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Cytotoxic and anti-inflammatory active plicamine alkaloids from *Zephyranthes grandiflora*

Hong-Yong Wang[a], Sheng-Ming Qu[b], Ying Wang[c], Hai-Tao Wang[d,*]

[a] Department of Radiotherapy, Second Hospital of Jilin University, Changchun 130041, China
[b] Department of Dermatology, Second Hospital of Jilin University, Changchun 130041, China
[c] Department of Gastroenterology, First Hospital of Jilin University, Changchun 130021, China
[d] Department of E.N.T., Second Hospital of Jilin University, Changchun 130041, China

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ABSTRACT

Phytochemical investigation on the 95% EtOH extract of the whole plants of *Zephyranthes grandiflora* resulted in the isolation of six new 4a-*epi*-plicamine-type alkaloids, zephygranditines A-F (**1–6**), including three novel 11,12-*seco*-plicamine-type alkaloids. The structures of the isolated compounds were established based on 1D and 2D (^{1}H−^{1}H COSY, HMQC, and HMBC) NMR spectroscopy, in addition to high resolution mass spectrometry. The isolated alkaloids were tested *in vitro* for cytotoxic potential against seven malignant melanoma cell lines and inhibitory activity for nitric oxide (NO) production and Cox-1/Cox-2. As a result, alkaloids **1–3** exhibited some cytotoxic activity against all the tested tumor cell lines with IC_{50} values < 20 μM and **1** and **2** displayed anti-inflammatory activity in both assay of inhibitory activity for nitric oxide production and Cox-1/Cox-2.

1. Introduction

The genus *Zephyranthes*, belonging to the family of Amaryllidaceae, consists of 60 species which distribute mainly in the warm-temperate regions of Western Hemisphere [1,2]. Plants of this family are well known for their ornamental value and medicinal properties [3]. Traditionally, *Z. candida* has been used in Africa for anti-diabetes and *Z. parulla* appears in the history of Peru for treating tumors, *Z. rosea* and *Z. flava* are used for variety of therapeutic purposes in India [4–6]. To date, > 600 Amaryllidaceae alkaloids representing 22 skeletal types have been reported [7], and some of them exhibit a wide variety of biological activities including acetylcholinesterase (AChE) inhibitory, analgesic, antibacterial, antifungal, antimalarial, antitumor, antiviral, and cytotoxic activities [8–11]. Thus, the Amaryllidaceae alkaloids are an important resource for new drug discovery [12]. *Zephyranthes grandiflora* mainly distributes in the temperate zone of Western Hemisphere and is used as an ornamental and medicinal plant in China [13]. Chemical investigations have discovered the presence of Amaryllidaceae alkaloids [3,13,14]. To find more structurally interesting substances of the genus *Zephyranthes*, a phytochemical investigation on the 95% ethanol extract of the whole plants of *Z. grandiflora* afforded six new alkaloids, including three 4a-*epi*-plicamine-type alkaloids, zephygranditines A-C (**1–3**), and three 11,12-*seco*-plicamine-type alkaloids, zephygranditines D-F (**4–6**) (Fig. 1). The novel plicamine-type alkaloids with *N*-deformyl-11,12-*seco*-5,6-dihydroplicane and 11,12-*seco*-5,6-dihydroplicane skeleton, which were obtained only from *Z. candida*, were formed by the cleavage between C-11 and C-12 of 4a-*epi*-plicamine-type alkaloids. This paper described the isolation and structure elucidation of the new compounds, as well as their *in vitro* cytotoxic and anti–inflammatory activities.

2. Experimental part

2.1. General

Optical rotations were determined with a JASCO P2000 digital polarimeter. Ultraviolet (UV) and infrared (IR) spectra were obtained on JASCO V-650 and JASCO FT/IR-4100 spectrophotometers, respectively. ECD spectra were recorded using JASCO *J*-810 instruments (JASCO Corporation, Tokyo, Japan). The NMR spectra were recorded on a Varian Unity INOVA 500 FT-NMR spectrometer (Varian Medical Systems, Salt Lake City, UT, USA; 500 MHz for ^{1}H; 125 MHz for ^{13}C, respectively). Chemical shifts were reported using residual $CDCl_3$ (δ_H 7.26 and δ_C 77.0 ppm) and CD_3OD (δ_H 3.30 and δ_C 49.0 ppm) as internal standard. High resolution ESI-MS spectra were obtained on a LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA, USA) spectrometer. Silica gel 60 (Merck, Darmstadt, Germany, 230–400 mesh), LiChroprep RP-18 (Merck, 40–63 μm), and Sephadex LH-20 (Amersham

* Corresponding author.
E-mail address: wanghaitao1912@sina.com (H.-T. Wang).

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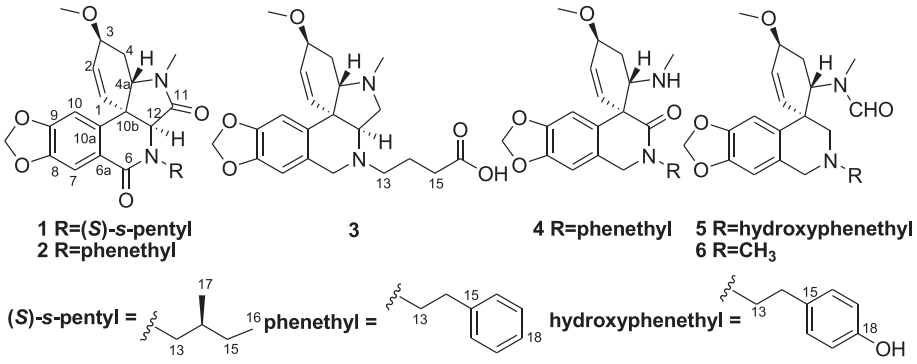

Fig. 1. Structures of compounds **1–6.**

Pharmacia Biotech., Roosendaal, The Netherlands) were used for column chromatography (CC). Precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) and precoated RP-18 F_{254s} plates (Merck) were used for analytical thin-layer chromatography analyses. HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector, with a Prevail (250 × 10 mm i.d.) preparative column packed with C_{18} (5 μm).

2.2. Plant material

The whole plants of *Z. grandiflora* were collected in June 2017 at the suburb of Changchun, Jilin Province of China. A specimen (No. 20170601ZF) was identified by one of the authors (H.T. Wang) and deposited at the Natural Product Laboratory of Medical School, Jilin University, China.

2.3. Extraction and isolation

The air-dried whole plants of *Z. grandiflora* (10.0 kg) were cut into small pieces and were extracted with 95% EtOH (25 L × 3) at room temperature for 24 h each time. After removal of EtOH under reduced pressure at 55 °C, the aqueous brownish syrup (1 L) was suspended in H_2O (4 L) and then partitioned with chloroform (5 L × 3) to afford chloroform soluble fraction (55.3 g). The chloroform soluble fraction was further fractionated through a silica gel column (200–300 mesh, 10 × 80 cm, 500 g) using increasing a proportion of methanol in chloroform (100:1, 50:1, 30:1, 15:1, 10:1, 7:1, 5:1, 3:1, 1:1, v/v, each 2.5 L) as the eluent to give 6 fractions according to TLC analysis. Fraction 3 (methanol-chloroform 15:1, 3.6 g) was applied to an ODS MPLC column (8 cm × 40 cm, 150 g) and eluted with MeOH-H_2O (20:80, 30:70, 40:60, each 500 mL) to yield 4 subfractions (Fr. 3–1 and Fr. 3–4). Subfraction 3–2 (MeOH-H_2O, 520 mg) was purified by preparative RP-HPLC (ODS column, 250 × 20 mm) using MeOH-H_2O (20:80) as mobile phase to obtain **5** (71 mg, 240 nm, retention time: 13.3 min). Subfraction 3–3 (MeOH-H_2O, 350 mg) was purified by preparative RP-HPLC (ODS column, 250 × 20 mm) eluting with MeOH/H_2O (22:78) to get **6** (57 mg, 240 nm, retention time: 14.7 min). Subfraction 3–4 (MeOH-H_2O 40:60, 210 mg) was purified by preparative RP-HPLC (ODS column, 240 nm, 250 × 20 mm) eluting with MeOH/H_2O (22% of MeOH-H_2O) to get **3** (55 mg, 240 nm, retention time: 14.6 min). Fraction 4 (methanol-chloroform 7:1, 2.9 g) was applied to an ODS MPLC column (8 cm × 40 cm, 150 g) and eluted with MeOH-H_2O (20:80, 30:70, 40:60, each 500 mL) to yield 3 subfractions (Fr. 4–1 and Fr. 4–3). Subfraction 4–1 (MeOH-H_2O 20:80, 226 mg) was repeatedly chromatographed on silica gel (150 g, 60 × 2.8 cm, chloroform-methanol, 20:1 → 10:1, each 500 mL) and then purified by preparative RP-HPLC (ODS column, 250 × 20 mm) using MeOH-H_2O (25:75) as mobile phase to obtain **2** (61 mg, 240 nm, retention time: 13.5 min). Subfraction 4–2 (MeOH-H_2O, 50:50, 265 mg) was chromatographed by a Sephadex LH-20 column (2 × 200 cm, 150 g) eluted with MeOH-H_2O (50% of MeOH-H_2O), and purifed on preparative RP-HPLC (ODS column, 250 × 20 mm) using MeOH-H_2O (30:70) as mobile phase to yield **4** (78 mg, retention time: 15.0 min). Subfraction 4–3 was purified by preparative RP-HPLC (ODS column, 250 × 20 mm) eluting with MeOH/H_2O (20:80) to get **1** (77 mg, 240 nm, retention time: 15.5 min).

2.3.1. Zephygranditine A (1)

Colorless oil; [α] +77.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 208 (4.18), 226 (4.35), 235 (4.18), 302 (3.66) nm; ECD (MeOH) 206 (Δε + 12.91), 226 (Δε +29.39), 255 (Δε +1.95), 263 (Δε +2.76) nm; IR (KBr) ν_{max} 2954, 2931, 1708, 1648, 1473, 1266, 1084, 1035, 933 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HR-ESI-MS *m/z*: 413.2079 ($C_{23}H_{29}N_2O_5$ $[M + H]^+$, calc. 413.2076).

2.3.2. Zephygranditine B (2)

Colorless oil; [α] +70.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 208 (4.70), 227 (4.33), 237 (3.89), 292 (3.78), 303 (3.68) nm; ECD (MeOH) 207 (Δε +12.27), 225 (Δε +27.67), 257 (Δε +1.90), 264 (Δε +2.74) nm; IR (KBr) ν_{max} 2952, 2929, 2892, 1707, 1650, 1501, 1483, 1387, 1267, 1085, 1037, 932 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HR-ESI-MS *m/z*: 447.1924 ($C_{26}H_{27}N_2O_5$ $[M + H]^+$, calc. 447.1920).

2.3.3. Zephygranditine C (3)

Colorless oil; [α] +36.1 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 208 (4.24), 223 (3.94), 242 (3.70), 292 (3.56) nm; ECD (MeOH) 207 (Δε +17.72), 224 (Δε +9.72) nm; IR (KBr) νmax 2951, 2929, 2868, 1762, 1614, 1502, 1484, 1388, 1244, 1097, 934 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HR-ESI-MS *m/z*: 401.2079 ($C_{22}H_{29}N_2O_5$ $[M + H]^+$, calc. 401.2076).

2.3.4. Zephygranditine D (4)

Colorless oil; [α] +19.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 208 (4.30), 225 (3.79), 237 (3.87), 291 (3.57) nm; ECD (MeOH) 205 (Δε +18.97), 237 (Δε +4.17), 288 (Δε +1.73) nm; IR (KBr) νmax 3333, 2953, 2930, 1688, 1643, 1483, 1425, 1237, 1089, 1037, 935 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HR-ESI-MS *m/z*: 421.2133 ($C_{25}H_{29}N_2O_4$ $[M + H]^+$, calc. 421.2127).

2.3.5. Zephygranditine E (5)

Colorless oil; [α] +104.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 208 (4.36), 226 (4.28), 242 (3.75), 291 (3.60) nm; ECD (MeOH) 205 (Δε +19.27), 234 (Δε +5.51), 287 (Δε +2.47) nm; IR (KBr) νmax 3332, 2952, 2933, 2871, 2820, 2764, 1684, 1513, 1482, 1239, 1081, 1036, 931 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HR-ESI-MS *m/z*: 451.2228 ($C_{26}H_{31}N_2O_5$ $[M + H]^+$, calc. 451.2233).

2.3.6. Zephygranditine F (6)

Colorless oil; [α] +77.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε):