Fitoterapia xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Fitoterapia

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

Monoterpenoid indole alkaloids from the leaves of *Alstonia scholaris* and their NF-κB inhibitory activity

Ji Yang[a], Jing Fu[a], Xin Liu[a], Zhi-Hong Jiang[a,b,*], Guo-Yuan Zhu[a,**]

[a] State Key Laboratory of Quality Research in Chinese Medicine, Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Macau, China

[b] International Institute for Translational Chinese Medicine, Guangzhou University of Chinese Medicine, Guangzhou, China

ARTICLE INFO

Keywords:
Alstonia scholaris
Monoterpenoid indole alkaloids
Scholarisines P-S
Nuclear factor-kappa B

ABSTRACT

Four new monoterpenoid indole alkaloids (MIAs), scholarisines P-S (**1**–**4**), and 14 known MIAs (**5**–**18**) were isolated from the leaves of *Alstonia scholaris* (L) R. Br. (Apocynaceae). Their structures were elucidated by analyzing their HRESIMS data and NMR spectroscopic data. All of the isolated MIAs were evaluated for their Nuclear Factor-kappa B (NF-κB) inhibitory activity in HepG2-NF-κB-Luc cells. Among them, five compounds (**4**, **7**, **8**, **13** and **16**) exhibited significant NF-κB inhibitory activity.

1. Introduction

Alstonia scholaris (L) R. Br. (Apocynaceae) is an evergreen tree that is widely distributed in the tropical regions of Asia and Africa [1,2]. The dried leaves and stem barks of this tree are extensively used as traditional folk medicine for the treatment of cough, asthma, and chronic bronchitis in China [3,4]. Previous phytochemical studies of *A. scholaris* indicated that this plant is a rich source of mototerpenoid indole alkaloids (MIAs) with diverse structures [5], such as alistonitrine A [6], scholarisine A [7], (19,20)-*E*-alstoscholarine [8]. MIAs from this plant were reported to possess anti-inflammatory [9,10], antineoplastic [11,12], antibacterial [13–15], and analgesic [9] effects. It has been reported that the alkaloidal patterns in *A. scholaris* in different ecological regions have remarkable difference. *A. scholaris* from China, India, Pakistan, Thailand and other continental countries contain picrinine-type MIAs as major alkaloids, while those from Indonesia and the Philippines are predominantly confined to angustilobine-type alkaloids [16]. In our previous study, we reported the isolation of a novel caged MIA possessing an unprecedented 6/5/6/5/5/6 ring skeleton from the leaves of *A. scholaris* collected from Shenzhen, China [6]. As a continuing phytochemical investigation on this plant, this paper reports the isolation and identification of four new MIAs, named scholarisines P-S (**1**–**4**), as well as fourteen known alkaloids (**5**–**18**). These compounds were evaluated for their NF-κB inhibitory activity in HepG2-NF-κB-Luc cells.

2. Experimental

2.1. General experimental procedures

Ultraviolet (UV) and CD spectra were measured on a Jasco J-1500 Circular Dichroism Spectrometer. IR spectra were recorded on an Agilent Cary 660 series FT-IR spectrometer (KBr). Optical rotations were obtained on a Rudolph Research Analytical Autopol I automatic polarimeter. HRESIMS spectra were carried on an Agilent 6230 MS spectrometer. 1D and 2D NMR spectra were performed on a Bruker Ascend 600 NMR spectrometer. The chemical shifts were expressed in δ (ppm) with TMS or solvent signals as an internal reference. Semi-preparative HPLC was conducted on an Agilent 1100 liquid chromatography instrument with a Waters Xbridge Prep C_{18} column (10 × 250 mm, 5 μm). MPLC was performed using a Buchi Sepacore flash system with a RP-18 column (SilicBond C18, 36 × 460 mm ID, 40–63 μm particle size (Silicycle)). UHPLC analyses were conducted on an Agilent 1290 system using a ZORBAX RRHD Eclipse Plus C18 column (1.8 μm 2.1 × 50 mm, Agilent). Column chromatography (CC) was performed on silica gel (40–60 mesh, Grace, USA). Thin layer chromatography (TLC) was determined on precoated silica gel 60 F_{254} plates (200 μm thick, Merck KGaA, Germany).

2.2. Plant material

The leaves of *A. scholaris* were collected from Shenzhen of

* Corresponding author at: State Key Laboratory of Quality Research in Chinese Medicine, Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Macau, China.

** Corresponding author.

E-mail addresses: zhjiang@must.edu.mo (Z.-H. Jiang), gyzhu@must.edu.mo (G.-Y. Zhu).

http://dx.doi.org/10.1016/j.fitote.2017.10.018
Received 4 September 2017; Received in revised form 13 October 2017; Accepted 20 October 2017

Guangdong Province, People's Republic of China, in August 2013. The species was identified by one of authors, Dr. Zhu G-Y. A voucher specimen (AS-201308) was deposited at State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology.

2.3. Extraction and isolation

The air-dried leaves of *A. scholaris* (15 kg) were refluxed three times with 80% ethanol (150 L) for 1 h each time. The ethanol was removed under reduced pressure at 60 °C. The brownish residue was dissolved in 1% HCl and the solution was then centrifuged at 5000 rpm for 10 min to remove dark brown precipitates. The acidic water-soluble material was basified to pH 9–10 with 10% ammonia solution. The solution was then partitioned with EtOAc to afford an EtOAc-soluble fraction (136.0 g), which was subjected to silica gel CC eluted with a $CHCl_3$-MeOH gradient (from 1:0 to 2:1, v/v). Eight fractions (Fr.A–Fr.H) were obtained according to their TLC behaviors. Fr·B (10.2 g) was chromatographed by MPLC with RP-18 column and eluted with MeCN/0.1% diethylamine in H_2O (from 20:80 to 100:0, v/v) to yield 11 subfractions (Fr·B1–Fr·B11). Fr·B6 (6.1 g) and Fr·B9 (200 mg) were further purified by RP-HPLC eluted with MeCN/0.1% diethylamine in H_2O (35:65 or 50:50 v/v) to obtain **7** (850.0 mg), **8** (3200.0 mg) and **9** (7.2 mg). Fr·C (5.2 g) was separated by MPLC with RP-18 column and eluted with MeCN/0.1% diethylamine in H_2O (from 1:4 to 1:0, v/v) to obtain 13 subfractions (Fr·C1–Fr·C13). Fr·C6 (322.0 mg) and Fr·C9 (87.0 mg) were purified by RP-HPLC with MeCN/0.1% diethylamine in H_2O (36:64 or 26:74 v/v) to yield **1** (3.1 mg), **4** (2.2 mg) and **6** (4.5 mg). Fr.D (6.7 g) was subjected to MPLC eluted with MeCN/0.1% diethylamine in H_2O (from 10:90 to 100:0, v/v) to afford three subfractions (Fr.D1–Fr.D3). Fr.D1 (710.0 mg), Fr.D2 (2.2 g) and Fr.D3 (1.0 g) were further isolated by RP-HPLC with MeCN/0.1% diethylamine in H_2O (28:72 or 26:74 or 25:75 v/v) to provide **2** (6.2 mg) **5** (3.3 mg), **10** (4.8 mg), **11** (3.1 mg), **12** (9.6 mg), **15** (2.9 mg) and **16** (7.5 mg). Fr.E (9.0 g) was fractioned via CC eluted with a $CHCl_3$–MeOH gradient (from 4:1 to 1:1, v/v) to afford **13** (71.6 mg). Fr·F (12.0 g) was separated via silica gel CC eluted with $CHCl_3$-MeOH (from 5:1 to 1:1) to provide 11 subfractions (Fr·F1–Fr·F11). Then, Fr·F7 (3.2 g) was purified on silica gel CC eluted with $CHCl_3$-MeOH (5:1) to obtain **14** (1.8 g). The Fr·F8 (25.0 mg) and Fr·F9 (3.7 g) were applied to RP-HPLC using a mobile phase of MeCN/0.1% diethylamine in H_2O (18:82 or 25:75 v/v) to give **3** (3.2 mg), **17** (4.5 mg) and **18** (6.0 mg).

2.3.1. Scholarisine P (1)

White amorphous powder; $[\alpha]_D^{25}$ +21.7 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε): 219 (4.53), 262 (3.87) nm; IR (KBr) cm^{-1}: 3422, 2924, 1736, 1627, 1599, 1439, 1385, 1196, 1171, 1115, 774; ^1H NMR ^{13}C NMR ($CDCl_3$) data see Table 1; HR-ESI-MS *m/z*: 353.1866 $[M + H]^+$ (calcd for $C_{21}H_{25}N_2O_3$, 353.1860).

2.3.2. Scholarisine Q (2)

White amorphous powder; $[\alpha]_D^{25}$ +125.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 203 (4.31), 237 (3.74), 283 (4.24) nm; IR (KBr) cm^{-1}: 3394, 2934, 1715, 1454, 1343, 1382, 1298 1223, 1100, 1061, 1035, 1001, 844, 740; ^1H NMR and ^{13}C NMR ($CDCl_3$) data see Table 1; HR-ESI-MS *m/z*: 355.1300 $[M + H]^+$ (calcd for $C_{19}H_{19}N_2O_5$, 355.1288).

2.3.3. Scholarisine R (3)

Yellow amorphous powder; $[\alpha]_D^{25}$ −2.7 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 201 (4.59), 224 (4.70), 280 (4.06) nm; IR (KBr) cm^{-1}: 3404, 2925, 1702, 1628, 1594, 1450, 1383, 1238, 1162, 1078, 1041, 746; ^1H NMR and ^{13}C NMR (CD_3OD) data see Table 1; HR-ESI-MS *m/z*: 517.2544 $[M + H]^+$ (calcd for $C_{27}H_{37}N_2O_8$, 517.2543).

2.3.4. Scholarisine S (4)

White amorphous powder; $[\alpha]_D^{25}$ +86.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.41), 258 (3.88) nm; IR (KBr) cm^{-1}: ν_{max} 3422, 2937, 1744, 1589, 1458, 1360, 1196, 1221, 1071, 1005, 750 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$) data see Table 1; HR-ESI-MS *m/z*: 353.1496 $[M + H]^+$ (calcd for $C_{20}H_{20}N_2O_4$, 353.1494).

2.4. Cell culture

The HepG2-NF-κB-Luc cell line was stably-transfected with the NF-κB-luciferase gene, which was kindly provided by Dr. C.H. Leung (University of Macau). Cells were cultivated with DMEM medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C with 5% CO_2 and 95% air incubator.

2.5. NF-κB luciferase assay

The NF-κB activity was determined by NF-κB luciferase assay as described in our previous publication with a slight modification [17]. Briefly, HepG2-NF-κB-Luc cells were seeded on a 96-well microplate with 1×10^4 cells/well and cultured at 37 °C with 5% CO_2 incubator for 18 h. Then, cells were pretreated with compounds (25 μM) for 12 h and induced with TNF-α (10 ng/mL) for 4 h. Ammonium pyrrolidine-dithiocarbamate (PDTC) was used as a positive control. The firefly luciferase activities were measured with the Bright-Glo Luciferase Reporter Assay System (Promega) according to the manufacturer's instruction using a multimode reader (Infinite 200 PRO, Tecan).

2.6. Determination of the absolute configuration of glucose

The absolute configuration of glucose in **3** was determined according to the reported procedure [18]. Concisely, compound **3** (0.5 mg) was dissolved in MeOH (0.1 mL) and then added 0.1 mL of 1 N HCl. The solution was heated at 80 °C for 4 h. After evaporated by N_2, the residue and L-cysteine methyl ester hydrochloride (0.5 mg) was dissolved in pyridine (0.1 mL) and heated at 60 °C for 60 min. Then, phenyl isothiocyanate (20 μL) was added to the mixture and heated at 60 °C for another 60 min. 20 μL of the reaction mixture was diluted to 1000 μL in MeOH and then analyzed by UHPLC-TOF MS with a ZORBAX RRHD Eclipse Plus C18 column (1.8 μm 2.1 × 50 mm, Agilent), a mobile phase of MeCN: H_2O (18: 72 V/V), and a flow rate of 0.35 mL/min. The retention time of glucose derivative obtained from acid hydrolysate of compound **3** was 17.05 min. Treated in the same way, standard D-glucose (Alfa Aesar) and L-glucose (Sigma) gave peaks at 17.01 and 17.65 min, respectively.

3. Results and discussion

3.1. Chemistry

Compound **1** was obtained as a white amorphous powder. The molecular formula of **1** was determined to be $C_{21}H_{24}N_2O_3$ on the basis of its HRESIMS data, with 11 degrees of unsaturation. The IR spectrum of **1** showed absorption bands at 3424 cm^{-1} for hydroxyl and 1736 cm^{-1} for carbonyl functionality. The ^1H NMR spectrum of **1** (Table 1) showed four aromatic protons [δ_H 7.58 (1H, d, J = 7.5 Hz, H-12), 7.36 (1H, t, J = 7.5 Hz, H-11), 7.23 (1H, t, J = 7.5 Hz, H-10) and 7.17 (1H, d, J = 7.5 Hz, H-9)]. By comparing with those of MIAs from this plant, these aromatic protons were assigned to the indole ring. Besides the indole ring signals, **1** possesses an *N*-methyl signal [δ_H 2.49 (3H, s)], a methyl signal [δ_H 0.98 (3H, t, H-18)], and a hydroxymethyl group [δ_H 3.44 (1H, d, H-22a) and 2.56 (1H, d, H-22b)]. The ^{13}C NMR (Table 1) and DEPT spectroscopic data showed the presence of 21 carbons attributable to two methyls, five methylenes, seven methines and seven quaternary carbons. The 1D NMR spectroscopic data of **1** is highly similar to scholarisine I [19], expect for that the methine at C-16