Brief Report

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

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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

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 Bio TOF-Q 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 GF_{254} 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-dexrose-agar (PDA) medium. The fermentation culture was extracted with EtOAc/MeOH/AcOH (80:15:5), and the extract partitioned between H_2O/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/H_2O (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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Table 1. The NMR assignments for compound 1 in CD_3OD. Recorded at 600/150 MHz (δ in ppm, *J* in Hz)

Position	^{13}C	^{1}H (mult, *J* in Hz)	HMBC	$^{1}H,^{1}H$ 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			
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
18	162.4 s			
19	122.7 s	/	/	/
20	128.0 d	7.71 (d,8.1,1H)	C18, C24, C22, C24	/
21	136.0 d	7.86 (dd,8.1,7.2,1H)	C23, C24	H22, H20
22	128.7 d	7.60 (m,1H)	C19	H21
23	127.7 d	8.24 (t,7.2,1H)	C21	H22
24	148.6 s	/	/	
26	148.6 d	8.23 s	/	/
27	13.3 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/H_2O 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 10^6 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 DEME and 3 μL TNF-α and 2 μL compound in DMSO, and 97 μL DEME and 3 μL DMSO as negative control; and 97 μL DEME 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 = $(OD_{control} - OD_{treated})/OD_{control} \times 100\%$.

3. Results and Discussion

3.1. *Elucidation of structure*

Compound **1**, $[\alpha]_D^{20} = -27.9$ (*c* 0.43, MeOH), was obtained as white powder, and was determined to have the molecular formula $C_{22}H_{18}N_4O_4$ by HR-ESI-MS (403.1251 $[M + H]^+$, 425.1031 $[M + Na]^+$) and ^{13}C-NMR.

Its ^{1}H-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 ^{13}C-NMR and DEPT spectra (Table 1) displayed signals of three carbonyls (δ 173.6, 167.5, 162.4), five quaternary sp^2 (δ 167.5, 148.6, 140.4, 137.4, 122.7), nine methine sp^2 (δ 148.6, 136.0, 131.3, 128.7, 128.0, 127.7, 127.0, 125.7, 116.2), one quaternary sp^3 (δ 78.1), two methine sp^3 (δ 84.6 and 61.4), one methylene sp^3 (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-26 (δ 8.23, s) and C-24 (δ 148.6) as well as between the signals of H-20 (δ 7.71, d, 8.1,) and C-18 (δ 162.4), C-24 (δ 148.6), C-19 (δ 122.7) 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 H_3-27 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 mainly difference is that the five-membered spirolactone 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 isotryptoquivaline 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 (EC_{50} = 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

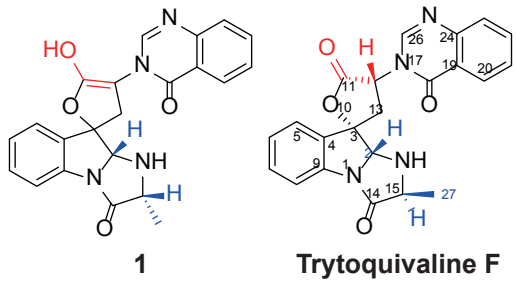

Figure 1. The chemical structures of compound 1 and trytoquivaline F.

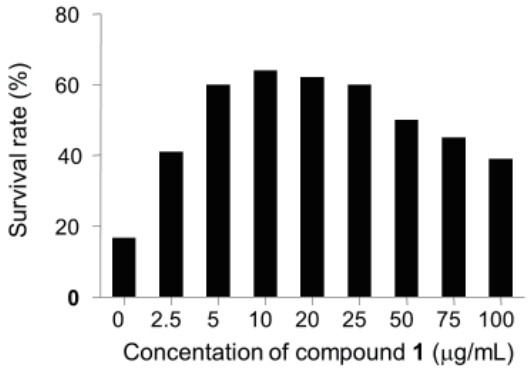

Figure 2. Dose-dependent action of compound 1.

(*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 cytochalasan 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 Isotryptoquivaline F, that was prepared from an endophytic strain *A.* 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. Juanlimycins 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 gdmAI-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 NXZ-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, Maebayashi 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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