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Novel monoterpenoid indole alkaloids from *Melodinus yunnanensis*

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ABSTRACT

Five monoterpenoid indole alkaloids, namely meloyines A-B (**1**–**2**), meloyines II-III (**3**–**4**), and 10-*O*-glucosyl-scandine (**5**) together with thirty-four known alkaloids were isolated from leaves and twigs of *Melodinus yunnanensis*. Alkaloid **1** was characterized as an unprecedented skeleton with a 6/5/5/6/6/4 ring system, and alkaloids **3**–**4** were dimeric monoterpenoid quinolone alkaloids. Their structures were elucidated based on 1D and 2D- NMR, FTIR, UV, and MS spectroscopic data. The cytotoxic activity of new alkaloids were evaluated against three human cancer cell lines.

1. Introduction

Monoterpenoid quinolone alkaloids (MQAs) were a special kind of natural products, which were proposed to arise by rearrangement of monoterpenoid indole alkaloids (MIAs). A possible route for MQAs biosynthesis was that the N_1–C_2 or C_2–C_7 bond cleaved in the indole heterocyclic ring and then generated new amine and/or keto functions. Then a new quinolone heterocycle would be formed by this nucleophilic reaction. Natural MQAs mainly were distributed in plants of *Alstonia* (corialstonine[1] and scholarisine I-II[2]), *Melodinus* (meloscandonine and scandine derivatives,[3] rhazimine,[4] meloyunine C[5]) and *Gardneria* (gardquinolone[6]), *Tabernaemontana* (voastrictine[7] and voaharine[8]) genera among family Apocynaceae. Pharmacological investigations on MQAs have demonstrated their significant bioactivities. Additionally, famous drugs, camptothecin from plants of both *Camptotheca* and *Nothapodytes* genera, and quinine from *Cinchona* genus were belonged to MQAs. Previous research indicated MQAs derived from Aspidosperma-type MIAs are mainly alkaloids in plants of *Melodinus* genus.[5,9,10] Likewise, another small subtype in Aspidosperma types, with C–C new bond such as C_2–$C_{18/19}$ (vindolinine and venalstonine)[11] and C_{17}–C_{21} (pandine),[12] were widely distributed in *Alstonia* and *Tabernaemontana* genera. To discover novel and bioactive alkaloids, the first studied on *M. yunnanensis* distributed in south of Yunnan Province, China, led to ten new alkaloids.[13] Since the production of plant secondary metabolites were influenced by variable environments,[14] phytochemical research on same plant from different place was undertaken again. Herein, this paper described the isolation, structural determination, and cytotoxic activities of new alkaloids (**1**–**5**) together with the thirty four known ones, namely 15α-hydroxy-meloscandonine (**6**),[15] 10-hydroxyscandine (**7**),[16] scandine (**8**),[9] epimeloscine (**9**),[17] meloscandonine (**10**),[18] 19-epimeloscandonine (**11**),[19] tubotaiwine (**12**),[20] 19*R*-hydroxytabersonine (**13**),[21] 11-methoxytabersonine (**14**),[22] tabersonine (**15**),[23] 11-methoxy-19-hydroxytabersonine (**16**),[24] 11-hydroxytabersonine (**17**),[25] pachysiphine (**18**),[25] lochnericine (**19**),[26] 11-hydroxylochnericine (**20**),[27] venalstonine (**21**),[11] 17α-hydroxyvenalstonine (**22**),[28] venalstonidine (**23**),[29] 19β-hydroxyvenalstonidine (**24**),[30] kopsinine (**25**),[31] kopsiloscine G (**26**),[32] 19*R*-vindolinine (**27**),[11] 16β-hydroxy-19*R*-vindolinine (**28**),[19] 19*S*-vindolinine (**29**),[11] 19,20-dihydroakuammicine (**30**),[33] strictcine (**31**),[34] rhazimol (**32**),[35] scholarisin VII (**33**),[36] picrinine (**34**),[37] 14-hydroxymeloyunine (**35**),[13] 14,15-dehydrovincamine (**36**),[38] 16-hydroxymethylpleiocarpamine (**37**),[39] akuammidine (**38**),[40] voaphylline (**39**).[41]

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2. Results and discussion

The MeOH extract of leaves and twigs of *M. yunnanensis* was partitioned between H_2O and EtOAc and column chromatography was used to separate the alkaloidal fraction into 39 alkaloids.

Alkaloid (**1**) was isolated as a white powder. Its molecular formula was determined as $C_{21}H_{24}N_2O_3$ by the HRESIMS (*m/z* 353.1859 $[M + H]^+$, calcd. 353.1865) in association with the ^1H and ^{13}C NMR spectroscopic data, indicating 11° of unsaturation. Its UV spectrum showed the characteristic absorptions of the indoline chromophore at 210, 248 and 289 nm,[42] and IR absorption bands at 3423, 3356, 1710 and 1640 cm^{-1} suggested the presence of NH, OH, aromatic ring and ester carbonyl functionalities. The ^{13}C NMR and DEPT data suggested that alkaloid **1** possessed 21 carbons including seven quaternary carbons, eight methines, four methylenes, one methoxycarbonyl and one methyl (Table 1), close to tabersonine skeleton. The ^1H NMR spectrum of **1** displayed proton signals for an unsubstituted indole A ring [δ_H 7.10 (d, J = 7.8 Hz, H-9), 6.58 (t, J = 7.8 Hz, H-10), 6.92 (t, J = 7.8 Hz, H-11), 6.63 (d, J = 7.8 Hz, H-12)], two olefin protons [δ_H 5.80 (dd, J = 11.9, 4.3 Hz, H-14), 5.66 (d, J = 11.9 Hz, H-15)], one methoxyl (δ_H 3.49, s) and one methyl (δ_H 0.63, d, J = 7.4 Hz, H-18) (Table 1). In the ^{13}C NMR spectrum, the downfield shifts at δ_C 50.0 (t), 53.5 (t) and 75.4 (d) were easy to assigned as CH_2-3/5 and CH-21, respectively. The characteristic quaternary carbon at δ_C 52.0 was assigned as C-7 according to its correlations from H-9 and H-5 in the HMBC spectrum (Fig. 2). In addition, the HMBC correlations also indicated the connections of C_7–C_6–C_5, C_3–C_{14}–C_{15} and C_{15}–C_{20}–C_{21}. The doublets methyl at δ_H 0.63 was assigned as C-18 on the basis of the cross peaks from H-18 to C-19 and C-20. The methylene signals (δ_H 2.36, 1.98) were assigned as H_2-6, which was supported by the correlations from δ_H 2.36 to C-8 and C-21. And the correlations from δ_H 2.32 to C-19 and C-21 suggested the signals (δ_H 2.32, 2.09) were belonged to H_2-17. Furthermore, the correlations from H_2-17 (δ_H 2.32, 2.09), H-18 (δ_H 0.63), and OH (δ_H 4.35) to signal of δ_C 89.0 and the correlations from H-15 (δ_H 5.66), H_2-17, OH (δ_H 4.35) to C-19 revealed the connection of C_{16}–C_{19}. The chemical formula and the HMBC correlations from N–H (δ_H 5.61), H_2-6, H_2-17 and H-21 (δ_H 2.13) to the signal at δ_C 60.5 (s) suggested that the quaternary carbon was C-2 which was substituted by the methoxycarbonyl.

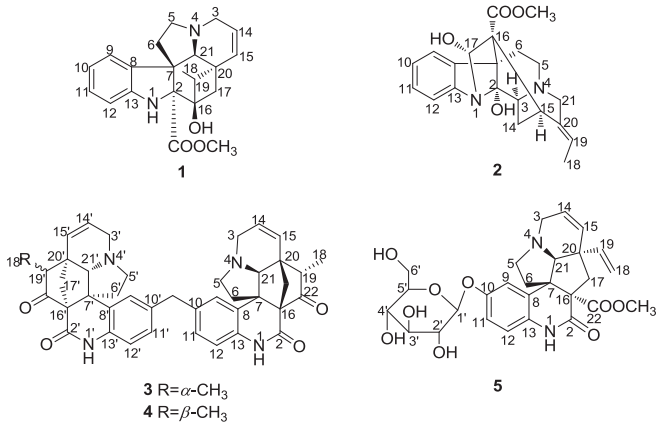

Fig. 1. Structures of alkaloids **1**–**5** from *M. yunnanensis*.

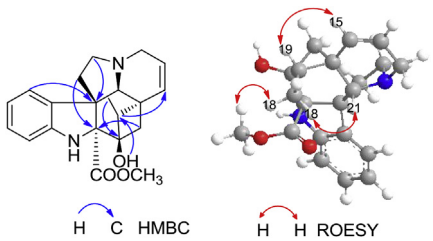

Fig. 2. The key HMBC and NOE correlations of alkaloid **1**.

Table 1
^1H and ^{13}C NMR spectroscopic data from alkaloids **1**–**2** in acetone-d_6 (δ in ppm, J in Hz).

No	**1**[a]		**2**[b]	
	δ_H	δ_C	δ_H	δ_C
NH	5.61, s			
2		60.5 s		112.0 s
3	3.36, dd (16.4, 4.3) 3.11, d (16.4)	50.0 t	3.35, overlap	59.4 d
5	3.20, t (7.2) 2.96, overlap	53.5 t	3.52, m 2.64, m	49.2 t
6	2.36, m 1.98, m	37.4 t	2.82, m 2.20, m	24.2 t
7		52.0 s		50.7 s
8		125.9 s		127.8 s
9	7.10, d (7.8)	126.7 d	7.26, d (8.0)	128.5 d
10	6.58, t (7.8)	117.4 d	6.68, t (8.0)	118.9 d
11	6.92, t (7.8)	127.3 d	6.95, t (8.0)	128.4 d
12	6.63, d (7.8)	113.8 d	6.51, d (8.0)	115.6 d
13		143.4 s		144.5 s
14	5.80, dd (11.9, 4.3)	130.6 d	2.29, m 1.93, m	28.3 t
15	5.66, d (11.9)	129.1 d	3.57, m	37.8 d
16		89.0 s		58.1 s
17	2.32, d (10.1) 2.09, overlap	42.6 t	4.88, s	85.0 d
18	0.63, d (3H, 7.4)	9.3 q	1.45, d (3H, 7.0)	12.9 q
19	2.27, q (7.4)	53.4 d	5.32, q (7.0)	117.6 d
20		47.8 s		143.8 s
21	2.13, s	75.4 d	3.86, d (16.8) 2.88, d (16.8)	55.0 t
$COOCH_3$	3.49, s (3H)	51.4 q	3.52, s (3H)	51.6 q
$COOCH_3$		171.9 s		171.2 s
OH	4.35, s			

[a] ^1H NMR recorded at 600 MHz, ^{13}C NMR recorded at 150 MHz.
[b] ^1H NMR recorded at 400 MHz, ^{13}C NMR recorded at 100 MHz.

The configuration of alkaloid **1** was determined by NOE correlations (Fig. 2). The absolute configurations at C-7, C-20, C-21 were determined as *R*, *R*, *S*, for compound **1** was originated from tabersonine-type alkaloid.[23] The absolute configurations were then determined as 2*S*, 7*R*, 16*R*, 19*S*, 20*R*, 21*S*, by the correlations between H-18/H-21, H-18/carbomethoxy and H-19/H-15, which was further confirmed by ECD calculation method. The geometry was optimized at B3LYP/6–31 + g(d,p) level in methanol using the continuum polarizable continuum model (CPCM). Harmonic vibration frequencies were then calculated to confirm the stability of these conformers. The theoretical ECD spectrum of (2*S*, 7*R*, 16*R*, 19*S*, 20*R*, 21*S*)-**1** (**1a**) and (2*R*, 7*R*, 16*R*, 19*S*, 20*R*, 21*S*)-**1** (**1b**) was calculated in methanol using Time-dependent Density functional theory (TD-DFT) at the B3LYP/6–311 + g(d,p) level of the Gaussian 09 program package.[43] The experimental ECD spectrum of alkaloid **1** and that the calculated ECD spectrum for the molecular having 2*S*, 7*R*, 16*R*, 19*S*, 20*R*, 21*S* (**1a**) were in good agreement, which validated the absolute configurations of **1** (Fig. 3). Thus, the structure of alkaloid **1** was elucidated and alkaloid **1** was named subsequently meloyine A (Fig. 1).

Alkaloid **2** possessed molecular formula of $C_{21}H_{24}N_2O_4$ as established by HRESIMS ($[M + H]^+$ at *m/z* 369.1816) as well as the NMR data. In the ^1H-NMR spectrum (Table 1) of **2**, an unsubstituted indole ring [δ_H 7.26 (d, J = 8.0 Hz), 6.95 (t, J = 8.0 Hz), 6.68 (t, J = 8.0 Hz) and 6.51 (d, J = 8.0 Hz)], one methyl ester group

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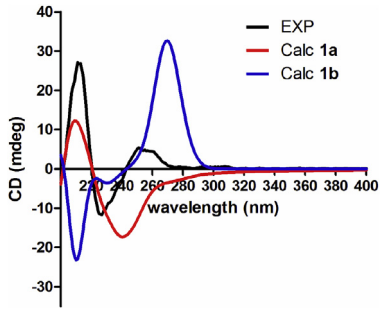

Fig. 3. Calculated ECD spectrum of (2*S*, 7*R*, 16*R*, 19*S*, 20*R*, 21*S*)-**1a** (red); (2*R*, 7*R*, 16*R*, 19*S*, 20*R*, 21*S*)-**1b** (blue); experimental ECD spectrum of **1** (black).

resonance signal at δ_H 3.52 (s) and a methyl resonance signal at δ_H 1.45 (d, J = 7.0 Hz) were observed. In comparison of NMR spectra (Table 1) with those of rhazimal,[44] the formyl group at C-17 and N_1H of rhazimal were absent in **2**, instead, appearance of a new hemiacetal (δ_C 85.0, δ_H 4.88) displayed the linkage between C-17 and N-1. This new five-membered ring in **2** was confirmed by the HMBC cross-peaks from H-17 (δ_H 4.88) to C-16 (δ_C 58.1), C-15 (δ_C 37.8), C-13 (δ_C 144.5), C-7 (δ_C 50.7) (Fig. 4). The additional hydroxyl was located at C-2 in **2** through the HMBC correlations of H-3 (δ_H 3.35), H-6 (δ_H 2.82), H-14 (δ_H 1.93), and H-17 (δ_H 4.88) with quaternary carbon δ_C 112.0 (C-2). In the ROESY spectrum, the NOE correlations of H-18 (δ_H 1.45) with H-15 (δ_H 3.57) and of H-19 (δ_H 5.32) with H-21 (δ_H 2.88) suggested *E* configuration of the double bond C-19/20. The cross-peaks of H-17/H-15 revealed H-17 as α-orientation. The molecular model of alkaloid **2** indicated that the 2-OH in the rigid skeleton was α-oriented. Thus, the structure of **2** was established and subsequently named meloyine B.

Alkaloid **3** was isolated as a white powder. The UV absorption at 212 and 254 nm showed the presence of quinolone rings,[9] and the IR spectrum indicated the presence of NH (3343 cm^{-1}), carbonyl (1712 cm^{-1}), and benzene ring (1650 cm^{-1}). The molecular formula of alkaloid **3** was determined as $C_{41}H_{40}N_4O_4$ by its HRESIMS and NMR data. The ^1H NMR spectrum (Table 2) displayed two NH signals [δ_H 9.28 (2H, s)], six aromatic protons [δ_H 6.94, 6.93, 6.88, 6.87, 7.05 and 7.09] and two doublet methyls (δ_H 0.80 and 1.06). Its ^{13}C and DEPT NMR spectra indicated that **3** possessed 41 carbons, including two methyls, nine methylenes, 14 methines and 16 quaternary carbons. Combination of HRESIMS at *m/z* 653.3125 ($C_{41}H_{41}N_4O_4$ [M + H]$^+$) and NMR spectra suggested that **3** was a dimeric alkaloid. The presence of three pairs of sp^3 quaternary carbons (δ_C 68.6, 68.6, 57.3, 55.4, 46.2, and 45.2) in the ^{13}C and DEPT spectra, suggesting that alkaloid **3** contained two meloscandonine analogues (Fig. 1).[17] In the ^1H NMR spectra, the major difference between two units was the chemical shifts of H-18/18′, H-19/19′, H-21/21′ (Table 1), suggesting that the two units were meloscandonine and its 19-epimer.[19] The presence of an additional methylene [δ_C 41.2, δ_H 3.82 (2H, m)] connected two moieties at C-10/10′ supported by the HMBC correlations (Fig. 5) from δ_H 3.82 to C-9 (δ_C 124.5), C-9′ (δ_C 124.8), C-10 (δ_C 136.7), C-10′ (δ_C 136.8), C-11 (δ_C 128.5) and C-11′ (δ_C 128.6). The CH_3-18/18′ were determined as α and β orientation by their proton signal chemical shifts in ^1H NMR spectra[19] and ROESY correlations of H-18/21 and H-19′/21′. Thus, the structure of the dimeric alkaloid was elucidated and called as meloyine II because meloyine I, a first dimer alkaloid was found from same plant.[13]

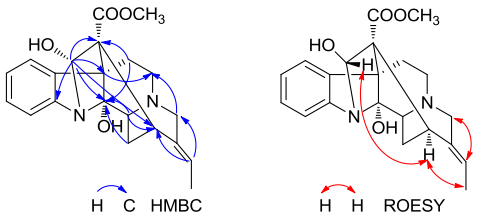

Fig. 4. Key HMBC and NOE correlations of alkaloid **2**.

Table 2
^1H and ^{13}C NMR spectroscopic data of alkaloid **3** in acetone-d_6 (δ in ppm, J in Hz)[a].

No	δ_H	δ_C	No	δ_H	δ_C
NH	9.28, s		N′H	9.28, s	
2		168.3 s	2′		168.4 s
3	3.71, overlap 3.32, overlap	47.8 t	3′	3.72, overlap 3.34, overlap	48.0 t
5	3.13, overlap 3.05, overlap	55.2 t	5′	3.13, overlap 3.05, overlap	55.4 t
6	2.27, m 1.79, m	39.3 t	6′	2.26, overlap 1.77, overlap	39.5 t
7		57.3 s	7′		55.4 s
8		131.9 s	8′		132.2 s
9	7.05, s	124.5 d	9′	7.09, s	124.8 d
10		136.7 s	10′		136.8 s
11	6.93, overlap	128.5 d	11′	6.94, overlap	128.6 d
12	6.87, overlap	116.6 d	12′	6.88, overlap	116.7 d
13		136.9 s	13′		137.0 s
14	5.91, overlap	129.0 d	14′	5.94, overlap	129.3 d
15	5.94, overlap	126.9 d	15′	5.99, overlap	125.2 d
16		68.6 s	16′		68.6 s
17	2.16, overlap 2.01, overlap	40.9 t	17′	2.16, overlap 2.01, overlap	36.8 t
18	0.80, d (3H, 7.2)	8.8 q	18′	1.06, d (3H, 7.8)	11.2 q
19	2.30, q (7.2)	52.8 d	19′	1.73, q (7.8)	51.1 d
20		46.2 s	20′		45.2 s
21	3.51, s	62.4 d	21′	3.30, s	70.9 d
22		210.6 s	22′		211.4 s
CH_2	3.82, m (2H)	41.2 t			

[a] ^1H recorded at 600 MHz and ^{13}C NMR recorded at 150 MHz.

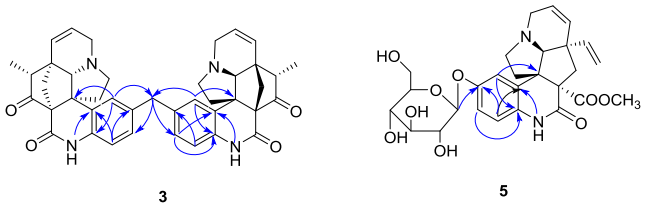

Fig. 5. Key HMBC correlations of alkaloids **3** and **5**.

Similar to alkaloid **3**, the UV and IR spectra of **4** also suggested the presence of the quinolone rings.[9] Its ^{13}C and DPET NMR data showed a methyl, five methylenes, seven methines and seven quaternary carbons, one more methylene than meloscandonine. However, alkaloid **4** had a same molecular formula as **3** based on the HRESIMS, indicating it was also a dimers with both same units. Its chemical shift of CH_3-18 (δ_H 0.68, δ_C 8.8) observed in alkaloid **4** supported the unit was 19-epimeloscandonine other than meloscandonine.[19] Dimeric coupling via a methylene linkage at C-10 and C-10′ was supported by the HMBC correlations from $-CH_2$ (δ_H 3.71) to C-9/9′ (δ_C 124.6), C-10/10′ (δ_C 136.9) and C-11/11′ (δ_C 128.5). Thus, the structure of alkaloid **4** was established and named meloyine III.

The ^1H-, ^{13}C-NMR and DEPT spectra displayed a glucose unit on basis of a proton signal at δ_H 4.78 (1H, d, J = 7.5 Hz), an anomeric carbon [δ_C 102.3 (d)], a methylene [δ_C 62.6 (t)], and other 4 methine signals between δ_C 70 and δ_C 80. The singlet peak at δ_H 4.78 indicated the β-orientation of the anomeric proton of the glucosyl residue. The identification of the sugar residue was continued by hydrolysis with 10% HCl to afford a D-glucose which was confirmed

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by comparison with authentic samples and determination of their optical rotation values ($[\alpha]_D^{21} = +21°$).[45] The glycositatic position was unambiguously determined to be at C-10 from the HMBC correlations (Fig. 5) from H-1' (δ_H 4.78) and singlet H-9 (δ_H 7.02) to C-10 (δ_C 154.4), and from H-9 (δ_H 7.02) to C-7 (δ_C 58.5). So alkaloid **5** was named as 10-*O*-glucosyl-scandine.

Moreover, other alkaloids (**6**–**39**) were identified by comparison of their NMR spectroscopic data with the literature. Alkaloids **1**–**5** were evaluated for their cytotoxicity against three human cancer cell lines. No one showed cytotoxicity under these conditions ($IC_{50} > 25$ μM).

3. Experimental section

3.1. General information

Optical rotations were measured with either a Horiba SEPA-300 polarimeter (Horiba Scientific, Kyoto, Japan) or JASCO DIP-370 digital polarimeter (Jasco International Co., Tokyo, Japan). UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer (Shimadzu Corp., Kyoto, Japan). Scanning IR spectroscopy was performed on a Tenor 27 spectrophotometer using KBr pellets. MS data were recorded on an Agilent G6230 TOF MS (Applied Biosystems, Ltd., Warrington, UK). 1D- and 2D- NMR spectra were obtained on Bruker Avance III-600, DRX-500, and AM-400 spectrometers (Bruker BioSpin GmBH, Rheinstetten, Germany) with TMS as an internal standard. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qing-dao Haiyang Chemical Co., Ltd, Qingdao, China) and C_{18}-silica gel (20–45 μm, Fuji Silysia Chemical Ltd.). Fractions were monitored by TLC on silica gel plates (GF254, Qingdao Haiyang Chemical Co., Ltd.) and spots visualized with Dragendorff's reagent spray. Medium pressure liquid chromatography (MPLC) was employed using a Buchi pump system coupled with C_{18}-silica gel-packed glass column (15 × 230 and 26 × 460 mm). High performance liquid chromatography (HPLC) was performed using a Waters 600 pump (Waters Corp., Milford, MA, USA) coupled with Sunfire analytical, semi-preparative, or preparative C_{18} columns (150 × 4.6, 150 × 10 mm, and 250 × 19 mm, respectively). The HPLC system employed a Waters 2996 photodiode array detector and a Waters fraction collector II (Waters Corp.).

3.2. Plant material

Leaves and twigs of *M. yunnanensis* Tsiang & P. T. Li were collected in July, 2013 in Gengma, Yunnan Province, P. R. China, and identified by Dr. Cheng Liu. A voucher specimen (Cai20130627) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

13 kg of *M. yunnanensis* leaves and twigs were extracted with MeOH (3 × 25 L) at room temperature for a week and the solvent removed *in vacuo*. The residue was dissolved in 0.3% aqueous hydrochloric acid (v/v), basified with 5% aqueous ammonia to pH 9–10, and partitioned with EtOAc. The EtOAc phase (80 g) was subjected to CC over silica gel (1.0 kg) and eluted with a $CHCl_3$-acetone gradient (from 1:0–0:1, v/v) to produce five fractions (I–V). Fraction I (4.0 g) was purified on a C_{18} MPLC column with a MeOH–H_2O gradient from 40% to 80% to yield four fractions I-1–2. I-1 (1.6 g) was further purified on the silica gel CC to afford alkaloids **8** (510 mg) and **9** (320 mg). I-2 (0.8 g) was purified on the silica gel CC with a petroleum ether-acetone gradient (8:1, v/v) to afford alkaloids **10** (137 mg) and **11** (158 mg). Fr. II (11 g) was purified on a C_{18} MPLC column with a MeOH–H_2O gradient from 40% to 80% to yield five fractions II-1–5. II-1(2.2 g) was purified on a C_{18} MPLC column with a gradient of MeOH–H_2O from 20% to 40% to yield alkaloids **8** (310 mg), **12** (530 mg) and **13** (543 mg). Fraction II-2 (2.0 g) was further purified on a C_{18} MPLC column with a gradient of MeOH–H_2O from 20% to 40% to afford fractions II-2-1–3. II-2-3 was further purified on a C_{18} HPLC column with a gradient of MeOH–H_2O from 65% to 75% to afford alkaloid **16** (48 mg). Alkaloid **14** (215 mg) was crystallized from fraction II-3. II-4 (1.5 g) was further purified on a C_{18} MPLC column with a gradient MeOH–H_2O 50%–70% to afford three fractions. II-4-1 was further purified on C_{18} HPLC column with a gradient MeOH–H_2O (55:45–65:35, v/v) to yield alkaloid **15** (47 mg). II-4-2 was further purified on C_{18} HPLC column with a gradient MeOH–H_2O (55:45–65:35, v/v) to yield alkaloids **17** (51 mg) and **19** (17 mg). II-4-3 was further purified on the silica gel CC with a petroleum ether-acetone gradient (10:1, v/v) to afford alkaloid **18** (89 mg). Fraction III (17.1 g) was purified on a C_{18} MPLC column with a MeOH–H_2O gradient eluent from 20% to 65% to yield fractions III-1–5. III-1 (5.5 g) was further purified on a C_{18} MPLC column with a MeOH–H_2O gradient eluent from 20% to 65% to afford four fractions. III-1-1 (1.1 g) was purified on a silica gel CC with a petroleum ether-acetone gradient (8:1, v/v) to afford alkaloids **17** (361 mg) and **20** (220 mg). III-1-2 (1.0 g) was purified on a silica gel CC with a petroleum ether-acetone gradient (8:1, v/v) to obtain alkaloids **20** (122 mg) and **23** (136 mg). III-1-3 was purified on a preparative C_{18} HPLC column with a gradient MeOH–H_2O (55:45–65:35, v/v) to yield alkaloid **21** (19 mg). III-1-4 (2.1 g) was purified on a silica gel CC with a petroleum ether-acetone gradient (8:1, v/v) to obtain alkaloids **22** (45 mg) and **24** (236 mg). III-2 (1.5 g) was further purified on a C_{18} MPLC column with a MeOH–H_2O gradient eluent from 30% to 45% to afford three fractions. Each of the fractions was purified on a C_{18} HPLC column with a gradient MeOH–H_2O (55:45–65:35, v/v) to afford alkaloids **20** (33 mg), **22** (18 mg) and **24** (52 mg). III-3 (6.2 g) was purified on a C_{18} MPLC column with a MeOH–H_2O gradient eluent from 30% to 65% to yield fractions III-3-1–3. III-3-1 (0.8 g) was purified on a C_{18} HPLC column with a gradient MeOH–H_2O (35:65–45:55, v/v) to afford alkaloid **1** (3 mg). III-3-2 (2.5 g) was purified on a silica gel CC with a petroleum ether-acetone gradient (8:1, v/v) to afford alkaloids **27** (1.2 g) and **28** (437 mg). III-3-3 (2.0 g) was further purified on a silica gel CC with a petroleum ether-acetone gradient (8:1, v/v) to afford alkaloids **25** (310 mg) and **26** (117 mg). III-4 (1.7 g) was purified on a C_{18} MPLC column with a MeOH–H_2O gradient eluent from 30% to 65% to yield two fractions. The fractions were purified on a C_{18} HPLC column with a gradient MeOH–H_2O (55:45–65:35, v/v) to afford alkaloids **29** (56 mg) and **30** (21 mg). III-5 (2.3 g) was further purified on a silica gel CC with a $CHCl_3$-acetone gradient (15:1, v/v) to afford two fractions. III-5-1 was purified on a C_{18} HPLC column with a gradient MeOH–H_2O (60:40–70:30, v/v) to afford alkaloids **3** (12 mg) and **4** (15 mg). III-5-2 (98 mg) was purified on a LH-20 with a MeOH–H_2O eluent (70%) to afford III-5-2-1. III-5-2-1 was purified on a C_{18} HPLC column with a gradient MeOH–H_2O (55:45–65:35, v/v) to afford alkaloid **7** (23 mg). Fraction IV (8.3 g) was purified on a C_{18} MPLC column with a MeOH–H_2O gradient eluent from 10% to 50% to yield fractions IV-1–2. IV-1 (0.5 g) was purified on a C_{18} MPLC column with a MeOH–H_2O gradient eluent from 10% to 20% to yield two fractions. Each of them was purified on a C_{18} HPLC column with a gradient MeOH–H_2O (25:75–35:65, v/v) to afford alkaloids **2** (19 mg), **5** (8 mg), **32** (12 mg) and **33** (21 mg). IV-2 (1.0 g) was purified on a silica gel CC with a $CHCl_3$-acetone gradient (15:1, v/v) to afford alkaloid **31** (126 mg). Fraction V (4.3 g) was purified on a C_{18} MPLC column with a MeOH–H_2O gradient eluent from 10% to 50% to yield fractions V-1–2. V-1 (0.3 g) was purified on a C_{18}

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HPLC column with a gradient MeOH–H_2O (20:80–30:70, v/v) to afford alkaloids **6** (21 mg), **35** (31 mg) and **36** (17 mg). V-2 (1.2 g) was purified on a C_{18} MPLC column with a MeOH–H_2O gradient eluent from 20% to 35% to afford two fractions. Alkaloids **34** (17 mg) and **37** (43 mg) were purified on a C_{18} HPLC column with a gradient MeOH–H_2O (20:80–30:70, v/v) from V-2-1 and alkaloids **38** (26 mg) and **39** (20 mg) was isolated from V-2-2 with the same condition.

3.4. Spectroscopic data

Meloyine A (**1**): white powder; $[\alpha]_D^{20}$ 117 (*c*, 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.81), 248 (3.43) and 289 (3.12) nm; IR (KBr) ν_{max} 3423, 3356, 1710, 1640 cm^{-1}; The ^1H and ^{13}C NMR data (acetone-d_6), see Table 1; positive HRESIMS *m/z* 353.1859 $[M + H]^+$ (calcd for $C_{21}H_{25}N_2O_3$, 353.1865).

Meloyine B (**2**): white powder; $[\alpha]_D^{20}$ 120 (*c*, 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 221 (3.49) and 288 (3.10) nm; IR (KBr) ν_{max} 1756, 1651, 1602 cm^{-1}; The ^1H and ^{13}C NMR data (acetone-d_6), see Table 1; positive HRESIMS *m/z* 369.1816 $[M + H]^+$ (calcd for $C_{21}H_{25}N_2O_4$, 369.1814).

Meloyine II (**3**): white powder; $[\alpha]_D^{20}$ 55 (*c*, 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 212 (3.42) and 254 (3.14) nm; IR (KBr) ν_{max} 3343, 1712, 1650 cm^{-1}; The ^1H and ^{13}C NMR data (acetone-d_6), see Table 2; positive HRESIMS *m/z* 653.3125 $[M + H]^+$ (calcd. for $C_{41}H_{41}N_4O_4$, 653.3128).

Meloyine III (**4**): white powder; $[\alpha]_D^{20}$ 9 (*c*, 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 212 (3.89) and 254 (3.95) nm; IR (KBr) ν_{max} 3358, 1678, 1644 cm^{-1}; The ^1H and ^{13}C NMR data (acetone-d_6), see Table 3; positive HRESIMS *m/z* 653.3125 $[M + H]^+$ (calcd for $C_{41}H_{41}N_4O_4$, 653.3128).

10-*O*-glucosylscandine (**5**): white powder; $[\alpha]_D^{20}$ 82 (*c*, 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 212 (3.45) and 254 (3.07) nm; IR (KBr) ν_{max} 3483, 1686, 1668, 1598 cm^{-1}; The ^1H and ^{13}C NMR data (acetone-d_6), see Table 3; positive HRESIMS *m/z* 529.2184 $[M + H]^+$ (calcd for $C_{27}H_{33}N_2O_9$, 529.2186).

Table 3
^1H and ^{13}C NMR spectroscopic data of alkaloid **5** in acetone-d_6 (δ in ppm, *J* in Hz)[a].

No	**4**		**5**	
	δ_H	δ_C	δ_H	δ_C
NH	9.19, s		9.36, s	
2		168.3 s		168.3 s
3	3.58, d (18.6)	47.8 t	3.17, m	48.7 t
	3.21, m		2.91, m	
5	3.05, m	55.3 t	3.05, m	54.3 t
	2.96, m		2.87, m	
6	2.12, m	39.6 t	2.24, m	41.9 t
	1.71, m		1.78, m	
7		57.3 s		58.5 s
8		131.9 s		130.6 s
9	6.98, s	124.6 d	7.02, d (2.5)	116.6 d
10		136.9 s		154.4 s
11	6.82, d (8.1)	128.5 d	6.78, dd (8.7, 2.5)	116.5 d
12	6.75, d (8.1)	116.7 d	6.71, d (8.7)	116.4 d
13		136.9 s		131.0 s
14	5.83, overlap	129.3 d	5.58, m	124.4 d
15	5.83, overlap	126.9 d	5.46, m	132.0 d
16		68.6 s		64.6 s
17	2.05, d (11.3)	40.9 t	2.68, br. s (2H)	45.2 t
	1.90, d (11.3)			
18	0.68, d (3H, 7.1)	8.8 q	4.77, overlap	114.8 t
			4.67, d (10.8)	
19	2.19, q (7.1)	52.7 d	5.51, t (6.7)	143.1 d
20		46.3 s		48.3 s
21	3.41, s	62.4 d	2.78, s	85.2 d
22		210.5 s		
$\underline{C}OOC\underline{H}_3$			3.45, s (3H)	52.6 q
$\underline{C}OOCH_3$				171.4 s
CH_2	3.71, s (2H)	41.2 t		
1′			4.78, d (7.5)	102.3 d
2′			3.38, m	77.8 d
3′			3.33, m	74.6 d
4′			3.30, m	71.3 d
5′			3.31, m	77.9 d
6′			3.77, d (11.8)	62.6 t
			3.57, d (11.8)	

[a] ^1H NMR recorded at 400 MHz, ^{13}C NMR recorded at 100 MHz.

3.5. Cytotoxicity assay

Three human cancer cell lines, HeLa, SGC-7901 gastric cancer, and A-549 lung cancer, were used for cytotoxic assays. Cells were cultured in RPMI-1640 (Sigma-Aldrich, St. Louis, MO, USA) or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37 °C. Cytotoxicity assays were performed according to the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) method in 96-well microplates. Briefly, 100 μL of adherent cell types were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before the addition of test compounds. Suspended cell types were seeded at an initial density of 1×10^5 cells/mL just before drug addition. Each tumor cell line was exposed to a test compound at concentrations of 0.04, 0.20, 1.00, 5.0, and 25.0 μM in DMSO in triplicate for 48 h, with cisplatin (Sigma-Aldrich) as the positive control. After treatment, cell viability was assessed, cell growth graphed, and IC_{50} values calculated by Reed and Muench's method.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2017.08.008.

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