SHORT REPORT

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Fusacintone A, a New Polyketide from the Endophytic Fungus *Fusarium tricinctum* from *Fritillaria monantha*

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Abstract: A previously undescribed polyketide (**1**) and four known compounds (**2-5**) isolated from the endophytic fungus *Fusarium tricinctum* from *Fritillaria monantha*. Compound **1** showed anti-inflammatory activity by inhibiting NO production with an IC_{50} value of 17.6±0.46 μM.

Keywords: *Fusarium tricinctum*; endophytic fungus; polyketide; structural elucidation; anti-inflammatory.

1. Fungal Source

The endophytic fungus *Fusarium tricinctum* was isolated from fresh and healthy *Fritillaria monantha* Migo collected from Sunhe Township, Enshi Tujia and Miao Autonomous Prefecture, Hubei Province. This isolate was identified according to the ITS sequence (GenBank Accession No. KX058063.1, query cover 100%, maximum identity 99%). Subsequently it was deposited in the Research Group of Medicinal Fungi and Ethnic Medicine, School of Pharmacy, South Central Minzu University.

2. Previous Studies

Endophytic fungi, commonly residing within healthy plant tissues, not only refrain from inducing diseases in their host plants but also engage in a symbiotic relationship that is mutually advantageous [1]. These fungi are integral to the global ecosystem, significantly contributing to the advancement of plant and animal life, as well as other microorganisms, fostering co-evolution and enhancing biodiversity [2]. Primarily existing in a symbiotic state, plant endophytic fungi adapt to their distinctive environments by synthesizing a diverse array of secondary metabolites characterized by novel structures and potent biological activities [3]. Consequently, research into plant endophytic fungi and their secondary metabolites holds substantial importance [4].

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Fusacintone A, a new polyketide

Fusarium is one of the most important species of fungi, which exists in soil and plant tissues through parasitism and saprophytosis etc. *Fusarium sp.* are rich in variety of morphology and widely distributed, which has high research value. The secondary metabolites of *Fusarium* such as deoxynivalenol (DON) and zearalenone (ZEN) are the most important mycotoxins in the world [5-6]. To date, a variety of components, including terpenoids [7], alkaloids [7], pyranone [8] and naphthoquinone dimers [9], have been isolated from *Fusarium sp*. As part of our ongoing efforts to discover bioactive metabolites derived from endophytic fungi, a chemical investigation on the cultural broth of *F.tricinctum* was carried out.

3. Present Study

This study reports the isolation, structural elucidation, and biological activities of the isolates were reported. As a result, a new polyketone, fusacintone A (**1**), with four known compounds (**2-5**) were obtained (Figure 1). Herein, we report the isolation, structural elucidation and biological activities of the isolates.

The fermentation broth was extracted five times with 2-fold volume of EtOAc, and concentrated under reduced pressure to obtain 54 g of crude extract. The EtOAc extract was treated with 200-300 mesh normal-phase silica gel and eluted with a gradient of CH_2Cl_2-MeOH (100:0-0:100, v/v) to obtain six fractions (A-F).

Fraction B (12.0 g) was subjected to C18 MPLC using MeOH–H_2O (20:80–100:0, v/v), yielding nine subfractions (B-1~B-9). Fraction B-3 (180 mg) by normal-phase silica gel column chromatography in a continuous gradient of petroleum ether-EtoAc (10:1-0:1, v/v), resulting in six fractions (B-3-1~B-3-6). Fraction B-3-1 was isolated and purified by semipreparative C18 HPLC with a gradient of MeCN−H_2O (57:43-62:38, v/v) to obtain **1** (7.9 mg, t_R = 15.3 min). Fraction B-3-6 was isolated and purified by semipreparative C18 HPLC with a gradient of MeCN−H_2O (65:35-70:30, v/v) to obtain **2** (4.1 mg, t_R = 18.4 min). Fraction B-5 (79 mg) by normal-phase silica gel column chromatography in a continuous gradient of petroleum ether-EtoAc (10:1-0:1, v/v), resulting in five fractions (B-5-1~B-5-5). Fraction B-5-2 was isolated and purified by semipreparative C18 HPLC with a gradient of MeOH−H_2O (68:32-73:27, v/v) to obtain **5** (3.9 mg, t_R = 20.5 min).

Fraction C (11 g) was subjected to C18 MPLC using MeOH–H_2O (20:80–100:0, v/v), yielding seven subfractions (C-1~C-7). Fraction C-7 (121 mg) by normal-phase silica gel column chromatography in a continuous gradient of petroleum ether-EtoAc (10:1-0:1, v/v), resulting in seven fractions (C-7-1~C-7-7). Fraction C-7-2 was isolated and purified by semipreparative C18 HPLC with a gradient of MeCN−H_2O (68:32-73:27, v/v), to obtain **4** (5.4 mg, t_R = 19.3 min). Fraction C-2-1 was isolated and purified by semipreparative C18 HPLC with a gradient of MeCN−H_2O (63:37-62:38, v/v), to obtain **3** (5.2 mg, t_R = 16.9 min).

Equipment: The HPLC model is an Agilent 1260 liquid chromatograph with a DAD detector and a Zorbax SB-C18 preparative column (4.6 mm×150 mm, 5 μm). The model of medium-pressure liquid chromatography was Biotage SP1 (Biotage, Sweden) with an RP-18 column (Fuji Silysia Chemical Ltd., Japan). Column chromatography was conducted using silica gel (80–100 mesh and 200–300 mesh, Qingdao Marine Chemical Factory, China), Sephadex LH-20 (Pharmacia Fine Chemical Co, Sweden). Thin-layer chromatography (TLC) was performed on GF254 plates (Qingdao Marine Chemical Factory, China).1D and 2D spectra were produced by a Bruker spectrometer (Bruker, Germany, model AM600). HR-ESI-MS data were collected by A Q Exactive HF (Thermo Fisher Scientific, USA).

*Fusacintone A (**1**)*: yellow powder; UV (MeOH): 230 (3.49); HR-ESI-MS (pos.): 233.04445 $[M + H]^+$ (calcd. for $C_{12}H_9O_5^+$, 233.04443); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 6.89 (1H, s, H-4), 6.21 (1H, s, H-8), 5.30 (3H, s, H-12), 2.45 (2H, s, H-10); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) = 161.6 (C, C-

1), 107.1 (C, C-2), 153.5 (C, C-3), 100.5 (CH, C-4), 160.5 (C, C-5), 110.2 (C, C-6), 183.3 (C, C-7), 110.4 (CH, C-8), 168.4 (C, C-9), 20.6 (CH_3, C-10), 167.5 (C, C-11), 68.8 (CH_2, C-12).
*2,5-Dimethyl-7-hydroxychromone (**2**)*: white amorphous powder; ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 2.32 (3H, d, J = 0.7 Hz, 2-CH_3), 2.71 (3H, s, 5-CH_3), 6.00 (1H, d, J = 0.8 Hz, H-3), 6.62 (1H, dd, J = 1.0 Hz, H-6), 6.64 (1H, d, J = 2.4 Hz, H-8); ^{13}C NMR (150 MHz, CD_3OD): δ (ppm) = 182.1 (C, C-4), 166.7 (CH, C-2), 163.2 (C, C-7), 161.5 (C, C-8a), 143.7 (C, C-5), 118.0 (CH, C-6), 115.6 (C, C-4a), 111.4 (CH, C-3), 101.7 (CH, C-8), 23.2 (5-CH_3), 19.9 (C-2-CH_3).The above data is consistent with literature data [10].

*(10Z)-12-Acetoxy-cyclonerodiol (**3**)*: Yellow oil; ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 1.01 (3H, d, J = 6.9 Hz, H-1), 1.63 (1H, m, H-2), 1.73 (1H, m, H-4a), 1.58 (2H, m, H-4b/5b), 1.83 (2H, m, H-5a/6), 1.55 (2H, m, H-8), 2.14 (2H, m, H-9), 5.44 (1H, m, H-10), 4.61 (2H, d, J = 7.9 Hz, H-12), 1.29 (3H, s, H-13), 1.22 (3H, s, H-14), 1.69 (3H, s, H-15), 2.03 (3H, s, H-2'); ^{13}C NMR (150 MHz, CD_3OD): δ (ppm) = 173.4 (C, C-1'), 132.5 (C, C-11), 131.6 (CH, C-10), 82. 6 (C, C-3), 76.00 (C, C-7), 64.6 (CH_2, C-12), 56.1 (CH, C-6), 46.0 (CH, C-2), 42.5 (CH_2, C-4), 41.9 (CH_2, C-8), 26.6 (CH_3, C-13), 25. 7 (CH_2, C-5), 25.1 (CH_3, C-14), 24.0 (CH_2, C-9), 22.1 (CH_3, C-15), 21.3 (CH_3, C-2'), 15.9 (CH_3, C-1).The above data is consistent with literature data [11].

*Indazole (**4**)*: white amorphous powder; ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 8.13 (1H, d, J = 7.7 Hz, H-4), 7.89 (1H, s, H-3), 7.41 (1H, dd, J = 7.4, 1.5 Hz, H-7), 7.17 (1H, m, H-6), 7.15 (1H, m, H-5); ^{13}C NMR (150 MHz, CD_3OD): δ (ppm) = 138.2 (C, C-8), 132.5 (CH, C-3), 127.9 (CH, C-6), 123.2 (C, C-9), 122.3 (CH, C-4), 121.9 (CH, C-5), 112.7 (CH, C-7).The above data is consistent with literature data [12].

*Cyclo(glycyltryptophyl) (**5**)*: white amorphous powder; ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 7.60 (1H, m, H-8), 7.32 (1H, d, J = 8.1 Hz, H-5), 7.10 (1H, dd, J = 8.2, 1.2 Hz, H-7), 7.05 (1H, s, H-2), 7.01 (1H, s, H-6), 4.20 (1H, t, J = 4.2 Hz, H-19b), 3.47 (dd, J = 14.7, 3.9 Hz, H-19a), 3.14 (1H, dd, J = 14.7, 4.5 Hz, H-15a), 2.50 (1H, dd, J = 17.6, 1.1 Hz, H-15b); ^{13}C NMR (150 MHz, CD_3OD): δ (ppm) = 171.1 (C, C-17), 168.9 (C, C-20), 137.9 (C, C-4), 128.7 (C, C-9), 126.1 (CH, C-2), 122.6 (CH, C-6), 120.2 (CH, C-8), 119.7 (CH, C-7), 112.2 (CH, C-5), 109.0 (C, C-3), 57.4 (CH, C-16), 44.8 (CH_2, C-19), 31.1 (CH_2, C-15).The above data is consistent with literature data [13].

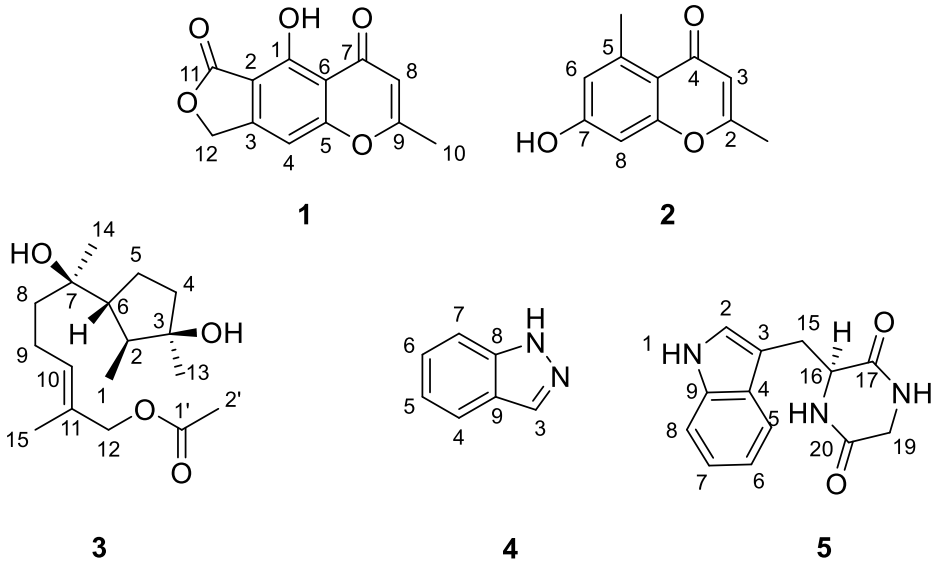

Figure 1. The chemical structures of compounds **1**–**5**

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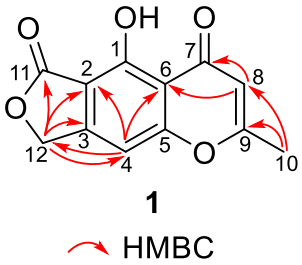

Figure 2. Key HMBC correlations of compound **1**

Fusacintone A (**1**) was obtained as a yellow powder. The molecular formula $C_{12}H_8O_5$ was indicated by the HR-ESI-MS at *m/z* 233.04443 $[M + H]^+$ (calcd for $C_{12}H_8O_5H^+$ 233.04445), indicating a molecular unsaturation index of nine. The ^{1}H NMR spectrum of this compound shows one methyl proton [δ_H 2.45 (s)], two olefinic protons [δ_H 6.21 (s), 6.89 (s)], and two oxygenated methylene protons [δ_H 5.30 (s)]. ^{13}C NMR and DEPT data revealed twelve carbons, which included one methyl (δ_C 20.6), one methylene (δ_C 68.8), two olefinic carbons (δ_C 100.5, 110.4), eight quaternary carbons (δ_C 107.1, 110.2, 153.5, 160.5, 161.6, 168.4, 167.5, 183.8). Above-mentioned NMR pattern of **1** was similar to those of the known synthetic compound 5-hydroxy-2-methylchromone-6,7-di-carboxylic anhydride [14]. The major difference was that the C-12 ester group in reported compound was replaced by a methylene group at the same position in 1. This presumption was confirmed by HMBC correlations from H-12 to C-2, C-4 and C-11, and H-4 to C-12. Furthermore, the existence of intermediate aromatic ring linked to the pyranone ring was established through HMBC correlations from H-4 to C-2, C-6 and C-5, as well as H-8 to C-6 and C-7. The HMBC correlations of H-10 with C-8 and C-9, and of H-8 to C-10 confirmed a methyl group was linked at C-9 (Figure 2).

In vitro anti-inflammatory activity: [15] NO production was assessed indirectly by analyzing the supernatant. RAW264.7 cells were plated in 96-well plates and incubated for 24 hours. Afterward, the medium was replaced, and the cells were exposed to sample solutions at concentrations of 1, 5, 10, 20, 30, and 40 μM for 1 hour. The IC_{50} values of the tested compounds were calculated by harvesting the supernatant following a 12-hour incubation period with LPS, which was utilized to quantify NO production. The absorption at 540 nm was measured by a microplate reader at room temperature. The IC_{50} values were calculated by GraphPad Prism 6 software.

$$\text{NO Relative Expression Level (\%)} = \frac{\text{(Experimental group - Control group)}}{\text{(LPS-treated group - Control group)}} \times 100\ \%$$

Compound **1** was assessed for its anti-inflammatory properties by inhibiting NO release in LPS-activated RAW264.7 cells. As a result, compound **1** showed inhibitory activity against NO production with an IC_{50} value of 17.6±0.46 μM.

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Supporting Information

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