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An endophytic Fungi of *Ginkgo biloba* L. produces antimicrobial metabolites as potential inhibitors of FtsZ of *Staphylococcus aureus*

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ABSTRACT

A total of 58 fungal isolates, belonging to 24 genera, were obtained from the leaves, stems and roots of *Ginkgo biloba* L.. Among them, one endophytic fungal strain, *Penicillium cataractum* SYPF 7131, displayed the strongest antibacterial activity. Four new compounds (**1–4**) were isolated from the strain fermentation broth together with four known compounds (**5–8**). These structures were determined on the basis of 1D and 2D NMR and $[Rh_2(OCOCF_3)_4]$-induced electronic circular dichroism (ECD) spectroscopic analyses. All the isolated compounds were screened for their in vitro antimicrobial activities. Compound **3** and **4** showed moderate inhibitory activity against *Staphylococcus aureus*. Compound **7** exhibited significant inhibitory activity against *S. aureus* with MIC value of 10 μg/mL. Further, the in silico molecular docking studies of the active compounds was used to explore the binding interactions with the active site of filamentous temperature-sensitive protein Z (FtsZ) from *Staphylococcus aureus*. The docking results revealed that compounds **3**, **4** and **7** showed high binding energies, strong H-bond interactions and hydrophobic interactions with FtsZ from *S. aureus* validating the observed antimicrobial activity. Based on antimicrobial activities and docking studies, compounds **3**, **4** and **7** were identified as promising antimicrobial lead molecules.

1. Introduction

Ginkgo biloba L. is one of the most ancient plants on earth with fossil records dating back more than 200 million years [1, 2]. It has been widely used as an important and traditional medicine for various ailments [3]. It is scarcely infected with plant diseases and insect pests during a long life and has been used as traditional medicine for centuries in Asian [1]. Endophytic fungi play a key role in plant defense and can be used as a promising source for biocontrol agents [4]. During the whole life, plant endophytic fungi protect their host from infectious agents and also adapt to survive in adverse environmental conditions [5, 6]. Considering the unique specific symbiotic relationships between host plants and their associated endophytes, endophytic fungi may have special biochemical metabolites that may have great potential for pharmacological applications.

Microbial secondary metabolites represent a huge quantity of diverse chemical entities that have been developed as commercial products for human medicines [7–9]. Currently, the search for novel classes of antimicrobial metabolites represents one of the most important strategies for new drug development [10]. One purpose of this study was to examine the diversity of endophytic fungi isolated from the leaves, stems, and roots of *G. biloba*. In total, 58 fungi isolates were obtained and cultured to examine the antimicrobial activities of crude extracts. Fifteen percent of the isolates displayed antimicrobial activities against at least one microorganism. One isolate, *Penicillium cataractum* SYPF 7131, presented the strongest antibacterial activity.

The genus *Penicillium* is one of the most promising sources of physiologically active compounds, including antibiotics, hormones, mycotoxins, alkaloids, etc [11, 12]. The bioactive metabolites of this genus are attractive to exploring antimicrobial prodrugs [13, 14]. In our previous research, we have successive research on the discovery of the secondary metabolites with good antimicrobial and anticancer activities [15, 16]. This study aimed to isolate antimicrobial secondary metabolites of endophytic fungi from *G. biloba*. The investigation of the fermentation broth of *Penicillium cataractum* SYPF 7131 has led to the isolation of four new compounds (**1–4**) and four known compounds (**5–8**) (Fig. 1). In this paper, their structural elucidations were determined by 1D and 2D NMR and $[Rh_2(OCOCF_3)_4]$-induced electronic circular dichroism (ECD) spectroscopy. All compounds were evaluated for their antimicrobial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*. As results, compound **3**, **4** and **7** showed antibacterial activity

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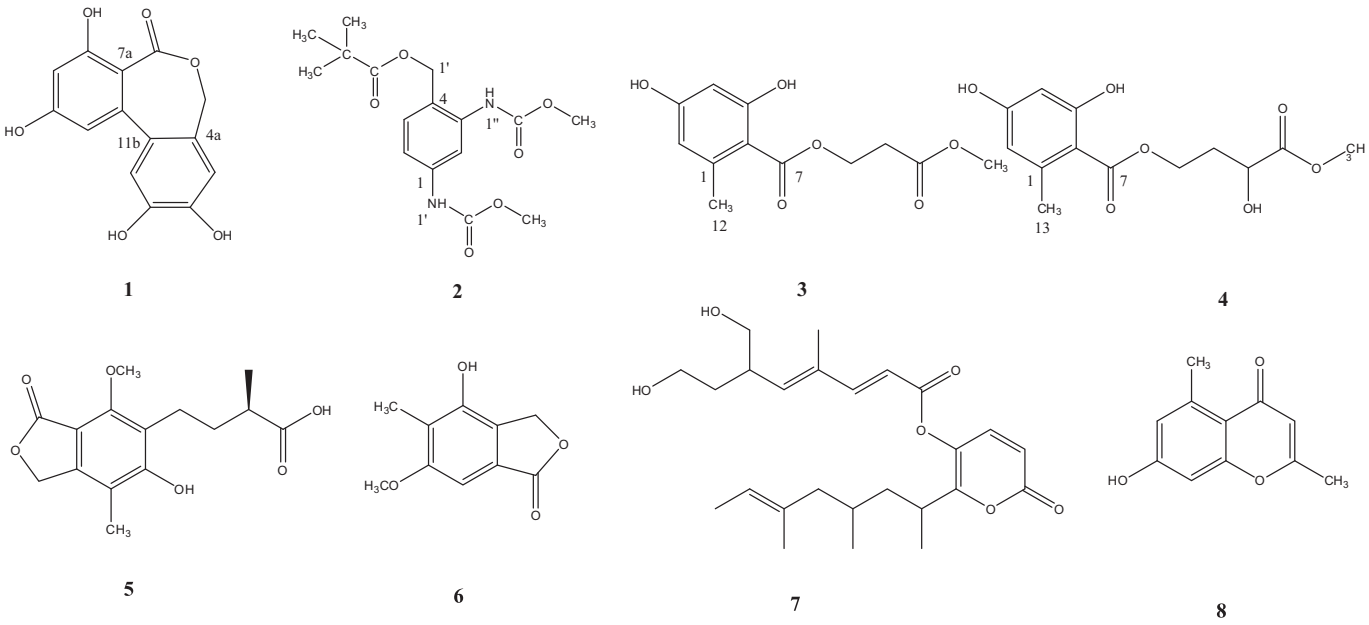

Fig. 1. Chemical structures of compounds **1–8**.

toward *S. aureus* (MIC 65 μg/mL, 59 μg/mL and 10 μg/mL).

Generally, almost all of the drugs used to treat bacterial infections target to one of four processes: bacterial protein synthesis, nucleic acid synthesis, cell-wall synthesis and folate synthesis [17]. However, the development of antibiotic resistance is a growing problem and bacteria can rapidly acquire resistance to the known drugs, which lead an urgency to discover new kind of antibiotics with novel mechanisms of actions [18]. Bacterial cell division is an essential process that is not yet targeted by clinically approved entities, but is an area of untapped potential with antibacterial activities now well underway [19]. Microbial filamentous temperature-sensitive protein Z (FtsZ) is homologous to eukaryotic tubulin that polymerizes into ring like structure at the mid cell and plays a central role in the protein network [19]. FtsZ is also the first protein to move to the division site and recruits other proteins that produce a new cell wall between the dividing cells [18]. Recently, FtsZ is a highly promising target for new antibacterial drugs because of its central role in bacterial cell division [19–21]. The inhibition of FtsZ can restrain bacterial cellular fission, further leading to bacterial apoptosis [22]. At the same time, the antimicrobial compound would not disturb the eukaryotic tubulin structure due to the evolutionary distance between bacterial FtsZ and eukaryotic tubulin [23]. So, molecular docking in this study was accomplished to explore the possible mechanism between the lead compounds and FtsZ from *S. aureus* (PDB ID: 3VOB) [19].

2. Experimental method

2.1. General experimental procedures

Optical rotations were measured with a P-2000 Digital Polarimeter (JASCO, United Kingdom). IR spectra were obtained with an Equinox55 spectrophotometer in KBr discs (Bruker Optik BmbH, Ettlingen, Germany). The NMR spectra were run on Bruker AVANCE-125 or AVANCE-600 NMR spectrometers (Rheinstetten, Germany). HR-ESI-MS data were obtained on a Bruker Customer micr OTOF-Q 125 mass spectrometer (MA, Germany). All solvents were obtained from Tianjin Kemiou Chemical Reagent Company (Tianjing, China), CH_3CN and MeOH for HPLC analysis were chromatographic grade (Merck, Darmstadt, Germany). Silica gel (200–300 mesh) was purchased from Qingdao Marine Chemical Factory (Qingdao, China).

2.2. Isolation and identification of endophytic Fungi

The general isolation and identification of endophytic fungi have been described in our previous research [10]. The samples of healthy branch of *G. biloba* were collected from Dandong city, Liaoning Province, northeast of China. After surface sterilization with 75% EtOH for 60 s, the branch of *G. biloba* was rinsed in sterile water. Then the branch was aseptically cut into small pieces and pressed onto potato dextrose agar plates. The growing fungi was further isolated, purified and cultured. The ITS sequence of strain SYPF 7131 was amplified submitted and deposited at GenBank with accession number MG779617. A BLAST search showed that the isolate has 100% identity to *Penicillium cataractum*. Its morphological features were also identical to *Penicillium cataractum* according to Persoonia et al. [15]. A voucher specimen (SYPF 7131) was deposited at the School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, China.

2.3. Fermentation and extraction

Sterile water (53 mL) and rice (40 g) were added to an Erlenmeyer flask (250 mL), and autoclaved at 121 °C for 30 min. The spore of *Penicillium cataractum* SYPF 7131was cultured in each Erlenmeyer flask for solid fermentation, 120 of Erlenmeyer flask were incubated at 28 °C for 30 days. The fermented material was soaked with ethyl acetate (12 L) for 3 times to afford the crude extract (26 g). Then it was dissolved using 90% MeOH–H_2O (1 L), and extracted by hexane (1 L) for 3 times to obtain the residue (18.2 g).

2.4. Isolation of secondary metabolites

The extract (18.2 g) was separated by silica gel for column chromatography (CC) and eluted with petroleum ether-EtOAc (80:1 to 0:100), yielding five fractions (A–E). Fraction C (6.27 g) was separated by ODS (MeOH-H_2O, 10:90 to 90:10) to afford eight fractions (C1-C8). Fraction C3 (1.2 g) was subjected to a silica gel CC using petroleum ether-EtOAc (10:1 to 1:1) as an eluent, yielding nine subfractions (C3-1–C3-9) and compound **5** (10 mg). Subfraction C3-4 (100 mg) was purified by preparative TLC (petroleum ether-EtOAc, 5:1) and preparative HPLC (MeOH–H_2O, 74%) to afford compound **1** (7.0 mg). Compound **2** (8 mg) was afforded from subfraction C-4-3 (20 mg)