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α-Pyrones, secondary metabolites from fungus *Cephalotrichum microsporum* and their bioactivities

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ABSTRACT

Cephalotrichum microsporum (SYP-F 7763) was a fungus isolated from the rhizosphere soil of traditional Chinese medicine *Panax notoginseng*. The EtOAc extract of *Cephalotrichum microsporum* cultivated on sterilized moistened-rice medium was separated by various chromatographic techniques, which yielded 11 metabolites (**1–11**) of this fungus. On the basis of the widely spectroscopic data, the chemical structures of isolated metabolites were determined, most of which were α-pyrones, including 5 compounds (**4–7**, and **10**) unreported. In the anti-bacterial bioassay, compound **1** displayed significant inhibitory effects on three pathogenic bacteria, MR *S. aureus*, *S. aureus*, and *B. cereus*. α-Pyrones **2**, **3**, and **5–7** also displayed moderate inhibitory effects on MR *S. aureus*, *S. aureus*, and *B. subtilis*, which could be the major anti-bacterial constituents of *Cephalotrichum microsporum*. Additionally, compounds **1**, **4**, and **5** displayed significant cytotoxicity on five human cancer cell lines, with the IC_{50} values $< 20\,\mu M$, which are more effective than positive control 5-fluorouracil. Therefore, α-pyrones were important secondary metabolites of *Cephalotrichum microsporum*, which displayed anti-bacterial and anti-tumor activities.

1. Introduction

Fungi are widely distributed in the world and play an important role for the ecosystem, towards plants, human, even other animals [1]. In the entire growth cycle of fungi, various bioactive secondary metabolites were produced, which displayed the significant bioactivities, such as antitumor, antifungal, and antibacterial. Now, a lot of these bioactive substances have been used clinically, such as the antibacterial agents penicillins, antifungal drug echinocandin B, immunosuppressive drug cyclosporin A, and cholesterol-lowering agent lovastatin [2]. Therefore, it has been attracted more and more attentions to explore the bioactive substances from the metabolites of fungi.

Cephalotrichum microsporum (*C. microsporum*) (SYP-F 7763) previously known as *Doratomyces microsporum* belongs to the genus *Cephalotrichum*, with the characterizations of dry-spored, indeterminate synnemata and enteroblastic percurrent conidiogenesis [3–4]. It is well known to produce an extracellular keratinase, which is closely related to proteinase K [5–8]. Additionally, various antibacterial indole and aromatic compounds were obtained from *C. microsporum*, which could be deduced by valproic acid [9].

In our previous research, a *Cephalotrichum microsporum* strain was obtained from the rhizosphere soil of traditional Chinese medicine *Panax notoginseng*. In this study, five new secondary metabolites (**4–7** and **10**), together with six known analogues (**1–3, 8, 9,** and **11**) of *C. microsporum* were isolated from the cultivated culture of using various chromatographic techniques (Fig. 1). Their structures were determined by spectroscopic analyses. The anti-bacterial and cytotoxic activities of **1–11** were evaluated. Combined with the bioassays about anti-bacterial, and anti-tumor, the bioactive metabolites were obtained from *C. microsporum*.

2. Materials and methods

2.1. General methods

The optical rotations were recorded using Perkin-Elmer 241

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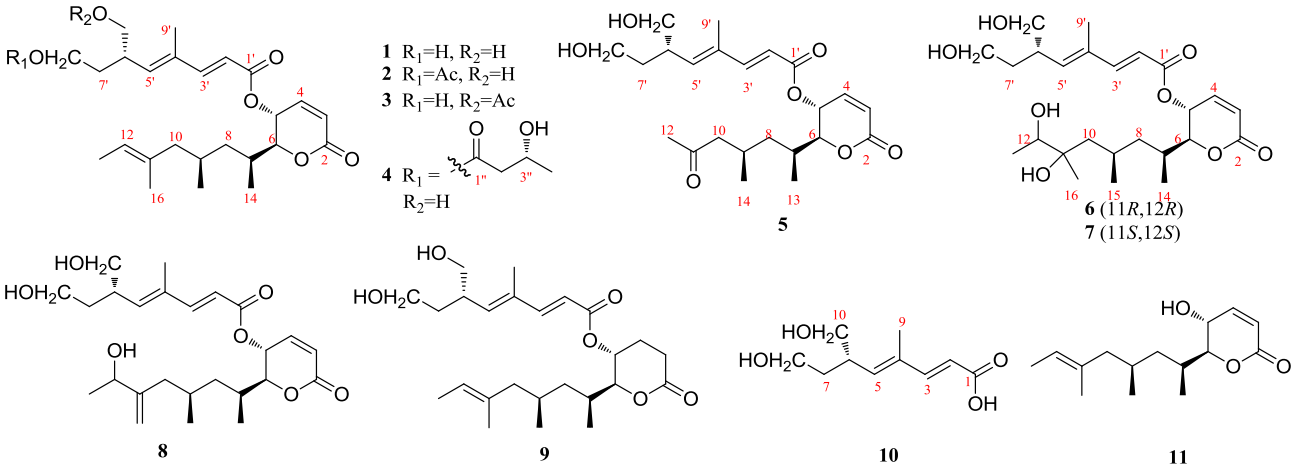

Fig. 1. Metabolites **1–11** isolated from the cultivated culture of *Cephalotrichum microsporum*.

polarimeter (Perkin-Elmer, Waltham, USA). UV spectra were measured on a Shimadzu UV 2201 spectrophotometer (Shimadzu, Japan). ECD spectra were recorded on a Bio-Logic Science MOS-450 spectrometer. 1D- and 2D-NMR spectra were obtained at 600 for ^{1}H and 150 MHz for ^{13}C, respectively, on a Burker 600 MHz spectrometer with solvent peaks as references. HRESIMS data were acquired on an Agilent 6210 TOF mass spectrometer. Analytical HPLC experiments were conducted on a Dionex UltiMate 3000 instrument (Thermo Scientific) equipped with a diode array detector and a Waters XBridge RP C18 column (250 × 4.6 mm, 5 μm). Preparative HPLC was performed on an Agel MPLC instrument with an UV detector (Tianjin, China) and a YMC C18 column (250 mm × 10 mm, 5 μm). 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). TLC was performed using precoated silica gel GF254 plates (5 × 10 cm, 2.5 × 7.5 cm, Qingdao Marine Chemical Inc.). The spots were visualized under UV light or by spraying the plates with 10% sulfuric acid in EtOH, followed by heating at 105 °C.

2.2. Fungal strain

SYP-F 7763 fungal strain was isolated from the rhizosphere soil of *Panax notoginseng* (Wenshan, Yunnan province of China). The DNA of the strain were sequenced, and deposited at the GenBank with accession numbers MH04912. NCBI-BLAST result revealed high identity with *C. microsporum* (100%).

2.3. Fermentation and extraction

The sterilized moistened-rice medium was prepared with sterile water (53 mL) and rice (40 g) in each Erlenmeyer flask (250 mL), which were autoclaved at 121 °C for 30 min. The spore of *C. microsporum* (2.0 mL) was inoculated in each Erlenmeyer flask (250 mL × 120), and cultivated at 30 °C for 30 days. The fermented material was extracted with ethyl acetate (12 L × 3), which gave the crude extract (19.0 g) after the evaporation of ethyl acetate.

2.4. Isolation of compounds

The extract (19.0 g) was subjected to silica-gel column chromatography, eluted by petroleumether-EtOAc (80:1 to 1:1), affording ten sub-fractions (Fr.1–Fr.10). After the analysis of fractions 1–10 using HPLC and TLC, sub-fraction Fr. 6 was separated using pre-HPLC eluted by gradient CH_3CN-H_2O (10:90–90:10, 70 min), at 10 mL/min flow rate, detected at 210 nm and 254 nm. As a result, compounds **1** (Rt. 34 min, 12.3 mg), **5** (Rt. 38 min, 3.5 mg), **6** (Rt. 25 min, 5.4 mg), and **7** (Rt. 27 min, 2.5 mg) were obtained. After the separation of sub-fraction Fr. 5 by pre-HPLC, compounds **2** (Rt. 46 min, 9.5 mg), **3** (Rt. 49 min, 6.2 mg), **4** (Rt. 57 min, 5.8 mg), **8** (Rt. 26 min, 3.5 mg), **9** (Rt. 35 min, 4.4 mg), and **11** (Rt. 30 min, 3.8 mg) were purified. Compound **10** (Rt. 25 min, 5.2 mg) were isolated from the sub-fraction Fr. 8 using pre-HPLC.

2.4.1. 8′-O-(3R-Hydroxy-butyryl)-rasfonin (4)

White amorphous powder; [α] – 19.5 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 272.6 nm; ECD (CH_3OH) $\Delta\varepsilon_{267}$ – 9.8, $\Delta\varepsilon_{243}$ – 9.6, $\Delta\varepsilon_{214}$ – 16.4; ^{1}H NMR (600 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (150 MHz, $CDCl_3$) see Table 2; (+)-HRESIMS *m/z* 543.2934 $[M + Na]^+$ (calcd. for 543.2928, $C_{29}H_{44}NaO_8$).

2.4.2. Cemironin A (5)

White amorphous powder; [α] – 25.4 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 204.8 (3.20), 275.5 (4.53) nm; ECD (CH_3OH) $\Delta\varepsilon_{265}$ – 8.2, $\Delta\varepsilon_{241}$ – 14.9, $\Delta\varepsilon_{217}$ –15.9; ^{1}H NMR (600 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (150 MHz, $CDCl_3$) see Table 2; (+)-HRESIMS *m/z* 445.2197 $[M + Na]^+$ (calcd. for 445.2197, $C_{23}H_{34}NaO_7$).

2.4.3. Cemironin B (6)

White amorphous powder; [α] – 15.1 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 205.1 (3.04), 275.8 (4.57) nm; $Mo_2(OAc)_4$-induced ECD $\Delta\varepsilon_{313}$ + 9.5; ^{1}H NMR (600 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (150 MHz, $CDCl_3$) see Table 2; (–)-HRESIMS *m/z* 503.2421 $[M + Cl]^-$ (calcd. for 503.2417, $C_{25}H_{40}ClO_8$).

2.4.4. Cemironin C (7)

White amorphous powder; [α] – 22.5 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 205.3 (3.13), 275.7 (4.62) nm; $Mo_2(OAc)_4$-induced ECD $\Delta\varepsilon_{313}$ – 15.1; ^{1}H NMR (600 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (150 MHz, $CDCl_3$) see Table 2; (–)-HRESIMS *m/z* 503.2414 $[M + Cl]^-$ (calcd. for 503.2417, $C_{25}H_{40}ClO_8$).

2.4.5. 4-Methyl-8,10-dihydroxy-caprylic acid (10)

White amorphous powder; [α] – 5.8 (*c* 0.1, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD) see Table 1; ^{13}C NMR (150 MHz, CD_3OD) see Table 2; (–)-HRESIMS *m/z* 199.0971 $[M - H]^-$ (calcd for $C_{10}H_{15}O_4$, 199.0976).

2.5. Hydrolysis of 4

Compound **4** (2 mg) was dissolved in 95% MeOH (1.0 mL), and

Table 1
The ^{1}H NMR spectroscopic data of compounds **4–7**, and **10** ($CDCl_3$, 600 MHz, δ in ppm, J in Hz).

No.	4	5	6	7	10
2	–	–	–	–	5.72 d (15.6)
3	6.22 d (9.6)	6.22 d (9.6)	6.24 d (9.6)	6.22 d (9.6)	7.17 d (15.6)
4	7.04 dd (9.6,6.0)	7.04 dd (9.6,6.0)	7.01 dd (9.6,6.0)	7.03 dd (9.6,6.0)	–
5	5.36 dd (6.0,2.4)	5.42 dd (6.0,2.4)	5.49 dd (6.0,2.4)	5.45 dd (6.0,2.4)	5.75 d (10.2)
6	4.14 dd (8.4,2.4)	4.17 dd (9.0,2.4)	4.13 dd (9.0,2.4)	4.14 dd (9.6,2.4)	2.68 m
7	2.17 m	2.04 m	2.05 m	2.13 m	1.67 m 1.31 m
8	1.21 m 1.04 m	1.31 m 1.06 m	1.23 m 1.09 m	1.52 m 0.98 m	3.33 m
9	1.68 m	2.7 m	1.80 m	1.79 m	1.75 s
10	2.05 m; 1.44 dd (13.2,10.2)	2.39 m 2.07 m	1.23 m 0.99 m	1.39 m 0.96 m	3.33 m
12	5.12 q (6.0)	2.08 s	3.44 m	3.53 m	
13	1.55 d (6.6)	1.15 d (6.6)	1.03 d (6.6)	1.06 d (6.6)	
14	1.15 d (6.6)	0.91 d (6.6)	1.16 d (6.6)	1.17 d (6.6)	
15	0.78 d (6.6)	–	1.05 d (5.4)	0.99 d (6.6)	
16	1.53 s	–	1.13 s	1.12 s	
2′	5.82 d (15.6)	5.83 d (15.6)	5.81 d (15.6)	5.85 d (15.6)	
3′	7.35 d (15.6)	7.37 d (15.6)	7.38 d (15.6)	7.37 d (15.6)	
5′	5.72 d (9.6)	5.81 d (9.6)	5.82 d (15.6)	5.82 d (15.6)	
6′	2.84 m	2.89 m	2.90 m	2.88 m	
7′	1.94 m 1.61 m	1.80 m 1.62 m	1.79 m 1.59 m	1.80 m 1.61 m	
8′	4.17 m 4.01 m	3.74 m 3.62 m	3.71 m 3.60 m	3.71 m 3.62 m	
9′	1.82 s	1.85 s	1.83 s	1.84 s	
10′	3.63 m 3.57 m	3.61 m	3.65 m 3.56 m	3.58 m	
2″	2.44 m				
3″	4.19 m				
4″	1.22 d (6.6)				

NaOH (5 mg) was added. The solution was stirred at 40 °C for 24 h, acidified with HCl (2 N), and then evaporated under reduced pressure. The residue was dissolved in MeOH and subjected to HPLC analysis.

2.6. Anti-bacterial assay

Bacterial strains used to evaluate the anti-bacterial effects of isolated compounds were MR *Staphylococcus aureus* (186), *Staphylococcus aureus* (3150), *Bacillus cereus* (3152), and *Bacillus subtilis* (3154) (Table S1). The antimicrobial activity testing was performed according to previously description [10–11]. The bacterial were cultivated at 37 °C for 24 h in LB media until the absorbance OD_{600} = 0.3. The bacterial culture was diluted using liquid LB medium (1%). All of the isolated compounds were dissolved with DMSO (final concentration of 0.5 g/mL). 198 μL bacterial cultures and 2 μL sample solution were mixed up, which was transferred to 96-well plate. The 96-well plate was measured by a multimode reader after 12 h of incubation at 37 °C. Ampicillin (final solution of 0.02 g/mL), kanamycin (0.02 g/mL), and gentamicin (final solution of 0.64 g/mL) were used as the positive control groups.

Minimal inhibitory concentration (MIC) of compound **1** was assessed against bacteria MRSA186. Compound **1** was dissolved with DMSO with the final concentrations of 0.6, 0.3, 0.15, 0.075, and 0.0375 g/L for the MIC determination. DMSO and gentamicin (0.64 g/L) were used as the negative control and positive control, respectively [12].

Table 2
The ^{13}C NMR spectroscopic data of compounds **4–7**, and **10** ($CDCl_3$, 150 MHz, δ in ppm).

No.	4	5	6	7	No.	10
2	163.3	163.2	163.2	163.4	1	167.9
3	125.0	125.0	125.1	125.0	2	116.4
4	140.6	140.5	140.3	140.5	3	149.0
5	61.8	61.6	61.0	61.4	4	133.0
6	83.3	82.8	83.3	83.4	5	144.4
7	31.4	31.7	31.3	31.6	6	38.3
8	40.0	39.5	39.8	40.7	7	34.5
9	27.9	30.4	25.8	25.6	8	58.7
10	46.3	49.8	41.2	41.7	9	12.5
11	134.2	208.5	75.2	74.9	10	64.4
12	120.1	26.5	75.1	74.8		
13	13.4	16.0	17.4	17.4		
14	15.9	21.1	15.9	16.5		
15	20.6	–	23.5	24.1		
16	15.6	–	23.4	23.9		
1′	166.0	166.0	166.0	166.2		
2′	115.4	114.9	114.8	115.1		
3′	150.6	151.2	151.3	151.1		
4′	135.6	134.6	134.8	134.8		
5′	142.4	143.8	144.2	143.7		
6′	38.7	39.3	39.2	39.3		
7′	30.3	34.7	34.5	34.6		
8′	62.5	60.6	60.5	60.6		
9′	12.7	12.7	12.7	12.7		
10′	65.6	65.8	65.9	65.9		
1″	172.8					
2″	42.8					
3″	64.3					
4″	15.6					

2.7. Cytotoxicity assay

The cytotoxicity assay of isolated compounds were performed on five human cancer cell lines, hepatocellular carcinoma cell (HepG2), human cervical carcinoma cell (Hela), human lung cancer cell (A549), human breast cancer cell (MCF-7), and human renal carcinoma cell (786-O) using MTT assay [13–16]. 5-fluorouracil (Sigma, U.S.A.) was used as positive control, and the negative control group was used as equal volume medium culture. Every experiment was performed in three biological replicates.

3. Results and discussion

Compound **4** was obtained as an amorphous powder. The molecular formula was determined as $C_{29}H_{44}O_8$ by HRESIMS at m/z 543.2934 $[M + Na]^+$ (calcd for $C_{29}H_{44}O_8Na$, 543.2928), combined with the NMR data (Table 1 and 2). The UV spectrum showed the maximum absorption at 272.6 nm for the conjugated unsaturated system. The ^{1}H NMR spectrum of **4** exhibited resonances attributable to one (*Z*)-olefinic bond at δ_H 6.22 (d, J = 9.6 Hz, H-3) and 7.04 (dd, J = 9.6, 6.0 Hz, H-4); one (*E*)-olefinic bond at δ_H 5.82 (d, J = 15.6 Hz, H-2′) and 7.35 (d, J = 15.6 Hz, H-3′); two oxygenated methylenes at δ_H 4.17 (m, H-8′), 4.01 (m, H-8′), 3.63 (m, H-10′), and 3.57 (m, H-10′); and three oxygenated methines at δ_H 5.36 (dd, J = 6.0, 2.4 Hz, H-5), 4.14 (dd, J = 8.4, 2.4 Hz, H-6), and 4.19 (m, H-3″). Additionally, two methyls with singlet [δ_H 1.53 (s, H-16) and 1.82 (s, H-9′)] and four methyls with doublets [δ_H 1.66 (d, J = 6.6 Hz, H-13), 1.15 (d, J = 6.6 Hz, H-14), 0.78 (d, J = 6.6 Hz, H-15), and 1.22 (d, J = 6.6 Hz, H-4″)] were observed in the ^{1}H NMR spectrum. The ^{13}C NMR and HSQC spectra displayed 29 signals (Table 2) including three carbonyl (δ_C 172.8, 166.0, and 163.3), five oxygenated carbons (δ_C 83.3, 65.6, 64.3, 62.5, and 61.8), together with eight olefinic carbons (δ_C 150.6, 142.4, 140.6, 135.6, 134.2, 125.0, 120.1, and 115.4). Analyses of the spectroscopic data indicated that compound **4** was similar to the known fungal metabolites rasfonin (**1**) [17], except for the ester substitute unit. The

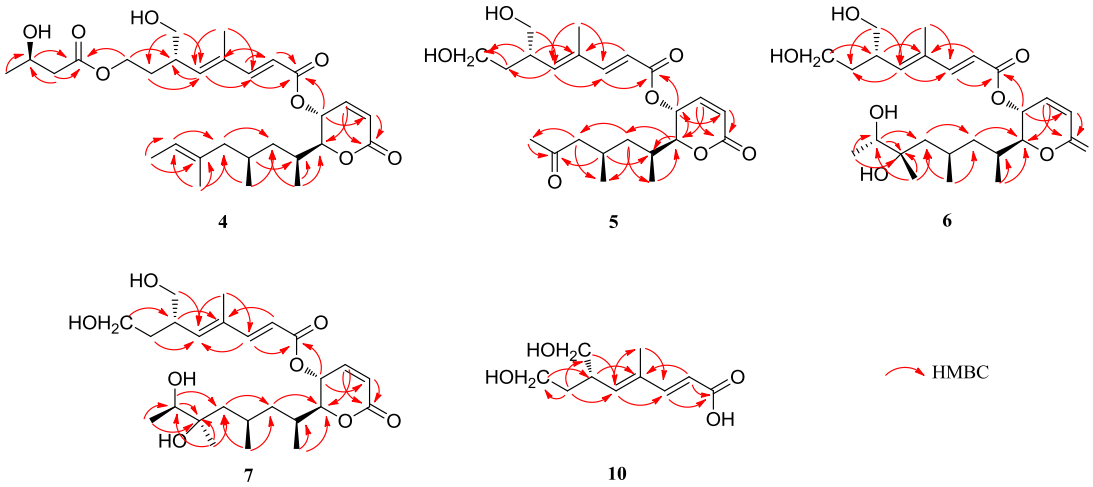

Fig. 2. Key HMBC (H → C) correlations of compounds **4–7**, and **10**.

substructure of **4** was further determined to be rasfonin by HMBC correlations of H-3/C-2, H-4/C-2, H-6/C-7, and H-5/C-1′ (Fig. 2). The NMR data [δ_H 1.22 (d, J = 6.6 Hz, H-4″), 2.44 (m, H-2″), and 4.19 (m, H-3″); δ_C 172.8, 42.8, 64.3, and 22.5] for the ester unit of **4** were almost indistinguishable from those of (*R*)-methyl 3-hydroxybutanoate [18]. Thus, the ester unit was determined to be 3(*R*)-hydroxy-butyryl, which was also confirmed by the correlations of H-3″/ C-1″, H-2″/ C-1″, H-2″/ C-3″, and H-4″/C-3″ observed in the HMBC spectrum of **4**. On the basis of the long range HMBC correlations from H-8′ to C-1″, the 3(*R*)-hydroxy-butyryl group was located at C-8′.The standard (*R*)-3-Hydroxybutanoic acid, (*S*)-3-Hydroxybutanoic acid and hydrolysate of **4** were also subjected to HPLC analysis with chiralcolumn. On the basis of the retention time, the ester unit was determined to be 3(*R*)-hydroxy-butyryl group. In the ECD spectrum, the observed negative Cotton effects at $\Delta\varepsilon_{267}$ (–9.8), $\Delta\varepsilon_{243}$ (–9.6), and $\Delta\varepsilon_{214}$ (–16.4) were similar to those of rasfonin and trichurusin J (**2**) [17], together with chemical shifts of these proton and carbon resonances, which suggested compound **4** has the same absolute stereochemistry as rasfonin and trichurusin J. Therefore, compound **4** was determined to be 8′-*O*-(3*R*-Hydroxy-butyryl)-rasfonin.

Compound **5**, a white amorphous powder, has a molecular formula of $C_{23}H_{34}O_7$ as determined by HRESIMS and NMR data (Table 1 and 2), which showed 12 less mass units than rasfonin. The 1D NMR spectra displayed compound **5** was a nor-rasfonin analogue [17]. The NMR spectrum of **5** exhibited resonances attributable to the *α*-Pyrone substructure at δ_C 163.2, 125.0, 140.5, 61.6, and 82.8; δ_H 6.22 (d, J = 9.6 Hz, H-3), 7.04 (d, J = 9.6, 6.0 Hz, H-4), 5.42 (dd, J = 6.0, 2.4 Hz, H-5), and 4.17 (dd, J = 9.0, 2.4 Hz, H-6). Similarly, the NMR data (Table 1 and 2) of unsaturated capryl group of **5** were also determined to be same as that of rasfonin [17]. Comparison of the NMR data between **5** and rasfonin, it was obvious that the difference existed in the side chain located at C-6. The ^{13}C NMR data of C-9, C-10, and C-11 in **5** were deshielded by $\Delta\delta_C$ +2.5, +3.5, and +74.3 ppm, respectively, whereas C-12 were shielded by $\Delta\delta_C$ −93.6 ppm. The other carbon resonances were shifted by $\Delta\delta_C \leq \pm 2.0$ ppm. This spectroscopic data indicated that the $\Delta^{11(12)}$ olefinic bond of rasfonin was oxidized to a carbonyl group, which was confirmed by the HMBC correlations of H-12 /C-11, H-10/C-12, and H-9/C-11 (Fig. 2). In the ECD spectrum of **5**, the negative Cotton effect at $\Delta\varepsilon_{265}$ (–8.2), $\Delta\varepsilon_{241}$ (–14.9), and $\Delta\varepsilon_{217}$ (–15.9) were similar to those of rasfonin analogues [17], together with chemical shifts of these proton and carbon resonances, which determined the absolute configuration of **5** was same as that of rasfonin. Therefore, compound **5** was elucidated to be Cemironin A.

Compounds **6** and **7** had the same molecular formula $C_{25}H_{40}O_8$ as determined by HRESIMS and NMR data (Table 1 and 2). The 1D NMR spectra displayed similar pattern to rasfonin, while the major difference was the $\Delta^{11(12)}$ olefinic bond in rasfonin was replaced by two hydroxy group in **6** and **7**, consistent with the NMR data displayed the extra oxygenated methine (δ_C 75.2), oxygenated methylene (δ_H 3.44, δ_C 75.1) in compound **6** and the extra oxygenated methine (δ_C 74.9), oxygenated methylene (δ_H 3.53, δ_C 74.8) in compound **7** [17], which were also proved by the 2D NMR data (Fig. 2). In the HMBC spectrum, the long range correlations of H-16/C-11, H-12/C-11, and H-13/C-12 established the 11,12-diol group for compound **6** and **7**. Thus, compounds **6** and **7** were established as the oxygenated derivatives of rasfonin [17]. The absolute configuration of the vicinal 11,12-diol moiety in compounds **6** and **7** were determined by the induced electronic circular dichroism (ECD) spectra of its in situ complex with $Mo_2(OAc)_4$ in DMSO solution (Snatzke's method) [19–20]. On the basis of the empirical helicity rule relating the sign of the Cotton effect of the diagnostic O—C—C—O moiety, the positive Cotton effect of **6** and negative Cotton effect of **7** at 313 nm indicated a 11*R*,12*R* and 11*S*,12*S* configuration for **6** and **7** respectively. Therefore, compounds **6** and **7** were determined to be Cemironin B and Cemironin C, respectively.

Compound **10** was obtained as a white powder. Its molecular formula was determined to be $C_{10}H_{16}O_4$ based on the HRESIMS ion at *m/z* 199.0971 $[M-H]^-$ (calcd for $C_{10}H_{15}O_4$, 199.0976) and NMR data (Table 1 and 2). Analyses of the spectroscopic data of compound **10** deduced that it was the ester substitute unit at 5-OH of rasfonin, which also revealed **10** has the same relative configuration [17]. The ^{1}H NMR spectrum (Table 1) displayed three olefinic groups at δ_H 5.72 (d, J = 15.6 Hz, H-2), 7.17 (d, J = 15.6 Hz, H-3), and 5.75 (d, J = 10.2 Hz, H-5); two oxygenated methylene groups at δ_H 3.33 (m, H-8 and H-10); one methyl group at δ_H 1.75 (s, H-9). In the ^{13}C NMR spectrum, the above mentioned moieties were also confirmed. In the HMBC experiment (Fig. 2), the long range correlations determined structure of **10** to be 4-methyl-8,10-dihydroxy-caprylic acid, which was the substituent of rasfonin.

The six known compounds were identified as rasfonin (**1**) [17], trichurusin J (**2**) [17], trichurusin K (**3**) [17], trichurusin D (**8**) [17], trichurusin B (**9**) [17], (5*R*,6*R*)-5,6-Dihydro-5-hydroxy-6-[(1*S*,3*R*,5*E*)-1,3,5-trimethyl-5-hepten-1-yl]-2H pyran-2-one (**11**) [21].

All of the compounds were tested for their anti-bacterial effects using four pathogenic bacteria, MR *Staphylococcus aureus*, *Staphylococcus aureus*, *Bacillus cereus*, and *Bacillus subtilis* with the concentrations 50 *μ*g/mL. As shown in Fig. 3, compound **1** displayed significant inhibitory effects on three pathogenic bacteria, MR *S. aureus*, *S. aureus*, and *B. cereus*. Especially, compound **1** could inhibit MR *S. aureus* significantly, with the IC_{50} value of 10 *μ*g/mL. *α*-Pyrones **2**, **3**, and **5–7** also displayed the moderate inhibitory effects on *S. aureus* and *B. subtilis*. Above all, the metabolites of *C. microsporum* displayed anti-bacterial effects, among which, *α*-pyrones could be the major bioactive substances.

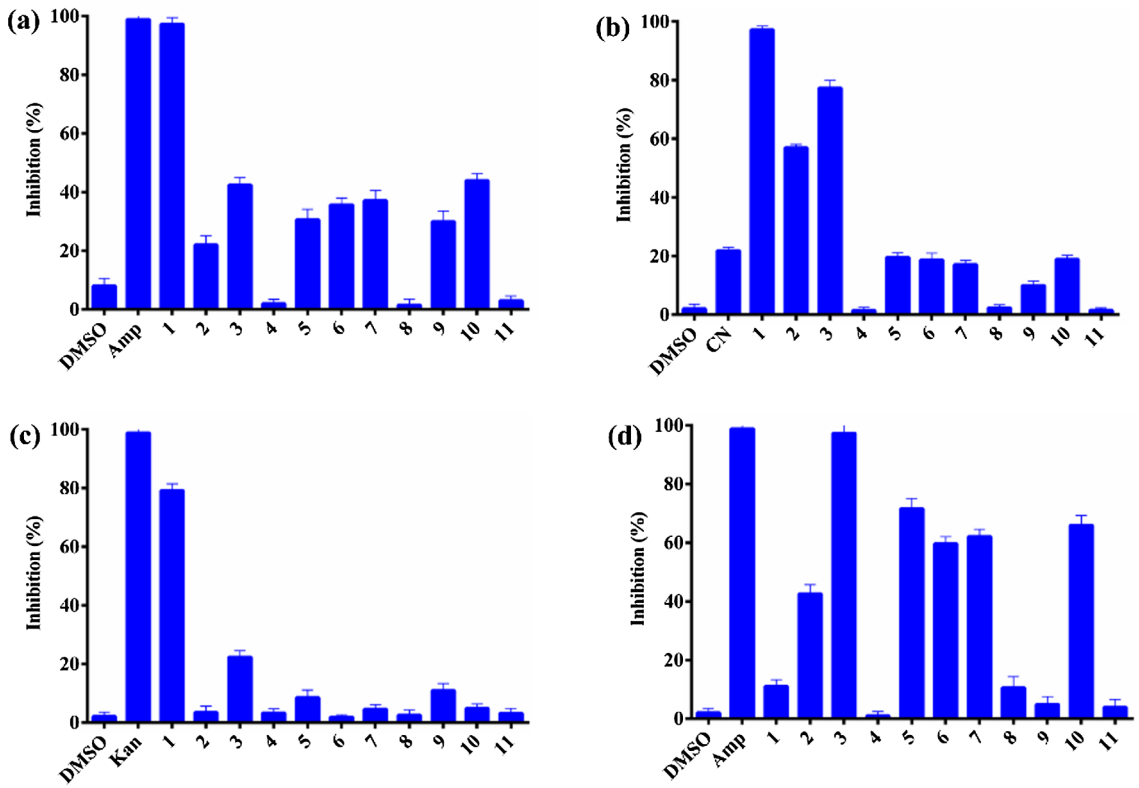

Fig. 3. The anti-bacterial effects of compounds **1–11**. (a) *Staphylococcus aureus*, (b) MR *Staphylococcus aureus*, (c) *Bacillus cereus*, (d) *Bacillus subtilis*. Kan (Kanamycin), CN (Vancomycin), Amp (Ampicillin).

The cytotoxic effects against five human cancer cell lines were evaluated on the isolated compounds **1–11** (Table 3). The current results suggested that compounds **1–5** and **7** showed good activity in a dose dependent manner against all five human cancer cell lines, with the IC_{50} values $< 100\,\mu M$. Especially, compounds **1**, **4** and **5** displayed more effective than positive control (5-fluorouracil) against five human cancer cell lines.

4. Conclusions

The bioactive metabolites investigation about the fungus *C. microsporum* was performed in the present work. 11 metabolites (**1–11**) were isolated from the cultivated culture of using various chromatographic techniques. On the basis of widely spectroscopic data, including 1D-NMR, 2D-NMR, ECD, and HRESIMS, the structures of isolated compounds were determined. Compounds **1–9**, and **11** were *α*-pyrones, among which, compounds **4–7**, and **10** were undescribed compounds. In the anti-bacterial bioassay, compound **1** displayed significant inhibitory effects on three pathogenic bacteria, MR *S. aureus*, *S. aureus*, and *B. cereus*. *α*-Pyrones **2**, **3**, and **5–7** also displayed moderate inhibitory effects on MR *S. aureus*, *S. aureus*, and *B. subtilis*, which could be the major anti-bacterial constituents of *C. microsporum*. Additionally, compounds **1**, **4**, and **5** displayed significant cytotoxicity on all five human cancer cell lines, with the IC_{50} values $< 20\,\mu M$, and could be considered to be potential as antitumor agents, in which they could significantly inhibit the cancer cells growth in a dose dependent manner. Therefore, *α*-pyrones were important secondary metabolites of *C. microsporum*, which displayed anti-bacterial and anti-tumor activities.

Table 3
The cytotoxic activity of isolated compounds against five human cancer cell lines. (IC50 μM).

Compounds	786-O IC50 (μM)	A549 IC50 (μM)	HepG2 IC50 (μM)	HeLa IC50 (μM)	MCF-7 IC50 (μM)
1	7.56	2.2	3.9	1.12	7.0
2	16.9	20.6	73.47	45.8	24.2
3	49.2	20.7	61.24	6.6	20.7
4	6.76	7.67	14.39	4.9	6.7
5	8.9	7.0	7.48	4.5	11.5
6	> 100	> 100	> 100	> 100	> 100
7	87.6	35.6	> 100	37.9	99.2
8	> 100	85.3	> 100	74.5	> 100
9	> 100	> 100	> 100	57.2	> 100
10	> 100	> 100	> 100	> 100	> 100
11	> 100	> 100	> 100	> 100	> 100
5-Fu[a]	38.55	15.73	21.12	28.57	14.69

[a] Positive control.

Conflict of interest

The authors declare no competing financial interest.

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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.2018.10.022.

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