Jian-Wen Liu[a,b], Zong-Qing Huo[b], Qian Zhao[b], Xiao-Jiang Hao[b], Hong-Ping He[a,*], Yu Zhang[b,*]

[a] Yunnan University of Traditional Chinese Medicine, Kunming 650500, PR China
[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, PR China

ARTICLE INFO

Keywords:
Melodinus tenuicaudatus
Monoterpenoid indole alkaloids
Melotenuines A-E
Aspidosperma-meloscandonine type

ABSTRACT

Five new monoterpenoid indole alkaloids, melotenuines A-E (**1–5**), along with 18 known indole alkaloids, were isolated from the twigs and leaves of *Melodinus tenuicaudatus*. The structures of the new alkaloids were determined by a combination of MS, NMR and ECD analysis. Melotenuine A (**1**) represents the first example of aspidosperma-meloscandonine type bisindole alkaloids characterized by a methylene bridge between the two monomers, while melotenuine B (**2**) possessed a rare eburnamine-melsocandonine skeleton. All of the new indole alkaloids were evaluated for in vitro cytotoxicities against five human cancer cell lines. Among them, alkaloid **4** showed specific cytotoxicity against HL-60 cell line with IC_{50} value (5.15 ± 0.16 μM) comparable with that of positive control.

1. Introduction

Monoterpenoid indole alkaloids (MIAs) are characteristic secondary metabolites found mainly in the plants of Rubiaceae, Loganiaceae, and Apocynaceae families [1]. The genus *Melodinus* (Apocynaceae) comprises about 50 species, and is distributed in tropical, or subtropical Asia and Australia [2]. Previous studies demonstrated that this genus is a rich source of MIAs and a series of alkaloids with structural complexity and significant biological activities had been identified [3–8]. As our further investigation on structurally interesting and bioactive MIAs [9–13], melotenuines A-E (**1–5**), along with 18 known indole alkaloids were isolated from the twigs and leaves of *Melodinus tenuicaudatus*. Reported herein are the isolation, structure elucidation, and cytotoxicity evaluation of the new alkaloids.

2. Experimental

2.1. General experimental procedures

Optical rotations were surveyed on a JASCO P-1020 digital polarimeter. A Bio-Rad FTS-135 was used for IR spectra as KBr pellets, while the ECD spectral data were recorded by an Applied Photophysics Chariscan Spectrometer. HRESIMS and ESI were recorded on Aglient 1290 UPLC/6540 Q-TOF spectrometer and a Waters Xevo TQ-S instrument, respectively. The 1D and 2D NMR spectra were measured with TMS as the internal standard for the Bruker 500, 600 and 800 MHz spectrometers. Silica gel (80–100 and 100–200 mesh, Qingdao Marine Chemical Inc., China), silica gel H (10–40 μm, Qingdao Marine Chemical Inc., China), and Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB), were used for column chromatography. Semipreparative HPLC was performed on a YMC Triart C_{18} (5 μm; 10 × 250 mm) reversed-phase column.

2.2. Plant material

The twigs and leaves of *M. tenuicaudatus* were collected in Xishuangbanna, Yunnan Province, People's Republic of China, in July 2017. The samples were identified by Mr. Yu Chen, Kunming Botanical Garden. A specimen (no. ZY20170712) was deposited at State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Science (CAS).

2.3. Extraction and isolation

The dried twigs and leaves of *M. tenuicaudatus* (17 kg) were powdered and extracted three times with methanol. The extract was diluted with water and the pH was adjusted to 2–3 with hydrochloric acid (5%) and then extracted three times with petroleum ether. The water fraction was basified to pH 9–10 with sodium hydroxide (10%), then extracted with chloroform to get the crude alkaloids. The crude alkaloids (300 g)

* Corresponding authors.
E-mail addresses: hehongping@yahoo.com (H.-P. He), zhangyu@mail.kib.ac.cn (Y. Zhang).

https://doi.org/10.1016/j.fitote.2019.104347
Received 27 July 2019; Received in revised form 26 August 2019; Accepted 26 August 2019
Available online 27 August 2019
0367-326X/

Table 1
^{1}H NMR Data of melotenuines A–C (**1–3**).

Position	**1**[a]	**2**[b]	**3**[b]
3a	3.39 (1H, dd, 4.5, 1.5)	3.02 (1H, ddd, 18.0, 4.2, 2.4)	3.32 (1H, dd, 15.6, 5.4)
3b	3.19 (1H, m)	2.86 (1H, m)	3.03 (1H, m)
5a	2.96 (1H, t, 8.0)	3.36 (1H, dd, 13.2, 7.2)	3.15 (1H, t, 14.4)
5b	2.66 (1H, m)	3.32 (1H, dd, 13.2, 6.0)	3.03 (1H, m)
6a	1.96 (1H, m)	3.19 (1H, m)	3.68(1H, m)
6b	1.66 (1H, dd, 11.0, 4.0)	2.53 (1H, ddd, 15.8, 7.2, 6.0)	2.70 (1H, m)
9	7.02 (1H, s)	7.34 (1H, d, 7.8)	7.28 (1H, dd, 7.8, 1.2)
10		6.89 (1H, td, 7.8, 1.2)	7.05 (1H, t, 7.8)
11		6.71 (1H, td, 7.8, 1.2)	6.85 (1H, td, 7.8, 1.2)
12	6.75 (1H, s)	6.22 (1H, d, 7.8)	6.28 (1H, d, 7.8)
14	5.76 (1H, ddd, 10.0, 4.5, 1.5)	5.58 (1H, ddd, 10.0, 4.2, 2.4)	5.84 (1H, m)
15	5.68 (1H, d, 10.0)	5.64 (1H, d, 10.0)	5.61 (1H, d, 10.0)
16		4.72 (1H, dd, 12.0, 3.6)	6.47 (1H, dd, 12.0, 2.4)
17a	2.44 (1H, d, 15.0)	2.23 (1H, dd, 13.8, 3.6)	2.59 (1H, m)
17b	2.51 (1H, d, 15.0)	1.92 (1H, br d, 13.8)	2.28 (1H, dd, 13.8, 2.4)
18	0.62 (3H, t, 7.5)	0.96 (3H, t, 7.8)	0.82 (3H, t, 7.8)
19a	0.97 (1H, m)	1.92 (1H, m)	1.52 (1H, m)
19b	0.77 (1H, m)	1.60 (1H, m)	1.38 (1H, m)
21	2.57 (1H, br s)	4.11 (1H, br s)	2.93 (1H, br s)
11-O<u>Me</u>	3.78 (3H, s)		
16-CO_2<u>Me</u>	3.69 (3H, s)		
3′a	3.70 (1H, m)	3.54 (1H, br d, 18.0)	3.45 (1H, m)
3′b	3.35 (1H, dd, 6.0, 1.2)	3.27 (1H, m)	3.15 (1H, t, 14.4)
5′a	3.17 (1H, m)	3.19 (1H, m)	3.03 (1H, m)
5′b	3.08 (1H, dd, 17.0, 9.0)	3.11 (1H, dd, 16.8, 8.4)	2.69 (1H, m)
6′a	2.25 (1H, ddd, 9.5, 7.5, 2.0)	2.30 (1H, m)	2.02 (1H, m)
6′b	1.82 (1H, td, 9.5, 9.0)	1.92 (1H, m)	1.73 (1H, dd, 11.4, 4.2)
9′	7.10 (1H, d, 1.5)	7.20 (1H, s)	7.60 (1H, s)
11′	6.98 (1H, dd, 8.0, 1.5)	7.21 (1H, d, 7.8)	
12′	6.86 (1H, d, 8.0)	7.07 (1H, d, 7.8)	6.58 (1H, s)
14′	5.95 (1H, m)	5.87 (1H, td, 8.4, 1.2)	5.84 (1H, td, 7.2, 1.2)
15′	5.94 (1H, br s)	5.86 (1H, td, 8.4, 1.2)	5.85 (1H, td, 7.2, 1.2)
17′a	2.17 (1H, dd, 6.0, 4.0)	2.15 (1H, br d, 11.0)	3.05 (1H, br d, 14.4)
17′b	2.02 (1H, br d, 6.0)	2.04 (1H, br d, 11.0)	2.50 (1H, br d, 14.4)
18′	0.81 (3H, d, 7.0)	0.56 (3H, br s)	0.94 (3H, d, 6.6)
19′	2.31 (1H, q, 7.0)	2.27 (1H, q, 7.2)	3.46 (1H, q, 6.6)
21′	3.51 (1H, s)	3.27 (1H, s)	2.87 (1H, br s)
23′a	3.83 (1H, br d, 15.0)		
23′b	3.77 (1H, br d, 15.0)		
11′-O<u>Me</u>			3.39 (3H, s)
16′-CO_2<u>Me</u>			3.68 (3H, s)

The underline indicated the specific atom.
[a] 500 MHz, acetone-d_6.
[b] 600 MHz, acetone-d_6.

were separated on a silica gel column (100–200 mesh), and eluded with a gradient of chloroform-methanol (40:1–20:1–10:1–5:1–1:1) to yield 5 fractions (A-E). The fraction A (18.1 g) was purified by a reversed phase chromatography on a C_{18} column (MeOH/H_2O, 30:70 → 100:0, *v*/v) to give four subfractions (AI-AIV). Subfraction AI (7.2 g) was purified by a reversed phase chromatography on a C_{18} column (MeOH/H_2O, 30:70 → 100:0, *v*/v) and followed by semipreparative HPLC using YMC Triart C_{18} (5 μm; 10 × 250 mm) column with MeOH/H_2O (83:17, 0.1‰ v/v diethylamine) to give 11-methoxytabersonine (30.6 mg, t_R 35.0 min), and 3*α*-acetonyltabersonine (2.2 mg, t_R 42.5 min). Subfraction AII (4.9 g) was purified by a reversed phase chromatography on a C_{18} column (MeOH/H_2O, 30:70 → 100:0, *v*/v) and followed by semipreparative HPLC using YMC Triart C_{18} (5 μm; 10 × 250 mm) column with MeOH/H_2O (70:30, 0.1‰ v/v diethylamine) to afford Δ^{14}-vincamenine (1.8 mg, t_R 27.5 min), *O*-methyl-Δ^{14}-vincanol (11.8 mg, t_R 38.0 min), and 19(*R*)-acetoxytabersonine (5.2 mg, t_R 48.5 min). Subfraction AIII (226.5 mg) was further separated by semipreparative HPLC using YMC Triart C_{18} (5 μm; 10 × 250 mm) column with MeOH/H_2O (56:44, 0.1‰ *v*/v diethylamine) to afford (−)-lochnerinine (1.9 mg, t_R 38.0 min), voaphylline (17.7 mg, t_R 48.2 min), 16-epi-Δ^{14}-vincanol (10.5 mg, t_R 56.4 min), and **5** (1.5 mg, t_R 67.4 min). Fraction B (16.9 g) was purified by a reversed phase chromatography on a C_{18} column (MeOH/H_2O, 30:70 → 100:0, *v*/v) to give two subfractions (BI-BII). Subfraction BI (8.2 g) was subjected to a silica gel CC eluting with petroleum ether/acetone (50:1–2:1, *v*/v) and followed by semipreparative HPLC using YMC Triart C_{18} (5 μm; 10 × 250 mm) column with MeOH/H_2O (38:62, 0.1‰ v/v diethylamine) to give kopsiyunnanine H (2.3 mg, t_R 30.0 min), *O*-methyl-Δ^{14}-epivincanol (30.0 mg, t_R 45.0 min), Δ^{14}-vincanol (0.8 mg, t_R 60.0 min), Δ^{14}-vincinone (0.5 mg, t_R 75.0 min), 14-epi-16-decarbomethoxy-vincapusine (36.0 mg, t_R 90.0 min), 19-epimeloscandonine (46.2 mg, t_R 95.0 min), and kopsiyunnanine K (1.3 mg, t_R 110.0 min). Subfraction BII (6.7 g) was subjected a silica gel CC eluting with petroleum ether/acetone (35:1–2:1, *v*/v) and a Sephadex LH-20 column (40–70 μm, Amersham Pharmacia Biotech AB) eluted with MeOH, then the fraction by semipreparative HPLC using YMC Triart C_{18} (5 μm; 10 × 250 mm) column with MeOH/H_2O (60:40, 0.1‰ v/v diethylamine) to afford *O*-methyl-epivincanol (1.0 mg, t_R 40.0 min), 14*β*,15*β*-epoxyscandine (2.0 mg, t_R 47.0 min), 14,15-dehydroepivincine (10.0 mg, t_R 55.0 min). Fraction D (8.6 g) was purified by a reversed phase chromatography on a C_{18} column (MeOH/H_2O, 30:70 → 100:0, *v*/v) to give three subfractions (DI-DIII). Subfraction DI (2.1 g) was subjected to a series of silica gel CC eluting with petroleum ether/acetone (15:1–2:1, *v*/v) and followed by semipreparative HPLC using YMC Triart C_{18} (5 μm; 10 × 250 mm) column with MeOH/H_2O (85:15, 0.1‰ v/v diethylamine) to afford **2** (2.1 mg, t_R 45.0 min). Subfraction DII (3.5 g) was

Table 2
^{13}C NMR Data of melotenuinines A-C (**1–3**).

Position	**1**[a]	**2**[b]	**3**[b]
2	167.9	135.7	173.4
3	51.0	44.6	54.3
5	51.2	49.8	53.4
6	45.7	17.5	43.4
7	55.7	105.9	203.8
8	130.3	130.0	140.5
9	123.5	118.3	128.6
10	122.2	119.7	126.1
11	158.3	121.1	130.1
12	95.1	112.9	127.6
13	143.9	137.8	140.0
14	126.1	128.0	125.6
15	133.4	127.9	132.5
16	92.2	57.8	53.4
17	29.1	45.4	41.4
18	7.5	8.9	8.3
19	27.3	34.8	34.4
20	42.3	38.2	39.6
21	70.7	58.6	72.7
11-OMe	55.8		
16-CO_2Me	168.7		
16-CO_2Me	50.9		
2′	168.3	168.6	167.2
3′	47.8	47.5	51.5
5′	55.3	55.5	51.5
6′	39.7	39.2	46.0
7′	57.3	57.5	55.5
8′	131.7	132.7	130.8
9′	124.5	122.4	121.6
10′	137.1	138.1	119.3
11′	128.5	127.1	158.9
12′	116.4	117.1	94.9
13′	136.8	138.5	146.2
14′	129.3	126.8	127.0
15′	127.0	129.1	130.9
16′	68.6	68.7	93.8
17′	40.9	40.7	28.0
18′	9.0	8.7	18.9
19′	52.8	52.7	65.6
20′	46.3	46.2	48.2
21′	62.5	62.0	67.1
22′	210.5	210.8	
23′	35.7		
11′-OMe			55.3
16′-CO_2Me			168.9
16′-CO_2Me			51.0

The underline indicated the specific atom.
[a] 125 MHz, acetone-d_6.
[b] 150 MHz, acetone-d_6.

subjected to a silica gel CC eluting with petroleum ether/acetone (12:1–1:1, *v*/v) and followed by semipreparative HPLC using YMC Triart C_{18} (5 μm; 10 × 250 mm) column with MeOH/H_2O (75:25, 0.1‰ v/v diethylamine) to give **3** (1.3 mg, t_R 30.0 min) and **1** (4.7 mg, t_R 42.0 min). Subfraction DIII (1.5 g) was subjected to Sephadex LH-20 column (40–70 μm, Amersham Pharmacia Biotech AB) eluted with MeOH, and followed by semipreparative HPLC using YMC Triart C_{18} (5 μm; 10 × 250 mm) column with MeOH/H_2O (49:51, 0.1‰ *v*/v diethylamine) to give **4** (1.2 mg, t_R 60.0 min).

2.4. Melotenuine A (1)

yellow amorphous powder; $[\alpha]_D^{25}$ -58.5 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 245 (3.88), 327 (3.83) nm; ECD (0.00013 M, MeOH) $\lambda_{max}(\Delta\varepsilon)$ 200 (+5.83), 252 (+6.47), 328 (−4.55) nm; IR (KBr) ν_{max} 3440, 2924, 2854, 1631, 1439, 1384, 1156 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 699.3557 $[M + H]^+$ (calcd. for $C_{43}H_{46}N_4O_5$, 699.3541).

Table 3
^{1}H and ^{13}C NMR Data of melotenuines D-E (**4–5**).

Position	**4**[a]		**5**[b]	
	δ_H	δ_C	δ_H	δ_C
2		164.7		171.7
3a		171.7	2.99 (1H, m)	51.0
3b			2.67 (1H, td, 12.8, 3.2)	
5a	4.05 (1H, dd, 13.2, 7.8)	43.9	2.89 (1H, m)	51.6
5b	3.42 (1H, ddd, 13.2, 12.0, 6.0)		2.81 (1H, m)	
6a	2.05 (1H, dd, 12.0, 7.8)	41.0	2.80 (1H, m)	34.0
6b	1.74 (1H, dd, 12.0, 6.0)		2.80 (1H, m)	
7		57.5	4.00 (1H, br d, 9.6)	46.8
8		129.3		135.8
9	7.14 (1H, d, 8.4)	123.0	7.24 (1H, dd, 8.0, 1.0)	124.2
10	6.44 (1H, dd, 8.4, 2.4)	106.6	7.04 (1H, td, 8.0, 1.0)	124.7
11		162.3	7.16 (1H, td, 8.0, 1.0)	128.0
12	6.64 (1H, d, 2.4)	98.0	8.13 (1H, d, 8.0)	115.3
13		146.3		143.9
14a	2.65 (1H, dd, 15.0, 3.0)	40.8	2.08 (1H, m)	20.1
14b	2.49 (1H, dd, 15.0, 4.8)		1.67 (1H, dt, 14.4, 3.2)	
15a	4.11 (1H, t, 3.8)	70.1	1.71 (1H, m)	
15b			1.41 (1H, td, 13.6, 3.2)	
16		91.3	4.02 (1H, br d, 9.6)	71.8
17a	2.70 (1H, d, 15.6)	22.9	1.96 (1H, m)	38.0
17b	2.18 (1H, d, 15.6)		1.47 (1H, dd, 8.0, 1.6)	
18	0.76 (3H, t, 7.8)	8.1	0.71 (3H, t, 7.2)	7.4
19a	0.97 (1H, dq, 30.0, 7.8)	28.0	1.34 (1H, m)	31.9
19b	0.97 (1H, dq, 30.0, 7.8)		0.97 (1H, m)	
20		45.2		41.4
21	3.48 (1H, br s)	69.9		99.1
11-OMe	3.85 (3H, s)	55.9		
16-CO_2Me		169.8		
16-CO_2Me	3.78 (3H, s)	51.3		

The underline indicated the specific atom.
[a] ^{1}H NMR measured at 600 MHz, ^{13}C NMR measured at 150 MHz in methanol-d_4.
[b] ^{1}H NMR measured at 800 MHz, ^{13}C NMR measured at 200 MHz in acetone-d_6.

2.5. Melotenuine B (2)

yellow amorphous powder; $[\alpha]_D^{25}$ +91.7 (c 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.19), 262 (3.90) nm; ECD (0.00015 M, MeOH) $\lambda_{max}(\Delta\varepsilon)$ 212 (−14.28), 233 (−3.10), 262 (+12.46) nm; IR (KBr) ν_{max} 3440, 2924, 2854, 1632, 1456, 1384, 1045 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 597.3227 $[M + H]^+$ (calcd. for $C_{39}H_{40}N_4O_2$, 597.3224).

2.6. Melotenuine C (3)

colorless oily substance; $[\alpha]_D^{25}$ -38.6 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.04), 326 (3.91) nm; ECD (0.00012 M, MeOH) $\lambda_{max}(\Delta\varepsilon)$ 234 (+26.22), 325 (−6.37) nm; IR (KBr) ν_{max} 3432, 2923, 2853, 1630, 1490, 1384, 1052 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 691.3502 $[M + H]^+$ (calcd. for $C_{41}H_{46}N_4O_6$, 691.3490).

2.7. Melotenuine D (4)

yellow oily substance; $[\alpha]_D^{25}$ -78.2 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 241 (4.03), 325 (3.83) nm; ECD (0.00027 M, MeOH) $\lambda_{max}(\Delta\varepsilon)$ 205 (+15.43), 239 (+8.19), 323 (−10.05) nm; IR (KBr) ν_{max} 3444, 2926, 2855, 1669, 1488, 1384, 1064 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; HRESIMS *m/z* 421.1731 $[M + Na]^+$ (calcd. for

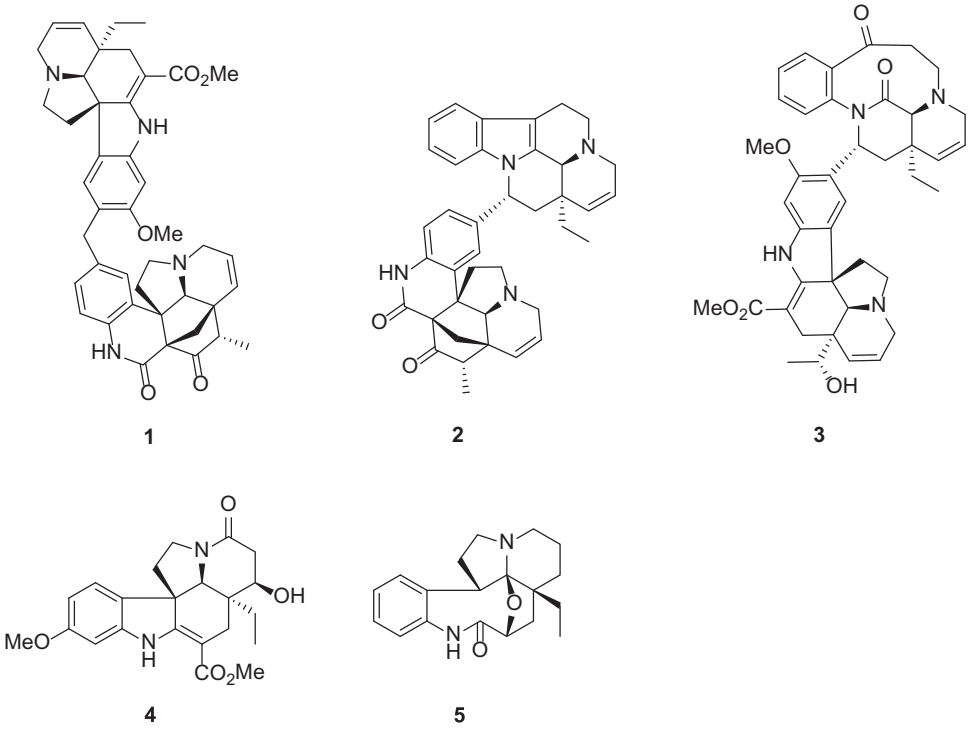


Fig. 1. Structures of compounds **1–5**.

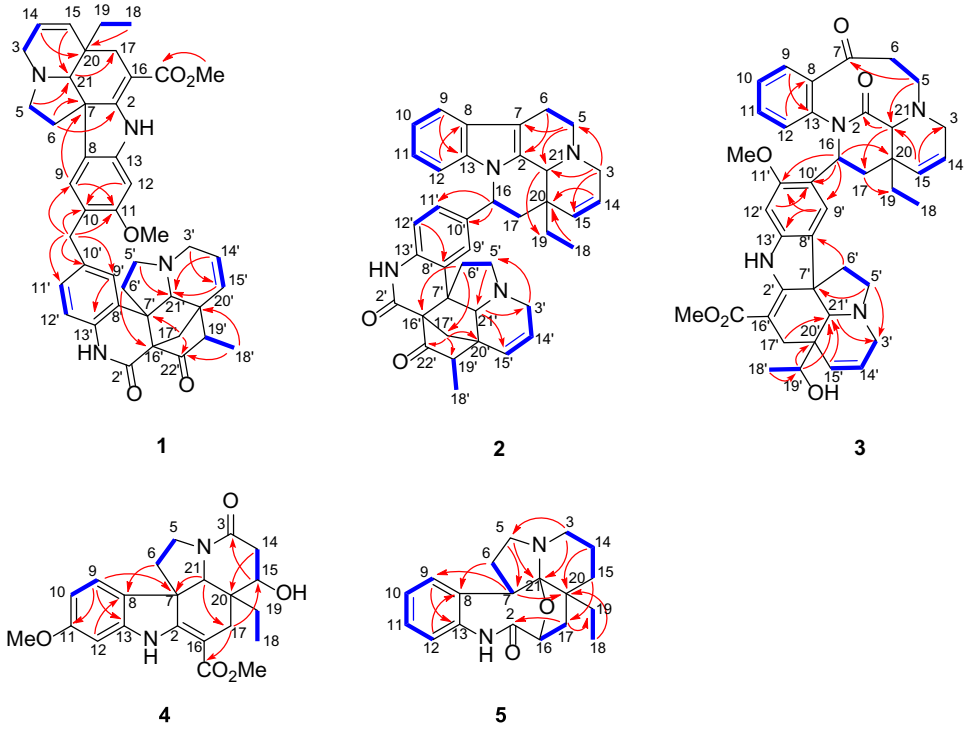


Fig. 2. Selected HMBC and $^{1}H-^{1}H$ COSY correlations of compounds **1–5**.

$C_{22}H_{26}N_2O_5$, 421.1734).

2.8. Melotenuine E (5)

white oily substance; $[\alpha]_D^{25}$-119.6 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε): 210 (4.27), 252 (3.90), 282 (3.49) nm; ECD (0.00032 M, MeOH) $\lambda_{max}(\Delta\varepsilon)$ 200 (−7.78), 240 (+3.83), 264 (−3.19), 289 (+1.79) nm; IR (KBr) ν_{max} 3428, 2924, 2852, 1675, 1481, 1391, 1096 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; HRESIMS *m/z* 313.1911 $[M + H]^+$ (calcd. for $C_{19}H_{24}N_2O_2$, 313.1910).

2.9. Cytotoxicity assays

Cytotoxicity evaluations were performed according to the previously described protocol [11].

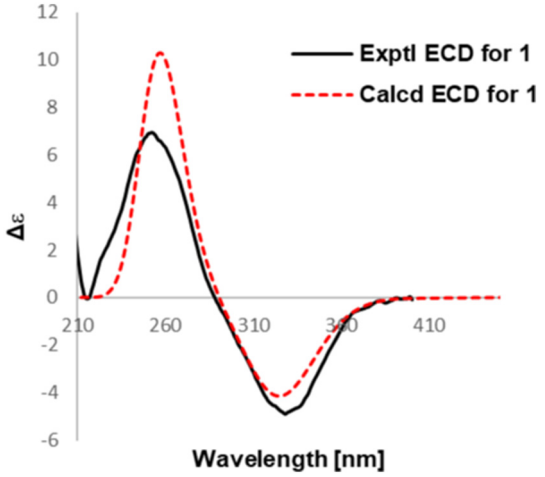


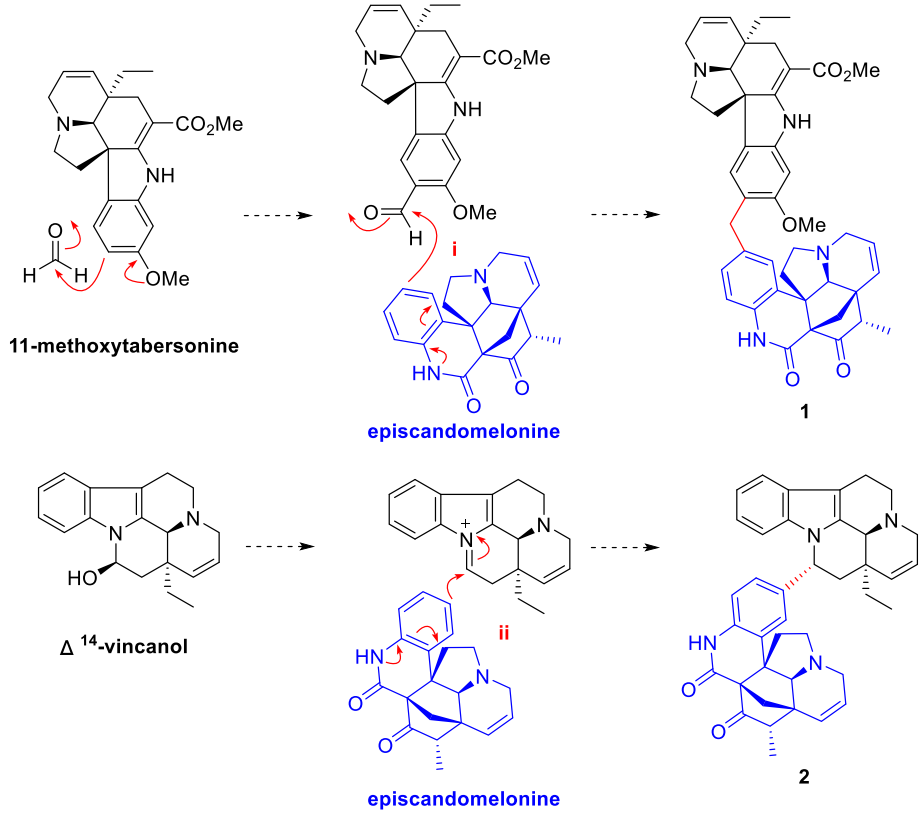
Fig. 3. Comparison of the experimental ECD and calculated ECD spectra of **1**.

3. Results and discussion

Melotenuine A (**1**) was obtained as a yellow amorphous powder. Its molecular formula was determined as $C_{43}H_{46}N_4O_5$ by HRESIMS at m/z 699.3557 $[M + H]^+$ (calcd 699.3541), with 23 degrees of unsaturation. The IR absorptions at 3440 and 1631 cm^{-1} suggested the presence of amino or hydroxyl and acrylamide functions, respectively. The ^{13}C NMR and DEPT data (Table 2) displayed that **1** possessed 43 carbon signals, which were classified as four methyls, 10 methylenes, 12 methines, and 17 quaternary carbons. Detailed analysis of the NMR spectra indicated that **1** was an aspidosperma-meloscandoine type bisindole alkaloid consists of 11-methoxytabersonine and 19-epimeloscandonine monomers, similar as that of episcandomelonine [14]. The major differences were the presence of an additional methylene (δ_H 3.83, br d, J = 15.0 Hz; 3.77, br d, J = 15.0 Hz; δ_C 35.7) and the linkage pattern of the two monomers. The HMBC correlations of from δ_H 3.83 to C-9 (δ_C 123.5), C-10 (δ_C 122.2), C-11 (δ_C 158.3), C-9′ (δ_C 124.5), C-10′ (δ_C 137.1), and C-11′ (δ_C 128.5) established the two monomers were connected to each other at C-10/10′ via a methylene bridge. Further 2D NMR spectroscopic data (HSQC, HMBC, and $^1H-^1H$ COSY) confirmed the above elucidation and thus established the planar structure of melotenuine A (**1**) as shown in Fig. 2. The ROESY correlations of H-19a/H-21, and H-21′/CH_3–18′ indicated that the two monomers in **1** had the same relative configurations with those of 11-methoxytabsersonine and 19-epimeloscandonine, respectively (Fig. S6). The absolute configuration of **1** was finally resolved by comparison of the calculated electronic circular dichroism (ECD) spectra with the experimentally recorded ECD spectra (Fig. 3). Therefore, the absolute configuration of **1** could be defined unequivocally as shown (Fig. 1).

Melotenuine B (**2**) was obtained as a yellow amorphous powder. The molecular formula of **2** was established as $C_{39}H_{40}N_4O_2$ by HRESIMS (*m/z* 597.3227, $[M + H]^+$, calcd 597.3224), with 22 degrees of unsaturation. The ^{13}C NMR and DEPT data (Table 2) demonstrated that **2** possessed 39 carbon signals, which were classified as two methyls, nine methylenes, 15 methines, and 13 quaternary carbons. Detailed analysis of the NMR spectra indicated that **2** was also a bisindole alkaloid and contains Δ^{14}-vincanol and episcandomelonine monomers. A linkage between the two monomers via C-10′ and C-16 was established by the key HMBC correlations from H-16 (δ_H 4.72, dd, J = 12.0, 3.6 Hz) to C-10′ (δ_C 138.1) and C-11′ (δ_C 127.1). Analysis its 2D NMR spectroscopic data (HSQC, HMBC, $^1H-^1H$ COSY, and ROESY) confirmed the other parts of the molecule were the same as those of 16-epi-Δ^{14}-vincanol and episcandomelonine, respectively. The coupling constants of H-16 (dd, J = 12.0, 3.6 Hz) established the β-configuration of H-16 [12]. It is noteworthy that alkaloid **2** represents the first example of bisindole alkaloid possess an eburnamine-melsocandonine skeleton.

Melotenuine C (**3**) was obtained as a colorless oily substance. Its molecular formula, $C_{41}H_{46}N_4O_6$, was determined by analysis the peak



Scheme 1. Plausible biogenetic pathway for compounds **1** and **2**.

Table 4
Cytotoxicity of compounds **1–4** (IC_{50}[a], μM).

Compounds	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	> 40	20.95 ± 0.91	> 40	36.34 ± 1.35	23.89 ± 0.99
2	37.09 ± 0.84	24.97 ± 2.32	> 40	26.26 ± 0.32	23.49 ± 0.84
3	30.35 ± 2.65	31.94 ± 3.73	> 40	> 40	22.17 ± 0.53
4	5.15 ± 0.16	22.47 ± 0.77	> 40	21.44 ± 1.12	> 40
5	> 40	> 40	> 40	> 40	> 40
Cisplatin[b]	3.41 ± 0.06	7.77 ± 0.34	33.71 ± 1.22	15.29 ± 1.11	15.45 ± 0.32

[a] IC_{50}: 50% inhibitory concentration.
[b] Positive control.

at *m/z* 691.3502 $[M + H]^+$ (calcd 691.3490) in the HRESIMS, which showed 21 degrees of unsaturation. The IR absorption bands suggested the presence of amino or hydroxyl (3432 cm^{-1}), and ester carbonyl (1630 cm^{-1}) functions. The ^1H and ^{13}C spectra (Tables 1 and 2) showed the 41 carbon signals including four methyls, nine methylenes, 14 methines, and 14 quaternary carbons, which were analogous to those of mekongenine A [13]. The striking difference was the presence of one oxygenated methine (δ_C 65.6; δ_H 3.46, 1H, q, J = 6.6 Hz) in **3**. The key HMBC correlation of CH_3–18′ (δ_H 0.94, d, J = 6.6 Hz) with the oxygenated methine (δ_C 65.6), as well as the ^1H–^1H COSY correlation of CH_3–18′ and δ_H 3.46 (1H, q, J = 6.3 Hz) suggested that a hydroxy group was located at C-19′ in **3** (Fig. 2). The relative configurations of **3** were identical with those of mekongenine A on the basis of their high similarity in ROESY spectrum and ECD curves (Figs. S24 and 27). The absolute configuration at C-19′ was confirmed to be *R* according to NMR data that paralleled those of (19*R*)-hydroxytabersonine [15].

The molecular formula of melotenuine D (**4**) was determined as $C_{22}H_{26}N_2O_2$ by HRESIMS peak at *m/z* 421.1731 $[M + Na]^+$ (calcd 421.1734), with 11 degrees of unsaturation. Detailed analysis of the NMR spectra indicated that **4** was a hydrolysate of alstoyunine G [16]. The key HMBC correlations of H_2–17 (δ_H 2.70, d, J = 15.6 Hz; 2.18, d, J = 15.6 Hz) to δ_C 70.1 indicated that the oxygenated methine (δ_C 70.1) was assigned as C-15 (Fig. 2). 2D NMR spectra (HSQC, HMBC, and ^1H–^1H COSY) confirmed the other parts of the structure were same as those of alstoyunine G. The ROESY correlations of H-15/CH_2-19, and CH_2-19/H-21 established these protons were α-orientation (Fig. S33).

Melotenuine E (**5**) had a molecular formula of $C_{19}H_{24}N_2O_2$, as established by HRESIMS signal at m/z 313.1911 $[M + H]^+$ (calcd 313.1910), with nine degrees of unsaturation. The IR spectrum at 1675 cm^{-1} suggested the presence of amide moiety. The ^1H and ^{13}C spectra (Table 3) showed the 19 carbon signals, which were classified as one methyl, seven methylenes, six methines, and five quaternary carbons. Detailed analysis of the NMR spectra implied that **5** was closely related to melokhanine *J* [5], except for the presence of an oxygenated methine (δ_C 71.8; δ_H 4.02, 1H, br d, J = 9.6 Hz). The key HMBC correlation of H-17a (δ_H 1.96, 1H, m) with the oxygenated methine carbon (δ_C 71.8) and the ^1H–^1H COSY correlation of CH_2–17 with the oxygenated methine proton (δ_H 4.02, 1H, br d, J = 9.6 Hz) demonstrated that the oxygenated methine was located at C-16. Though the key HMBC correlation of H-16 with C-21 was not observed, the oxygen bridge between C-16 and C-21 was established by its molecular weight, which is 2 mass units less than that of melokhanine *J*. Thus, the structure of melotenuine E (**5**) was established as shown (Fig. 1).

Since compounds **1** and **2** represent a new class of MIAs, a plausible biogenetic pathway for their generation was proposed as shown (Scheme 1). Alkaloid **1** might be originated from 11-methoxytabersonine, which could be transformed into key intermediate **i** by combined with formaldehyde. Then intermediate **i** and episcandomelonine generated **1** via key intermolecular nucleophilic addition reaction. Meanwhile, Δ^{14}-vincanol could convert to its iminium form **ii**, the subsequent intermolecular nucleophilic addition reaction between intermediate **ii** and episcandomelonine could finally transformed into **2** (Scheme 1).

The 18 known alkaloids were identified as 11-methoxytabersonine [17], 3α-acetonyltabersonine [18], Δ^{14}-vincamenine [19], 19(*R*)-acetoxytabersonine [20], (−)-lochnerinine [17], voaphylline [21], *O*-methyl-Δ^{14}-vincanol [22], kopsiyunnanine H [23], 16-epi-Δ^{14}-vincanol [24], *O*-methyl-Δ^{14}-epivincanol [22], Δ^{14}-vincanol [19], Δ^{14}-vincinone [24], 14-*epi*-16-decarbomethoxy-vincapusine [25], 19-epimeloscandonine [26], kopsiyunnanine K [27], *O*-methyl-epivincanol [28], 14β,15β-epoxyscandine [29], and 14,15-dehydroepivincine [30], respectively.

3.1. Cytotoxic activity

All new compounds were evaluated for their cytotoxicities against five human cancer cell lines: HL-60, SMMC-7721, A549, MCF-7, and SW480 using the MTS method, with cisplatin as a positive control [31]. Among them, compounds **1–4** showed weak and moderate cytotoxicities against five human cancer cell lines (Table 4). Interestingly, alkaloid **4** showed specific cytotoxicity against HL-60 cell line with IC_{50} value (5.15 ± 0.16 μM) comparable with that of positive control.

4. Concluding remarks

In this study, five new monoterpenoid indole alkaloids, melotenuines A-E (**1–5**), along with 18 known compounds, were isolated from the twigs and leaves of *M. tenuicaudatus*. Melotenuine A (**1**) is an unusual aspidosperma-meloscandonine type bisindole alkaloids characterized by a methylene bridge between the two monomers, while melotenuine B (**2**) had a rare eburnamine-melsocandonine skeleton. The discoveries of melotenuines A-E (**1–5**) would expand the diversity and complexity of MIAs, their presence maybe also helpful for further chemical investigations on the genus *Melodinus*. The cytotoxicities against several human cancer cell lines of all new alkaloids were also investigated, and some of them exhibited potent cytotoxicities.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (81874295 and 21432010), Yunnan Applied Basic Research Projects (2018FA049), CAS "Light of West China" Program (to Y. Zhang), and the Young Academic and Technical Leader Raising Foundation of Yunnan Province (to Y. Zhang).

Declaration of Competing Interest

Authors declare that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.104347.

References

[1] P.M. Dewick, Medicinal Natural Products: A Biosynthetic Approach, 3rd edition,

Chichester, UK, 2009, pp. 369–380.
[2] Y. Tsiang, P.T. Li, Flora of China, 16 Science Press, Beijing, 1995, pp. 147–150.
[3] Y.P. Liu, G.L. Yue Grace, K.M. Lee Julia, T. Feng, Y.L. Zhao, Y. Li, B.S. Lau Clara, X.D. Luo, Melodinine V, an antitumor bisindole alkaloid with selective cytotoxicity from *Melodinus henryi*, Bioorg. Med. Chem. Lett. 26 (2016) 4895–4898.
[4] Y.P. Liu, Y.L. Zhao, T. Feng, G.G. Cheng, B.H. Zhang, Y. Li, X.H. Cai, X.D. Luo, Melodinines A-H, cytotoxic bisindole alkaloid derivatives from *Melodinus suaveolens*, J. Nat. Prod. 76 (2013) 2322–2329.
[5] G.G. Cheng, D. Li, B. Hou, X.N. Li, L. Liu, Y.Y. Cheng, P.K. Lunga, A. Khan, Y.P. Liu, Z.L. Zuo, X.D. Luo, Melokhanines A-J, bioactive monoterpenoid indole alkaloids with diverse skeletons from *Melodinus khasianus*, J. Nat. Prod. 79 (2016) 2158–2166.
[6] Y.L. Zhou, J.H. Ye, Z.M. Li, Z.H. Huang, Study on the alkaloids of *Melodinus tenuicaudatus*, Planta Med. 54 (1988) 315–317.
[7] T. Feng, Y. Li, Y.Y. Wang, X.H. Cai, Y.P. Liu, X.D. Luo, Cytotoxic indole alkaloids from *Melodinus tenuicaudatus*, J. Nat. Prod. 73 (2010) 1075–1079.
[8] T. Feng, Y. Li, Y.P. Liu, X.H. Cai, Y.Y. Wang, X.D. Luo, Melotenine A, a cytotoxic monoterpenoid indole alkaloid from *Melodinus tenuicaudatus*, Org. Lett. 12 (2010) 968–971.
[9] Y.X. Yuan, Y. Zhang, L.L. Guo, Y.H. Wang, M. Goto, S.L. Morris-Natschke, K.H. Lee, X.J. Hao, Tabercorymines A and B, two vobasinyl − ibogan-type bisindole alkaloids from *Tabernaemontana corymbosa*, Org. Lett. 19 (2017) 4964–4967.
[10] Y. Zhang, Y.X. Yuan, M. Goto, L.L. Guo, X.N. Li, S.L. Morris-Natschke, K.H. Lee, X.J. Hao, Taburnaemines A − I, cytotoxic vobasinyl-iboga-type bisindole alkaloids from *Tabernaemontana corymbosa*, J. Nat. Prod. 81 (2018) 562–571.
[11] Y. Zhang, L.L. Guo, G.M. Yang, F. Guo, Y.T. Di, S.L. Li, D.Z. Chen, X.J. Hao, New vobasinyl-iboga-type bisindole alkaloids from *Tabernaemontana corymbosa*, Fitoterapia 100 (2015) 150–155.
[12] L.L. Guo, H.P. He, Y.T. Di, S.F. Li, Y.Y. Cheng, W. Yang, Y. Li, Y.P. Liu, Y. Zhang, X.J. Hao, Indole alkaloids from *Ervatamia chinensis*, Phytochemistry 74 (2012) 140–145.
[13] Y.H. Fu, H.P. He, Y.T. Di, S.L. Li, Y. Zhang, X.J. Hao, Mekongenines A and B, two new alkaloids from *Bousigonia mekongensis*, Tetrahedron Lett. 53 (2012) 3642–3646.
[14] M. Daudon, M.H. Mehri, M.M. Plat, E.W. Hagaman, E. Wenkert, Carbon-13 nuclear magnetic resonance spectroscopy of naturally occurring substances. 48. Dimeric quinolinic *Melodinus* alkaloids, J. Org. Chem. 41 (1976) 3275–3278.
[15] N. Langlois, R.Z. Andriamialisoa, Studies on vindolinine. 6. Partial synthesis of aspidospermane-type alkaloids, J. Org. Chem. 41 (1979) 2468–2471.
[16] T. Feng, Y. Li, X.H. Cai, X. Gong, Y.P. Liu, R.T. Zhang, X.Y. Zhang, Q.G. Tan, X.D. Luo, Monoterpenoid indole alkaloids from *Alstonia yunnanensis*, J. Nat. Prod. 72 (2009) 1836–1841.
[17] S. Baassou, H. Mehri, M. Plat, Alcaloides de *Melodinus aeneus*, Phytochemistry 17 (1978) 1449–1450.
[18] W. Fahn, V. Kaiser, H. Schübel, J. Stöckigt, B. Danieli, Catharanthus roseus enzyme mediated synthesis of 3-hydroxyvoafrine A and B-A simple route to the voafrines, Phytochemistry. 29 (1990) 127–133.
[19] S. Baassou, H. Mehri, M. Plat, Alkaloids of *Melodinus celastroides*. Isolation of four new alkaloids, Ann. Pharm. Fr. 45 (1987) 49–56.
[20] Y.P. Liu, Y. Li, X.H. Cai, X.Y. Li, L.M. Kong, G.G. Cheng, X.D. Luo, Melodinines M-U, cytotoxic alkaloids from *Melodinus suaveolens*, J. Nat. Prod. 75 (2012) 220–224.
[21] E. Wenkert, E.W. Hagaman, N.Y. Wang, N. Kunesch, The C (7) stereochemistry of the chloroindolenines of cleavamines and quebrachamines, Heterocycles 12 (1979) 1449–1453.
[22] D.E. Pegnyemb, R.T. Ghogomu, B.L. Songdengam, Minor alkaloids from the seeds of Voacanga africana, Fitoterapia 70 (1999) 446–448.
[23] Y.Q. Wu, M. Kitajima, N. Kogure, Y.S. Wang, R.P. Zhang, Takayama. Two new aspidosperma indole alkaloids from Yunnan *Kopsia arborea*, Chem. Pharm. Bull. 58 (2010) 961–963.
[24] M. Zeches, J. Lounkokobi, B. Richard, M. Plat, L. Le Men-Olivier, T. Sevenet, J. Pusset, Alkaloids of *Melodinus guillauminii*, Phytochemistry 23 (1984) 171–174.
[25] X.H. Cai, Y. Li, Y.P. Liu, X.N. Li, M.F. Bao, X.D. Luo, Alkaloids from *Melodinus yunnanensis*, Phytochemistry 83 (2012) 116–124.
[26] L.W. Guo, Y.L. Zhou, Alkaloids from *Melodinus hemsleyanus*, Phytochemistry 34 (1993) 563–566.
[27] R. Tokuda, Y. Okamoto, T. Koyama, N. Kogure, M. Kitajima, H. Takayama, Asymmetric total synthesis of kopsiyunnanine K, a monoterpenoid indole alkaloid with a rearranged skeleton, Org. Lett. 18 (2016) 3490–3493.
[28] P. Pfaffli, H. Hauth, Additionsreaktionen an der alicyclischen doppelbindung von eburnamenin und apovincamin, Helv. Chim. Acta. 61 (1978) 1682–1695.
[29] T.T. Zhang, Z.W. Liu, W.J. Wang, Y.B. Tong, F.F. Xu, J.Q. Yuan, B. Liu, X.Q. Zhang, W.C. Ye, Alkaloids from *Melodinus suaveolens*, Heterocycles 87 (2013) 2047–2052.
[30] N. Neuss, H.E. Boaz, J.L. Occolowitz, E. Wenkert, F.M. Schell, P. Potier, C. Kan, M.M. Plat, M. Plat, The structure of Vincarodine, Helv. Chim. Acta. 56 (1973) 2660–2666.
[31] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.