(±)-Zanthonitidine A, a Pair of Enantiomeric Furoquinoline Alkaloids from *Zanthoxylum nitidum* with Antibacterial Activity

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Received: 4 May 2018 / Accepted: 18 May 2018 / Published online: 31 May 2018

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**Abstract**

A pair of new enantiomeric furoquinoline alkaloids, (±)-zanthonitidine A (1), together with nine known ones (2–10) were isolated from the radix of *Zanthoxylum nitidum*. Their chemical structures were elucidated based on the extensive spectroscopic analysis. The racemic mixture of 1 was separated by chiral column chromatography, and the absolute configurations of (±)-1 and (−)-1 were determined by the comparison of experimental and calculated electronic circular dichroism spectra. Antibacterial activities of compounds 1–9 were evaluated, and compounds (+)-1, (−)-1, 3, 7 and 8 showed antibacterial activities against *Bacillus subtilis, Enterococcus faecalis* or *Staphylococcus aureus*.

Li-Na Zhao and Xi-Xi Guo have contributed equally to this work.

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s13659-018-0169-7) contains supplementary material, which is available to authorized users.

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1 Introduction

The genus *Zanthoxylum* Linn of the Rutaceae family comprises of about 250 species in the world, mainly distributed in Asian, America, Africa, tropical and subtropical regions in Oceania. There are 39 species and 14 varietas in China [1], and the largest part occurs in south of the Yangtze River and south western provinces [2]. *Zanthoxylum nitidum* (Roxb.) DC. (Rutaceae) is a morphologically variable species with hooked prickly branchlets plant of the *Zanthoxylum* genus [3]. The radix of *Z. nitidum* was recorded as a traditional Chinese medicine, named “liangmianzhen” in Chinese Pharmacopeia (Version 2015), and has been widely used for the treatment of toothache, neuralgia, stomachache, sore throat, rheumatoid arthritis, turgescence and venomous snake bite [4]. It was main raw material of Chinese herbal toothpaste called “liangmianzhen”, and it is also used for some preparations, such as Jinji Tablet, Dieda Wanhua Oil. Several types of alkaloids including quinolines, isoquinolines, quinolones and benzophenanthridines, have previously been isolated from *Z. nitidum* [3, 5, 6], and some other kinds of compounds including coumarins and lignans, were also reported in this plant [5, 7]. Among them, alkaloids, especially benzophenanthridines, are considered as the main bioactive constituents, which show various pharmacological activity, including inhibiting DNA topoisomerase I [8], anti-inflammatory [6], anti-noceceptive [9], inhibiting the growth and inducing the pro-apoptosis [10]. With the purpose to discover more pharmacological alkaloids, we performed the phytochemical investigation on the radix of *Z. nitidum*. As a result, a pair of new enantiomeric furoquinoline alkaloids, (±)-zanthonitidine A (1) (Fig. 1), together with nine known alkaloids (2–10) (Fig. S1) were obtained. Herein, we report their isolation, structural elucidation, and antibacterial activity.

2 Results and Discussion

Zanthonitidine A (1) was obtained as a yellow powder. Its molecular formula was determined by HRESIMS ([M + H]⁺, 440.13478, calcd. 440.13399) as C₂₃H₂₁NO₈, implying fourteen degrees of unsaturation. The IR spectrum showed the absorptions at 3425 and 1624 cm⁻¹, indicating the existence of hydroxyl and phenyl groups. The ¹H NMR spectrum (Table 1) showed two pairs of AB doublets at δH 7.79 (1H, d, J = 9.2 Hz), 7.56 (1H, d, J = 2.3 Hz), 7.14 (1H, d, J = 9.2 Hz), 7.06 (1H, d, J = 2.3 Hz); two aromatic protons at δH 6.68 (2H, s); one methylene group at δH 3.86 (1H, dd, J = 12.6, 1.5 Hz), 3.71 (1H, dd, J = 12.6, 5.3 Hz); two methyne groups at δH 4.95 (1H, d, J = 8.1 Hz), 4.22 (1H, m); three methoxyl groups at δH 4.44 (3H, s), 3.91 (6H, s). The ¹³C NMR spectrum displayed 15 aromatic carbons at δC 163.8 (s), 158.2 (s), 147.7 (s), 147.7 (s), 143.8 (s), 137.0 (s), 135.8 (s), 135.8 (s), 127.2 (s), 116.5 (d), 115.1 (d), 114.6 (s), 104.6 (d), 104.6 (d), 102.8 (s); two olefinic carbons at δC

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### Table 1

| Compounds | E. faecalis | S. aureus |
|-----------|------------|-----------|
| (±)-1     | 21.97      | 21.97     |
| (−)-1     | 12.54      | 25.09     |
143.3 (d), 105.2 (d); one methylene group at δC 61.7 (t); two methyne groups at δC 79.4 (d), 77.4 (d); three methoxyl groups at δC 59.5 (q), 56.7 (q), 56.7 (q). Based on these data, 1 was presumed to be a furoquinoline containing glycerol and benzene moieties.

The structure was elucidated by detailed interpretation of 2D NMR correlations (Fig. 2). The HMBC correlations from δH 7.56 (H-a) to δC 163.8 (C-2) and δC 102.8 (C-3); from δH 7.14 (H-b) to δC 163.8 (C-2) and δC 102.8 (C-3); from δH 7.79 (H-5) to δC 158.2 (C-4), 143.8 (C-7), and 137.0 (C-8a); from δH 7.14 (H-6) to δC 143.8 (C-7), 135.8 (C-8), and 114.6 (C-4a); from δH 4.44 (4-OCH3) to δC 158.2 (C-4); together with the 1H-1H COSY correlations of H-a/H-b and H-5/H-6 gave a furoquinoline moiety. The HMBC correlations from δH 4.95 (H-9) to δC 158.2 (C-4); together with the 1H-1H COSY correlations of H-7/H-8 and H-5/H-6 gave a furoquinoline moiety. The HMBC correlations from δH 4.95 (H-9) to δC 158.2 (C-4); together with the 1H-1H COSY correlations of H-7/H-8 and H-5/H-6 gave a furoquinoline moiety. The HMBC correlations from δH 4.95 (H-9) to δC 158.2 (C-4); together with the 1H-1H COSY correlations of H-7/H-8 and H-5/H-6 suggested a glycerol segment at C-1 position of benzene moiety. In consideration of the degrees of unsaturation and the chemical shift of δC 143.8 (C-7) and δC 135.8 (C-8), the C-O bonds should exist in C-7/C-8a and C-8/C-7a, and formed a 1,4-dioxane moiety. Thus, the planar structure of 1 was established. There is no obvious

![Fig. 1 Chemical structures of (+)-zanthonitidine A (1)](image1)

![Fig. 2 Key 2D NMR correlations of (+)-zanthonitidine A (1)](image2)

Table 1 1H (600 MHz, δ in ppm, J in Hz) and 13C NMR (150 MHz, δ in ppm) data of zanthonitidine A (1) in CDCl3

| Position | δH (m, J, Hz) | δC |
|----------|--------------|----|
| 2        |              | 163.8 |
| 3        |              | 102.8 |
| 4        |              | 158.2 |
| 4a       |              | 114.6 |
| 5        | 7.79 (d, 9.2) | 115.1 |
| 6        | 7.14 (d, 9.2) | 116.5 |
| 7        |              | 143.8 |
| 8, 4'    |              | 135.8 |
| 8a       |              | 137.0 |
| 1'       |              | 127.2 |
| 2', 6'   | 6.68 (s)     | 104.6 |
| 3', 5'   |              | 147.7 |
| 7'a      | 3.71 (dd, 12.6, 1.5) | 61.7 |
| 7'b      | 3.86 (dd, 12.6, 5.3) | 79.4 |
| 8'       | 4.22 (m)     | 77.4 |
| 9'       | 4.95 (d, 8.1) | 143.3 |
| α        | 7.56 (d, 2.3) | 105.2 |
| β        | 7.06 (d, 2.3) | 59.5 |
| 4-OCH3   | 4.44 (s)     | 56.7 |
| 3', 5'-OCH3 | 3.91 (s) | 56.7 |
absorption of electronic circular dichroism, and the coupling constant between the H-8' and H-9' was 8.1 Hz, which indicated that 1 was proposed to be a racemate mixture. Further Chiralpak ID column chromatography was performed, and obtained the enantiomers, (+)-1 and (−)-1 (Fig. 3). The absolute configurations of the enantiomers were then determined by comparing the experimental electronic circular dichroism (ECD) to the calculated ECD using the time-dependent density functional theory (TD-DFT) of the Gaussian 09 program package. The ECD spectra for (8'R, 9'R)-1 and (8'S, 9'S)-1 were calculated at the same theory level. The experimental ECD spectra of (+)-1 and (−)-1 resembled the calculated spectra of (8'R, 9'R)-1 and (8'S, 9'S)-1, respectively (Fig. 4). Accordingly, the absolute configurations of (+)-1 and (−)-1 were then determined as (8'R, 9'R)-1 and (8'S, 9'S)-1.

The known compounds were identified as 8-methoxy-N-methylflindersine (2, zanthobungeanine) [11], 4-methoxyfuro[2,3-b]quinoline-8-ol (3, robustine) [12], 4-methoxyfuro[2,3-b]quinoline (4, dictamine) [13], 4,8-dimethoxy-2-quinolone (5, edulitine) [14], 4,7,8-trimethoxyfuro[2,3-b]quinoline (6, skimmianine) [15], 4-methoxy-N-methyl-2-quinolone (7) [16], trans-(-)-9,10-dihydroxy-9,10-dihydrozanthobungeanine (8, zanthodioline) [17], 4,8-dimethoxyfuro[2,3-b]quinoline (9, γ-fagarine) [18] and 4-methoxyfuro[2,3-b]quinoline-8-O-β-D-glucopyranoside (10) [19] by comparing their spectroscopic data with those reported in the literatures.

The antimicrobial activities of compounds 1–9 was tested on the gram-positive strains Bacillus subtilis, Enterococcus faecalis, and Staphylococcus aureus (Table 2); penicillin was used as the positive control. Both compounds (+)-1 and (−)-1 showed moderate inhibitory activities against Enterococcus faecalis and Staphylococcus aureus with MIC values of 21.97, 21.97 µg/mL and 12.54, 25.09 µg/mL, respectively. Compounds 3, 7 and 8 also showed inhibitory activities against Enterococcus faecalis.

In summary, the phytochemical investigation of the radix of Zanthoxylum nitidum in this study led to the identification of ten alkaloids (1–10) including a pair of
new enantiomeric furoquinoline alkaloids, (±)-zanthonitidine A (1), and nine known ones. Biological assay for the antibacterial activities of 1–9 was performed, and the results showed that (±)-1, (−)-1, 3, 7 and 8 possessed antibacterial activities.

### 3 Experimental

#### 3.1 General Experimental Procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. CD spectra were tested using Chirascan Circular Dichroism spectrometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. MS data were measured on Agilent G6230 TOF Mass spectrometer. 1D-NMR and 2D-NMR spectra were measured on a Bruker AM-400, DRX-500 or AVANCE III-600 at 298 K. Chemical shifts (δ) were expressed in parts per million (ppm) with reference to the solvent signals. Semi-preparative HPLC was performed on Waters HPLC system (1525 pump with 2998 photodiode array detector and 2707 autosampler) coupled with Zorbax Eclipse-C18 (9.4 mm × 250 mm; 5 μm) for purification or DAICEL Chiralpak ID column (4.6 mm × 250 mm; 5 μm) for chiral analysis. Column chromatography was performed on silica gel (100–200 mesh and 200–300 mesh, Qingdao Yu-Ming-Yuan Chemical Co. Ltd., Qingdao, China), Sephadex LH-20 (Pharmacia Fine Chemical Co., Uppsala, Sweden) or Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany). Thin layer chromatography (TLC) was performed on silica gel GF254 on glass plates (Qingdao Yu-Ming-Yuan Chemical Co. Ltd.) with detection by visualization with a UV lamp at 254 and 365 nm, and spots were visualized under ultra-violet light and 5% sulfuric acid–ethanol reagent.

#### 3.2 Plant Material

The radix of Zanthoxylum nitidum (Roxb.) DC. (Rutaceae) was purchased from Bozhou Herbal Medicine Market (Anhui, China) in December 2016 and authenticated by Prof. Min-Jian Qin of China Pharmaceutical University. A voucher specimen was deposited in the Herbarium of China Pharmaceutical University.

#### 3.3 Extraction and Isolation

The air-dried and milled radix of Z. nitidum (10 kg) was extracted three times with methanol (3 × 20 L) under reflux, and the resulting solution was evaporated under reduced pressure to yield the methanol extract (688.4 g). All amount of the extract was separated by a silica gel column chromatography (CC) (100–200 mesh), eluted with a gradient of CHCl3–MeOH (100:0, 95:5, 9:1, 8:2, 7:3, 1:1, 0:1) to yield eleven fractions (Fr. 1–Fr. 11). Fr. 3 (23.7 g) was subjected to silica gel CC (200–300 mesh), eluted with a gradient of petroleum ether-acetone (100:0, 30:1, 10:1, 7:1; 3:1, 1:1) to yield eight subfractions (Fr. 3–1 to Fr. 3–8). Fr. 3.5 (10.0 g) was further purified by RP-18 gel CC (20–100% MeOH–H2O), Sephadex LH-20 CC (CHCl3–MeOH, 1:1), and silica gel CC (200–300 mesh) (petroleum ether-chloroform, 1:3) to afford 8-methoxy-N-methylflindersine (2) (20.1 mg), 4-methoxyfuro[2,3-b]quinoline-8-ol (3) (18.0 mg), and 4,7,8-trimethoxyfuro[2,3-b]quinoline (6) (22.5 mg). Fr. 3.6 (5.1 g) was further purified by RP-18 gel CC (20–100% MeOH–H2O) and Zorbax Eclipse C18 column (41% acetonitrile–H2O) to afford 4-methoxy-N-methyl-2-quinolone (7) (10.8 mg).

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### Table 2

| Compounds | Bacillus subtilis (MIC, μg/mL) | Enterococcus faecalis (MIC, μg/mL) | Staphylococcus aureus (MIC, μg/mL) |
|-----------|-------------------------------|----------------------------------|----------------------------------|
| (+)-1     | –                             | 21.97                            | 21.97                            |
| (−)-1     | –                             | 12.54                            | 25.09                            |
| 2         | –                             | –                                | –                                |
| 3         | –                             | 5.37                             | –                                |
| 4         | –                             | –                                | –                                |
| 5         | –                             | –                                | –                                |
| 6         | –                             | –                                | –                                |
| 7         | –                             | 18.91                            | –                                |
| 8         | –                             | 37.83                            | –                                |
| 9         | –                             | –                                | –                                |
| Penicillin | 5.92                          | < 2.96                           | < 2.96                           |

*a*Inactive (MIC > 50 μg/mL)

*b*Penicillin: positive control
**3.3.1 Zanthonitidine A (I)**

Yellow powder; UV (MeOH) \( \lambda_{max} \) (log \( e \)) 207.5 (4.67), 254.5 (4.86), 323.0 (3.80), 407.5 (2.62), 589.5 (2.58) nm; IR (KBr) \( \nu_{max} \) 3425, 2928, 1624, 1516, 1487, 1461, 1409, 1370, 1341, 1298, 1260, 1238, 1156, 1097, 1060, 979 cm\(^{-1}\); \( ^1 \)H (600 MHz) and \( ^{13} \)C (150 MHz) NMR data, see Table 1; ESIMS (positive): \( m/z \) 440.33 [M + H]+; HRESIMS (positive): \( m/z \) 440.13478 (calcd for \( C_{23}H_{22}NO_8 \), 440.13399).

**3.3.2 (+)-Zanthonitidine A ((+)-1)**

Yellow powder; [\( \pi \)D]\(^20\) +20.0 (c 0.02, MeOH); ECD (0.23 mM, MeOH) \( \lambda_{max} \) (\( \Delta \epsilon \)) 207 (−2.5), 213 (−2.2), 223 (1.2), 239 (−0.8), 254 (4.0) nm; HRESIMS (positive): \( m/z \) 440.13399 (calcd for \( C_{23}H_{22}NO_8 \), 440.13399).

**3.3.3 (−)-Zanthonitidine A ((−)-1)**

Yellow powder; [\( \pi \)D]\(^20\) −93.3 (c 0.01, MeOH); ECD (0.46 mM, MeOH) \( \lambda_{max} \) (\( \Delta \epsilon \)) 203 (4.3), 210 (7.1), 226 (−3.6), 241 (2.0), 253 (−11.0) nm; HRESIMS (positive): \( m/z \) 440.13338 (calcd for \( C_{23}H_{22}NO_8 \), 440.13399).

**3.4 ECD Calculation**

Conformational analysis was initially performed using Confab at MMFF94 force field for two configurations for I. Room-temperature equilibrium populations were calculated according to Boltzmann distribution law. The conformers with Boltzmann-population of over 1% were subjected to ECD calculations. The theoretical calculation was carried out using Gaussian 09 [20]. The conformers was initially optimized at PM6 using semiempirical theory method, and then optimized at the B3LYP/6-311G (d, p) in MeOH using the IEPFCM model. The theoretical calculation of ECD was conducted in MeOH using Time-dependent Density Functional Theory (TD-DFT) at the same theory level.

**3.5 Antibacterial Assay**

_Bacillus subtilis, Enterococcus faecalis_, and _Staphylococcus aureus_ were cultured in Luria–Bertani (LB) broth. The broth microdilution assay was applied for the antibacterial activity screening according to CLSI guidelines (CLSI 2015). _Bacillus subtilis, E. faecalis_, and _S. aureus_ were propagating in the Mueller–Hinton broth (0.20%, w/v, beef extract; 1.75%, w/v, acid digest of casein; 0.15%, w/v, starch). After incubation with various concentrations of 1–9 at 37 °C for 24 h, the 96-well plates were checked by visual inspection; penicillin was used as the positive control. The MICs were determined as the lowest concentration for no visible growth of bacteria.

**Acknowledgements**

This work was supported by the National Natural Science Foundation of China (31470428, 21702231), the National New Drug Innovation Major Project of China (2017ZX09309027), the Program for Jiangsu Province Innovative Research Team, the Fund for Introduction of High-level Talents from China Pharmaceutical University and the Fundamental Research Funds for the Central Universities (2632017PY19).

**Compliance with Ethical Standards**

Conflict of interest The authors declare no conflict of interest.

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