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Bioactive monoterpene indole alkaloids from *Nauclea officinalis*

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ABSTRACT

Two new monoterpene indole alkaloids, naucleaoffines A (**1**) and B (**2**), together with six known alkaloids (**3–8**), were isolated from the stems and leaves of *Nauclea officinalis*. The structures of **1** and **2** were elucidated by extensive spectroscopic methods and the known compounds were identified by comparisons with the data reported in literature. All isolated compounds were evaluated for their anti-inflammatory activities and anti-HIV-1 activities. Compounds **1–8** exhibited significant inhibitory activities on nitric oxide (NO) production induced by lipopolysaccharide in mouse macrophage RAW 264.7 cells *in vitro* with IC_{50} values comparable to that of hydrocortisone. In addition, compounds **1–8** showed significant anti-HIV-1 activities with EC_{50} ranged from 0.06 to 2.08 μM. These findings suggest that the discoveries of these indole alkaloids with significant anti-inflammatory activities and anti-HIV-1 activities isolated from *N. officinalis* could be of great importance to the development of new anti-inflammatory and anti-HIV agents.

1. Introduction

The genus *Nauclea* (Rubiaceae) comprising about 35 species are widely distributed in tropical regions of Asia, Africa and Oceania. There are two species of this genus, namely, *N. officinalis* and *N. orientalis*, growing mainly in Hainan, Yunnan, Guangxi and Guangdong Provinces in China. Among them, *N. officinalis* is a native plant, while *N. orientalis* is an introduced and cultivated plant [1]. As a Li folk medicine named as Danmu, *N. officinalis* had been used as an anti-inflammatory and antibacterial agent in China. Currently, the preparations from Danmu extract, including tablets and injection, are clinically used for the treatment of inflammatory ailments [2]. Earlier chemical and pharmacological studies on *N. officinalis* had showed that its alkaloids account for its anti-inflammatory activities [3–7]. Our preliminary experimental results showed that the EtOH extract of the stems and leaves of *N. officinalis* exhibited significant inhibitory activity on nitric oxide (NO) production induced by lipopolysaccharide in mouse macrophage RAW 264.7 cells with the IC_{50} value of 8.80 μg/mL and anti-HIV-1 reverse transcriptase (RT) activity with an EC_{50} value of 7.16 μg/mL. As a part of our ongoing research into structurally and biologically interesting alkaloids from tropical medicinal plants in China [8–12], a chemical investigation on *N. officinalis* was thus undertaken and had led to the isolation and characterization of two new monoterpene indole alkaloids, naucleaoffines A (**1**) and B (**2**), together with six known alkaloids. Their structures were elucidated on the basis of extensive spectroscopic analyses. In addition, all isolated compounds were evaluated for their anti-inflammatory activities and anti-HIV-1 activities. Herein, we describe the isolation, structure elucidation, anti-inflammatory activities and anti-HIV-1 activities of these compounds.

2. Experimental

2.1. General experiment procedure

Optical rotations were measured with a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. IR spectra were recorded on a Nicolet Nexus 470 spectrophotometer in KBr discs. NMR spectra were recorded on Bruker 400 MHz spectrometers using TMS as an internal standard, with chemical shifts recorded as δ values. HR-ESI-MS spectra were measured on a Micromass Q-TOF Ultima Global GAA076 LC mass spectrometer. Semi-preparative HPLC was performed on an Agilent 1260 LC series

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with a DAD detector using an Agilent Eclipse XDB-C_{18} column (250 × 9.4 mm, 5 μm). Silica gel (300–400 mesh, Qingdao Marine Chemical Inc., China), Silica gel H (10–40 μm, Qingdao Marine Chemical Inc., China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), and Sephadex LH-20 (40–70 μm, Amersham Biosciences, Sweden) were used for column chromatography (CC).

2.2. Plant material

The stems and leaves of *N. officinalis* were collected from Bawangling Nature Reserve, Hainan Province China, in May 2015, and identified by Prof. Qiong-Xin Zhong, College of Life Science, Hainan Normal University. A voucher specimen (No. 20150508) has been deposited at the Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, Hainan Normal University.

2.3. Extraction and isolation

The powdered air-dried stems and leaves of *N. officinalis* (30.0 kg) were extracted with 85% EtOH at room temperature for three times, each for a week. The solvent was combined and condensed *in vacuum* to yield a crude extract. After suspended in water (10.0 L), the crude extract was extracted successively with petroleum ether (10.0 L × 3) and ethyl acetate (10.0 L × 3), to obtain the petroleum ether extract and the EtOAc extract. The EtOAc extract (860.0 g) was subjected to silica gel CC, eluted with petroleum ether/acetone (95:5 to 10:90, *v/v*) yielding eight fractions (Fr.1–Fr.8). Fr.3 (33.6 g) was further chromatographed over a RP-18 gel medium-pressure CC (CH_3OH/H_2O, 40:60 to 100:0, *v/v*) to give seven fractions (Fr.3A–Fr.3G). Fr.3B (4.6 g) was purified using Sephadex LH-20 gel CC, eluted with CH_3OH, then separated by semi-preparative HPLC (Agilent Eclipse XDB-C_{18} column, i.d. 250 × 9.4 mm, 5 μm, 50% CH_3OH, 3.0 mL/min, t_R 28.6, 33.2, 39.7 and 45.2 min) to afford **1** (32.1 mg), **4** (43.7 mg), **5** (25.9 mg) and **8** (64.2 mg). Fr. 3C (3.2 g) was purified using Sephadex LH-20 gel CC, eluted with CH_3OH, then separated by semi-preparative HPLC (Agilent Eclipse XDB-C_{18} column, i.d. 250 × 9.4 mm, 5 μm, 45% CH_3CN, 3.0 mL/min, t_R 21.7, 32.4, 36.8 and 40.3 min) to afford **2** (19.5 mg), **3** (35.7 mg), **6** (46.5 mg) and **7** (73.3 mg).

2.4. Naucleaoffine A (1)

Yellow amorphous powder; $[\alpha]_D^{19}$-67.3 (c 0.13, CH_3OH); IR (KBr) ν_{max} 3419, 2918, 1631, 1592, 1442, 1431, and 1170 cm^{-1}; UV (MeOH) λ_{max} (log ε) 225 (4.32), 290 nm (3.87); ^1H and ^{13}C NMR data (see Table 1); ESIMS *m/z* 363 $[M + Na]^+$; HRESIMS *m/z* 363.1679 (M + Na; calcd for $C_{20}H_{24}N_2O_3Na$, 363.1679).

2.5. Naucleaoffine B (2)

Yellow amorphous powder; $[\alpha]_D^{19}$-42.8 (c 0.11, CH_3OH); IR (KBr) ν_{max} 3424, 2923, 1627, 1595, 1448, 1433, and 1173 cm^{-1}; UV (MeOH) λ_{max} (log ε) 223 (4.26), 287 (3.73) nm; ^1H and ^{13}C NMR data (see Table 1); ESIMS *m/z* 363 $[M + Na]^+$; HRESIMS *m/z* 363.1680 (M + Na; calcd for $C_{20}H_{24}N_2O_3Na$, 363.1679).

2.6. Anti-inflammatory bioassays

The RAW 264.7 cells were incubated in RPMI 1640 medium containing 10% fetal bovine serum, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cell concentration was adjusted to 5 × 10^5 cells/mL, and 200 μL of cell suspension was seeded in each well of a 96-well plate. After 1 h incubation, cells were treated with LPS (1 μg/mL) and test samples were dissolved in DMSO at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM (final DMSO concentration 0.2%, *v/v*) for 24 h at 37 °C. A 100 μL sample of the culture supernatant was determined by the Griess reaction [13] The Griess reagent (50 μL of 1% sulfanilamine in 5% H_3PO_4, and 50 μL of 0.1% *N*-1-naphthylethylenediamine dihydrochloride) was added to each well. After 10 min, the reaction products were colorimetrically quantitated at 540 nm using a microplate reader. The experiments were performed in triplicate. Hydrocortisone was used as a positive control; The cytotoxicity assay was performed using the MTT method in 96-well microplates [14]. An MTT solution (200 μg/mL) was added after the 24 h treatment and then incubated for another 4 h at 37 °C. The reduced MTT-formazan was solubilized with 150 μL of DMSO, and the absorbance of the MTT-formazan solution at 570 nm was measured by an immunoreader. The percentage of suppression was calculated by comparing the absorbance of sample treated cells with that of nontreated cells.

2.7. Anti-HIV-1 activity bioassays

Cytotoxicity against C8166 cells (CC_{50}) was assessed using the MTT method, and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}) as described in the literature and earlier researches [15–17]. Briefly, cells were seeded on a microtiter plate in the absence or presence of various concentrations of compounds in triplicate and incubated at 37 °C in a humid atmosphere of 5% CO_2 for three days. 20 μL MTT reagent (5 mg/mL in PBS) was added to each well, then incubated at 37 °C for 4 h, 50% DMF-20% SDS (100 μL) was added. After the formazan was dissolved completely, the plates were read on a Bio-Tek ELx 800 ELISA reader at 595 nm/630 nm (A595/630). The cytotoxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was calculated from dose–response curve. In 100 μL various concentrations of compounds, C8166 cells (4 × 10^5/mL) were infected with virus (HIV-1_{IIIB}) at a multiplicity of infection (M.O.I) of 0.06. The final volume per well was 200 μL. Control assays were performed without the testing compounds in HIV-$1I_{IIB}$ infected and uninfected cultures. AZT (3′-azido-3′-deoxythymidine) was used as a positive control. After three days of culture, the cytopathic effect (CPE) was measured by counting the number of syncytia (multinucleated giant cell). Percentageinhibition of syncytia formation was calculated and 50% effective concentration (EC_{50}) was calculated. The therapeutic index (TI) was calculated from the ratio of CC_{50}/EC_{50}.

3. Results and discussion

3.1. Phytochemical investigation

The 85% EtOH extract of the stems and leaves of *N. officinalis* was suspended in water and extracted successively with petroleum ether and EtOAc. The EtOAc extract fraction was repeatedly subjected to silica gel, Sephadex LH-20, reversed-phase C_{18} silica gel CC and semi-preparative HPLC, to yield eight alkaloids, including two new ones, as shown in Fig. 1.

Naucleaoffine A (**1**) was obtained as a yellowish amorphous powder with a specific rotation of $[\alpha]_D^{19}$-67.3 (c 0.13, CH_3OH). Its molecular formula, $C_{20}H_{24}N_2O_3$, was established by HRESIMS (*m/z* 363.1679 $[M + Na]^+$, calcd 363.1679), with an index of hydrogen deficiency of 10. Its IR spectrum showed the presence of hydroxyl groups (3419 cm^{-1}), amide carbonyl group (1631 cm^{-1}) and phenyl group (1592, 1442, and 1431 cm^{-1}). The UV absorption bands at 225 and 290 nm were characteristic of indole alkaloid [18]. The ^{13}C NMR and DEPT data revealed the presence of 20 carbon atoms, including 11 sp^2 carbon atoms, five sp^3 methylenes and four sp^3 methine. In addition, the 11 sp^2 carbon atoms were attributable to one indole ring group, one monosubstituted double bond group and one amide carbonyl group. The above data revealed that the structure of **1** was similar to that of naucleamide A [18]. The only difference between them was the presence of the double bond between C-18 and C-19 in **1** instead of the double bond between C-19 and C-20 in naucleamide A [18], which was supported by the HMBC correlations of H-14, H-16 and H-18 to C-20 (δ_C 47.3), as well as H-15 and H-19 to C-21 (δ_C 62.6), together with the ^1H-^1H correlations

Table 1
^{1}H and ^{13}C NMR data of naucleaoffines A (**1**) and B (**2**) in DMSO-d_6.

Position	naucleaoffine A (**1**)		naucleaoffine B (**2**)	
	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]
2		134.8 s		135.1 s
3	4.88 (1H, overlapped)	50.1 d	4.95 (1H, m)	53.1 d
5*α*	4.89 (1H, overlapped)	39.8 t	4.79 (1H, dd, *J* = 12.2, 4.8 Hz)	41.9 t
5*β*	2.77 (1H, ddd, *J* = 11.9, 4.2, 3.6 Hz)		2.87 (1H, dd, *J* = 12.2, 3.6 Hz)	
6*α*	2.66 (1H, overlapped)	20.9 t	2.74 (1H, ddd, *J* = 12.2, 4.8, 3.6 Hz)	20.6 t
6*β*	2.58 (1H, m)		2.60 (1H, dd, *J* = 12.2, 3.6 Hz)	
7		107.0 s		108.1 s
8		126.3 s		126.7 s
9	7.38 (1H, d, *J* = 7.8 Hz)	117.6 d	7.36 (1H, d, *J* = 7.8 Hz)	117.5 d
10	6.96 (1H, dd, *J* = 7.8, 7.6 Hz)	118.5 d	6.96 (1H, dd, *J* = 7.8, 7.6 Hz)	118.5 d
11	7.05 (1H, dd, *J* = 8.0, 7.6 Hz)	120.8 d	7.05 (1H, dd, *J* = 8.0, 7.6 Hz)	120.8 d
12	7.32 (1H, d, *J* = 8.0 Hz)	111.1 d	7.32 (1H, d, *J* = 8.0 Hz)	111.2 d
13		136.1 s		135.9 s
14*α*	2.65 (1H, overlapped)	26.6 t	2.50 (1H, m)	27.1 t
14*β*	1.86 ((1H, ddd, *J* = 14.0, 4.2, 3.8 Hz)		2.32 (1H, overlapped)	
15	2.17 (1H, m)	30.7 d	1.83 (1H, m)	32.5 d
16	2.47 (1H, ddd, *J* = 10.6, 5.2, 3.8 Hz)	47.1 d	2.32 (1H, overlapped)	46.6 d
17*α*	3.58 (1H, dd, *J* = 11.2, 3.8 Hz)	62.6 t	3.82 (1H, dd, *J* = 11.0, 4.8 Hz)	59.5 t
17*β*	3.51 (1H, dd, *J* = 11.2, 5.2 Hz)		3.74 (1H, dd, *J* = 11.0, 5.0 Hz)	
18*α*	5.11 (1H, d, *J* = 9.8 Hz)	116.8 t	5.07 (1H, d, *J* = 10.2 Hz)	116.2 t
18*β*	5.06 (1H, d, *J* = 17.2 Hz)		5.03 (1H, d, *J* = 17.4 Hz)	
19	5.72 (1H, ddd, *J* = 17.2, 9.8, 9.8 Hz)	140.4 d	5.68 (1H, ddd, *J* = 17.3, 9.8, 9.8 Hz)	140.0 d
20	2.26 (1H, m)	47.3 d	2.32 (1H, overlapped)	47.1 d
21*α*	3.66 (2H, d, *J* = 3.9 Hz)	62.6 t	3.62 (1H, dd, *J* = 10.8, 4.0 Hz)	62.8 t
21*β*			3.45 (1H, dd, *J* = 10.8, 6.4 Hz)	
22		168.6 s		170.9 s
N_1-H	11.01 (1H, s)		10.90 (1H, s)	
17-OH	4.72 (1H, br s)		4.62 (1H, br s)	
21-OH	4.57 (1H, br s)		4.40 (1H, br s)	

[a] Measured at 400 MHz.
[b] Measured at 100 MHz.

from H-18 to H-21 and H-15 with H-20. Detailed analysis of 2D NMR (HSQC, HMBC and ^{1}H-^{1}H COSY) spectra confirmed the planar structure of **1** as shown in Fig. 2. The relative configurations of **1** were elucidated on the basis of the ROESY correlations (Fig. 2). The ROESY correlations of N_1-H, H-16 and H-20 with H-3 and H-14*a*, as well as H_2-17 with H-14*β* and H-15 suggested that H-3 and H-16 were cofacial and were arbitrarily assigned as *α*-orientation, while H-15 was assigned as *β*-oriented. Thus, the structure of **1** was determined as shown in Fig. 1.

Naucleaoffine B (**2**) was obtained as a yellow amorphous powder. Its molecular formula was established as $C_{20}H_{24}N_2O_3$ by HRESIMS (*m/z* 363.1680, $[M+Na]^+$; calcd: 363.1679), which was the same with that of **1**. The ^{1}H and ^{13}C NMR data (Table 1) suggested that **2** possessed 20 carbon signals which show similar structural features to those of **1**, except for the signals around C-16. Detailed analysis of 2D NMR (HSQC, HMBC and ^{1}H-^{1}H COSY) spectra confirmed that **2** shared the same planar structure with **1** (see Fig. 3). The specific rotation of **2**, $[\alpha]_D^{19}$-42.8 (c 0.11, CH_3OH), suggested that its configurations should be different from that of **1** ($[\alpha]_D^{19}$-67.3). The relative configurations of **2** were elucidated on the basis of the ROESY correlations (Fig. 3). The ROESY correlations of N_1-H and H-20 with H-3 and H-14*a*, as well as H-16 with H-14*β* and H-15 suggested that H-15 and H-16 were cofacial and were assigned as *β*-orientation, while H-3 was assigned as *α*-oriented. Therefore, the structure of **2** was determined as shown in Fig. 1.

In addition to the new alkaloids, naucleaoffines A (**1**) and B (**2**), six known alkaloids were isolated and identified as naucleactonin A (**3**)

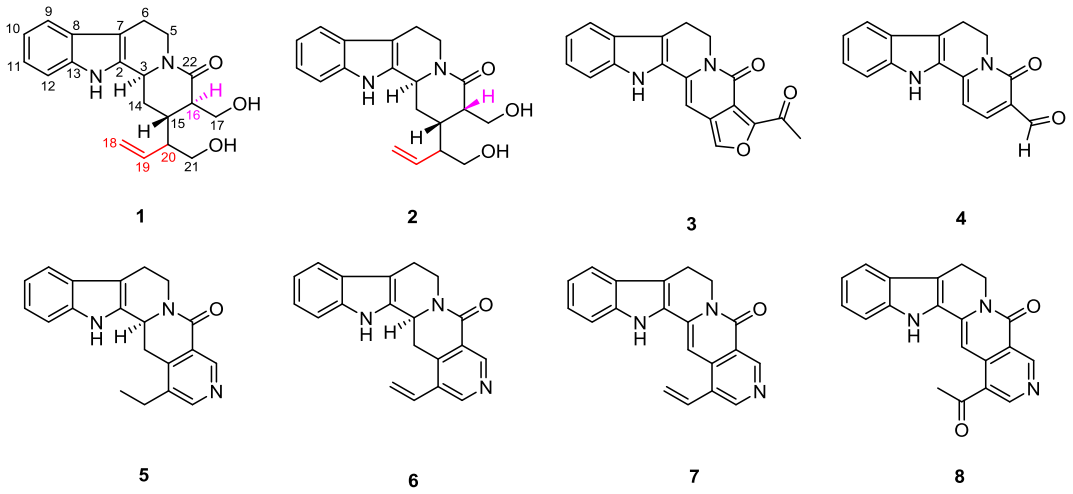

Fig. 1. Structures of compounds **1–8** isolated from *N. officinalis*.

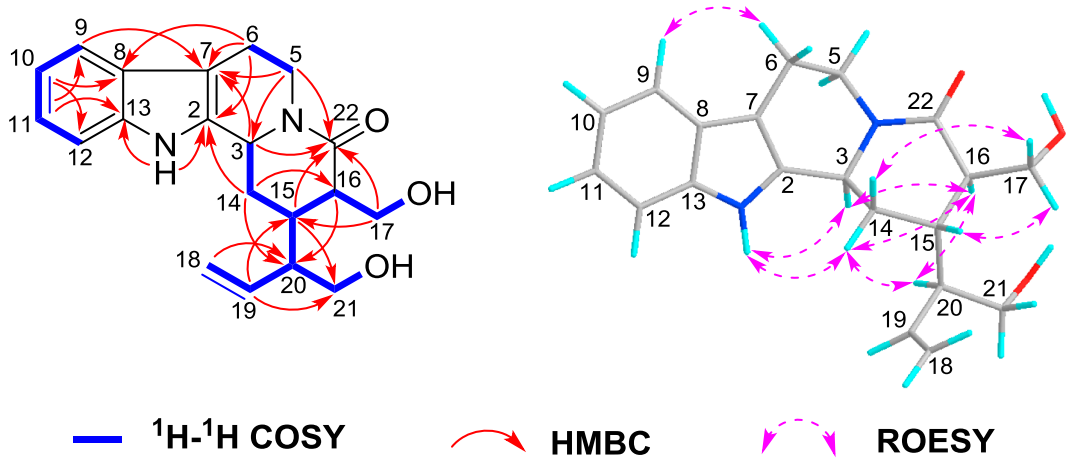

Fig. 2. Selected 2D NMR correlations for naucleaoffine A (**1**).

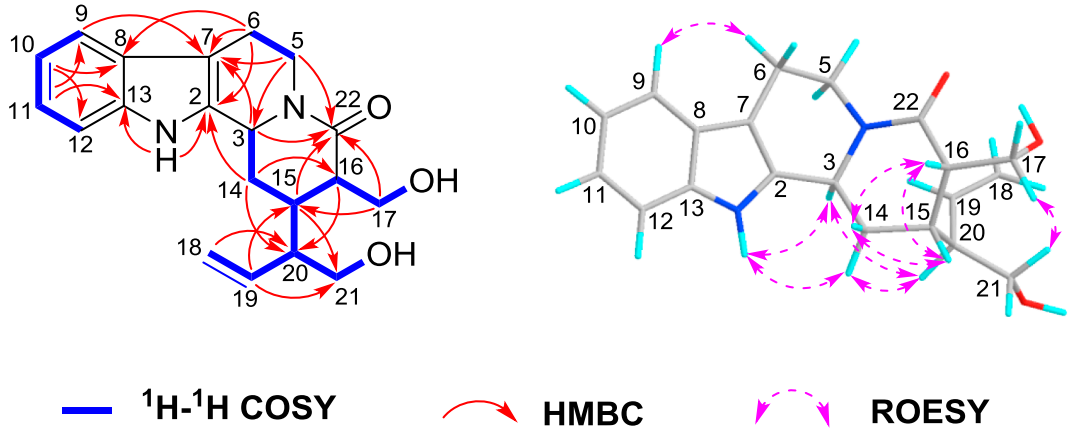

Fig. 3. Selected 2D NMR correlations for naucleaoffine B (**2**).

[19], nauclefidine (**4**) [20], 3,14-dihydroangustine (**5**) [3], 3,14,18,19-tetrahydroangustine (**6**) [3], angustine (**7**) [3] and naucletine (**8**) [6], by comparing their experimental and reported physical data.

3.2. Anti-inflammatory activity

All isolated alkaloids were evaluated their anti-inflammatory activities *via* examining the inhibitory activities on nitric oxide (NO) production induced by lipopolysaccharide in mouse macrophage RAW 264.7 cells *in vitro*. As a result, compounds **1–8** showed significant inhibitory activities with the IC_{50} value of 1.18 ± 0.10 μM, 3.86 ± 0.09 μM, 1.36 ± 0.07 μM, 1.82 ± 0.10 μM, 2.02 ± 0.08 μM, 2.56 ± 0.12 μM, 1.28 ± 0.06 μM and 4.89 ± 0.11 μM, respectively. While the positive control, hydrocortisone, showed a inhibitory activity with the IC_{50} value of at 3.26 ± 0.12 μM. No cytotoxicity was observed in compounds **1–8** treated cells (cell viability > 90%).

3.3. Anti-HIV-1 activity

All isolated compounds were evaluated for their anti-HIV-1 activities. They were evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}) and cytotoxicity assay against C8166 cell line (CC_{50}) by MTT methods. Compounds **1–8** showed significant activities with EC_{50} of 0.06, 0.23, 0.48, 0.39, 0.28, 0.72, 0.89 and 2.08 μM, respectively (see Table 2).

Table 2
Anti-HIV-1 activities of compounds **1–8**.

No.	CC_{50} (μM)[a]	EC_{50} (μM)[b]	TI[c]
1	> 200	0.06	> 3333.33
2	> 200	0.23	> 869.57
3	> 200	0.48	> 416.67
4	> 200	0.39	> 512.82
5	> 200	0.28	> 714.29
6	> 200	0.72	> 277.78
7	> 200	0.89	> 224.72
8	> 200	2.08	> 96.15
AZT[d]	3695.04	0.01889	195608.26

[a] CC_{50}: 50% Cytotoxic concentration.
[b] EC_{50}: 50% Effective concentration.
[c] TI (therapeutic index) = CC_{50}/EC_{50}.
[d] AZT (3′-azido-3′-deoxythymidine) was used as a positive control.

4. Conclusions

In this study, the chemical investigation on *N. officinalis* was undertaken and had led to the isolation and characterization of two new monoterpene indole alkaloids, naucleaoffines A (**1**) and B (**2**), together with six known alkaloids (**3–8**). The discovery of compounds **1** and **2** are not only a further addition to diverse and complex array of monoterpene indole alkaloids, but also, their presence as characteristic marker may be helpful in chemotaxonomical classifications. The inhibitory activities on nitric oxide (NO) production induced by lipopolysaccharide in mouse macrophage RAW 264.7 cells and anti-HIV-1 activities of all isolated compounds were also investigated, and found to

be quite potent. The significant inhibitory activities on nitric oxide (NO) production of compounds **1–8** may be used as an explanation of the clinical use of *N. officinalis*, which was used as an anti-inflammatory drug in China. These findings also suggest that these monoterpene indole alkaloids with significant inhibitory activities on nitric oxide (NO) production and anti-HIV-1 activities isolated from *N. officinalis* could be used for the development of new anti-inflammatory and anti-HIV agents.

Conflict of interest

The authors have declared that there is no conflict of interest.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.10.013.

References

[1] Editorial Committee of Flora of China, Flora Reipublicae Popularis Sinicae, vol. 71(1) (1997) 258–260.
[2] Editorial Committee of Chinese Herbs, Chinese Herbs, vol. 6 (1999) 456.
[3] C.A.J. Erdelmeier, U. Regenass, T. Rali, O. Sticher, Planta Med. 58 (1992) 43–48.
[4] Z. Zhang, H.N. ElSohly, M.R. Jacob, D.S. Pasco, L.A. Walker, A.M. Clark, J. Nat. Prod. 64 (2001) 1001–1005.
[5] J. Sichaem, S. Surapinit, P. Siripong, S. Khumkratok, J. Jong-aramruang, S. Tippyang, Fitoterapia 81 (2010) 830–833.
[6] S.Y. Liew, M.R. Mukhtar, A.H.A. Hadi, K. Awang, M.R. Mustafa, K. Zaima, H. Morita, M. Litaudon, Molecules 17 (2012) 4028–4036.
[7] H.Y. Wang, R.X. Wang, Y.X. Zhao, K. Liu, F.L. Wang, J.Y. Sun, Chem. Biodivers. 12 (2015) 1256–1262.
[8] Y.H. Fu, Y.T. Di, H.P. He, S.L. Li, Y. Zhang, X.J. Hao, J. Nat. Prod. 77 (2014) 57–62.
[9] Y.H. Fu, Y. Zhang, H.P. He, L. Hou, Y.T. Di, S.L. Li, X.D. Luo, X.J. Hao, J. Nat. Prod. 75 (2012) 1987–1990.
[10] Y.H. Fu, H.P. He, Y.T. Di, S.L. Li, Y. Zhang, X.J. Hao, Tetrahedron Lett. 53 (2012) 3642–3646.
[11] A.H. Chen, Q.L. Liu, Y.L. Ma, Z.H. Jiang, J.Y. Tang, Y.P. Liu, G.Y. Chen, Y.H. Fu, W. Xu, Nat. Prod. Res. 31 (2017) 1490–1494.
[12] Y.L. Ma, Y.P. Liu, C. Zhang, W.H. Zhao, S. Shi, D.N. He, P. Zhang, X.H. Liu, T.T. Han, Y.H. Fu, Bioorg. Chem. 76 (2018) 359–364.
[13] Z.H. Jiang, L. Chai, Y.P. Liu, H.C. Qiu, T.T. Wang, X.Y. Feng, Y.T. Wang, Z.H. Huang, B.M. Liu, Y.H. Fu, Phytochemistry Lett. 22 (2017) 194–198.
[14] T. Mosmann, J. Immunol. Methods. 65 (1983) 55–63.
[15] Y.T. Zheng, K.L. Ben, S.W. Jin, Acta Pharmacol. Sin. 21 (2000) 179–182.
[16] J.H. Wang, S.C. Tam, H. Huang, D.Y. Ouyang, Y.Y. Wang, Y.T. Zheng, Biochem. Biophys. Res. Commun. 317 (2004) 965–971.
[17] Y.P. Liu, W.H. Zhao, X.Y. Feng, Z.J. Zhang, K. Zong, Z.G. Sun, Y.T. Zheng, Y.H. Fu, Bioorg. Chem. 79 (2018) 111–114.
[18] H. Shigemori, T. Kagata, H. Ishiyama, F. Morah, A. Ohsaki, J. Kobayashi, Chem. Pharm. Bull. 51 (2003) 58–61.
[19] W.D. Xuan, H.S. Chen, J.L. Du, S. Liang, T.Z. Li, D.G. Cai, J. Asian Nat. Prod. Res. 8 (2006) 719–722.
[20] M. Lin, X. Liu, D.Q. Yu, Planta Med. 50 (1984) 459–461.