FULL PAPER

Anti-inflammatory Activity of Two New Indole Alkaloids from the Stems of *Nauclea officinalis*

by **De-Li Chen**[a])[1]), **Guo-Xu Ma**[a])[c])[1]), **Min-Jun He**[a]), **Yang-Yang Liu**[a]), **Xiao-Bing Wang***[b]), and **Xin-Quan Yang***[a])

[a]) Hainan Branch Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Haikou 570311, P. R. China (phone: +86-13876521118; e-mail: yangxinquan@sina.com)

[b]) College of Materials and Chemical Engineering, Hainan University, Haikou 570228, P. R. China (phone: +86-13707509296; e-mail: wangxiaobing4000@163.com)

[c]) Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 151, Malianwa North Road, Haidian District, Beijing 100193, P. R. China (phone: +86-13627547028; e-mail: chendeli9999@163.com)

Two new indole alkaloid derivatives (**1**, **2**), together with six known indole alkaloids (**3** – **8**) were isolated from the 70% EtOH/H_2O extract of the stem of *Nauclea officinalis*. Their structures were determined on the basis of extensive analyses of spectroscopic data (IR, MS, 1D- and 2D-NMR). All the isolates were evaluated for their anti-inflammatory activities on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW 264.7, and all the compounds showed significant inhibitory activities with the IC_{50} values of 0.82, 6.45, 9.75, 1.34, 3.40, 2.69, 1.58, and 1.96 μM, compared to the positive drug control group aminoguanidine with an IC_{50} value of 1.80 μM, especially compound **1** had the most significant activity.

Keywords: *Nauclea officinalis*, Nauclealises A and B, Indole alkaloid, Anti-inflammatory.

Introduction

Nauclea officinalis Pierre ex Pitard belongs to the genus *Nauclea* and the family Rubiaceae, which is widely used a folk Chinese Herb, mainly distributed throughout subtropical and tropical areas including southern regions of China, such as Hainan, Guangdong, Guangxi, and Yunnan, and is widely cultivated in Hainan [1][2]. The stems and barks of *N. officinalis* are found throughout the year, and are cut into small pieces and dried for medical purposes. These are cold natured and taste bitter. The plant, also called 'Danmu' in China, has been developed as traditional Li medicine products for years including Danmu injection and Danmu extract tablet, and are clinically used for the treatment of inflammatory ailments, containing acute tonsillitis, exogenous fever, bronchitis, enteritis, pharyngitis, pneumonia, diarrhea, dysentery, conjunctivitis, and upper respiratory tract infection [3][4].

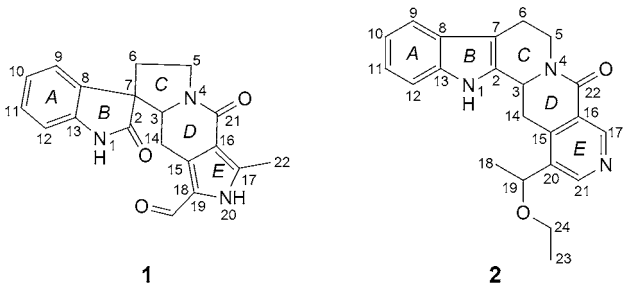
In recent years, dozens of alkaloids have been isolated from *N. officinalis* [5 – 10]. In our continuous effort of searching for interesting anti-inflammatory agents from 'Danmu', two new indole alkaloids together with six known indole alkaloids have been isolated from the stems of *N. officinalis*. In this article, the isolation and structural elucidation of these two new indole alkaloids were identified based on spectral analysis and by comparison of their spectral data with those reported in the literature (*Fig. 1*). Furthermore, these compounds were evaluated for their nitric oxide (NO) inhibitory activities on lipopolysaccharide (LPS)-induced NO production in mouse macrophage RAW 264.7 cells. We reported herein the isolation and structural elucidation of two new alkaloids and their bioactivities.

Results and Discussion

Compound **1** was isolated as pale-yellow amorphous powder. The molecular formula was established as $C_{19}H_{17}N_3O_3$ by its HR-ESI-MS at *m*/*z* 358.1245 ([*M* + Na]$^+$, $C_{19}H_{17}N_3NaO_3^+$; calc. 358.1241). The IR spectrum displayed characteristic absorptions attributing to a CHO group (2745 cm^{-1}), a C=O group (1725 and 1650 cm^{-1}), amide groups (1635 cm^{-1}), and an aromatic ring (1625, 1515, and 1420 cm^{-1}), respectively. The ^{1}H-NMR spectrum of **1** (*Table 1*) showed four aromatic H-atom signals, of which two appeared as *triplet*s at δ(H) 7.34 and 7.29 and were attributed to H–C(10) and H–C(11), two were *doublet*s at δ(H) 6.97 and 7.08 and were assigned to H–C(12) and H–C(9), verified by the substitution pattern for ring *A*. The two CH_2 groups at δ(H) 2.35 (*m*, CH_2(6)) and 3.96 (*t*, *J* = 9.6, CH_2(5)) attributed to the presence of

[1]) These authors contributed equally to this work.

 DOI: 10.1002/hlca.201600159



1 **2**

Fig. 1. Structures of compounds **1** and **2**.

the sequence CH_2–CH_2–N at ring *C*, and one CH_2 group at δ(H) 2.82 (*dd*, *J* = 5.4, 16.2, 1 H), 2.65 (*t*, *J* = 15.0, 1 H) could be assigned to CH_2(14) for ring *D*. Also, a pyrrole ring (ring *E*) was observed and connected to ring *D* with one CHO and Me H-atoms at δ(H) 9.75 (*s*, 1 H) and 2.51 (*s*, 3 H), respectively. The ^{13}C-NMR spectrum of **1** showed the resonance of all 19 C-atoms, one Me, two amide C=O, one CHO, three CH_2, four CH, and eight quaternary C-atoms (*Table 1*). The presence of amide C=O C-atoms were observed at δ(C) 181.5 (C(2)) and 160.4 (C(21)). The signals at δ(C) 11.7 (Me(22)) could be assigned as the resonance of the Me group. The HMBCs (*Fig. 2*) from H–C(9) to C(8) (δ(C) 129.9), C(7) (δ(C) 58.2), and C(13) (δ(C) 143.6), H–C(12) to C(8) and C(13), CH_2(6) to C(7) and C(2), H–C(3) (δ(H) 4.48) to C(2), and C(7), CH_2(14) (δ(H) 2.82, 2.65) to C(3) (δ(C) 66.9), C(7), C(15) (δ(C) 145.0), and C(16) (δ(C) 120.4) indicated the presence of four rings *A*/*B*/*C*/*D*, which were similar to the known compounds naucleoxoside A and nauclealotide A [11][12]. In fact, the differences between them were due to the additional pyrrole ring (ring *E*) in compound **1**. In the HMBC, the connections from CH_2(14) to C(15), C(16), and C(19) (δ(C) 128.0) confirmed the existence of pyrrole ring and the connectivity of C(15)/C(16) between rings *D* and *E* (*Fig. 2*). Furthermore, the correlations from H–C(18) to C(15) and C(19), and Me(22) to C(17) and C(16) indicated that the CHO and Me groups were linked to C(19) and C(17), respectively. Based on the above data and comprehensive 2D-NMR experiments, structure **1** was assigned as shown, and was named nauclealise A.

Compound **2** was isolated as pale-yellow amorphous powder. The molecular formula was established as $C_{22}H_{23}N_3O_2$ by its HR-ESI-MS at *m*/*z* 361.1835 ([*M* + Na]$^+$, $C_{22}H_{23}N_3NaO_2^+$; calc. 361.1831). The IR spectrum displayed characteristic absorptions attributing to C=O group (1715 cm^{-1}), amide group (1640 cm^{-1}), and aromatic ring (1620, 1520, and 1430 cm^{-1}), respectively. The ^{1}H-NMR spectrum of **2** (*Table 1*) showed two *triplet*s at δ (H) 7.03 (H–C(10)) and 7.12 (H–C(11)), two *doublet*s at δ (H) 7.35 (H–C(12)) and 7.48 (H–C(9)), one *singlet* H-atom at δ(H) 5.05 (H–C(3)), and three CH_2 at δ(H) 2.90 (*m*, CH_2(6)), 5.13 (*dd*, *J* = 4.8, 12.6, H_a–C(5)), 3.06 (*td*, *J* = 7.8, 12.0, H_b–C(5b)), and 3.90 (*m*, CH_2(14)). The two

Table 1. ^{1}H- and ^{13}C-NMR (600, 150 MHz) data of compounds **1** and **2** (CD_3OD). δ in ppm, *J* in Hz

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	–	–	–	–
2	–	181.5	–	133.5
3	4.48 (*dd*, *J* = 5.4, 14.4, 1 H)	66.9	5.05 (*t*, *J* = 12.0, 1 H)	52.8
4	–	–	–	–
5	3.96 (*t*, *J* = 9.6, 2 H)	44.2	5.13 (*dd*, *J* = 4.8, 12.6, 1 H), 3.06 (*td*, *J* = 7.8, 12.0, 1 H)	40.9
6	2.33 – 2.36 (*m*, 2 H)	34.6	2.88 – 2.94 (*m*, 2 H)	21.9
7	–	58.2	–	109.7
8	–	129.9	–	128.0
9	7.08 (*d*, *J* = 7.8, 1 H)	124.0	7.48 (*d*, *J* = 7.8, 1 H)	119.3
10	7.34 (*t*, *J* = 7.8, 1 H)	124.2	7.03 (*t*, *J* = 7.8, 1 H)	120.4
11	7.29 (*t*, *J* = 7.8, 1 H)	130.3	7.12 (*t*, *J* = 7.8, 1 H)	123.1
12	6.97 (*d*, *J* = 7.8, 1 H)	111.3	7.35 (*d*, *J* = 7.8, 1 H)	112.3
13	–	143.6	–	138.2
14	2.82 (*dd*, *J* = 5.4, 16.2, 1 H), 2.65 (*t*, *J* = 15.0, 1 H)	23.7	3.86 – 3.94 (*m*, 2 H)	32.0
15	–	145.0	–	145.5
16	–	120.4	–	126.4
17	–	145.0	9.05 (*s*, 1 H)	149.0
18	9.75 (*s*, 1 H)	187.8	1.46 (*d*, *J* = 6.6, 3 H)	23.3
19	–	128.0	4.92 (*q*, *J* = 7.2, 1 H)	74.5
20	–	–	–	137.8
21	–	160.4	8.68 (*d*, *J* = 6.0, 1 H)	151.2
22	2.51 (*s*, 3 H)	11.7	–	165.2
23	–	–	1.24 (*t*, *J* = 7.2, 3 H)	15.9
24	–	–	3.53 – 3.59 (*m*, 2 H)	65.9

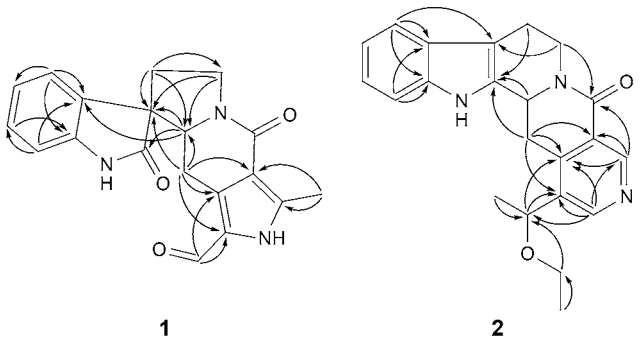


Fig. 2. Key HMBCs of compounds **1** and **2**.

CH H-atoms at δ(H) 9.05 (*s*, 1 H) and 8.68 (*d*, 1 H) were assigned to H–C(17) and H–C(21), respectively. The presence of a EtOCH(Me) group at C(20) was observed by the H-atom signals at δ(H) 1.46 (*d*, J = 6.6, Me(18)), 4.92 (*q*, J = 7.2, H–C(19)), 1.24 (*t*, J = 7.2, Me(23)), and 3.53 (*m*, CH_2(24)), and the C-atom signals at δ(C) 23.3 (C(18)), 74.5 (C(19)), 15.9 (C(23)), and 65.9 (C(24)), together with the HMBCs from Me(18) and CH_2(24) to C(19). The ^{13}C-NMR data indicated that the structure possessed one lactam C=O, eight aromatic C-atoms, one Me, three CH_2, and one EtO (*Table 1*). The HMBCs (*Fig. 2*) from H–C(3) to C(2) (δ(C) 133.5), CH_2(6) to C(7) (δ(C) 109.7), H–C(9) to C(7), C(8) (δ(C) 128.0), and C(13) (δ(C) 138.2), H–C(10) to C(8), and H–C(12) to C(8) and C(13) indicated the presence of a tetrahydro-β-carboline ring (rings *A*, *B*, and *C*). Specifically, a δ-lactam ring (ring *D*) was identified by the correlations between CH_2(5) (δ(H) 5.13 and 3.06) and C(22) (δ(C) 165.2), CH_2(14) (δ(H) 3.90) and C(15) (δ(C) 145.5), and C(16) (δ(C) 126.4) and C(20 (δ(C) 137.8) in the HMBC spectrum of **2**. Furthermore, the HMBCs from CH_2(14), Me(18) to C(20), H–C(19) to C(15) and C(20), and H–C(21) (δ(H) 8.68) to C(17) (δ(C) 149.0), C(15), C(20), and C(19) indicated that the ring *D* was connected to a pyridine ring (ring *E*). Comparing the spectroscopic data, compound **2** was similar to the known compounds latifoliamide D [13] and 3,14-dihydroangustoline [14]. Based on the above analyses, compound **2** was determined to be 3,14-dihydro-19-*O*-ethylangustoline, assigned with a trivial name nauclealise B.

The six known compounds, naucleofficine D (**3**) [5], latifoliamide D (**4**) [13], latifoliamide B (**5**) [13], angustoline (**6**) [3], 3,14-dihydroangustine (**7**) [14], and 3,14,18,19-tetrahydroangustine (**8**) [14] (*Fig. 3*) were identified by comparing their ^{1}H- and ^{13}C-NMR data with the those in literature.

Considering this medicinal herb as an anti-inflammatory agent, all the isolated compounds were examined for their anti-inflammatory activities on LPS-induced NO production in RAW 264.7. The IC_{50} values are summarized in *Table 2*. The results showed that compounds **1** – **8** showed significant inhibitory activities with IC_{50} value of 0.82, 6.45, 9.75, 1.34, 3.40, 2.69, 1.58, and 1.96 μM comparing with the positive group aminoguanidine. From the biological results, compounds **1** – **8** deserve further exploration as potential anti-inflammatory candidates.

Conclusions

Two new indole alkaloid derivatives (**1** and **2**), together with six known indole alkaloids (**3** – **8**) were isolated from the 70% EtOH/H_2O extract of the stems of *N. officinalis*. All compounds were evaluated for their anti-inflammatory activities on LPS-induced NO production in RAW 264.7, and all compounds showed significant inhibitory activities with the IC_{50} values of 0.82, 6.45, 9.75, 1.34, 3.40, 2.69, 1.58, and 1.96 μM compared with the positive drug control group aminoguanidine with an IC_{50} value of 1.80 μM. Compound **1** had the most significant activity, which could be a candidate compound of anti-inflammatory drug. The significant anti-inflammatory activity of compounds also confirmed the clinical curative effect of *N. officinalis*.

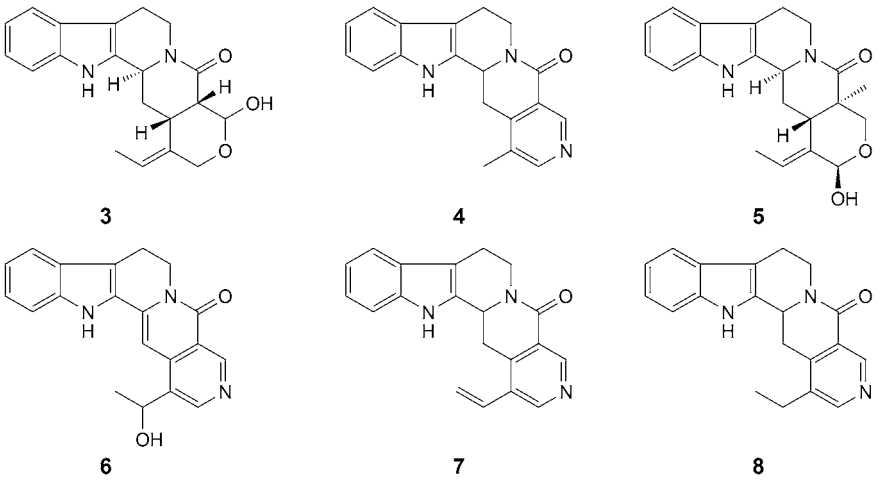


Fig. 3. Structures of known compounds.

Table 2. Inhibitory activity of compounds on LPS-induced NO production in RAW 264.7 macrophages

Compound	IC_{50} [μM]
1	0.82
2	6.45
3	9.75
4	1.34
5	3.40
6	2.69
7	1.58
8	1.96
Aminoguanidine	1.80

This work was supported by the *Program of Hainan Association for Science and Technology Plans to Youth Academic Innovation* (No. 201517) and the *Major Science and Technology Program of Hainan Province* (No. ZDZX2013008-1). The authors have declared no conflict of interest.

Experimental Part

General

All solvents used were of anal. grade (*Beijing Chemical Works*). Thin-layer chromatography (TLC): precoated SiO_2 GF_{254} plates (*Zhi Fu Huang Wu Pilot Plant of Silica Gel Development*, Yantai, P. R. China); visualized by spraying with 5% H_2SO_4 in EtOH. Column chromatography (CC): ODS (12 nm, 50 μm, *YMC Co. Ltd*., Japan), and SiO_2 (100 – 200 and 300 – 400 mesh, *Qingdao Marine Chemical Plant*, Qingdao, P. R. China). Semiprep. LC: *Lumtech K-1001* anal. LC equipped with two pumps of *K-501*, a UV detector of *K-2600*, and an *YMC Pack* C_{18} column (250 mm × 10 mm, i.d., 5 μm, *YMC Co. Ltd.*, Japan) eluted with MeOH/H_2O at a flow rate of 2 ml/min. Optical rotations: *Perkin-Elmer 341* digital polarimeter. IR Spectra: *FTIR-8400S* spectrometer; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AV III 600* NMR spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-ESI-MS: *LTQ-Obitrap XL* spectrometer; in m/z.

The stems of *N. officinalis* were collected from Tongzha Town, Wuzhishan City, Hainan Province, P. R. China, in April 2015. The botanical identification of the plant was done by Prof. *Xi-long Zheng*, Hainan Branch Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, where a voucher specimen (No. DM20150416) was deposited.

The dried and powdered stems of *N. officinalis* (50 kg) ware extracted with 70% aq. EtOH (500 l × 3) under refluxing for 2 h. The filtrate was concentrated to dryness under reduced pressure to yield a semisolid residue (2042 g) that constituted the crude extract. The crude extract was triturated successively with H_2O (3 l) and treated with 2% HCl until the pH was 2.0, which was stewed for 12 h before filtering. The filtrate was treated with 10% NaOH until the pH was 9.0, and then subjected to further extraction to afford petroleum ether-soluble fraction (*Fr. A*), CH_2Cl_2-soluble fraction (*Fr. B*), AcOEt-soluble fraction (*Fr. C*), and BuOH-soluble fraction (*Fr. D*) and a residue fraction (*Fr. E*), resp. A part of *Fr. B* (80.0 g) was subjected to CC over SiO_2 (200 – 300 mesh, 200 g) and eluted with CH_2Cl_2/MeOH 100:0 in increasing polarity to give a total of 70 fractions, *Frs. 1 – 70* (500 ml each).

Fr. 50 (5.8 g) was separated by semiprep. LC using a MeOH/H_2O 60:40 system to yield **1** (10.7 mg) and **3** (10.4 mg). *Fr. 70* (3.4 g) was separated by semiprep. LC using a MeOH/H_2O 42:58 system to yield **2** (9.8 mg), **4** (10.2 mg), **5** (9.8 mg), **6** (11.3 mg), **7** (14.4 mg), and **8** (8.5 mg). The entire detection was under UV 210 nm and the flow rate was 2 ml/min.

The structures of compounds **1** – **8** were determined by IR, ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HSQC, HMBC, NOESY, and HR-ESI-MS.

Nauclealise A (= **1,2,2′,4′,6′,7′,8′a,9′-Octahydro-3′-methyl-2,4′-dioxospiro[3*H*-indole-3,8′-[8*H*]pyrrolo[3,4-*f*]indolizine]-1′-carboxaldehyde**; **1**). Pale-yellow amorphous powder. $[\alpha]_D^{20}$ = –20.8 (c = 0.15, MeOH). IR (KBr): 2745, 1725, 1650, 1635, 1625, 15155, 1420. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (150 MHz, CD_3OD): see *Table 1*. HR-ESI-MS: 358.1245 ([M + Na]$^+$, $C_{19}H_{17}N_3NaO_3^+$; calc. 358.1241).

Nauclealise B (= **1-(1-Ethoxyethyl)-8,13,13b,14-tetrahydroindolo[2′,3′:3,4]pyrido[1,2-*b*][2,7]naphthyridin-5(7*H*)-one**; **2**). Pale-yellow amorphous powder. $[\alpha]_D^{20}$ = –35.2 (c = 0.12, MeOH). IR (KBr): 2950, 2920, 2855, 1715, 1640, 1620, 1520, 1430, 1405, 1295. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (150 MHz, CD_3OD): see *Table 1*. HR-ESI-MS: 361.1836 ([M + Na]$^+$, $C_{22}H_{23}N_3NaO_2^+$; calc. 361.1831).

The *in vitro* anti-inflammatory activity was evaluated by determining the nitrite concentration in the medium and the proliferation of RAW 264.7 cells as described in a previous study with some modifications [15][16]. Briefly, the cells (10^5 cells/well) were coincubated with drugs and LPS (1 μg/ml) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the cultured RAW 264.7 macrophage supernates with *Griess* reagent. Aliquots of supernates (100 μl) were incubated, in sequence, with 50 μl of 1% sulfanilamide and 50 μl of 0.1% naphthylethylenediamine in 2.5% H_3PO_4 soln. The absorbance was recorded on a microplate reader at a wavelength of 570 nm.

REFERENCES

[1] H.-Y. Wang, K. Liu, R.-X. Wang, S.-H. Qin, F.-L. Wang, J.-Y. Sun, 'Two new triterpenoids from *Nauclea officinalis*', *Nat. Prod. Res.* **2015**, *7*, 644 – 649.

[2] N. Li, L. Cao, Y. Cheng, Z.-Q. Meng, Z.-H. Tang, W. J. Liu, Z.-Z. Wang, G. Ding, W. Xiao, 'In vivo anti-inflammatory and

analgesic activities of strictosamide from *Nauclea officinalis*', *Pharm. Biol.* **2014**, *11*, 1445 – 1450.
[3] W. D. Xuan, J. Bian, H. S. Chen, 'Alkaloidal constituents of *Nauclea officinalis*', *Chin. Tradit. Herb. Drugs* **2007**, *38*, 170 – 173.
[4] W. D. Xuan, H. S. Chen, Z. X. Yuan, P. Zhu, 'Chemical constituents of *Nauclea officinalis*', *Chin. J. Nat. Med.* **2005**, *3*, 181 – 183.
[5] J. Y. Sun, H. X. Lou, S. J. Dai, H. Xu, F. Zhao, K. Liu, 'Indole alkoloids from *Nauclea officinalis* with weak antimalarial activity', *Phytochemistry* **2008**, *69*, 1405 – 1410.
[6] S. Y. Liew, M. R. Mukhtar, A. H. A. Hadi, K. Awang, M. R. Mustafa, K. Zaima, H. Morita, M. Litaudon, 'Naucline, a New Indole Alkaloid from the Bark of *Nauclea officinalis*', *Molecules* **2012**, *17*, 4028 – 4036.
[7] W.-D. Xuan, H.-S. Chen, J.-L. Du, S. Liang, T.-Z. Li, D.-G. Cai, 'Two new indole alkaloids from *Nauclea officinalis*', *J. Asian Nat. Prod. Res.* **2006**, *8*, 719 – 722.
[8] M. Lin, X. Liu, D. Q. Yu, 'Alkaloids of *Nauclea officinalis*', *Planta Med.* **1985**, *6*, 459 – 461.
[9] S. Y. Liew, K. Y. Khaw, V. Murugaiyah, C. Y. Looi, Y. L. Wong, M. R. Mustafa, M. Litaudon, K. Awang, 'Natural indole butyrylcholinesterase inhibitors from *Nauclea officinalis*', *Phytomedicine* **2015**, *1*, 45 – 48.
[10] J. Y. Sun, H. X. Lou, H. Xu, S. J. Dai, K. Liu, 'Two new indole alkaloids from *Nauclea officinalis*', *Chin. Chem. Lett.* **2007**, *9*, 1084 – 1086.
[11] F. Long, Ph.D. Studies on chemical constituents of the leaves of *Nauclea officinalis*, Jinan University at Guangzhou, 2010.
[12] W. D. Xuan, Ph.D. Studies on bio-active constituents of *Nauclea officinalis* and *Ervatamia yunnanensis*, Second Military Medical University at Shanghai, 2005.
[13] A. A. Agomuoh, A. Ata, C. C. Udenigwe, R. E. Aluko, I. Irenus, 'Novel Indole Alkaloids from *Nauclea latifolia* and Their Renin-Inhibitory Activities', *Chem. Biodiversity* **2013**, *3*, 401 – 410.
[14] C. A. J. Erdelmeier, U. Regenass, T. Rali, O. Sticher, 'Indole Alkaloids with in vitro Antiproliferative Activity from the Ammoniacal Extract of *Nauclea orientalis*', *Planta Med.* **1992**, *1*, 43 – 48.
[15] X.-J. Huang, J.-Q. Tang, M.-M. Li, Q. Liu, Y.-L. Li, C.-L. Fan, H. Pei, H.-N. Zhao, Y. Wang, W.-C. Ye, 'Triterpenoid saponins from the rhizomes of *Anemone flaccida* and their inhibitory activities on LPS-induced NO production in macrophage RAW264.7 cells', *J. Asian Nat. Prod. Res.* **2014**, *9*, 910 – 921.
[16] G.-X. Ma, X.-P. Zhang, P.-F. Li, Z.-H. Sun, N.-L. Zhu, Y.-D. Zhu, J.-S. Yang, D.-L. Chen, H.-F. Wu, X.-D. Xu, 'Four new phenolic acid with unusual bicyclo[2.2.2]octane moiety from *Clerodendranthus spicatus* and their anti-inflammatory activity', *Fitoterapia* **2015**, *105*, 61 – 65.

Received May 31, 2016
Accepted July 20, 2016

