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BRIEF COMMUNICATION

Polyketides from two *Chaetomium* species and their biological functions

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Abstract

Four new secondary metabolites, chaetosemins G-J (**1**−**4**), along with 11 known ones (**5**−**15**) were isolated from the culture of *C. seminudum* C208 and *Chaetomium* sp. C521. Their structures were determined by extensive NMR spectroscopic analyses. These metabolites were evaluated in vitro for antifungal, antioxidant, toxicity, and α-glucosidase inhibitory activities. Chaetosemin J (**4**) and monaschromone (**5**) significantly inhibited the growth of four plant pathogenic fungi *Botrytis cinerea*, *Alternaria solani*, *Magnaporthe oryzae*, and *Gibberella saubinettii* with the minimum inhibitory concentrations (MIC) values ranging from 6.25 to 25.0 μM. Moreover, both epicoccone B (**11**) and flavipin (**14**) exhibited the DPPH free radical scavenging ability with IC_{50} values of 10.8 and 7.2 μM, respectively, and had more potent α-glucosidase inhibition than the drug acarbose with IC_{50} values of 27.3 and 33.8 μM, respectively. Monaschromone (**5**) might act as the lead compound of pesticide.

Introduction

Fungi are considered as a potential repository that we depend on in our endeavors for the discovery of pharmacologically important molecules with notable bioactivities or druggability to some extent. *Chaetomium* genus, containing more than 100 species, is a common fungal genus that ubiquitously inhabited in soil and decomposed plant materials. A large number of structurally diverse metabolites have recently been characterized from *Chaetomium* species, including chaetoglobosins, xanthones, anthraquinones, azaphilones, terpenoids, and steroids [1, 2]. These structures display a wide range of biological activities, such as anticancer, antimicrobial, enzyme inhibitory, antimalarial, and antioxidant [1].

Previously, we reported the isolation and characterization of biologically active metabolites from *Chaetomium* species, including anticancer molecules from *Chaetomium globosum* [3], and antifungal agents from *Chaetomium seminudum* [4]. Encouraged by these discoveries, two strains namely *C. seminudum* C208 and *Chaetomium* sp. C521 were selected for further investigation. After repeated column chromatograph, 15 polyketide metabolites **1**−**15** including four new ones chaetosemins G-J (**1**−**4**) were obtained (Fig. 1). In details, compounds **1**−**9** were produced by *C. seminudum*, and **10**−**15** were isolated from *Chaetomium* sp., respectively. Herein, we addressed the isolation, structure determination, and bioactivity evaluation of these metabolites.

Results and discussion

Chaetosemin G (**1**) obtained as a white powder has the molecular ion at *m/z* 243.0424 ($[M+H]^+$) by its HR-ESIMS, indicating its molecular formula to be $C_{11}H_{11}O_4Cl$. The ^1H NMR spectrum showed the presence of one methine signal at δ_H 4.67, one methylene group at δ_H 2.79 (dd, $J = 16.6$, 11.6 Hz) and 3.15 (dd, $J = 16.8$, 3.3 Hz), and two methyl groups at δ_H 1.56 (d, $J = 6.3$ Hz) and δ_H 2.19 (Table 1). The ^{13}C NMR spectrum combined DEPT and HSQC experiments demonstrated that **1** was similar to

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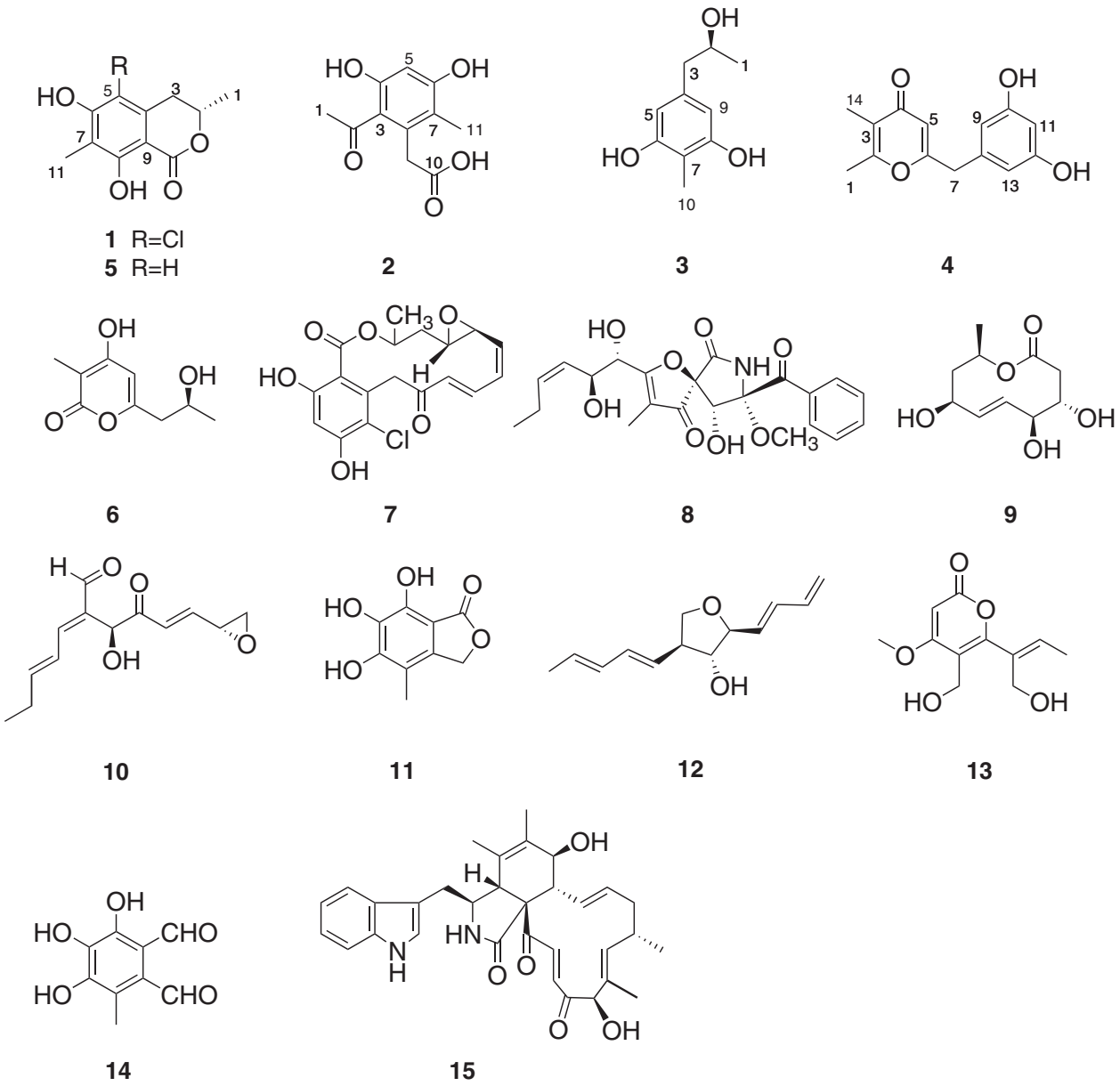

Fig. 1 Structures of compounds **1**−**9** from *C. seminudum*. C208 and **10**−**15** from *Chaetomium* sp. C521

monaschromone (**5**) except for the downfield movement of C-5 (from δ_C 105.5 to 109.2). Further analysis of 2D NMR spectrum including $^1H-^1H$ COSY, HSQC, and HMBC spectra indicated that 1 was a chlorinated monaschromone (**5**) [5] at C-5, which rationalized all the ^1H and ^{13}C NMR spectral data. The stereochemistry of **1** was determined as 2*S*-configuration since the optical rotation of **1** ($[\alpha]_D^{28}$ +124.16, CH_3OH) agreed with that of monaschromone ($[\alpha]_D^{25} = +34.4$, CH_3Cl; $[\alpha]_D^{20} = +74.55$, CH_3OH) [5].

Chaetosemin H (**2**), isolated as a white powder, possessed a molecular formula of $C_{11}H_{12}O_5$ based on the $[M + Na]^+$ peak at *m/z* 247.0572 in its HRESIMS. The ^1H NMR spectrum of **2** gave altogether four singlets at δ_H 6.35, 3.69, 2.54, and 2.07, and its ^{13}C NMR spectrum displayed 11 resonance lines ascribed to six aromatic carbons, two carbonyls and two methyl groups (Table 1). Close analysis of the ^1H and ^{13}C NMR spectra of **2** disclosed that they resembled those of 6-methylcurvulinic acid [6]. This observation was supported by the subsequent 2D NMR (COSY, NOESY, HMQC and HMBC), leading to the unambiguous assignment of all ^1H and ^{13}C NMR data of **2**. In particular, the key HMBC correlations of CH_3-11 (δ_H 2.07) with C-6 (δ_C 159.66) and C-8 (δ_C 134.69) demonstrated that **2** was 7-methylcurvulinic acid.

Chaetosemin I (**3**) was obtained as a colorless amorphous powder, and the molecular formula was deduced to be

Table 1 ^{1}H and ^{13}C NMR data for **1–4**

No.	1[a]		2[b]		3[b]		4[b]	
	δ_C	δ_H [c]	δ_C	δ_H [c]	δ_C	δ_H [c]	δ_C	δ_H [c]
1	20.75	1.56 (d, 6.3)	32.66	2.54 (s)	22.71	1.12 (d, 6.2)	17.74	2.35 (s)
2	74.92	4.67 (m)	207.91		69.89	3.90 (m)	164.84	
3	32.23	3.15 (dd, 16.8, 3.3) 2.79 (dd, 16.6, 11.6)	122.05		46.51	2.42 (dd, 13.2, 7.0) 2.64 (dd, 13.2, 6.3)	121.28	
4	134.11		157.05		138.24		182.37	
5	109.23		102.17	6.35 (s)	108.66	6.19 (s)	112.68	6.14 (s)
6	155.33		159.66		157.01		169.94	
7	111.87		117.56		109.90		40.49	3.72 (s)
8	161.01		134.69		157.01		138.69	
9	101.98		36.43	3.69 (s)	108.66	6.19 (s)	108.48	6.22 (d, 2.1)
10	169.67		175.16		8.31	2.00 (s)	159.94	
11	8.37	2.19 (s)	11.21	2.07 (s)			102.44	6.20 (t, 2.1)
12							159.94	
13							108.48	6.22 (d, 2.1)
14							9.68	1.92 (3 H, s)

[a] ^{1}H (400 MHz) NMR and 100 MHz for ^{13}C recorded of **1** in $CDCl_3$

[b] ^{1}H (400 MHz) NMR and 100 MHz for ^{13}C recorded of **2–4** in methanol-d_4

[c] mult., *J* in Hz

$C_{10}H_{14}O_3$ from its molecular ion at *m/z* 183.1013 ($[M + H]^+$) by HR-ESIMS. IR absorptions implied that **3** possessed hydroxy group (3381 cm^{-1}) and an aromatic ring (1627, 1594 cm^{-1}). The ^{1}H and ^{13}C NMR spectra (δ_H/δ_C2.00/8.31; δ_H/δ_C 6.19 × 2/108.66 × 2, 109.90, 138.24, and 157.01 × 2) indicated that **3** was a 1,2,3,5-tetrasubstituted benzene derivative with a 1,3-dihydroxy-2-methylphenol ring (Table 1), whose data were similar to those of the 1,3-dihydroxyphenol ring in orcinotriol [7]. ^{1}H–^{1}H COSY connectivities of H-1 to H-2 and H-2 to H-3 suggested the presence of a 2-hydroxypropyl group. The structure of **3** was next confirmed by the HSQC, $^{1}H-^{1}H$ COSY, and HMBC experiments, especially the correlations of CH_3-10 with C-6 and C-8, of H-5 with C-3, and of CH_3-1 with C-3. Thus, compound **3** was determined as 7-methylorcinotriol [7]. Comparison of the specific rotations of **3** ($[\alpha]_D^{28}$+12, CH_3OH) with that of (2*S*)-orcinotriol ($[\alpha]_D^{25}$+6, CH_3OH), which was isolated from the culture of the yeast *Aureobasidium pullulans* [7], suggested the absolute configuration at C-2 of **3** to be *S*.

Chaetosemin J (**4**), afforded as a white powder, has a quasi-molecular ion at *m/z* 245.0817 ($[M-H]^-$) by its HR-ESIMS, indicating its molecular formula to be $C_{14}H_{14}O_4$. The ^{1}H NMR spectrum showed three aromatic proton NMR signals at δ_H 6.22 (2 H, d, $J = 2.1$ Hz) and 6.20, suggesting the incorporation of a 1,3,5-trisubstituted benzene unit in the molecule (Table 1). The ^{13}C NMR resonance profile disclosed the presence of the α,β-unsaturated ketone moiety on the basis of the signals at δ_C 182.4, 164.8 and 169.9. Moreover, the ^{1}H and ^{13}C NMR spectra data indicated that it was close to macrocarpon C [8]. The only difference in **4** was the presence of a singlet at δ_H 1.92 (CH_3-14) in the ^{1}H NMR spectrum. The subsequent analysis of its 2D NMR spectra, especially the key HMBC correlations of CH_3-1 with C-3, of CH_3-14 with C-4, and of CH_2-7 with C-5, and C-9 pinpointed that compound **4** was a 3-methylated derivative of macrocarpon C.

In addition, the known compounds were identified as monaschromone (**5**) [5], chaetoquadrin F (**6**) [9], monorden A (**7**) [10], pseurotin A (**8**) [11], decarestrictine D (**9**) [12], mollipilin A (**10**) [13], epicoccone B (**11**) [14], (–)-aureonitol (**12**) [15], chaetoglocin A (**13**) [15], 1,2-benzenedicarboxaldehyde-3,4,5-trihydroxy-6-methyl (flavipin, **14**) [16], chaetoglobosin B (**15**) [17], by comparison of their spectroscopic data with the literature. Interestingly, decarestrictine D (tuckolide, **9**), isolated from *Polyporus tuberaster* [18] and *Penicillium corylophilum* [12] was found to exert inhibitory activity against cholesterol biosynthesis but no other effects, such as antibacterial or antifungal activities.

All the isolated compounds were subjected to bioactive assays, including antifungal, brine shrimp toxicity, antioxidant, and α-glucosidase inhibitory activity as previously reported method [4, 19, 20]. All 15 metabolites except **8** were tested in vitro for the antifungal activity against the phytopathogenic fungi *Botrytis cinerea, Alternaria solani, Magnaporthe oryzae*, and *Gibberella saubinettii*. Some of

them displayed varying degrees of antifungal activities against several plant pathogenic fungi. Among the tested compounds (**1**, **3**−**4**) showed significant antifungal activities (minimum inhibitory concentrations (MICs) = 6.25–50.0 μM). Compound **3** displayed moderate inhibitory activity against *B. cinerea* with the MIC value of 12.5 μM, and **4** exhibited inhibitory activity against *B. cinerea*, *A. solani*, *M. oryzae*, and *G. saubinettii*, with MIC values of 25, 12.5, 12.5, and 25 μM, respectively. Notably, compound **5** inhibited the growth of *B. cinerea*, *A. solani*, *M. oryzae*, and *G. saubinettii*, with the MIC values of 12.5, 12.5, 12.5, and 6.25 μM, which were comparable to the commonly used fungicide carbendazim, indicating that it could be used as a fungicide or as a lead compound of new fungicides.

In this assay, only compound **1** possessed a weak lethality activity toward brine shrimps (*Artemia salina*) with corrected mortality rate 57.8% at 100 μM, while the positive control toosendanin giving 100% corrected mortality rate at 1.0 μM, while others including **14** did not display any effects.

Compounds **11** and **14** presented the moderate antioxidant activity with regard to DPPH radical scavenging ability, with IC_{50} values of 10.8 and 7.2 μM, respectively, compared to that of the positive control ascorbic acid (IC_{50} = 6.5 μM). The antioxidant ability of compound **14** was consistent with the results reported [16].

Moreover, in the α-glucosidase inhibitory activity assay, both **11** and **14** inhibited this enzyme with an IC_{50} values of 27.3 and 33.8 μM, respectively, which were almost 30-fold stronger than that of acarbose (IC_{50} = 838.3 μM), a well known drug, while other compounds (**2–7**, and **9**) had no effects at 50 μM, and no test of compounds (**1**, **8**) was carried out due to lack of sufficient sample. To the best of our knowledge, this is the first time that both **11** and **14** were found to have α-glucosidase inhibitory activities.

In conclusion, 15 polyketides including four new ones, namely, chaetosemins G-J (**1**−**4**) were isolated and identified from the culture of *C. seminudum* C208 and of *Chaetomium* sp. C521, respectively. In the bioassays, both epicoccone B (**11**) and flavipin (**14**) exhibited an obvious antioxidant and α-glucosidase inhibitory activities, while chaetosemin J (**4**) and monaschromone (**5**) displayed the potential antifungal effects on the test fungal pathogens. Collectively, our findings indicated that chaetosemin J (**4**) and monaschromone (**5**) might act as the lead compounds of pesticide.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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