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Diphenyl ethers from *Aspergillus* sp. and their anti-Aβ_{42} aggregation activities

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ABSTRACT

Two new compounds with the character of diphenyl ether structure, oxisterigmatocystin D (**1**) and 9-acetyldiorcinol B (**6**), were isolated from the endolichenic fungal strain *Aspergillus* sp. (No. 16-20-8-1), along with six known compounds, oxisterigmatocystin A (**2**), oxisterigmatocystin C (**3**), sterigmatocystin (**4**), diorcinol B (**5**), violaceol-I (**7**), and violaceol-II (**8**). The structures of the new compounds were determined by extensive NMR spectroscopic data, and the absolute configuration of **1** was established by single-crystal X-ray diffraction analysis. Moreover, the Aβ_{42} aggregation inhibitory activities of **5**–**8** were evaluated by the standard thioflavin T (ThT) fluorescence assay using epigallocatechin gallate (EGCG) as the positive control. Compounds **7** and **8** displayed significant anti-Aβ_{42} aggregation activity with IC_{50} values of 5.1 and 2.3 μM, respectively. Preliminary structure–activity relationship of these diphenyl ethers as anti-Aβ_{42} aggregation inhibitors was proposed.

1. Introduction

The diphenyl ethers are reported from microorganisms, plantae and animalia, including *Aspergillus* sp. [1], *Cordyceps* sp. [2], *Dendrospora* sp. [3], *Dysidea* sp. [4–7], *Kirschsteiniothelia* sp. [8], *Neoplaconema* sp. [9], *Penicillium* sp. [10,11], *Leathesia nana* [12], *Phyllanthus atropurpureus* [13], *Symphyocladia latiuscula* [14], and so on. Diphenyl ethers exhibit a wide range of interesting biological activities, such as antimicrobial [1,3,6,8,10], cytotoxic [2,6,9], radical-scavenging [11], enzyme inhibitory [5], actin inhibitory [15], anti-HSV-1 [2], and phytocidal activities [16,17]. In early chemical investigation, we reported the isolation of two diphenyl ethers, 2-isopentenyldiorcinol (**9**) and diorcinol (**10**), from a strain of endolichenic fungus *Aspergillus* sp. (No. 16-20-8-1) [18]. In subsequent bioactive screening, diorcinol (**10**) showed significant Aβ_{42} aggregation inhibitory activity with IC_{50} value of 20.1 μM, while 2-isopentenyldiorcinol (**9**) was inactive. It is firstly discovered that diorcinol (**10**) with the character of diphenyl ether structure has anti-Aβ_{42} aggregation activity. However, not all of diphenyl ethers displayed anti-Aβ_{42} aggregation activity. This phenomenon arouses our interest in the structure–activity relationship of these diphenyl ethers as anti-Aβ_{42} aggregation inhibitors.

Alzheimer's disease (AD) is one of neurodegenerative diseases, which is characterized by the aggregation of amyloid β peptides (Aβ) into fibrillar plaques [19]. Therefore, blocking the neurotoxicity of Aβ is one of the strategies for AD treatment. In order to search more diphenyl ethers with Aβ_{42} aggregation inhibitory activity and to reveal their structure–activity relationship as anti-Aβ_{42} aggregation inhibitors, the solid culture of the strain *Aspergillus* sp. (No. 16-20-8-1) was re-fermented at the same scale. Further chemical investigation

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was carried out, which led to the isolation of eight compounds with the character of diphenyl ether structure, including four sterigmatocystins (**1**–**4**) and four diphenyl ethers (**5**–**8**). Among them, there were two new compounds, oxisterigmatocystin D (**1**), 9-acetyldiorcinol B (**6**), and six known compounds, oxisterigmatocystin A (**2**) [20], oxisterigmatocystin C (**3**) [20], sterigmatocystin (**4**) [21], diorcinol B (**5**) [22], violaceol-I (**7**) [23] and violaceol-II (**8**) [23] (Fig. 1).

Sterigmatocystins are a kind of toxins with toxicological, mutagenic and carcinogenic effects in animals, which have been recognized as a 2B carcinogen (possibly carcinogenic to humans) [24,25]. In view of the treatment of AD, the $A\beta_{42}$ aggregation inhibitory activities of **1**–**4** were not evaluated. The $A\beta_{42}$ aggregation inhibitory activities of **5**–**8** were evaluated by the standard thioflavin T (ThT) fluorescence assay using epigallocatechin gallate (EGCG) as the positive control. The ThT fluorescence assay showed that **7** and **8** displayed significant $A\beta_{42}$ aggregation inhibitory activity with IC_{50} values of 5.1 and 2.3 μM, respectively, while the inhibitory activities of **5** and **6** were weak. It is firstly reported that the diphenyl ethers (**7**, **8** and **10**) have anti-$A\beta_{42}$ aggregation activity. The isolation, characterization of **1**–**8** and anti-$A\beta_{42}$ aggregation effects of **5**–**10** are reported herein.

2. Experimental

2.1. General

Optical rotations were measured on a JASCO P-1020 digital polarimeter, and UV data were recorded on a JASCO V-550 UV/vis spectrometer. IR spectra were obtained using a JASCO FT/IR-480 plus spectrometer. ESI-MS spectra were recorded on a Finnigan LCQ Advantage MAX mass spectrometer. HR-ESI MS spectra were recorded using a Waters Synapt G2 mass spectrometer. 1D and 2D NMR spectra were recorded using a Bruker AV-400 spectrometer using solvent signals (DMSO-d_6: δ_H 2.50/δ_C 39.5) as the internal standards. The analytical HPLC was performed on a Dionex HPLC system equipped with an Ultimate 3000 pump, an Ultimate 3000 diode array detector (DAD), an Ultimate 3000 Column Compartment, an Ultimate 3000 autosampler (Dionex, USA) and an Alltech (Grace) 2000 ES evaporative light scattering detector (ELSD) (Alltech, USA) using a reversed-phase C18 column (5 μm, 4.6 × 250 mm; Phenomenex, Gemini). The semi-preparative HPLC was carried out on a Shimadzu LC-6AD Liquid Chromatography with a SPD-20A Detector (Shimadzu, Japan) using a reversed-phase C18 column (5 μm, 10.0 × 250 mm; Phenomenex, Gemini). Column chromatography (CC) was carried out on silica gel (200–300 mesh) (Qingdao Haiyang Chemical Group Corporation, Qingdao, China), Sephadex LH-20 (Pharmacia) and ODS (50 μm, YMC). TLC was performed on precoated silica gel plate (SGF254, 0.2 mm, Yantai Chemical Industry Research Institute, China).

2.2. Cultivation of *Aspergillus* sp. (No. 16-20-8-1)

The strain of *Aspergillus* sp. (No. 16-20-8-1) was isolated from *Peltigera elisabethae* var. *mauritzii* (Gyeln.) J. C. collected in Changbai Mountains, Jilin Province, China, in November

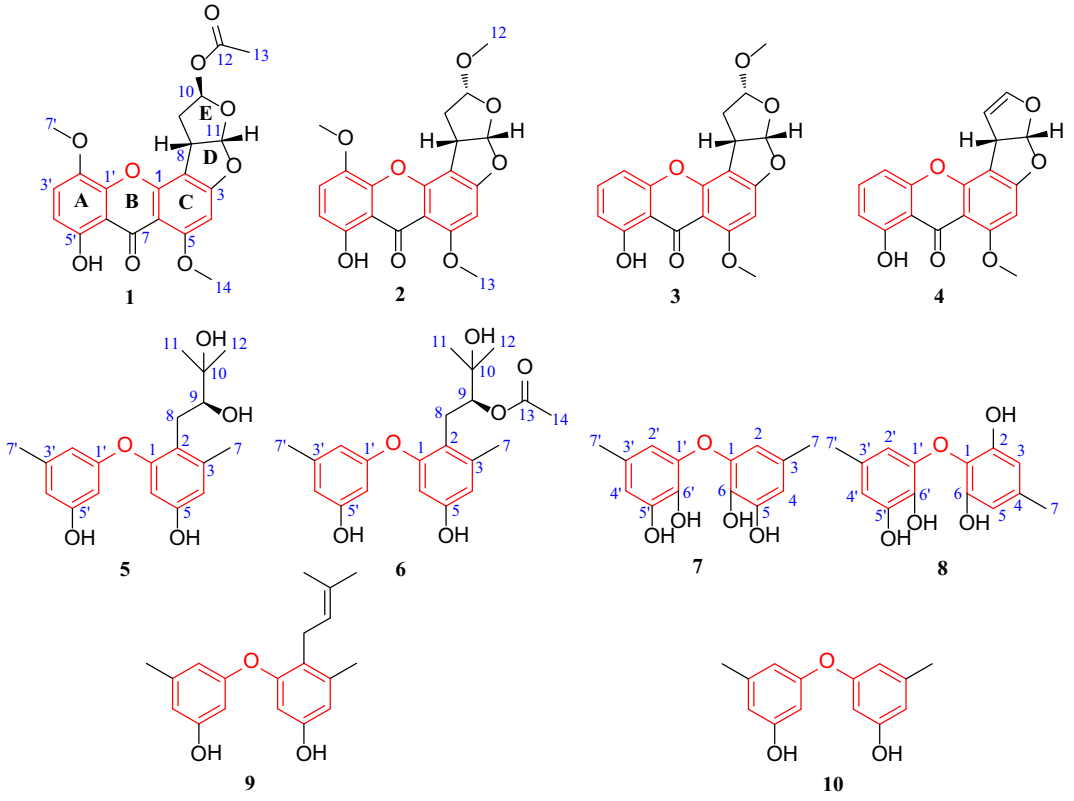

Fig. 1. Chemical structures of **1**–**10**.