Isotryptoquivaline F, a new quinazolinone derivative with anti TNF-α activity from Aspergillus sp. CM9a

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1. Introduction

Endophytes are a group of microorganisms living within plant internal tissues or organs without causing any apparent symptoms or diseases in the hosts. They can serve as important sources of bioactive compounds, presumably due to the symbiotic relationship with their hosts (1). More recently, endophytes have been considered to be a prolific source of pharmacologically active natural products with potential medicinal or agrochemical applications (2,3). And we have started to investigate endophytic fungi as a source for biologically active natural products, and isolated a series of new compounds from endophytic microorganisms (4-9).

As part of our continuous screening for more active secondary metabolites from endophytic microorganisms, 11 compounds have been identified from strain Aspergillus sp. CM9a (9), and this time, isotryptoquivaline F (1) (Figure 1) was obtained from the fermentation extracts of A. sp. CM9a and it showed good anti TNF-α activity.

2. Materials and Methods

2.1. General experimental procedures

Mass spectra were measured using a Bruker DRX-600 NMR spectrometer; NMR spectra were measured on Bruker DRX-600 NMR spectrometers with tetramethylsilane (TMS) as an internal standard. Reversed-phase (RP) C18 silica gel for column chromatography (CC) was obtained from Merck and Sephadex LH-20 was obtained from Amersham Biosciences. Silica gel (200-300 mesh) for CC and silica gel GF254 for TLC were purchased from Qingdao Marine Chemical Ltd., Qingdao, Shandong, China. DMEM culture media was purchased from Gibco BRL. TNF-α was purchased from Sigma. And Cell Counting Kit-8 (CCK-8) was obtained from Dojindo, Japan.

2.2. Microorganism specimens

The fungal stain Aspergillus sp. CM9a was isolated from the current-year stems of Cephalotaxus mannii collected from Xishuangbanna, Yunnan, China (9). It was deposited at China Center for Type Culture Collection (CCTCC No: M2011006).

2.3. Fermentation and isolation of compound 1

The strain was incubated for 14 d at 28°C on potato-dextrose-agar (PDA) medium. The fermentation culture was extracted with EtOAc/MeOH/AcOH (80:15:5), and the extract partitioned between H2O/EtOAc.

The EtOAc extract (4.2 g) was separated to nine fractions (Fr. A-H) by column chromatography (RP-18, 80 g), eluted with MeOH/H2O (0:100, 40:60, 60:40, and 100:0). These fractions were further purified by repeated column chromatography on Sephadex LH-20, RP-18.

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Summary

Isotryptoquivaline F (1) was isolated from Aspergillus sp. CM9a, an endophytic fungus of Cephalotaxus mannii. The structure was elucidated by extensively 1D and 2D NMR and HR ESI MS spectroscopy. It has good TNF-α antagonistic effect, and can be used for anti-inflammatory drugs or other bioactive leading drugs.

Keywords: Cephalotaxus mannii, TNF-α antagonistic effect, anti-inflammatory drugs
Table 1. The NMR assignments for compound 1 in CD3OD. Recorded at 600/150 MHz (δ in ppm, J in Hz)

| Position | 13C | 1H (mult, J in Hz) | HMBC | 1H, 1H COSY |
|----------|-----|--------------------|------|-------------|
| 2        | 84.6 d | 5.60 (s,1H) | C3 | / |
| 3        | 78.1 s | / | / | / |
| 4        | 137.4 s | / | / | / |
| 5        | 125.7 d | 7.49 (d,1.6,1H) | C3, C9, C4 | H6 |
| 6        | 127.0 d | 7.26 (t,7.6,1H) | C9, C5, C7, C8, C4 | / |
| 7        | 131.3 d | 7.43 (t,7.7,1H) | C9, C5, C6, C8, C4 | / |
| 8        | 116.2 d | 7.58 (m,1H) | C3, C9, C5, C6, C4 | H7 |
| 9        | 140.4 s | / | / | / |
| 10       | 167.5 s | / | / | / |
| 11       | 167.5 s | / | / | / |
| 12       | 167.5 s | / | / | / |
| 13       | 39.5 t | 2.64 (d,3.0,13.0,1H) | C14, C2, C3 | / |
| 14       | 173.6 s | / | / | / |
| 15       | 61.4 d | 4.60 (dd,7.0,6.4,1H) | C14, C27 | H27 |
| 16       | 162.4 s | / | / | / |
| 17       | 122.7 s | / | / | / |
| 18       | 128.0 d | 7.71 (d,8.1,1H) | C18, C24, C22, C24 | / |
| 19       | 136.0 d | 7.86 (dd,8.1,7.2,1H) | C23, C24 | / |
| 20       | 128.7 d | 7.60 (m,1H) | C19 | H21 |
| 21       | 127.7 d | 8.24 (t,7.2,1H) | C21 | H22 |
| 22       | 148.6 s | / | / | / |
| 23       | 148.6 s | / | / | / |
| 24       | 148.6 s | / | / | / |
| 25       | 133.3 q | 1.73 (d,6.9,3H) | C15, C14 | / |

Silica gel and silica gel.

Fr. C (699 mg) was separated by CC (RP-18, 80 g, MeOH/H2O 30:70; 40:60; 50:50) to give four fractions (Fr. C1-C4). Fr. C2 was separated to four fractions (Fr. C2a-C2d) by CC (Sephadex LH-20, MeOH). Fr. C2d was subjected to CC (Sephadex LH-20, acetone) to afford Fr. C2d3 (13 mg). Compound 1 (5 mg) was finally purified by Sephadex LH-20 eluted with acetone from Fr. C2d3.

2.4 Biological study

The anti TNF-α (Tumor necrosis factor-α) activity was evaluated against mouse fibroblast cell line L929 with TNF-α at 3 ng/mL for 24 h by WST-8 colorimetric assay (Cell Counting Kit, Dojindo, Japan).

The trypsin-dispersed cells L929 in 100 μL of DMEM culture medium containing 10% FBS were plated in each well of 96-well plates (Falcon, CA) at density of 10⁵ cells/mL. After growing for 24 h, the cells were washed with fresh culture media and then treated in triplicate with various concentrations of compound 1 (95 μL DME and 3 μL TNF-α and 2 μL compound in DMSO, and 97 μL DME and 3 μL DMSO as negative control; and 97 μL DME and 3 μL TNF-α as blank control) for 24 h at 37°C. Then 90 μL fresh DEME media and 10 μL CCK-8 (cell counting kit-8) solution were added directly to all wells and incubated for 2 h at 37°C.

The optical density of each well was measured with a microplate reader (M-3350, Bio-Rad) at 450 nm. Cell survival rate was calculated by the following equation: cell survival rate = (ODcontrol − ODtreated)/ODcontrol × 100%.

3. Results and Discussion

3.1. Elucidation of structure

Compound 1, [α]D20 = −27.9 (c 0.43, MeOH), was obtained as white powder, and was determined to have the molecular formula C22H18N4O4 by HR-ESI-MS (403.1251 [M + H]+, 425.1031 [M + Na]+) and 13C-NMR.

Its 1H-NMR spectrum exhibited one methyl doublet at δ 1.73 (3H, d, J = 6.9 Hz), one methylene signals at δ 2.64 (dd, J = 3.0, 13.0 Hz), two methine signals at δ 4.60 (dd, J = 7.0, 6.4 Hz), 5.60 (s), nine aromatic protons at δ 7.26 (1H, t, J = 7.6 Hz), 7.43 (1H, t, J = 7.6 Hz), 7.49 (1H, d, J = 1.6 Hz), 7.58 (1H, m), 7.60 (1H, m), 7.71 (1H, d, J = 8.1 Hz), 7.86 (1H, t, J = 8.1 Hz), 8.24 (1H, t, J = 7.2 Hz) and 8.23 (s). The 13C-NMR and DEPT spectra (Table 1) displayed signals of three carbonyls (δ 173.6, 167.5, 162.4), five quaternary sp² (δ 167.5, 148.6, 140.4, 137.4, 122.7), nine methine sp³ (δ 148.6, 136.0, 131.3, 128.7, 128.0, 127.7, 127.0, 116.2), one quaternary sp³ (δ 78.1), two methine sp³ (δ 84.6 and 61.4), one methylene sp³ (δ 39.5) and one methyl carbons (δ 13.3).

The coupling system of the aromatic protons observed in the COSY spectrum (Table 1) revealed the presence of two 1, 2-disubstituted benzene rings. Analysis of the HMBC spectrum (Table 1) indicated that one of the 1, 2-disubstituted rings was part of the quinazolin-4(3H)-one moiety while another belonged to the indole portion of the molecule. The HMBC correlations between the signals of H-20 (δ 7.71, d, 8.1,) and C-18 (δ 148.6) as well as between the signals of H-26 (δ 8.23, s) and C-24 (δ 148.6) permitted identification of the N-substituted quinazolin-4-one and a 6-5-5 gem-dimethyl imidazoindolone ring system was
evidenced by the HMBC correlations between the signals of H-2 and C-3, C-9; the signals of H-13 and C-2, C-3, C-11 as well as between the signals of H2-27 and C-15, and H-15 and C-14 and C-27. Above data suggested that compound 1 could correspond to the previously reported tryptoquivaline F or its C-12 epimer, tryptoquivaline J. (10,11). The main difference is that the five-membered spiro lactone in tryptoquivaline F turned into an olefin alcohol because of keto-enolic tautomerism, which further confirmed by the chemical shifts of C-11 and 12 (δ 170.8 and 57.0 in tryptoquivaline F (Figure 1); 167.5 and 167.5 in compound 1). The NOESY spectrum exhibited clearly correlations between the signals of H-2 and H-15. Whereas, the orientations of H-2 and H-15 are opposite in tryptoquivaline F (11).

Therefore compound 1 was identified as isotoryptoquivaline F because of the difference of the relative configuration of C-2 and C-15.

3.2. Biological study

The TNF-α inhibitory activity of 1 was dose-dependent manner (Figure 2), the survival rate of L929 cell lines rose from about 16.7% to 63.9% when the concentration of 1 changed from zero to 10 μg/mL (EC50 = 8.7 μM), which indicated that 1 had good activity against the necrotic cell death induced by TNF-α.

TNF-α is a pleiotropic cytokine that mediates biological activities in many immune-mediated inflammatory diseases such as rheumatoid arthritis, psoriasis, septic shock and inflammatory bowel disease (12). Blockage of the effect of TNF-α has been proved efficient for treating these diseases (13). However, the current clinically approved protein-based TNF-α inhibitors are capable of reducing TNF-α activity, but can have serious side effects (12).

Many natural compounds belonging to various classes such as phenolics, terpenes and alkaloids and cytchalasan have been found to inhibit the upstream signaling pathways to inhibit the expression of TNF-α (14,15), but there is no lead compound that can inhibit the excessive TNF-α or its downstream pathways. Here, we reported a new quinazolinone derivatives Isotoryptoquivaline F, that was prepared from an endophytic strain Aspergillus sp. CM9a and exhibited good anti-TNF-α activity.

This is the first report that quinazolinone derivative compound exhibit TNF-α inhibitory activity, while the detailed biological activity and identified target of 1 are on the way to elucidate.

Acknowledgments

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References

1. Gunatilaka AA. Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat. Prod. 2006; 69:509-526.
2. Strobel G, Daisy B, Castillo U, Harper J. Natural products from endophytic microorganisms. J Nat Prod. 2004; 67:257-268.
3. Tan RX, Zou WX. Endophytes: a rich source of functional metabolites. Nat Prod Rep. 2001; 18:448-459.
4. Zhao PJ, Wang HX, Li GH, Li HD, Liu J, Shen YM. Secondary metabolites from endophytic Streptomyces sp. Lz531. Chem Biodivers. 2007; 4:899-904.
5. Zhang J, Qian Z, Wu X, Ding Y, Li J, Lu C, Shen Y. Juchilymycin A and B, ansamycin macrodilactams from Streptomyces sp. Org Lett. 2014; 16:2752-2755.
6. Lu C, Li Y, Deng J, Li S, Shen Y, Wang H, Shen Y. Hygrocins C-G, cytotoxic naphthoquinone ansamycins from gdmAl-disrupted Streptomyces sp. LZ35. J Nat Prod. 2013; 76:2175-2179.
7. Tan QF, Yan XF, Lin X, Huang YJ, Zheng ZH, Song SY, Lu CH, Shen YM. Chemical constituents of the endophytic fungal strain Phomopsis sp NZX-05 of Camptotheca acuminata. Helv Chim Acta. 2007; 90:1811-1817.
8. Hu ZY, Li YY, Huang YJ, Su WJ, Shen YM. Three new sesquiterpenoids from Xylaria sp NCY2. Helv Chim Acta. 2008; 91:46-52.
9. Xue H, Lu CH, Liang LY, Shen YM. Secondary metabolites of Aspergillus sp CM9a, an endophytic fungus of Cephalotaxus mannii. Rec Nat Prod. 2012; 6:28-34.
10. Yamazaki M, Okuyama E, Machayashi Y. Isolation of some new tryptoquivaline-related metabolites from Aspergillus fumigatus. Chem Pharm Bull. 1979; 27:1611-1617.
11. Buttachon S, Chandrapatya A, Manoch L, Silva A, Gales L, Bruyere C, Kiss R, Kijjoa A. Sartorymensin, a new indole alkaloid, and new analogues of tryptoquivaline and fiscalins produced by Neosartorya siamensis (KUFC 6349). Tetrahedron. 2012; 68:3253-3262.

12. Palladino MA, Bahjat FR, Theodorakis EA, Moldawer LL. (2003) Anti-TNF-α therapies: the next generation. Nat Rev Drug Discov. 2003; 2:736-746.

13. Morel J, Berenbaum F. Signal transduction pathways: new targets for treating rheumatoid arthritis. Joint Bone Spine. 2004;71:503-510.

14. Paul AT, Gohil VM, Bhutani KK. Modulating TNF-α signaling with natural products. Drug Discov Today. 2006; 11:725-732.

15. Liu J, Hu Z, Huang H, Zheng Z, Xu Q. Aspochalasin U, a moderate TNF-α inhibitor from Aspergillus sp. J Antibiot. 2012; 65:49-52.

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