SHORT REPORT

A C G publications

Rec. Nat. Prod. X:X (2025) XX-XX

records of natural products

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

Qing Liu [1], Wei-Jie Tian [1], Lei-Lei He [1], Zheng-Hui Li [1] and Bao-Bao Shi [1,2*]

[1]*School of Pharmaceutical Sciences, South-Central MinZu University, Wuhan 430074, People's Republic of China*

[2] *International Cooperation Base for Active Substances in Traditional Chinese Medicine in Hubei Province, School of Pharmaceutical Sciences, South-Central Minzu University*

(Received March 17, 2025; Revised April 17, 2025; Accepted April 18, 2025)

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].

* Corresponding author: Email: shibb0505@163.com (B.-B. Shi)

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 202x EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.514.2503.3464
Available online: April 29, 2025

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].

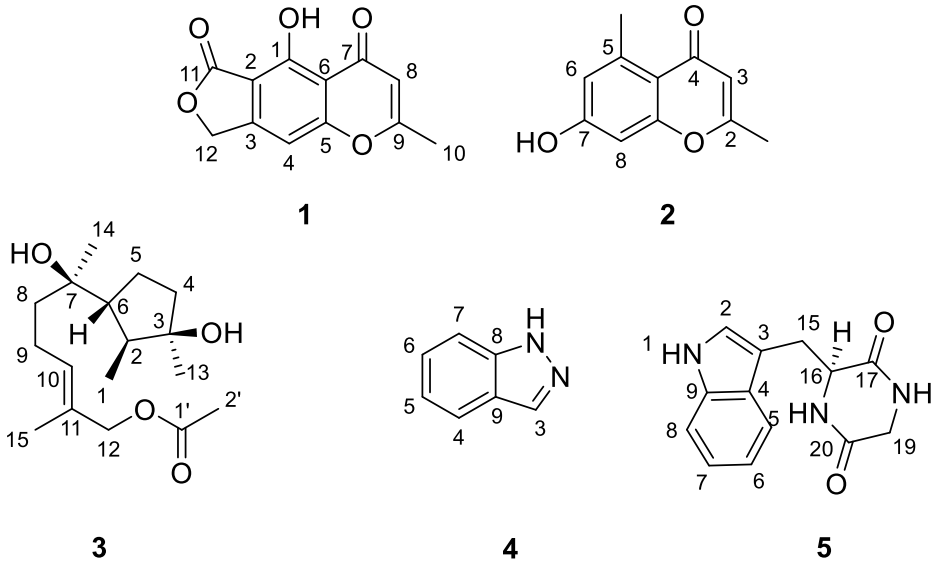

Figure 1. The chemical structures of compounds **1**–**5**

Fusacintone A, a new polyketide

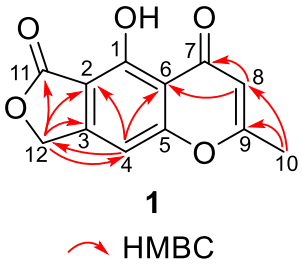

Figure 2. Key HMBC correlations of compound **1**

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.

Acknowledgments

This work was financially supported by the Hubei Provincial Natural Science Foundation of China (2022CFB920), and South-Central University for Nationalities (YZY24019).

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID

Qing Liu: 0009-0004-3573-0596
Wei-Jie Tian: 0009-0002-0041-7618

Lei-Lei He: 0009-0002-0632-2273
Zheng-Hui Li: 0000-0003-1284-0288
Bao-Bao Shi: 0000-0001-9138-5628

References

[1] S. Waqar, A. A. Bhat and A. A. Khan (2024). Endophytic fungi: unravelling plant-endophyte interaction and the multifaceted role of fungal endophytes in stress amelioration, *Plant Physiol. Biochem.* **206**, 108174.
[2] F. E. Vega, A. Simpkins, M. C. Aime, F. Posada, S. W. Peterson, S. A. Rehner and A. E. Arnold (2010). Fungal endophyte diversity in coffee plants from Colombia, Hawai'i, Mexico and Puerto Rico, *Fungal Ecol.* **3**, 122-138.
[3] M. Erb and D. J. Kliebenstein (2020). Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy, *Plant Physiol.* **184**, 39-52.
[4] B. Schulz, C. Boyle, S. Draeger, A. K. Römmert and K. Krohn (2002). Endophytic fungi: a source of novel biologically active secondary metabolites, *Mycological Res.* **106**, 996-1004.
[5] E. Feizollahi and M. S.Roopesh (2022). Mechanisms of deoxynivalenol (DON) degradation during different treatments: A review, *Crit. Rev. Food Sci. Nutr.* **62**(**21**), 5903-5924.
[6] H. Zhang, M. Dong, Q. Yang and X. Zhang (2016). Biodegradation of zearalenone by *Saccharomyces cerevisiae*: Possible involvement of ZEN responsive proteins of the yeast, *J. Proteomics* **143**, 416-423.
[7] C. X. Jiang, J. Li, J. M. Zhang, B. Yu, J. G. Fang and Q. X. Wu (2019). Isolation, identification, and activity evaluation of chemical constituents from soil fungus *Fusarium avenaceum* SF-1502 and endophytic fungus *Fusarium proliferatum* AF-04, *J. Agric. Food Chem.* **67**(**7**), 1839-1846.
[8] J. T. Ma, X. X. Qin, X. Wang and J. He (2022). Pyranones from kiwi-associated fungus *Fusarium tricinctum* and their antibacterial activity, *Phytochem. Lett.* **49**, 167-170.
[9] M. Moussa, W. Ebrahim, M. Bonus, H. Gohlke, A. Mándi, T. Kurtán and P. Proksch (2019). Co-culture of the fungus *Fusarium tricinctum* with *Streptomyces lividans* induces production of cryptic naphthoquinone dimers, *R.S.C. Adv.* **9**(**3**), 1491-1500.
[10] J. Phaopongthai, S. Wiyakrutta, D. Meksuriyen, N. Sriubolmas and K. Suwanborirux (2013). Azole-synergistic anti-candidal activity of altenusin, a biphenyl metabolite of the endophytic fungus *Alternaria alternata* isolated from *Terminalia chebula* Retz, *The J. Microbiol. Method.* **51**, 821-828.
[11] S. T. Fang, Y. J. Wang, X. Y. Ma, X. L. Yin and N. Y. Ji (2019). Two new sesquiterpenoids from the marine-sediment-derived fungus *Trichoderma harzianum* P1-4, *Nat. Prod. Res.* **33**, 3127-3133.
[12] P. L. Yuan, X. P. Wang, K. X. Chen, Y. M. Li and Q. Jia (2016). Chemical constituents from the stems of *Solanum torvum*, *Chinese Trad. Patent Medicin.* **38**, 104-107.
[13] J. P. Guo, J. L. Tan, Y. L. Wang, H. Y. Wu, C. P. Zhang, X. M. Niu and K. Q. Zhang (2011). Isolation of talathermophilins from the thermophilic fungus *Talaromyces thermophilus* YM3-4, *J. Nat.Prod*. **74**, 2278-2281.
[14] J. S. Gray, G. C. J. Martin and W. Rigby (1967). Aurofusarin, *J. Am. Chem. Soc*. 2580-2587.
[15] Y. Chen, L. T. Sun, H. X. Yang, Z. H. Li, J. K. Liu, H. L. Ai and T. Feng (2020). Depsidones and diaryl ethers from potato endophytic fungus *Boeremia exigua*, *Fitoterapia* **141**, 104483.