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TWO NEW C_{19}-DITERPENOID ALKALOIDS FROM *ACONITUM FRANCHETII* VAR. *VILLOSULUM*

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Abstract – Two new C_{19}-diterpenoid alkaloids, named villosudine A (**1**) and villosudine B (**2**), along with seven known diterpenoid alkaloids, were isolated from the root of *Aconitum franchetii* var. *villosulum*. Their structures were elucidated by extensive spectroscopic analyses including 1D, 2D NMR, and HR-ESI-MS. Compounds (**1**-**7**) were evaluated for their cytotoxicity against the MCF-7 and HepG2 human cancer cell lines.

Aconitum franchetii var. *villosulum* belongs to the family Ranunculaceae and has a wide distribution in Sichuan province of China. Our previous phytochemical studies on this herb have led to the isolation of eleven diterpenoid alkaloids.[1] Diterpenoid alkaloids are typical chemical components of the genus *Aconitum*, and display various bioactivities such as anti-inflammatory, anti-arrhythmia, antifungal, and cytotoxic properties, as well as antiviral, insecticidal and antifeedant activities.[2] To find more biologically active secondary metabolites, two new C_{19}-diterpenoid alkaloid, named villosudine A (**1**) and villosudine B (**2**), along with seven known diterpenoid alkaloids, were isolated from this plant. Meanwhile, compounds (**1**-**7**) were tested for their cytotoxicity against the MCF-7 and HepG2 human cancer cell lines. Herein, isolation, structural elucidation, as well as cytotoxicity of these diterpenoid alkaloids are reported. The hydrochloric acid extract of *A. franchetii* was alkalinized with 28% NH_4OH soln. and then extracted with CH_2Cl_2 to give the total alkaloid. The alkaloid fraction was chromatographed over silica gel to afford two new C_{19}-diterpenoid alkaloids, villosudine A (**1**) and villosudine B (**2**), together with seven known analogues (**3**-**9**). The structures of the isolated compounds were as shown in Figure 1, and the ^1H and ^{13}C NMR data of **1** and **2** were listed in Table. The known compounds, 6-epichasmanine (**3**),[3] ezochasmaconitine (**4**),[4] ezochasmanine (**5**),[4] pseudoaconitine (**6**),[5] ludaconitine (**7**),[6] leucanthumsine A

(**8**),[7] and vilmorisine (**9**)[8] were established by comparison of their spectroscopic data with these reported in the literature.

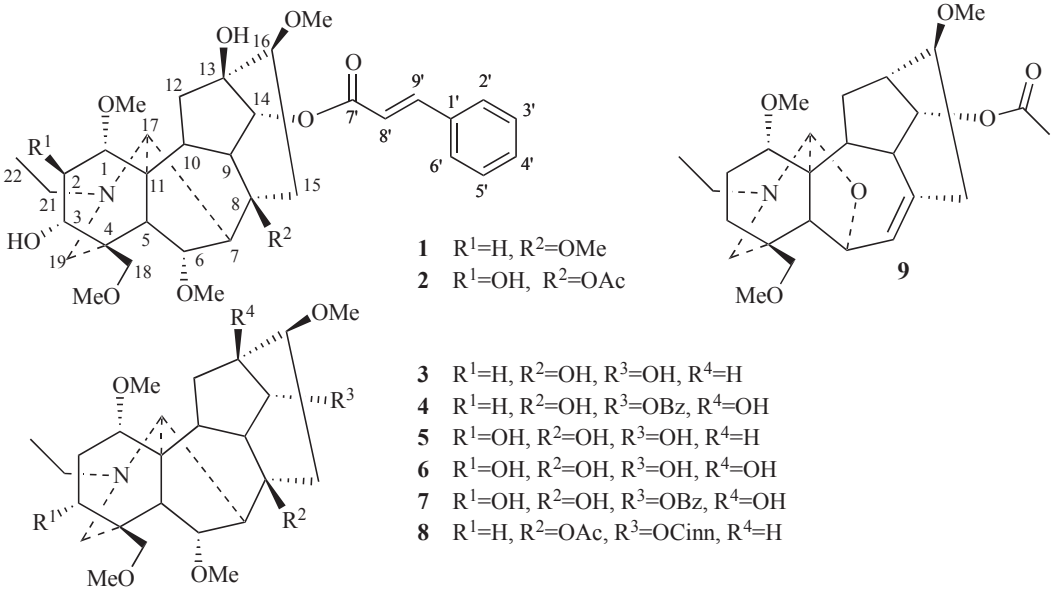

Figure 1. Structures of compounds **1-9**

Compound **1** was isolated as a white amorphous powder and gave a positive reaction to Dragendorff's reagent. Its molecular formula, $C_{35}H_{49}NO_9$ was deduced from the HR-ESI-MS *m/z*: 628.3488 $[M+H]^+$, calcd. for 628.3486 and ^{13}C NMR spectroscopic data. The IR (KBr) spectrum showed absorption bands for hydroxy group (3502 cm^{-1}) and ester carbonyl group (1713 cm^{-1}). The NMR data (Table) showed signals characteristic for an *N*-ethyl group [δ_H 1.10 (3H, t, J = 7.2 Hz), 2.50 (2H, overlapped); δ_C 48.7 t, 13.4 q], five methoxyl groups [δ_H 3.12, 3.23, 3.28, 3.29, and 3.52 (each 3H, s); δ_C 48.8 q; 56.0 q, 58.7 q, 58.9 q, and 59.2 q]. The quaternary carbon signals (δ_C 43.1 and 50.8) characteristic for C-4 and C-11, as well as an O-bearing methylene (δ_C 77.4, t) for C-18 suggesting compound **1** might be a typical C_{19}-diterpenoid alkaloid.[9] In addition, resonances of a *trans*-double bond [δ_H 6.42 (1H, d, J = 16.0 Hz) and 7.68 (1H, d, J = 16.0 Hz); δ_C 118.5 d and 144.9 d], an aromatic ring [δ_H 7.36-7.53 (5H, m); δ_C 128.2×2, 129.0×2 , 130.3 and 134.7], and a carboxyl group (δ_C 166.9) in the NMR spectra, along with the key HMBC correlations (Figure 2), H-9' (δ_H 7.68) to C-2' (δ_C 128.2) and C-7' (δ_C 166.9), suggested the presence of a *trans*-cinnamyl moiety.[7,10]

The *trans*-cinnamyl moiety was located at C-14 by the long-range correlation in the HMBC experiment from H-14 [δ_H 4.76 (1H, d, J = 5.2 Hz)] to C-7′ (δ_C 166.9). The existence of eight oxygenated carbons deduced from its HR-ESI-MS and ^{13}C-NMR spectrum suggested **1** possess two hydroxy groups. A hydroxyl group could be placed at C-13 based on the multiplicity of H-14 and the δ value of 16-OCH_3 (δ_H 3.52, s).[11] Another hydroxy group was positioned at C-3, due to the HMBC correlations from H-3 (δ_H

3.78, m) to C-1 (δ_C 82.6), C-5 (δ_C 48.0), and C-18 (δ_C 77.4). The NMR data of compound **1** were very similar to those of villosutine,[1] a known alkaloid isolated from this plant, except for lacking a signal for acetyl group. The chemical shift of C-8 at δ_C 85.8 in villosutine was shifted upfield to δ_C 78.7 in compound **1** suggesting that 8-OAc was substituted by a methoxyl group,[12] which was confirmed by the difference of 28 mass units between those two compounds. The remaining methoxyl groups were assigned to at C-1, C-6, C-16, and C-18, due to the long-range correlations between 1-OCH_3 (δ_H 3.23)/C-1 (δ_C 82.6), 6-OCH_3 (δ_H 3.28)/C-6 (δ_C 83.1), 16-OCH_3 (δ_H 3.52)/C-16 (δ_C 83.7), and 18-OCH_3 (δ_H 3.29)/C-18 (δ_C 77.4) in HMBC spectrum (Figure 2). Accordingly, the substitution pattern and assigned planar structure of **1** was confirmed by complete ^{1}H-^{1}H COSY, HMQC, and HMBC spectroscopic analyses.

The relative configuration of compound **1** was deduced from the vicinal coupling constants (Table) and NOESY experiment (Figure 2). The coupling constant between H-5 and H-6 (J = 6.4 Hz) confirmed the β-position of H-6,[13] which was further supported by the cross-peaks between H-6/H-9β in the NOESY spectrum. The β-methoxyl group at C-16 was demonstrated by the correlations between the H-16 and H-17α in the NOESY spectrum. Furthermore, the NOESY cross-peak between H-1/H-3, H-1/H-10β and H-10β/H-14 showed that H-1, H-3 and H-14 were β-oriented. Thus, the structure and absolute configuration of villosudine A (**1**) was determined as shown in Figure 1, and the full assignment of its spectroscopic data was achieved based on 1D and 2D NMR analyses.

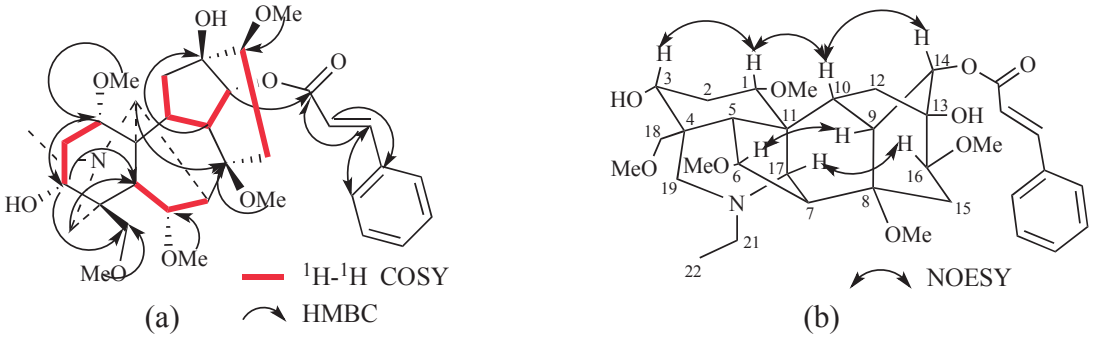

Figure 2. Key ^{1}H-^{1}HCOSY, HMBC (a) and NOESY (b) correlations of **1**

Compound **2** exhibited a pseudo-molecular-ion peak at *m/z* 672.3384 $[M+H]^+$ in the HR-ESI-MS, corresponding to the molecular formula $C_{36}H_{49}NO_{11}$. Compound **2** exhibited characteristic NMR (Table) spectral features of an aconitine-type C_{19}-diterpenoid alkaloid[14] bearing an *N*-ethyl group [δ_H 1.08 (3H, t, J = 7.2 Hz); δ_C 49.0 t, 12.6 q], four methoxyl groups [δ_H 3.26 and 3.50 (each 3H, s); 3.28 (6H, s); δ_C 55.4 q; 58.8 q, 58.9 q, and 59.1 q], and an acetyl group [δ_H 1.78 (3H, s); δ_C 22.3 q, 169.8 s]. The acetyl signal at higher field (δ_H 1.78) and the chemical shift of C-8 at δ_C 85.9 suggested that the acetyl group was

presented on C-8.[7] Comparison of the NMR data of **2** with those of the known compound atropurpursine[15] showed that the only difference between **2** and atropurpursine was a substitute group at C-14. The ^{1}H-NMR spectrum of **2** showed the presence of one CH=CH moiety at δ_H 6.58 (1H, d, J = 16 Hz) and 7.74 (1H, d, J = 16 Hz), together with those of five aromatic protons at δ_H 7.43-7.46 (3H, m) and 7.67-7.69 (2H, m), which were assigned to a *trans*-cinnamoyl group.[7,10] The doublet signal at δ_H 4.77 (J = 5.2 Hz) could be assigned to H_β-14, suggesting the presence of an ester function at C-14.[14] The long-rang correlations between H-14 (δ_H 4.77) and H-9' (δ_H 7.74, d, J = 16.0 Hz) with the carbonyl carbon signal at δ_C 166.9 (s) in HMBC confirmed the presence of the *trans*-cinnamoyl group at C-14 (Figure 3). In addition, compound **2** possessed a similar configuration to that of **1**, according to the deduction from its NOESY experiment (Figure 3). Accordingly, the structure of villosudine B (**2**) was confirmed by extensive analysis of its NMR spectra.

Table. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Data for Compounds **1** and **2** (δ in ppm, J in Hz)

No.	**1** in $CDCl_3$		**2** in $(CD_3)_2CO$		*No.*	**1** in $CDCl_3$		**2** in $(CD_3)_2CO$	
	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C	δ_H	δ_C
1	3.13 m	82.6 d	3.30 d (5.2)	83.8 d	19	a 2.43 d (12.4) b 2.91 d (12.4)	47.8 t	a 2.21 d (12) b 2.66 d (12)	46.5 t
2	a 1.90 m b 2.32 m	33.3 t	3.97 m	66.0 d					
3	3.78 m	71.8 d	3.43 m	68.5 d	21	2.50[a]	48.7 t	a 2.50 q (7.2) b 2.70 q (7.2)	49.0 t
4	—	43.1 s	—	44.7 s	22	1.10 t (7.2)	13.4 q	1.08 t (7.2)	12.6 q
5	2.39 d (6.4)	48.0 d	2.35 d (6.4)	46.5 d	1'	—	134.7 s	—	135.4 s
6	4.01 d (6.4)	83.1 d	4.15 d (6.4)	83.9 d	2'	7.50-7.53 m	128.2 d	7.67-7.69 m	129.0 d
7	2.07 s	46.1 d	3.12 s	50.5 d	3'	7.36-7.38 m	129.0 d	7.43-7.46 m	129.9 d
8	—	78.7 s	—	85.9 s	4'	7.36-7.38 m	130.3 d	7.43-7.46 m	131.3 d
9	2.58 m	45.9 d	2.88 m	46.8 d	5'	7.36-7.38 m	129.0 d	7.43-7.46 m	129.9 d
10	2.03[a]	41.4 d	2.28 m	41.3 d	6'	7.50-7.53 m	128.2 d	7.67-7.69 m	129.0 d
11	—	50.8 s	—	53.0 s	7'	—	166.9 s	—	166.9 s
12	a 2.03[a] b 2.50[a]	35.9 t	a 2.65 m b 2.09 m	38.8 t	8'	6.42 d (16.0)	118.5 d	6.58 d (16.0)	119.4 d
					9'	7.68 d (16.0)	144.9 d	7.74 d (16.0)	145.8 d
13	—	75.3 s	—	75.5 s	1-OMe	3.23 s	56.0 q	3.28 s	55.4 q
14	4.76 d (5.2)	79.1 d	4.77 d (5.2)	79.4 d	6-OMe	3.28 s	58.7 q	3.28 s	58.8 q
15	2.28 m	36.9 t	a 2.42 dd (6, 16) b 2.99 dd (8.8, 16)	40.5 t	8-OMe	3.12 s	48.8 q	—	—
16	3.38 t (8)	83.7 d	3.41 dd (6, 8.8)	84.6 d	16-OMe	3.52 s	58.9 q	3.50 s	59.1 q
17	2.77 br. s	61.2 d	2.82 br. s	60.6 d	18-OMe	3.29 s	59.2 q	3.26 s	58.9 q
18	a 3.48 ABq (8.8) b 3.58 ABq (8.8)	77.4 t	3.47 ABq (8.0) 3.63 ABq (8.0)	72.6 t	-COMe	—	—	—	169.8 s
					-CO*Me*	—	—	1.78 s	22.3 q

[a] Overlapped signals.

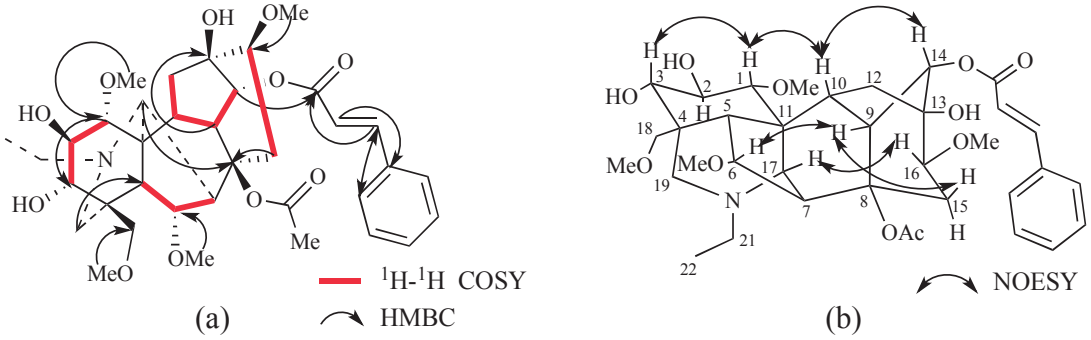

Figure 3. Key ^{1}H-^{1}HCOSY, HMBC (a) and NOESY (b) correlations of **2**

To evaluate the biological activities of these compounds isolated from the roots of *A. franchetii* for future applications, alkaloids **1-7** were tested for their *in vitro* cytotoxicity against the MCF-7 and HepG2 human cancer cell lines. Unfortunately, all of the compounds were inactive (IC_{50} > 50 μM, n = 3).

EXPERIMENTAL

General experimental procedure. Optical rotations were measured in $CHCl_3$ using a PerkinElmer polarimeter with a sodium lamp operating at 598 nm and 20 °C. The IR spectra were obtained using a Thermo Fisher Nicolet 6700 spectrometer. The HR-ESI-MS data were measured using a Q-TOF micro mass spectrometer (Waters). The NMR spectra were recorded on Bruker AV 400 spectrometer. The TLC plates were precoated with silica gel GF_{254} (Qingdao Haiyang Chemical Co., Ltd., China), and it was visualized under a UV lamp at 254 nm or by spraying with Dragendorff's reagent or iodine.

Plant materials. *Aconitum franchetii* var. *villosulum* were collected in Baoxing, Sichuan province of China, in August 2014 and identified by Prof. Liang-ke Song of the Institute of Life Science and Engineering, Southwest Jiaotong University. A voucher specimen (No. SC20140805) was deposited in the School of Life Science and Engineering, Southwest Jiaotong University, Sichuan, China.

Extraction and isolation. Dried and powdered roots of *A. franchetii* var. *villosulum* (9.2 kg) were extracted with 0.1 mol/L hydrochloric acid seven times at room temperature, for two days each soaking. The combined solvent was adjusted to pH 9-10 with 28% NH_4OH soln., and then extracted with CH_2Cl_2. The CH_2Cl_2 extracts were concentrated to produce the crude alkaloid extract (100 g). Column chromatography of the crude alkaloid extract over silica gel, using a CH_2Cl_2: MeOH (100:1, *v/v*) mixture with increasing polarity afforded fractions A–F based on TLC analysis. Fraction C was separated by silica gel CC (petroleum ether: Me_2CO: Et_2NH 20:1:0.1, *v/v/v*) to obtain **2** (13 mg) and **9** (1 mg). CC (silica gel, petroleum ether: Me_2CO: Et_2NH 15:1:0.1, *v/v/v*) of fraction D afforded **7** (12 mg) and **8** (1 mg). Fraction E was chromatographed on silica gel column and eluted with petroleum ether: Me_2CO: Et_2N (10:1:0.1-0:1:0.1, *v/v/v*) to afford **1** (9 mg), **5** (5 mg) and **3** (6 mg). Fraction F was subjected to CC on

silica gel and eluted with petroleum ether: CH_2Cl_2 (1:1-0:1, *v/v*) to give **6** (6 mg) and **4** (8 mg).

Villosudine A (1)

White amorphous powder; $[\alpha]_D^{20}$ +10.38 (*c* 0.55, $CHCl_3$); IR (KBr) ν_{max} 3502, 3060, 2963, 2931, 2889, 2821, 1713, 1638, 1578, 1496, 1450, 1385, 1335, 1309, 1281, 1202, 1176, 1089, 1043, 983, 921, 766, 709, 684, 491; ^{1}H NMR (400 MHz, $CDCl_3$) data and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table; HR-ESI-MS (*m/z*): 628.3488 $[M + H]^+$, calcd. for $C_{35}H_{50}NO_9$, 628.3486.

Villosudine B (2)

White amorphous powder; $[\alpha]_D^{20}$ +11.59 (*c* 0.55, $CHCl_3$); IR (KBr) ν_{max} 3503, 3060, 2971, 2927, 2890, 2820, 1716, 1637, 1577, 1495, 1450, 1369, 1277, 1228, 1175, 1128, 1098, 985, 964, 921, 880, 841, 769, 709, 685, 620, 498; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) data and ^{13}C NMR (100 MHz, $(CD_3)_2CO$) data, see Table; HR-ESI-MS (*m/z*): 672.3384 $[M + H]^+$, calcd. for $C_{36}H_{50}NO_{11}$, 672.3384.

Cell lines and cell culture and cytotoxicity assay

The *in-vitro* growth inhibitory activities of compounds **1**-**7** were assayed by the MTT method.[16] The HepG2 (human hepatic carcinoma) and MCF-7 (human breast cancer) cell lines were obtained from ATCC. Cells were plated in 96-well plates 24 h before treatment and continuously exposed to different concentrations of compounds for 72 h. DMSO (0.1%, *v/v*) was used as the negative controls and adriamycin (≥ 98%; Sigma Chemical Co., Ltd., Shanghai, China) was used as the positive control.

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REFERENCES

1. (a) W. Xu, L. Shan, S. Huang, S. Li, and X. Zhou, *Chin. J. Org. Chem.*, 2016, **36**, 2739; (b) J.-F. Zhang, L. Chen, S. Huang, L.-H. Shan, F. Gao, and X.-L. Zhou, *J. Nat. Prod.*, 2017, **80**, 3136.
2. (a) F.-P. Wang, Q.-H. Chen, and X.-Y. Liu, *Nat. Prod. Rep.*, 2010, **27**, 529; (b) F. O. A. Frejat, W. Xu, L. Shan, and X. Zhou, *Heterocycles*, 2017, **94**, 1903; (c) C. Li, Y. Hirasawa, H. Arai, H. A. Aisa, and H. Morita, *Heterocycles*, 2010, **80**, 607; (d) J. Zhang, L. Chen, and X. Zhou, *Heterocycles*, 2018, **96**, 304.
3. Z.-B. LI and F.-P. Wang, *Chin. Chem. Lett.*, 1996, **7**, 443.
4. H. Takayama, M. Ito, M. Koga, S.-i. Sakai, and T. Okamoto, *Heterocycles*, 1981, **15**, 403.
5. A. Klásek, V. Simánek, and F. Santavý, *Lloydia*, 1972, **35**, 55.

6. S. L. Shyaula, T. Tamang, N. Ghouri, A. Adhikari, S. Marasini, G. B. Bajracharya, M. D. Manandhar, and M. I. Choudhary, *Nat. Prod. Res.*, 2016, **30**, 2590.
7. H. Yan, D.-L. Chen, X.-X. Jian, and F.-P. Wang, *Helv. Chim. Acta*, 2007, **90**, 1133.
8. L. Ding, Y. Chen, and F. Wu, *Acta Chim. Sinica*, 1992, **50**, 405.
9. H. Ji, D.-L. Chen, and F.-P. Wang, *Heterocycles*, 2004, **63**, 2363.
10. (a) D.-L. Chen, L.-Y. Lin, Q.-H. Chen, X.-X. Jian, and F.-P. Wang, *J. Asian Nat. Prod. Res.*, 2003, **5**, 209; (b) L.-Y. Li, Q.-H. Chen, X.-L. Zhou, D.-L. Chen, F.-P. Wang, and C.-T. Che, *J. Nat. Prod.*, 2003, **66**, 269; (c) C.-S. Peng, F.-P. Wang, and X.-X. Jian, *J. Asian Nat. Prod. Res.*, 2000, **2**, 245; (d) R. Wang and Y.-Z. Chen, *Planta Med.*, 1987, **53**, 544.
11. (a) S. Zheng, L. Gao, X. Hao, X. Wang, and X. Shen, *Phytochemistry*, 1997, **46**, 951; (b) S. W. Pelletier, N. V. Mody, R. S. Sawhney, and J. Bhattacharyya, *Heterocycles*, 1977, **7**, 327.
12. F.-P. Wang, *J. Org. Chem.*, 1982, **3**, 161.
13. D. Csupor, P. Forgo, K. Csedő, and J. Hohmann, *Helv. Chim. Acta*, 2006, **89**, 2981.
14. S. W. Pelletier, N. V. Mody, B. S. Joshi, and L. C. Schramm, 'Alkaloids: Chemical and Biological Perspectives', Vol. 2, ed. by S. W. Pelletier, John Wiley Press, New York, 1984, pp. 205-462.
15. P. Tang, D. L. Chen, X. X. Jian, and F. P. Wang, *Chin. Chem. Lett.*, 2007, **18**, 704.
16. S. Huang, J. Zhang, L. Shan, Y. Zhang, and X. Zhou, *Heterocycles*, 2017, **94**, 485.