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Original Article

Metabolites isolated from *Penicillium* HDS-Z-1E, an endophytic fungal strain isolated from *Taxus cuspidata* and their activation effect of catalase

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ABSTRACT

Objective: To study the compounds isolated from *Penicillium* HDS-Z-1E, an endophytic fungal strain isolated from *Taxus cuspidata* and their activation effect of catalase (CAT).

Methods: The chemical constituents were isolated from *Penicillium* HDS-Z-1E, by using silica gel, Sephadex LH-20 and HPLC. The structural elucidations of five metabolites were elucidated on the basis of spectroscopic including ^{1}H-NMR, ^{13}C-NMR, HMBC and HSQC. Their activation sites of catalase have been investigated by molecular docking.

Results: Five metabolites, compounds (**1**–**5**) were isolated from *Penicillium* HDS-Z-1E and identified as 4-hydroxy-4-methyltetrahydro-2*H*-pyran-2-one (**1**), 4-hydroxymethyl-5, 6-dihydro-pyran-2-one (**2**), 5, 6-dihydro-2-oxo-2*H*-pyran-4-carboxylic (**3**), *N*-acetyl-hydrazinobenzoic acid (**4**), and methyl 2-(2, 5-dihydroxyphenyl) acetate (**5**).

Conclusion: Compound **3** is a new compound. Compounds **3** and **4** may have potential activators of catalase, providing a theoretical basis for the development of CAT activators.

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1. Introduction

As an important part of the medicinal plant microecosystem, the diversity of structure and activity of the secondary metabolites of medicinal plant endophyte were decided by the special living environment (Wang et al., 2015). The plant endophyte could promote the host plants to produce secondary metabolites, simultaneously produce bioactive substances that are the same or similar to the host plants, mainly including some alkaloids, terpenoids, steroids, quinones, cyclic peptides and fats (Lyu et al., 2018). It has found that plant endophytic fungi can promote the growth and development of host plants, increase the total biomass of host plants, enhance the resistance of host plants to the external environment, and promote the production of secondary metabolites of host plants (Guo et al., 2019). Therefore, medicinal plant endophytic microorganisms have great potential in protecting rare medicinal plant resources.

Taxus cuspidata Sieb. et Zucc is a variety of *Taxus cuspidata* var. *chinensis* (Pilger) Florin, which has the characteristics of short plant shape, many branches and fast growth (Lu et al., 2021). *Penicillium* sp. HDS-Z-1E is an endophytic fungal strain isolated from *T. cuspidata*. The strain was identified as *Penicillium* by sequencing. In this study, five compounds were isolated and purified from *Penicillium* sp. HDS-Z-1E, and their biological activities were studied. Compound **3** was found for the first time.

Catalase (CAT) is an important liver protective enzyme class of terminal oxidative enzymes widely found in animals, plants and microorganisms. Five compounds were separately docked with the different active sites of the catalase proteins. The interaction of compounds **1**–**5** with the target protein were evaluated with the "Glide-score" score function value, and the greater the absolute value indicates that the more stable the docking complex of the small molecules and the target protein and the better the matching binding effect.

2. Materials and methods

2.1. General

HPLC separation experiments were carried out using a system composed of Agilent 1260 (Agilent Technologies Inc., CA, USA) (XB C_{18} 10 mm × 250 mm, 5 μm) and LC-20AR (Shimadzu, Beijing, China) (XB C_{18} 10 mm × 250 mm, 5 μm) liquid chromatograph and the UV detection wavelength was 210 nm. Gel LH-20 (Sephadex, Sweden) and silica gel 100–200 mesh and 200–300 mesh (Qingdao Ocean Chemical Co., Ltd., Qingdao, China) for column chromatography (CC) were used. Precoated silica gel GF254 (Qingdao Marine

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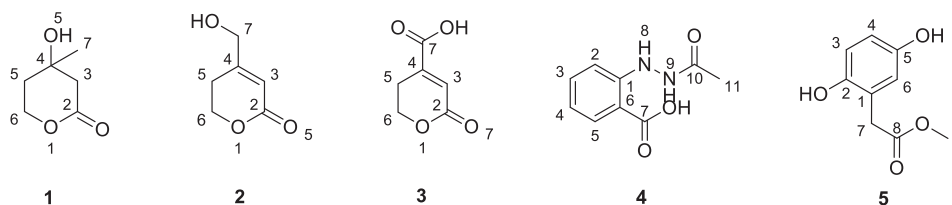

Fig. 1. Structures of compounds **1**–**5**.

Chemistry Co., Ltd., Qingdao, China) plates was used for TLC and PTLC. TLC results were observed with UV wavelengths of 254 nm and 365 nm. Using DMSO d_6 as solvent, the nuclear magnetic spectrum (^{1}H-NMR, ^{13}C-NMR, HMBC, HSQC etc.) of the separated compound samples were measured by Bruker AvanceⅡ(Bruker Analytical Instruments, Germany) 500 M nuclear magnetic resonance instrument. IKA RV8 / HB 10 rotary evaporator used to concentrate the extract was from German IKA company (Staufen, German). The reagents used for experimental separation and purification were analytical pure reagents, the reagents used for high performance liquid chromatography (HPLC) were preparation pure reagents, and the water was self-made ultrapure water and Watson purified water.

2.2. Materials

Plant material was collected from Changbai Mountain Area of Jilin Province, China. The plant endophytic fungu strain *Penicillium* sp. HDS-Z-1E which identified by Professor Huiguo Wang and used in this experiment was isolated from the branch part of *T. cuspidata*. It has been identified as *Penicillium* sp. by sequencing, serial number was KT876714.1. Now it preserved in the School of Life Science and Technology, Dalian University at Dalian City, China.

2.3. Extraction and isolation

The components of fungus No. 4 medium were 2% D-Mannitol, 2% glucose, 0.5% yeast extract, 1% peptone, 0.05% KH_2PO_4, 0.03% $MgSO_4 \cdot 7H_2O$, 0.1% corn pulp and 100 mL purified water. Strain HDS-Z-1E was inoculated into a conical flask (200 mL/ 500 mL) containing fungal medium No. 4 and cultured in a shaking table at 28 °C and 120 *r*/min for 7 d. Added 10 mL of fermentation broth and mycelium after 7 d of shaking culture into 500 mL conical flask with solid medium, inoculated 100 bottles in total, and cultured at room temperature for 40 d. The solid medium was consisted of 80 g rice and 120 mL purified water.

The same volume of methanol was added to the fermented rice medium for ultrasonic extraction for three times, 30 min each time. The extract was filtered with eight layers of gauze to remove impurities such as culture medium and mycelium. After the filtrate was concentrated to the extract, it was dissolved with water, extracted with the same amount of petroleum ether, ethyl acetate and *n*-butanol for three times, and the extract was concentrated to obtain the corresponding extract.

The Ethyl acetate extract (30.89 g) was selected for separation and CH_2Cl_2: MeOH (100:0–0:100) as mobile phase was used for gradient separation. According to the TLC results, the flow was divided into 11 sub streams (Frs.1–11), and further Sephadex.7 gel (methanol) elution, PTLC and LH-20 (Fr) were prepared for Fr.7, and compounds **1**–**4** were obtained. Compound **5** was prepared by PTLC on Fr.8. The structures of compounds **1**–**5** were shown in Fig. 1 and key HMBC correlations of compound **3** was shown in Fig. 2.

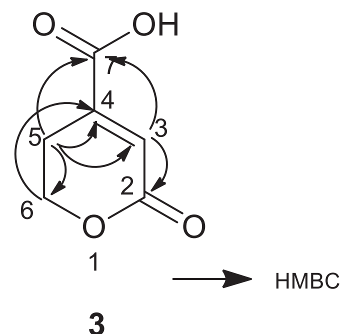

Fig. 2. Key HMBC correlations of compound **3**.

2.4. Molecular simulation

The amino acid sequence (P25819) of catalase (CAT) was obtained from the UniProt database (https://www.uniprot.org). AF-P25819-F1 is a type of CAT, and the structure of this protein is shown in Fig. 3. Alpha Fold structure prediction was obtained in the Alpha Fold Protein Structure Database (https://alphafold.ebi.ac.uk/). The modeled proteins were structure-optimized using the Maestro 11.5 "Protein Preparation Wiz-ard" tool to predict the potential active site of the target proteins using the "Sitmap" program. The predicted active site was determined using the "Receptor Grid Generation" program. The 3D structures of compounds **1**–**5** were constructed using software Chem Draw Ultra 8.0 and Chem 3D 17.1, then imported into Maestro 11.5, structure optimization and 3D structure transformation with "OPLC_2005" force field, and the optimized compounds **1**–**5** were saved as "mol2" file format for subsequent molecular docking. Compounds **1**–**5** were separately docked to the different active sites of the target protein, via the "Ligand Docking" tool. The interactions of compounds **1**–**5** with the target protein were evaluated with the

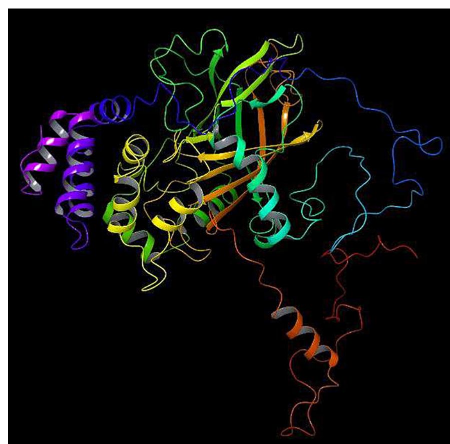

Fig. 3. Structure of AF-P25819-F1.

"Glide-score" score function value, which comprehensively considers hydrogen bond, hydrophobic, van der Waals force and the greater the absolute value indicates the more stable the docking complex with the target and the better the matching binding effect. 6BA was a positive drug. It was an activator of CAT. Compared the docking scores of compounds **1**–**5** to target protein with the docking scores of 6BA to target protein.

3. Results

3.1. Identification of compounds

Compound **1**: white amorphous powder (MeOH). ESI-MS *m*/*z* 153.053 2 [M + Na]$^+$ (calcd for $C_6H_{10}O_3Na$, 153.05). ^{1}H-NMR (500 MHz, DMSO d_6) δ: 2.52 (1H, d, *J* = 17.0 Hz, H-3a), 2.38 (1H, d, *J* = 17.0 Hz, H-3b), 4.97 (1H, s, 4-OH), 1.85 (1H, m, H-5a), 1.71 (1H, m, H-5b), 4.39 (1H, m, H-6a), 4.26 (1H, m, H-6b), 1.21 (3H, s, H-7a, H-7b, H-7c); ^{13}C-NMR (125 MHz, DMSO d_6) δ: 171.0 (C-2), 44.9 (C-3), 67.3 (C-4), 35.7 (C-5), 66.2 (C-6), 29.7 (C-7). The above data were consistented with those reported in literature (Varejão et al., 2013), and were identified as 4-hydroxy-4-methyltetrahydro-2*H*-pyran-2-one.

Compound **2**: white amorphous powder (MeOH). ESI-MS *m*/*z* 151.0369 [M + Na]$^+$ (calcd for $C_6H_8O_3Na$, 151.04). ^{1}H-NMR (500 MHz, DMSO d_6) δ: 5.84 (1H, s, H-3), 2.32 (2H, s, H-5a, 5b), 4.31 (2H, s, H-6a, 6b), 4.11 (2H, s, H-7a, 7b), 5.35 (1H, s, 7-OH); ^{13}C-NMR (125 MHz, DMSO d_6) δ: 164.6 (C-2), 112.4 (C-3), 163.7 (C-4), 24.8 (C-5), 62.7 (C-6), 66.3 (C-7). The above data were consistented with those reported in literature (Punya et al., 2012), and were identified as 4-(hydroxymethyl)-5, 6-dihydro-pyran-2-one.

Compound **3**: white amorphous powder (MeOH). The spectral data were as follows ESI-MS *m*/*z*: 165.0158 [M + Na] $^+$, theoretical data ESI-MS *m*/*z*: 165.03 [M + Na] $^+$. UV (MeOH) λ_{max} (logε): 251 nm; IR (KBr) ν_{max}: 2 950, 1 710, 1 670, 1 590 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO d_6) δ: 6.43 (1H, s, H-3), 2.60 (2H, brs, H-5), 4.39 (2H, brs, H-6); ^{13}C-NMR (125 MHz, DMSO d_6) δ: 164.5 (C-2), 123.5 (C-3), 149.6 (C-4), 24.1 (C-5), 67.0 (C-6), 166.6 (C-7). HSQC correlations about compound **3** from H-3 [6.43 (1H, s)] to C-5 (123.5), from H-5 [2.60 (2H, brs)] to C-5 (24.1), from H-6 [4.39 (2H brs)] to C-6 (67.0). HSQC correlations about compound **3** from H-3 [6.43 (1H, s)] to C-2 (164.5) and C-7 (166.6), from H-5 [2.60 (2H, brs)] to C-7 (166.6), C-4 (149.6), C-5 (123.5) and C-6(67.0), from H-6 [4.39 (2H brs)] to C-2 (164.5), C-4 (149.6) and C-5 (24.1). The compound was identified as a new compound named 5, 6-dihydro-2-oxo-2*H*-pyran-4-carboxylic.

Compound **4**: colorless flake crystal (MeOH). ESI-MS *m*/*z*: 217.059 2 [M + Na]$^+$ (calcd for $C_6H_{10}O_3N_2Na$, 217.06). ^{1}H-NMR (500 MHz, DMSO d_6) δ: 7.88 (1H, d, *J* = 8.1 Hz, H-2), 6.93 (1H, d, *J* = 8.3 Hz, H-3), 7.47 (1H, t, *J* = 7.1 Hz, H-4), 6.82 (1H, t, *J* = 7.3 Hz, H-5), 13.1 (1H, s, 7-COOH), 9.09 (1H, s, 8-NH), 9.98 (1H, s, 9-NH), 1.98 (3H, s, H-11a, H-11b, H-11c); ^{13}C-NMR (125 MHz, DMSO d_6) δ: 111.9 (C-1), 131.7 (C-2), 117.8 (C-3), 134.7 (C-4), 112.7 (C-5), 152.0 (C-6), 169.8 (C-7), 169.3 (C-10), 21.0 (C-11). Compared with reference (Zhang et al., 2016), it was determined to be *N*-acetyl-hydrazinobenzoic acid.

Compound **5**: white amorphous powder (MeOH). ESI-MS *m*/*z* 205.0473 [M + Na]$^+$ (calcd for $C_9H_{10}O_4Na$, 205.05). ^{1}H-NMR (500 MHz, DMSO d_6) δ: 8.66 (1H, s, 2-OH), 6.58 (1H, d, *J* = 8.6 Hz, H-3), 6.46 (1H, dd, *J* = 8.3, 1.9 Hz, H-4), 8.77 (1H, s, 5-OH), 6.52 (1H, s, H-6), 3.47 (2H, s, H-7a, H-7b), 3.58 (3H, s, H-9a, 9b, 9c); ^{13}C-NMR (125 MHz, DMSO d_6) δ: 122.1 (C-1), 148.2 (C-2), 115.8 (C-3), 114.7 (C-4), 150.0 (C-5), 118.0 (C-6), 35.6 (C-7), 172.2 (C-8), 51.9 (C-9). Consistented with the report in literature (Meng, Feng, & Zeng, 2013), it was identified as methyl 2-(2, 5-dihydroxyphenyl) acetate.

3.2. Molecular simulation results

The results showed that compounds **3** and **4** had good docking scores of −6.535 and −6.858, respectively, as shown in Figs. 4 and 5. Compound **3** formed hydrogen bonding with valine at position 63, arginine at position 62, and glutamine at position 352 on catalase, and a salt bridge with arginine at position 355. Compound **4** formed hydrogen bonding with arginine at position 62 on catalase and glutamine at position 352; And a salt bridge with arginine at position 355. As shown in Fig. 6, the docking score of 6BA was −5.703. Theoretical data suggest that compounds **3** and **4** can act as potential activators of catalase.

4. Discussion

Paclitaxel is a new anticancer drug with good efficacy in a range of cancers, the content of paclitaxel in *T. cuspidata* is relatively low, and endophytes metabolites can promote, inhibit, or adjust the (Wei et al., 2011) synthesis of host plant secondary metabolites. Five compounds were obtained by isolating the secondary metabolites of the *T. cuspidata* endophytic fungal, where compound **3** was not reported in other articles, as new compounds. The biological function of catalase is to promote the decomposition of hydrogen peroxide in cells, so that it will not further produce highly toxic hydroxyl radicals, thereby protecting the function of the antioxidant enzyme system. Compounds **1**–**5** were separately docked with the different active sites of the catalase proteins. Screened out compounds **3** and **4** may be potential activators of catalase. It

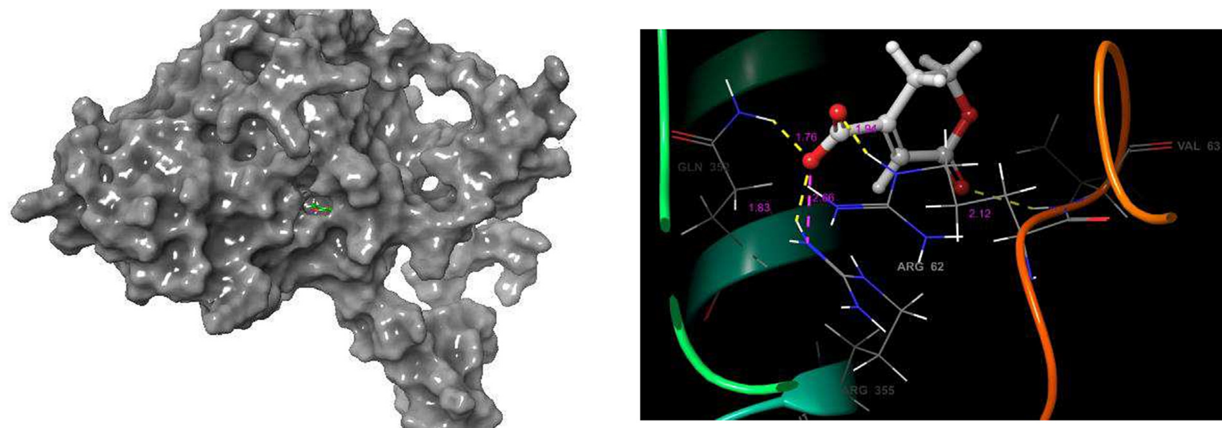

Fig. 4. Molecular docking simulated the interaction between compound **3** and CAT. Active pocket of compound **3** combined with CAT (left). Molecular docking of compound **3** with CAT (right).

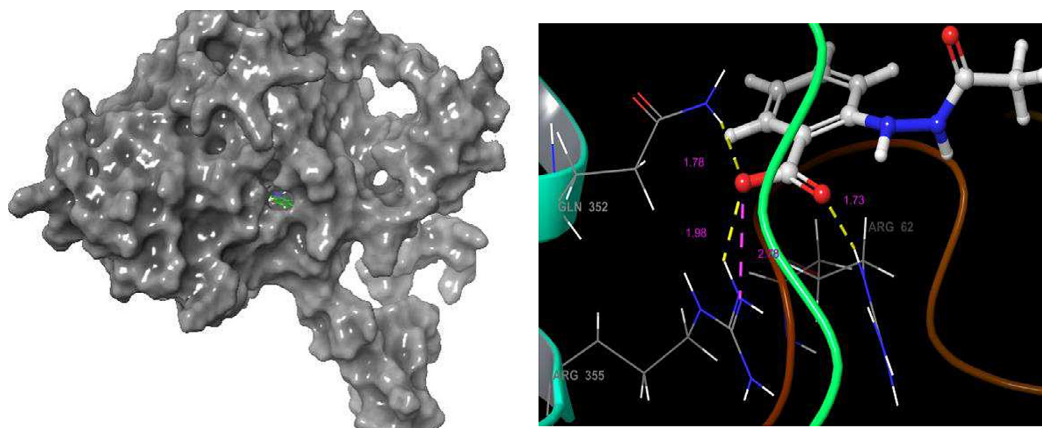

Fig. 5. Molecular docking simulated the interaction between compound **4** and CAT. The active pocket of compound **4** combined with CAT (left). Molecular docking of compound **4** with CAT (right).

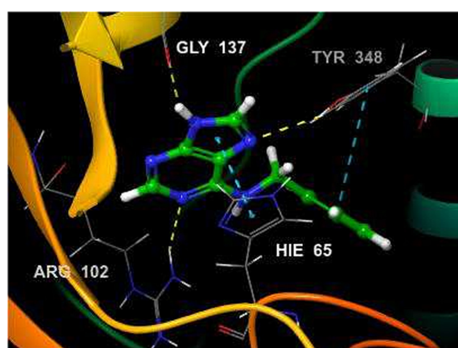

Fig. 6. Molecular docking simulated interaction between 6BA and CAT.

has great significance for the growth, development and metabolic activities of organisms.

5. Conclusion

In this study, five monomeric compounds were obtained through a series of isolation means, and compound **3** was not reported in other articles as a new compound. To take it step further, evaluation by molecular simulation experiments revealed that compounds **3** and **4** may be potential activators of catalase, providing a theoretical basis for the development of CAT activators.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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