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BRIEF COMMUNICATION

Peniazaphilin A, a new azaphilone derivative produced by *Penicillium* sp. CPCC 400786

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Abstract

A new azaphilone derivative, named peniazaphilin A (**1**) and one known isocoumarin, (*R*)-3-methyl-6-hydroxy-8-methoxy-3,4-dihydroisocoumarin (**2**) were isolated from the fungus *Penicillium* sp. CPCC 400786. Their structures were elucidated by means of extensive spectroscopic analysis. The absolute configuration of **2** was established by circular dichroism for the first time. Compounds **1** and **2** exhibited weak anti-HIV activities with the IC_{50} values of 60.4 and 69.3 μM, respectively.

Fungi has been demonstrated to be a rich source of secondary metabolites with various structural features and diverse biological activities [1, 2]. Azaphilones are a structurally variable class of fungal metabolites with highly oxygenated pyranoquinone bicyclic core from numerous fungal genera (such as *Penicillium*, *Aspergillus*, *Chaetomium*, *Phomopsis*, *Emericella*, *Monascus*) [3], which displayed a wide range of impressive biological activities such as cytotoxic, antibacterial, and antiviral activities [1, 2, 4]. The extract of fungus *Penicillium* sp. CPCC 400786 showed anti-HIV activities with inhibition rates of 99.9% at a concentration of 100 μg ml^{-1}. As part of our ongoing search for bioactive natural products, the EtOAc extract of this fungi was investigated, which led to the isolation of a new azaphilone derivative, peniazaphilin A (**1**), along with a known isocoumarin, (*R*)-3-methyl-6-hydroxy-8-methoxy-3,4-dihydroisocoumarin (**2**) (Fig. 1). Their structures and absolute configurations were elucidated by extensive spectroscopic analyses. Compounds **1** and **2** were tested for anti-HIV activities, and displayed weak anti-HIV activities. Herein, we report the isolation, structural elucidation, and biological activities for these two compounds.

The fungus *Penicillium* sp. CPCC 400786 was isolated from the soil, collected in Goddess Peak, Chongqing, China, in September 2006, and classified basing on morphological and molecular (ITS1-5.8S-ITS2 rRNA gene sequence) analyses. The strain was deposited at the China Pharmaceutical Culture Collection (No.: CPCC 400786).

The fungal strain was spread onto slants of modified potato dextrose agar (PDA) medium (potato 200 g, glucose 20 g, distilled water 1 l, KH_2PO_4 3 g, $MgSO_4$ 0.73 g, vitamin B_1 10 mg, agar 8 g, pH 6–6.3), and incubated at 28 °C for 8–10 days. The agar plugs were inoculated into 250 ml Erlenmeyer flasks containing 50 ml of PDA liquid medium at 28 °C on a rotary shaker (180 rpm) for 48 h to prepare the seed culture. Fermentation was carried out in Fernbach flasks (500 ml), each containing 80 g of rice. Distilled H_2O (120 ml) was added to each flask, and the contents were soaked overnight before autoclaving at 121 °C for 30 min. After cooling to room temperature, each flask was inoculated with 8 ml of the seed culture, and incubated at 28 °C for 30 days.

The fermented material (13 flasks) was extracted repeatedly with EtOAc (4 × 2 l), and the organic solvent was evaporated to dryness under vacuum to yield the crude extract (4.1 g), which was initially subjected to silica gel (Qingdao Marine Chemical Inc. Qingdao, China) column chromatography (CC) eluting with CH_2Cl_2–acetone gradient (100:0–50:50) to produce seven fractions (Fr.1–Fr.7). Fraction Fr.3 (300 mg) was applied to Sephadex LH-20

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Table 1 NMR data of peniazaphilin A (**1**) in DMSO-d_6

Position	δ_C, type	δ_H (J in Hz)
1	58.8, CH_2	4.15, br d (16.2) 4.08, br dd (16.2, 2.4)
2	129.9, C	
3	199.7, C	
4	76.1, C	
5	72.4, CH	3.81, m
6	34.9, CH_2	2.61, ddd (18.0, 2.4, 24.) 2.37, dddd (18.0, 6.6, 2.4, 2.4)
7	146.6, C	
8	76.3, CH	3.58, s
9	106.8, C	
10	33.2, CH_2	1.96, m
11	23.7, CH_2	1.90, m
12	68.3, CH_2	3.86, m
13	18.8, CH_3	1.13, s
14	57.0, CH_3	3.34, s
4-OH		5.24, s
5-OH		5.19, d (4.2)

(GE Healthcare, Uppsala, Sweden) CC eluting with $CHCl_3$–CH_3OH (50:50) to give six fractions (Fr.3.1–Fr.3.6), fraction Fr.3.4 (140 mg) was further purified by reversed-phase semi-preparative HPLC eluting with CH_3OH–H_2O (40:60) at 4 ml min^{-1} to obtain **2** (28 mg, $t_R = 14.8$ min). Fraction Fr.4 (280 mg) was subjected to Sephadex LH-20 CC eluting with $CHCl_3$–CH_3OH (50:50) to afford five fractions (Fr.4.1–Fr.4.5), fraction Fr.4.4 (50 mg) was isolated by reversed-phase semi-preparative HPLC eluting with CH_3OH–H_2O (55:45) at 2 ml min^{-1} to yield **1** (1.4 mg, $t_R = 7.8$ min).

Peniazaphilin A (**1**): White powder; $[\alpha]^{25}_D$ –47.5 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε): 228 (3561), 298 (499) nm; IR (ν_{max}): 3351, 2935, 1679, 1403, 1203, and 1138 cm^{-1}; CD (MeOH) $\Delta\varepsilon$ (nm): +1.31 (218), −3.04 (251), +0.24 (323); ESIMS *m/z* 283.3 $[M-H]^-$; HRESIMS *m/z* 307.1146 $[M+H]^+$ (calcd for $C_{14}H_{20}O_6Na$, 307.1152); ^1H and ^{13}C NMR data, see Table 1.

Peniazaphilin B (**2**): Pale yellow powder; $[\alpha]^{25}_D$ –122.8 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε): 214.5 (24,050), 263.5 (14,499), and 298 (7874) nm; IR (ν_{max}): 3181, 1685, 1612, 1587, 1351, 1248, 1082, and 841 cm^{-1}; CD (MeOH) $\Delta\varepsilon$ (nm): −5.97 (231), +0.47 (250.5), −4.81 (269.5); ESIMS *m/z* 209.1 $[M+H]^+$; HRESIMS *m/z* 209.0806 $[M+H]^+$ (calcd for $C_{11}H_{13}O_4$, 209.0808); ^1H NMR(600 MHz, CDCl3) δ: 6.40 (1 H, d, $J = 2.4$ Hz, H-7), 6.28 (1 H, d, $J = 2.4$ Hz, H-5), 4.50 (1 H, m, H-3), 3.84 (3 H, s, H-10), 2.81 (1 H, dd, $J = 16.2$, 11.4 Hz, H-4a), 2.74 (1 H, dd, $J = 16.2$, 3.6 Hz, H-4b), 1.43 (3 H, d, $J = 0.6$ Hz, H-9); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 163.6 (C-1), 73.7 (C-3), 36.2 (C-4), 143.9 (C-4a), 106.5 (C-5), 162.5 (C-6), 98.6 (C-7), 163.5 (C-8), 105.5 (C-8a), 20.6 (C-9). 55.9 (C-10).

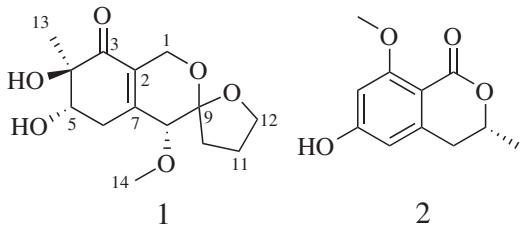

Fig. 1 Structures of **1** and **2**

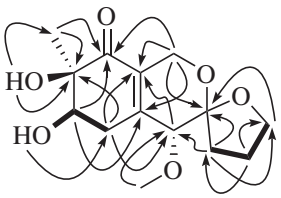

Fig. 2 key COSY (—) and HMBC (→) correlations of **1**

Compound **1** was isolated as white powder and gave a HRESIMS ion peak at *m/z* 307.1146 $[M+Na]^+$, corresponding to a molecular formula of $C_{14}H_{20}O_6$ with five degrees of unsaturation. The IR absorption bands at 3351 and 1679 cm^{-1} indicated the presence of hydroxyl and carbonyl groups. The ^{13}C NMR and DEPT spectra displayed 14 carbon resonances (Table 1), which consisted of five quaternary carbons (δ_C 199.7, 146.6, 129.9, 106.8, and 76.1, including one carbonyl and two olefinic carbons), two methine carbons (δ_C 76.3, 72.4, two oxygenated), five methylene carbons (δ_C 68.3, 58.8, 34.9, 33.2, and 23.7, including two oxygenated), and two methyl carbons (δ_C 57.0, 18.8, including one oxygenated). The HMBC correlations (Fig. 2) of H-1/C-2, C-3, C-7, and C-9; H-13/C-3, C-4, and C-5; H-6/C-2, C-4, C-5, C-7, and C-8; H-8/C-2, C-6, C-7, C-9, and C-14 indicated the presence of an isochromenone group. Furthermore, the HMBC correlations for H-10/C-8, C-9, C-11, and C-12; H-12/C-9, C-10, and C-11 along with the ^1H–^1H COSY correlations of H-10/H-11/H-12, suggested the tetrahydrofuran ring. Thus, the planar structure of **1** was determined to be a new azaphilone analog.

The relative configuration of **1** was established by NOESY spectrum. The NOESY correlations (Fig. 3) from H-6α to H-13, from H-6β to H-5 and H-8 indicated that H-5 and H-8 were *syn*-orientated, and H-13 was on the opposite side. The NOESY correlations from H-8 to H-10 was observed, but the distance of H-8 and H-10 were both close no matter that the configuration of C-9 was either *R* or *S* (Figure S1). In the CD spectrum (Figure S12), the positive Cotton effect at 218 nm, and negative Cotton effect at 251 nm were nearly identical to those of pestafolide A [5], suggesting the 4*R*, 5*S*, 8*R* configurations of **1**. The C-9 was

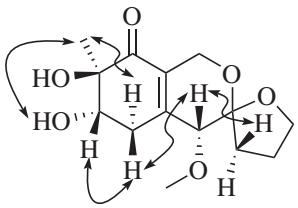

Fig. 3 key NOESY (↔) correlations of **1**

far away from the chromophore group, and has no effect on the CD spectrum of **1**, so that the absolute configuration of C-9 remains unsolved. The structure of **1** was established as peniazaphilin A.

Compound **2** was obtained as pale yellow powder. Its molecular formula of $C_{11}H_{12}O_4$ with six degrees of unsaturation was established by a HRESIMS ion peak at *m/z* 209.0806 $[M+H]^+$. Interpretation of ^1H NMR, ^{13}C NMR, DEPT, $^1H-^1H$ COSY, HSQC, and HMBC spectroscopic data (Fig. 3) assigned the planar structure of compound **2**, which was the same to known compound, 3-methyl-6-hydroxy-8-methoxy-3,4-dihydroisocoumarin (**2a**) [6], the optical rotation of **2** was similar to that of **2a**, indicating these two compounds were the same one. However, the CD spectrum or X-ray diffraction data of **2a** was lacking, its NMR data and optical rotation were not enough for determining the absolute configuration. So, the reported stereochemistry of **2a** was inaccurate. For the purpose of confirming the absolute configuration, the CD spectrum (Figure S22) was performed, and showed the negative Cotton effect at 231 and 269.5 nm, and positive Cotton effect at 250.5 nm, which was nearly identical to those of angelicoin A [7]. Therefore, the absolute configuration of **2** was assigned as the 3*R* configuration.

Compounds **1** and **2** were evaluated for anti-HIV activities (efavirenz as the positive control) [8]. Compounds **1** and **2** showed weak anti-HIV activities with the IC_{50} values of 60.4 and 69.3 μM, respectively, while the CC_{50} values of **1** and **2** were both more than 100 μM.

In summary, we isolated and fully characterized via extensive spectroscopic analyses a new azaphilone analog and a known isocoumarin from the fungus *Penicillium* species. These two compounds showed low anti-HIV activities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Schueffler A, Anke T. Fungal natural products in research and development. Nat Prod Rep. 2014;31:1425–48.
2. Aly AH, et al. Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Div. 2010;41:1–16.
3. Gao JM, Yang SX, Qin JC. Azaphilones: chemistry and biology. Chem Rev. 2013;113:4755–811.
4. Kharwar RN, et al. Anticancer compounds derived from fungal endophytes: their importance and future challenges. Nat Prod Rep. 2011;28:1208–28.
5. Ding G, et al. Antifungal metabolites from the plant endophytic fungus *Pestalotiopsis foedan*. J Nat Prod. 2008;71:615–8.
6. Dethoup T, et al. Merodrimanes and other constituents from *Talaromyces thailandiasis*. J Nat Prod. 2007;70:1200–2.
7. Shibano M, et al. Two isocoumarins from *Pleurospermum angelicoides*. Chem Pharm Bull. 2006;54:717–8.
8. Zhang Q, et al. High-throughput assay to identify inhibitors of Vpu-mediated down-regulation of cell surface BST-2. Antivir Res. 2011;91:321–9.