Chinese Chemical Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Original article

Periconones B–E, new meroterpenoids from endophytic fungus *Periconia* sp.

Q1 Ji-Mei Liu[a,1], De-Wu Zhang[a,b,1], Min Zhang[a], Ri-Dao Chen[a], Zheng Yan[a], Jian-Yuan Zhao[b], Jin-Lian Zhao[a], Nan Wang[a], Jun-Gui Dai[a,*]

[a] *State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China*
[b] *Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peaking Union Medical College, Beijing 100050, China*

ARTICLE INFO

Article history:
Received 12 June 2016
Received in revised form 14 July 2016
Accepted 24 July 2016
Available online xxx

Keywords:
Meroterpenoids
Periconia
Endophytic fungus
Annona muricata
Periconone

ABSTRACT

Periconones B–E (**1–4**), four new polyketide–terpenoid hybrid molecules were isolated from the endophytic fungus *Periconia* sp. F-31. Their structures and absolute configurations were established by extensive spectroscopic data analysis and electronic circular dichroism (ECD). Compound **4** exhibited *in vitro* cytotoxic activity against the human MCF-7 tumor cell line with an IC_{50} value of 4.2 μmol/L, and compound **1** displayed anti-HIV activity with an IC_{50} value of 18.0 μmol/L.

1. Introduction

The term "meroterpenoid" is referred to as natural product of mixed biosynthetic origin that is partially derived from terpenoids [1]. Moreover, the structural diversity of meroterpenoids can be grouped into two major classes based on their biosynthetic origins: polyketide–terpenoids and non-polyketide–terpenoids. In recent years, a huge range of structurally diverse meroterpenoids were isolated from fungi [2] and displayed important biological activities, such as antimicrobial [3], immunomodulatory [4–6], antiviral [7], antitumoral [8–10], and antiinsect [11]. As part of our ongoing work of searching for novel secondary metabolites with interesting biological activities from the endophytic fungus *Periconia* sp. F-31 [12–16], a further chemical investigation led to the isolation of four meroterpenoids Periconones B–E (**1–4**, Fig. 1). These compounds were proposed to be derived from typical polyketide–terpenoid hybrids pathway, which were formed from one acetyl-CoA starter and five malonyl-CoA extenders coupled with one C_5 unit [17–20]. Herein, we report their isolation, structural elucidation, biological activities, and plausible biogenetic pathway.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a PerkinElmer Model-343 digital polarimeter. The CD spectra were recorded on a JASCO J-815 spectropolarimeter. The UV absorption spectra were measured in MeOH on a Thermo Spectronic-Vision32 Software V1.25. IR spectra were acquired on a Nicolet 5700 FT-IR microscope spectrometer (FTIR Microscope Transmission). 1D and 2D NMR spectra were obtained at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR on VNOVA SYSTEM-600 and Bruker AVIIID 600, and 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR on Bruker AVIII 400 spectrometers. Chemical shifts (δ) are given in ppm, and coupling constants (J) are given in hertz (Hz). HRESIMS data were measured using an Agilent Technologies 6520 Accurate Mass Q-TOF LC/MS spectrometer. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., Qingdao, China) and Sephadex LH-20 gel (Amersham Biosciences, Sweden) were used for column chromatography (CC). Semi-preparative reversed phase and normal phase HPLC were performed on a Shimadzu HPLC instrument equipped with a Shimadzu RID-10A detector and a Grace Adsorbosphere C_{18} column (250 mm × 10 mm, i.d., 5 μm) by eluting with mixtures of methanol and H_2O at 3 mL/min, or a Grace Allsphere silica column (250 mm × 10 mm, i.d., 5 μm) by eluting with mixtures of *n*-hexane–isopropyl alcohol and *n*-hexane–EtOAC

* Corresponding author.
E-mail address: jgdai@imm.ac.cn (J.-G. Dai).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.cclet.2016.07.031

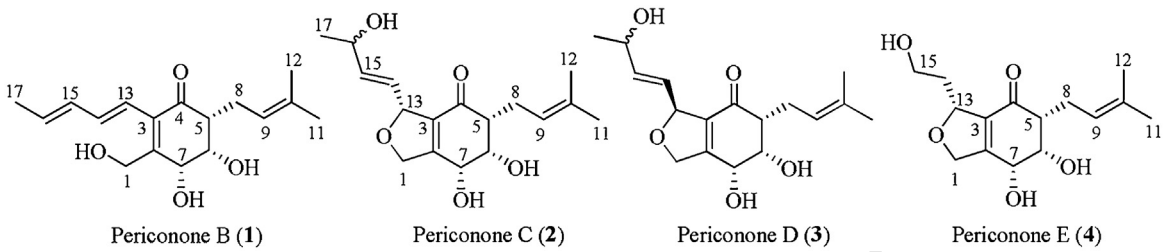

Fig. 1. Structures of Periconones B–E (**1**–**4**).

at 4 mL/min, respectively. Analytical TLC was carried out on pre-coated silica gel GF_{254} plates (Qingdao Marine Chemical Industry, Qingdao, China), and spots were visualized under UV light or by spraying with 10% H_2SO_4 in EtOH followed by heating at 120 °C.

2.2. Fungal material, fermentation, extraction and isolation

The fermentation, extraction, and isolation of the fungal strain *Periconia* sp. F-31 were performed as described previously [21]. The EtOAc extract (25.0 g) of the culture filtrate was subjected to silica gel CC eluting with a CH_2Cl_2–CH_3OH gradient (100:0–0:100) to produce eight fractions (Fr1–Fr8) on the basis of TLC analysis. Fr3 (4.5 g) was initially subjected to Sephadex LH-20 CC by eluting with CH_3OH to give four fractions (Fr3.1–Fr3.4). Fr3.4 (1.0 g) was then fractionated by reversed-phase semi-preparative HPLC eluting with CH_3OH–H_2O (65:35, v/v) to afford four fractions (Fr3.4.1–Fr3.4.4). Purification of Fr3.4.4 (78.9 mg) by reversed-phase semi-preparative HPLC (CH_3OH–H_2O, 70:30, v/v) resulted in **1** (48.0 mg, t_R 16.7 min). Fr4 (3.4 g) was fractionated by Sephadex LH-20 CC eluting with CH_3OH, yielding five fractions (Fr4.1–Fr4.5). Fr4.4 (500 mg) was further separated *via* normal-phase semi-preparative HPLC (*n*-hexane–EtOAC, 2:3, v/v) to furnish four fractions (Fr4.4.1–Fr4.4.4). Purification of Fr4.4.1 (120.0 mg) through reversed-phase semi-preparative HPLC (CH_3OH–H_2O, 30:70, v/v) followed by normal-phase semi-preparative HPLC afforded **2** (4.2 mg, t_R 16.2 min, *n*-hexane–isopropyl alcohol, 4:1, v/v), **3** (1.5 mg, t_R 28.5 min, *n*-hexane–isopropyl alcohol, 5:1, v/v), and **4** (2.2 mg, t_R 15.2 min, *n*-hexane–isopropyl alcohol, 4:1, v/v).

Periconone B (**1**). Yellow gum; $[\alpha]^{20}_{546}$ +21.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (logε) 206 (3.99) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 209 (−2.93), 254 (+1.64), 348 (−0.14) nm; IR (ν_{max}) 3410, 2971, 2913, 1677 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HR-ESIMS *m*/*z* 293.1741 $[M+H]^+$ ($C_{17}H_{25}O_4$, calcd. $[M+H]^+$ 293.1753).

Periconone C (**2**). Yellow gum; $[\alpha]^{20}_{546}$ +40.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (logε) 204 (4.12) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 207 (−4.45), 245 (+4.81), 335 (−0.34) nm; IR (ν_{max}) 3400, 2970, 2926, 1676 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HR-ESIMS *m*/*z* 331.1506 $[M+Na]^+$ ($C_{17}H_{24}O_5Na$, calcd. $[M+Na]^+$ 331.1516).

Periconone D (**3**). Yellow gum; $[\alpha]^{20}_{546}$ +73.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (logε) 204 (4.04), 243 (3.65) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 256 (+3.42), 328 (−0.54) nm; IR (ν_{max}) 3422, 2974, 2918, 1675 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HR-ESIMS *m*/*z* 331.1505 $[M+Na]^+$ ($C_{17}H_{24}O_5Na$, calcd. $[M+Na]^+$ 331.1516).

Periconone E (**4**). Yellow gum; $[\alpha]^{20}_{546}$ +21.4 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (logε) 248 (3.74) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 204 (−6.92), 255 (+5.14), 311 (−0.10) nm; IR (ν_{max}) 3404, 2921, 1673 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HR-ESIMS *m*/*z* 283.1533 $[M+H]^+$ ($C_{15}H_{23}O_5$, calcd. $[M+H]^+$ 283.1540).

2.3. Cytotoxicity bioassay [22]

The cytotoxicity of the compounds against the human tumor cell lines (HCT-8, Bel-7402, Hela, and MCF-7) was measured using the MTT assay. The cells were maintained in a RRMI S7 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μg/mL streptomycin. Cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Tumor cells were seeded in 96-well microtiter plates at 1200 cells/well. After 24 h, compounds were added to the wells. After incubation for 96 h, cell viability was determined by measuring the metabolic conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into purple formazan crystals by viable cells. The MTT assay results were read using an MK3 Wellscan (Labsystem Dragon, Helsinki, Finland) plate reader at 570 nm. All compounds were tested at five concentrations (10^{-5} mol/L, 10^{-6} mol/L, 10^{-7} mol/L, 10^{-8} mol/L, and 10^{-9} mol/L) in 100% DMSO with a final concentration of DMSO of 0.1% (v/v) in each well. Paclitaxel was used as a positive control. Each concentration of the compounds was tested in three parallels experiments. IC_{50} values were calculated using Microsoft Excel software.

2.4. HIV-inhibitory bioassay [23]

293T cells (2×10^5) were co-transfected with 0.6 μg of pNL-Luv-E^--Vpu^- and 0.4 μg of pHIT/G. After 48 h, the VSV-G pseudotyped viral supernatant (HIV-1) was harvested by filtration through a 0.45 μm filter and the concentration of viral capsid protein was determined by p24 antigen capture ELISA (Biomerieux). SupT1 cells were exposed to VSV-G pseudo typed HIV-1 (MOI = 1) at 37 °C for 48 h in the absence or presence of test compounds (Efavirenz was used as positive control). The inhibition rate was determined by using a firefly Luciferase Assay System (Promega).

3. Results and discussion

Periconone B (**1**) was obtained as a yellow gum and gave an HR-ESIMS ion peak at *m*/*z* 293.1741 $[M+H]^+$, corresponding to a molecular formula of $C_{17}H_{24}O_4$ with six degrees of unsaturation. The IR absorption bands at 3410 cm^{-1} and 1677 cm^{-1} indicated the presence of hydroxyl and conjugated carbonyl groups. The ^1H NMR spectrum of **1** (Table 1) in $CDCl_3$ showed five olefinic protons at δ_H 6.50 (dd, 1H, *J* = 15.6, 10.4 Hz), 6.12 (1H, m), 6.08 (d, 1H, *J* = 15.6 Hz), 5.80 (dq,1H, *J* = 14.8, 6.8 Hz), and 5.14 (1H, m); one oxygenated methylene protons at δ_H 4.58 (d, 1H, *J* = 13.6 Hz) and 4.53 (d, 1H, *J* = 13.6 Hz); two oxygenated methine protons at δ_H 4.67 (1H, brs) and 4.28 (1H, brs); one methylene protons at δ_H 2.62 (ddd, 1H, *J* = 14.8, 5.2, 5.2 Hz) and 2.30 (ddd, 1H, *J* = 14.8, 9.2, 9.2 Hz); one methine proton at δ_H 2.39 (ddd, 1H, *J* = 9.2, 4.4, 2.0 Hz); three methyl groups at δ_H 1.78 (d, 3H, *J* = 6.8 Hz), 1.71 (3H, s), and 1.65 (3H, s), and three hydroxyl groups at δ_H 2.97 (brs, 1H), 3.29 (brs, 1H), and 4.08 (brs, 1H). The ^{13}C NMR and DEPT spectra (Table 1) exhibited 17 carbon resonances, including four quaternary carbons (one α,β-unsaturated ketone at δ_C 198.3, three olefinic), eight methines (including five olefinic, two oxygenated), two methylenes (including one oxygenated), and three methyls. Among the 17 carbons, one carbonyl carbon and four double bonds accounted for five

Please cite this article in press as: J.-M. Liu, et al., Periconones B–E, new meroterpenoids from endophytic fungus *Periconia* sp., Chin. Chem. Lett. (2016), http://dx.doi.org/10.1016/j.cclet.2016.07.031