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Secondary Metabolites from the Coral-Derived Fungus *Aspergillus terreus* SCSIO41404 with Pancreatic Lipase Inhibitory Activities

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Abstract: Ten secondary metabolites were isolated from cultures of the marine coral-derived fungus *Aspergillus terreus* SCSIO41404. The compounds were identified as monacolin K (**1**), methyl ester of lactone ring-opened monacolin K (**2**), asperterreusine C (**3**), 4-hydroxybenzaldehyde (**4**), 4-hydroxy-3-(3-methylbut-2-en-1-yl) benzaldehyde (**5**), kojic acid (**6**), *p*-hydroxyphenylacetic acid methyl ester (**7**), *o*-hydroxyphenylacetic acid methyl ester (**8**), N-(2-hydroxyphenyl)-acetamide (**9**), and (S)-methyl 2-acetamido-3-phenylpropanoate (**10**), by comparing the spectroscopic data with the reported literature values. They were evaluated for their cytotoxic, antibacterial, and enzyme (pancreatic lipase and acetylcholinesterase) inhibitory activities. Monacolin K (**1**) and its derivative (**2**) were revealed with obvious pancreatic lipase (PL) inhibitory effects. The *in silico* molecular docking with PL protein was further performed to understand the binding effects, and it is suggested that the ring opening of the monacolin K facilitates for the PL inhibitory activities.

Keywords: Coral-derived fungus; *Aspergillus terreus*; secondary metabolites; pancreatic lipase; molecular docking.

1. Fungal Source

The strain SCSIO41404 was isolated from a soft coral sample collected in Sanya Bay of Hainan Island, China, in June 2019. It was stored on Muller Hinton broth (MB) agar (malt extract 15 g, sea salt 10 g, agar 15 g, H_2O 1 L, pH 7.4–7.8) slants at 4 °C. The strain was deposited in the CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology,

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Chinese Academy of Sciences, Guangzhou, China. The strain was identified as *Aspergillus terreus* based on the ITS region of the rDNA (Supporting Information).

2. Previous Studies

Coral-derived microorganisms participate in the host's defense system by producing special secondary metabolites, which could be considered as a prominent source of new bioactive natural products [1]. The fungal genus *Aspergillus* is one of the most important species of coral-derived microorganisms, with over 180 species that can produce bioactive terpenoids, polyketides, and alkaloids [2]. In our ongoing study for searching bioactive natural compounds, two *Aspergillus* strains, *A. terreus* SCSIO41404 and *Aspergillus* sp. SCSIO41405, were isolated from the coral sample, and the secondary metabolites of the strain SCSIO41405 were reported in our previous study [3].

A. terreus is a common fungal strain which could be found almost everywhere, in soil, plants or marine organism, and even in our living environment. It has frequently been described as an important human pathogen. Many natural products have been reported form the soil-derived fungus *A. terreus* all over the world. For example, from a Tibet plateau-derived *A. terreus* strain, a number of compounds, decanoic acid (2,2-dimethyl-1,3-dioxolan-4-yl) methyl ester, 4,5-dimethylresorcinol, indole-3-acetic acid, aspulvinone H, dihydroisoflavipucine, 14-hydroxyterezine D, terezine D, cyclo-[L-(4-hydroxyprolinyl)-L-leucine], *R*(–)-glycerol monolinoleate, 5α,8α-epidioxyergosta-6,22-dien-3β-ol, ergosterol, butyrolactone I, and lactariamide B were obtained [4]. Chemical study of a *A. terreus* strain collected from "paramo de Guasca (Cundinamarca, Colombia)" led to the isolation of mannitol, butyrolactone I and *R*(–)-6-hydroxymellein [5].

Secondary metabolites from marine-derived fungus *A. terreus* are receiving more and more attention. Two new sesquiterpenes aspterrics A and B, and aspterric acid, were isolated from the deep-sea-derived *A. terreus* YPGA10 [6]. From a coral-derived *A. terreus*, three new compounds, luteoride E, a butenolide derivative named versicolactone G, (3*E*,7*E*)-4,8-dimethyl-undecane-3,7-diene-1,11-diol, were obtained [7], together with nine new butenolides, including (±)-asperteretones A-D, and a racemate asperteretone E [8]. Seven thiodiketopiperazines, including two new ones emestrins L and M, and five reported dihydroisocoumarins, were obtained from the sea hare-associated *A. terreus* RA2905 [9]. Chemical epigenetic modification on this strain resulted in an obviously changed metabolic profile, and led to the isolation of unreported benzyl furanone and pyrones, such as (±)-asperfuranone, asperpyranones A and B [10]. Marine-derived fungus *A. terreus* are usually productive, and may produce different metabolites under different fermentation conditions.

3. Present Study

After fermentation (Supporting Information), the culture of *A. terreus* SCSIO41404 was soaked and extracted by ethyl acetate (EtOAc). Silica gel and octadecylsilyl (ODS) column chromatography and semi preparative high-performance liquid chromatography (HPLC), were used for separation and led to the isolation of 10 purified compounds (Figure 1).

Those compounds were identified as monacolin K (**1**) [11], methyl ester of lactone ring-opened monacolin K (**2**) [12], asperterreusine C (**3**) [13], 4-hydroxybenzaldehyde (**4**) [14], 4-hydroxy-3-(3-methylbut-2-en-1-yl) benzaldehyde (**5**) [15], kojic acid (**6**) [16], *p*-hydroxyphenylacetic acid methyl ester (**7**) [17], *o*-hydroxyphenylacetic acid methyl ester (**8**) [18], N-(2-hydroxyphenyl)-acetamide (**9**) [19], and (S)-methyl 2-acetamido-3-phenylpropanoate (**10**) [20], by comparing the spectroscopic data (Supporting Information) with the reported literature values.

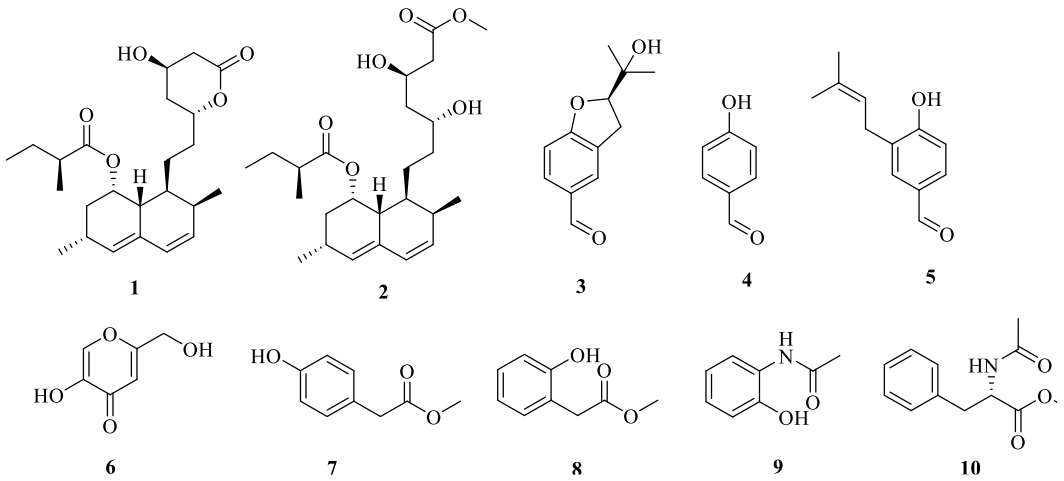

Figure 1. Structures of compounds **1-10.**

Compounds **1**–**10** were evaluated for their cytotoxic activities against human lung carcinoma cell line A549 cells and human hepatocellular carcinoma cell line HepG2 cells, but none of them showed obvious cytotoxicity with 50 μM. Then, those compounds were tested with antibacterial activities against five kinds of pathogenic bacteria, *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Klebsiella pneumoniae* (ATCC 13883), methicillin-resistant *Staphylococcus aureus* (MRSA, clinical strain) and methicillin-resistant *Staphylococcus epidermidis* (MRSE, clinical strain). Only **1** and **6** showed weak antibacterial activities with IC_{50} value of 50 μg/mL, against *Klebsiella pneumonia* and *Enterococcus faecalis*, respectively.

Moreover, the obtained compounds were screened for their enzyme inhibitory effects against pancreatic lipase (PL) and acetylcholinesterase (AChE) *in vitro*. As results, compounds **1** and **2** showed obvious inhibitory effects against PL with IC_{50} values 8.9 and 5.6 μM, respectively. Compounds **9** and **10** also showed weak activities against PL (IC_{50} value 23.5 μM) and AChE (IC_{50} value 18.6 μM), respectively. Orlistat and huperzine A were used as positive controls in the PL and AChE inhibitory assays, respectively (Table 1).

Table 1. The inhibitory activities of the compounds against pancreatic lipase and acetylcholinesterase

Compounds	**IC_{50} value (μM)**	
	PL	**AChE**
1	8.9 ± 0.64	>50
2	5.6 ± 0.61	>50
3-8	> 50	>50
9	23.5 ± 2.2	>50
10	> 50	18.6 ± 2.8
Positive controls	0.45 ± 0.08[a]	0.30 ± 0.06[b]

[a] Orlistat; [b] huperzine A

To better understand the molecular interactions between monacolin K (**1**) and its derivative (**2**) with PL protein, two main crystal structures was considered and subjected to molecular docking analysis *in silico*. One is the catalytic domain of human bile salt activated lipase (PDB: 1F6W, Resolution: 2.30 Å) [21], and another is human pancreatic lipase (PDB: 1LPB, Resolution: 2.46 Å) [22] from RCSB Protein Data Bank (www.rcsb.org/pdb). The Schrödinger 2017-1 suite (Schrödinger Inc., New York, NY) was employed, and crystal structures 1F6W and 1LPB were constructed following the Protein Prepare Wizard workflow in Maestro package. Orlistat, the only FDA approved drug that acts through inhibition of PL, was used as control [22]. The structures of **1**, **2** and Orlistat were generated in ChemBioOffice (v17.0), followed by an MM2 calculation to minimize the conformation energy. The binding site was produced using the Grid Generation procedure. The

prepared ligandswere then flexibly docked into the receptors using Glide (XP mode) with default parameters.

From the results of binding free energy values (Glide gscore values), crystal structures 1F6W is more suitable than 1LPB for binding with Orlistat (Figure S1). So, we also use the model of 1F6W to analysis the binding sites and the interaction details with **1** and 2. The molecules of **1** and 2 fit comfortably into the binding pocket with the similar binding positions in the models (Figure 2A and 2B), with the negative binding free energy values (Glide gscore values) of -6.714 (for **1**) and -6.848 (for **2**). In the predicted binding model of **1** with 1F6W (Figure 2C), the hydroxyl formed a hydrogen bond with the active-site residue ASP299, and the carbonyl in the lactone ring also formed hydrogen bond with the ILE229. While, in mode of the lactone ring-opened derivative (Figure 2D), there is a slight shift in the binding position. The extra hydroxyl group formed an extra hydrogen bond with the active-site residue PHE351 and THR354. Two carbonyl groups of **2** also interacted with the same active-site residue LYS231 by hydrogen bonds. The better binding effects in the docking mode of **2** with 1F6W might give us a rational explanation of the better PL inhibitory activities of the lactone ring-opened derivative of monacolin K (**2**).

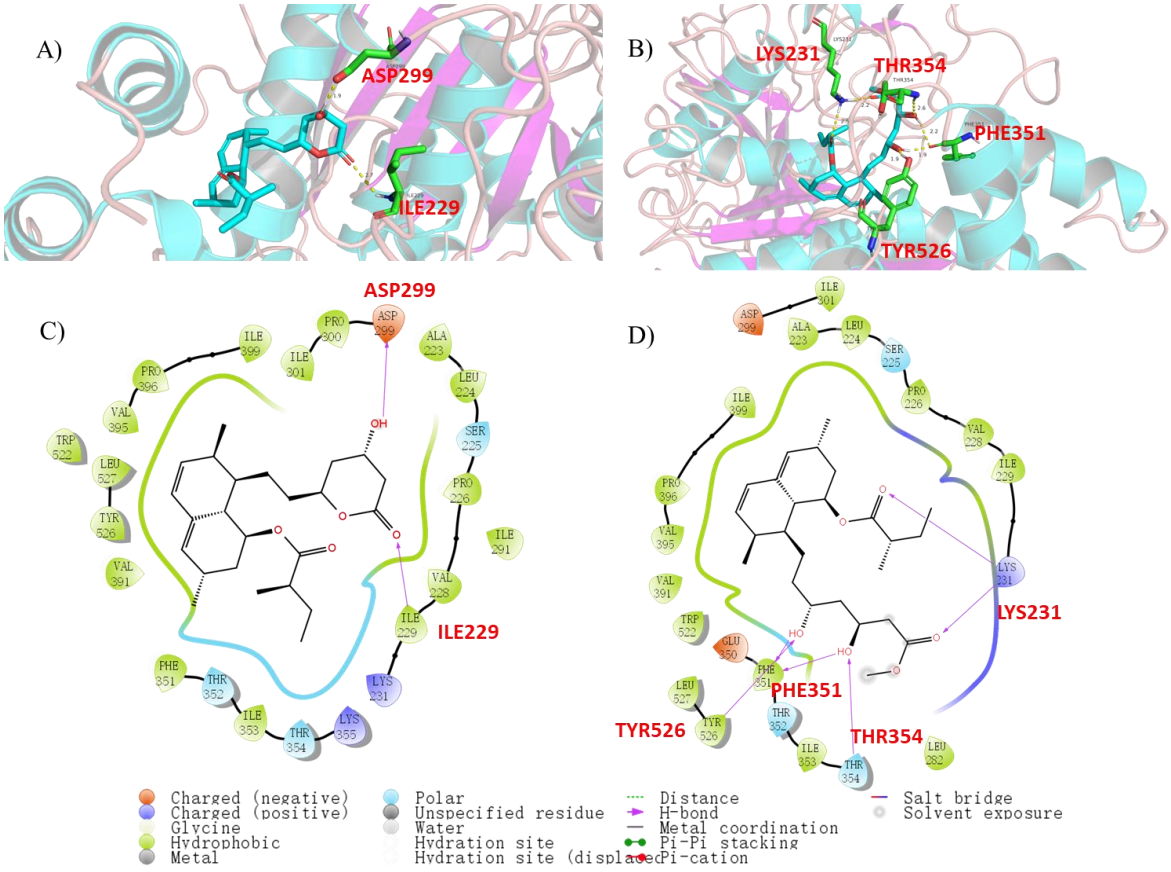

Figure 2. Molecular docking of **1** and **2** with PL (PDB code: 1F6W). A) Binding sites of the molecule **1** with the 1F6W crystal structures (part). B) Binding sites of the molecule **2** with the 1F6W crystal structures (part). C) The interaction details of the predicted binding mode of **1** with the 1F6W. D) The interaction details of the predicted binding mode of **2** with the 1F6W.

PL is considered as one of the suitable and important target for anti-obesity drug development. Monacolin K could inhibit the anabolism of lipid and affect lipid metabolism through SIRT1/AMPK pathway [23], however, whether it is also related to inhibiting PL is not clear. PL inhibitor could obviously interrupt the absorption and digestion of dietary lipids in a rat model [24]. Our study

revealed monacolin K (**1**) and its methyl ester of lactone ring-opened derivative (**2**) may act as PL inhibitors. Although the IC_{50} values of **1** and **2** against PL are inferior to that of the positive drug Orlistat, the activity intensity of this level is also of great concern, as inhibitors of natural origin [25]. This is of great significance for deeply understanding the lipid metabolism modulation of monacolin K.

In conclusion, 10 secondary metabolites were obtained and identified from the cultures of the marine coral-derived *Aspergillus terreus* SCSIO41404. After several bioassays, monacolin K (**1**) and its derivative (**2**) were revealed with obvious PL inhibitory activities. Molecular docking suggested that the ring opening of the monacolin K was facilitate for the PL inhibitory activities. The revealing of their PL inhibitory activities could help to understand the lipid metabolism modulation of monacolin K and its derivative.

Supporting Information

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

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