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Short communication

Bioactive cytosporone derivatives isolated from the mangrove-derived fungus *Dothiorella* sp. ML002

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ABSTRACT

Three new cytosporone derivatives dothiorelones K–M (**1**, **2 and 7**), together with six known ones (**3–6**, **8** and **9**) were isolated from the mangrove-derived fungus *Dothiorella* sp. ML002. Their structures were determined by comprehensive 1D, 2D NMR spectroscopic and HR-ESI-MS spectroscopic data. Compounds **1**, **2** and **5** displayed inhibitory activities against α-glucosidase with the IC_{50} values of 22.0, 77.9 and 5.4 μg/mL, respectively. Additionally, compounds **1**, **2**, and **5** also exhibited antibacterial activities against *Staphylococcus aureus* (ATCC 6538) with the same MIC values of 50 μg/mL, respectively. The results indicated that cytosporone derivatives will be useful to as diabetes control agents.

1. Introduction

Cytosporones are a class of octaketide phenolic lipids. In the last 20 years, more than 30 new cytosporones and similar compounds were isolated from a variety of fungi including *Acremonium*, *Aspergillus*, *Colletotrichum*, *Cytospora*, *Diaporthe*, *Leucostoma*, *Paraphaeosphaeria*, *Pestalotiopsis*, *Phomopsis* and *Trichoderma* [1-4]. Some cytosporones show various bioactivities, such as antivius [5], antibactierial [1], cytotoxic and anti-inflammatory activities [1]. And some of them has been biosynthesized or synthesized [1,6]. Among the cytosporone derivatives, about 16 compounds were isolated from the mangrove-derived fungus [7-12], such as antifungal cytosporones B and C [7], cytotoxic dothiorelone A and 2-(7′-hydroxyoxooctyl)-3-hydroxy-5-methoxybenzeneacetic acid ethyl ester [9,11]. In our ongoing effort to isolate bioactive metabolites from mangrove-derived fungi [13-16], a fungi *Dothiorella* sp. ML002 isolated from the medicinal mangrove *Xylocarpus granatum* Koenig, attracted our attention because its EtOAc extract showed significant inhibitory activity against α-glucosidase. Chemical investigation on the EtOAc extract of the fermentation broth led to the identification of three new cytosporone derivatives dothiorelones K–M (**1**, **2 and 7**), and six known ones (**3–6**, **8** and **9**) (Fig. 1) from the mangrove-derived fungus *Dothiorella* sp. ML002. Herein, we reported the isolation, structure elucidation, and bioactivities of these compounds.

2. Experimental section

2.1. General experimental procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a Thermo Nicolet 6700 (using KBr disks) spectrophotometer (Thermo Scientific, Madison, WI, USA). 1D and 2D NMR spectra were measured on a Bruker AV-400 (Bruker Corporation, Switzerland) instrument with TMS as the internal standard. HR-ESI-MS spectra were made on the Bruker Daltonics Apex-Ultra 7.0 T (Bruker Corporation, Billerica, MA, USA) and the Q-TOF Ultima Global GAA076 LC mass spectrometer. HPLC were used for Agilent 1260 prep-HPLC system with an Waters C18 anal. HPLC column (4.6 × 250 mm, 5 μm) and semi-prep. column (9.4 × 250 mm, 7 μm). Sephadex LH-20 (Pharmacia Co. Ltd., Sandwich, UK) and Silica gel (200–300 mesh, 300–400 mesh Qingdao Marine Chemical Factory, Qingdao, China) were used for column chromatography (CC). All

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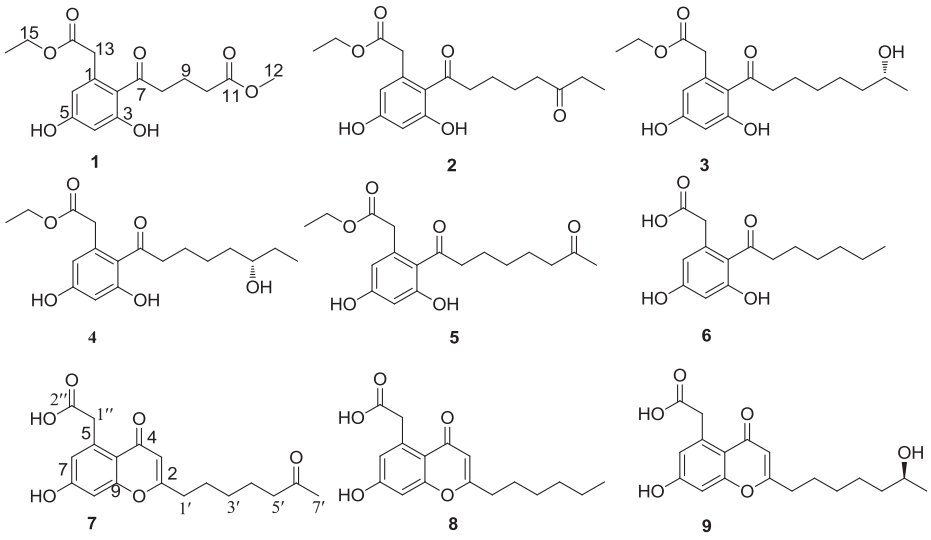

Fig. 1. Structures of compounds **1**–**9**.

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of **1** and **2** (δ in ppm, J in Hz) in $CDCl_3$.

	1		**2**	
Position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	117.2, C		117.7, C	
2	136.5, C		136.3, C	
3	162.9, C		161.9, C	
4	103.3, CH	6.22 (br s)	103.0, CH	6.21 (br s)
5	160.5, C		160.4, C	
6	112.9, CH	6.19 (br s)	112.6, CH	6.21 (br s)
7	205.3, C		206.6, C	
8	42.4, CH_2	2.92 (m)	43.3, CH_2	2.84 (m)
9	20.0, CH_2	2.05 (m)	24.2, CH_2	1.62 (m)
10	33.3, CH_2	2.43 (m)	36.0, CH_2	2.44 (m)
11	174.5, C		23.4, CH_2	1.60 (m)
12	51.9, CH_3	3.67 (s)	213.5, C	
13	41.6, CH_2	3.75 (s)	42.2, CH_2	2.41 (m)
14	172.3, C		7.83, CH_3	1.01 (t, 7.2)
15	61.7, CH_2	4.19 (q, 6.4)	41.1, CH_2	3.69 (s)
16	14.2, CH_3	1.28 (t, 6.4)	172.5, C	
17			61.6, CH	4.15 (q, 7.2)
18			14.1, CH_3	1.28 (t, 7.2)

solvents were purchased from Xilong Chemical Reagent Factory (Guangzhou, China).

2.2. Fungal materials

The fungal strain *Dothiorella* sp. ML002 was isolated from the stem of the mangrove *Xylocarpus granatum* Koenig, collected in the South China Sea in August 2015. The fungus was identified according to its morphological traits and a molecular protocol by amplification and sequencing of the DNA of the ITS region of the rRNA gene as described previously [14]. The strain was identified as *Dothiorella* sp. ML002 according to morphologic traits and molecular identification. Its 559 base pair ITS sequence had 99% sequence identity to that of *Dothiorella aegiceri* strain GX3-1A. The ITS sequence data have been submitted to GenBank with the accession number MK131322.

2.3. Fermentation, extraction, and isolation

The fungal strain *Dothiorella* sp. ML002 was cultivated in 20 L potato glucose liquid medium in sea water; 1 L Erlenmeyer flasks each containing 300 mL of culture broth at 26 °C without shaking for 4 weeks. The fungal cultures were filtered through cheesecloth, and the filtrate was extracted with EtOAc (3 × 20 L, 24 h each). The EtOAc extracts were concentrated in vacuo to yield residue (10.2 g), which was subjected to silica gel column chromatography (CC) (petroleum ether, EtOAc v/v, gradient 100: 0–0: 100) to generate seven fractions (Fr. 1 − Fr. 7). Fr. 3 was isolated by CC on silica gel eluted with petroleum ether–EtOAc, and then subjected to Sephadex LH-20 CC eluting with mixtures of petroleum ether $CHCl_3$: MeOH (v/v, 2: 1: 1), and further purified by using HPLC on an ODS semi-preparative column (Waters C18, 9.4 × 250 mm, 7 μm, 2 mL/min) eluted with 78% MeOH/H_2O to obtain **1** (3.0 mg). Fr. 4 was isolated by CC on silica gel eluted with petroleum ether–EtOAc, and then subjected to Sephadex LH-20 CC eluting with mixtures of $CHCl_3$–MeOH (v/v, 1: 1) to produce three subfractions (Fr. 4-1–Fr. 4-3). Fr. 4-2 were further purified by using HPLC eluted with 75% MeOH/H_2O to obtain **3** (6.5 mg) and **4** (5.3 mg). Compounds **5** (6.0 mg) and **6** (7.0 mg) were isolated from Fr. 4-3, which were purified by HPLC eluted with 75% MeOH/H_2O. Fr. 5 was subjected to petroleum ether–EtOAc to generate four fractions (Fr. 5-1 − Fr. 5-4). Fr. 5–2 were purified by Sephadex LH-20 CC (MeOH) and then further purified by using HPLC eluted with 70% MeOH/H_2O to obtain **2** (3.2 mg). Fr. 5-3 were purified by Sephadex LH-20 and HPLC to obtain **7** (6.2 mg), **8** (5.4 mg) and **9** (7.1 mg).

Dothiorelone K (**1**): colorless gum. UV (MeOH) λ max (log ε) 223 (3.18), 232 (3.10), 267 (2.99), 298 (3.42) nm; IR (KBr) ν_{max} 3290, 1736, 1606, 1465, 1367, 1232 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HR-ESI-MS m/z 325.1243 $[M+Na]^+$ (calcd for $C_{16}H_{21}O_7$, 325.1247).

Dothiorelone L (**2**): colorless gum. UV (MeOH) λ max (log ε) 212 (2.88), 232 (2.90), 265 (2.86), 289 (3.02) nm; IR (KBr) ν_{max} 3390, 1716, 1632, 1432, 1347, 1252 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HR-ESI-MS m/z 359.1463 $[M+Na]^+$ (calcd for $C_{18}H_{24}O_6$ Na, 359.1465).

Dothiorelone M (**3**): white amorphous solid. UV (MeOH) λ max (log ε) 221 (2.98), 241 (3.12), 250 (3.04) nm; IR (KBr) ν_{max} 3216, 1711, 1635, 1565, 1367 cm^{-1}; ^{1}H and ^{13}C NMR see Table 2; HR-ESI-MS m/z 333.1331 $[M+Na]^+$ (calcd for $C_{18}H_{21}O_6$, 333.1333).

2.4. Biological assays

The inhibition of α-glucosidase was assessed using adopted method [17], and acarbose was used as a positive control. Antibacterial activity was determined against five pathogenic bacteria, including *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 6633), *Vibrio alginolyticus* (ATCC 33787), and *V. parahemolyticus* (ATCC 17802), by the microplate assay method [18]. Ciprofloxacin was used as the positive control. Cytotoxic activities of all

Table 2
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of **7** (δ in ppm, J in Hz) in CD_3OD.

	7	
Position	δ_C	δ_H (J in Hz)
1		
2	170.1, C	
3	110.6, CH	6.01 (s)
4	181.6, C	
5	139.7, C	
6	119.4, CH	6.69 (br s)
7	163.2, C	
8	102.7, CH	6.75 (br s)
9	161.3, C	
10	115.8, C	
1′	34.3, CH_2	2.61 (t, 7.4)
2′	27.6, CH_2	1.72 (m)
3′	29.4, CH_2	1.39 (m)
4′	24.3, CH_2	1.61 (m)
5′	43.9, CH_2	2.50 (t, 7.4)
6′	211.8, C	
7′	29.7, CH_3	2.09 (s)
1″	49.0, CH_2	4.10 (s)
2″	173.0, C	

compounds against human A549, Hela and HepG2 cell lines were evaluated by the MTT method [19]. Adriamycin was used as a positive control.

3. Results and discussion

3.1. Structural elucidation

Compound **1** was obtained as a colorless gum. The molecular formula $C_{16}H_{20}O_7$ (seven degrees of unsaturation) of **1** was established by HR-ESI-MS. The ^{1}H NMR spectrum (Table 1) displayed characteristic signals for two *meta*-coupled aromatic protons of a 1,2,3,5-tetra-substituted benzene at δ_H 6.22 (br s) and 6.19 (br s), a ethoxyl group at δ_H (4.19, q, J = 6.4 Hz) and (1.28, t, J = 6.4 Hz), three methylene groups at δ_H 3.75 (s), 2.92 (m), 2.43 (m) and 2.05 (m), and one methoxyl group at δ_H 3.67 (s). In the ^{13}C NMR and DEPT spectral (Figs. S2 and S3), 16 signals were observed, including six aromatic carbons at δ_C 162.9 (C), 160.5 (C), 136.5 (C), 117.2 (C), 112.9 (CH) and 103.3 (CH), five methylenes carbons at (δ_C 61.7, 42.4, 41.6, 33.3 and 20.0), one methoxyl carbon at δ_C 51.9, and one methyl carbon at δ_C 14.2, one ketone carbonyl carbon at δ_C (205.3), and two ester carbonyl carbons at δ_C (174.5 and 172.3). Comparison of the ^{1}H, ^{13}C and DEPT NMR spectra of **1** with those of cytosporone A [19] indicated that both compounds shared the same basic skeleton. The main difference was that the propyl group in cytosporone A was replaced by a methyl formate group [δ_C 174.5 for C-11, and δ_H 3.67 (s) and δ_C 51.9 (CH_3) for C-12] in **1**. The presence of this moiety was confirmed by HMBC correlations from H-9 and H-12 to C-11 (Fig. 2). Finally, **1** was identified as a new compound and named as dothiorelone K.

Compound **2** was also obtained as a colorless gum, and had the molecular formula $C_{18}H_{24}O_6$ (7 degrees of unsaturation) as determined by the HR-ESI-MS. The 1D NMR spectra of **2** were very similar to dothiorelone B [5,6]. The most difference was that an oxygenated methine at δ_H 3.41 (m) and δ_C 73.8 (CH) for C-12 in dothiorelone B was replaced by a ketone carbonyl carbon at δ_C 213.5 (C) for C-12 in **2**. These conclusions were confirmed by the HMBC correlations of H-10, H-13 and H-14 to C-12 (Fig. 2). Thus, the structure of **2** was proposed as dothiorelone L.

Compound **7**, a white amorphous solid, has the molecular formula $C_{18}H_{20}O_6$ (nine degrees of unsaturation) established by HR-ESI-MS. The UV spectrum indicated a chromone derivative based on the characteristic absorbance observed at λ max (MeOH) 221, 241 and 250 nm [9,11]. The ^{1}H NMR spectrum (Fig. S8) displayed two aromatic meta-coupled protons at δ_H 6.75 (br s, H-8) and 6.69 (br s, H-6), an olefinic proton at δ_H 6.01 (s, H-3), a downfield shifted methylene group at δ_H 4.10 (s, C-1″), five methylene groups at δ_H 2.61 (t, 7.4, H-1′), 1.72 (m, H-2′), 1.39 (m, H-3′), 1.61 (m, H-4′) and 2.50 (t, 7.4, H-5′), and a methyl group at δ_H 2.09 (s, 7′-Me). The ^{13}C NMR and DEPT experiments showed one methyl group, seven methylenes, three olefinic methines, and seven quaternary carbons (including two carbonyls). The above NMR data (Table 1) of **7** were similar to the known compound pestalotiopsone C [9], and had the same chromone core. The obvious difference was the presence of one carbonyl group at δ_C 211.8 (C) for C-6′ in **7**, instead of a methylene group at δ_H (1.32, m) and δ_C 23.7 (CH_2) for C-6′ in pestalotiopsone C. These results were confirmed by the 2D NMR spectral (Fig. 2). Thus, compound **7** was identified as a new compound, and named as dothiorelone M.

The six known compounds were identified as dithiorelone A (**3**) [21], dithiorelone B (**4**) [20] and I (**5**) [2], cytoporone A (**6**) [6], pestalotiopsone C (**8**) [9] and 6′-hydroxypestalotiopsone C (**9**) [11], based on the spectroscopic analyses, and comparison with the literature data.

3.2. Biological activity

All the isolated compounds were tested for their inhibitory activities against α-glucosidase. Compounds **1**, **2** and **5** showed inhibitory activities with the IC_{50} values of 22.0, 77.9 and 5.4 μg/mL, respectively. Acarbose was used as a positive control with the IC_{50} value of 2.0 μg/mL.

They were also evaluated for their antibacterial activities against five pathogenic bacteria, including *S. aureus*, *B. cereus*, *E. coli*, *V. alginolyticus* and *V. Parahemolyticus*. Only compounds **1**, **2**, and **5** exhibited antibacterial activities against *S. aureus* with the same MIC values of 50 μg/mL, respectively.

Compounds **1**–**9** showed no cytotoxic activity against three human A549, Hela and HepG2 cell lines at the concentration of 10 μg/mL.

4. Conclusions

In summary, the chemical investigation on the medicinal mangrove-derived endophytic fungus *Dothiorella* sp. resulted in the isolation of three new cytosporone derivatives dothiorelones K–M (**1**, **2** and **7**), and six known ones (**3**–**6**, **8**, and **9**). Their inhibitory activities against α-glucosidase, antibacterial activity and cytotoxic activity were tested. Compound **5** showed potent inhibitory activity against α-glucosidase with the IC_{50} value of 5.4 μg/mL. Through this study, it is expected that the mangrove-derived fungus *Dothiorella* sp. and its active components could be useful to as diabetes control agents.

Declaration of interest

The authors report no conflicts of interests.

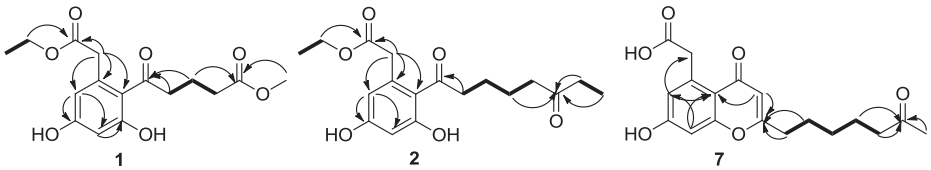

Fig. 2. Key ^{1}H-^{1}H-COSY (——) and HMBC correlations (H → C) of **1**, **2** and **7**.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.01.015.

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