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## Revision of the structure of isochaetoglobosin D<sub>b</sub> based on NMR analysis and biosynthetic consideration†

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Isochaetoglobosin D<sub>b</sub> is a new chaetoglobosin possessing a unique 3,4-substituted pyrrole ring isolated and named by Qiu *et al.*, and it is different from any one of the 14 sub-types in the macrocyclic ring of chaetoglobosins classified in our previous work. Its chemical shift values, coupling constants and biosynthetic consideration implied that the proposed structure of isochaetoglobosin D<sub>b</sub> was incorrect. In this report, based on detailed NMR data analysis together with biosynthetic consideration, the structure of isochaetoglobosin D<sub>b</sub> is suggested to be revised to that of penochalasin C. The NMR spectra of penochalasin C measured in the same solvent (DMSO-*d*<sub>6</sub>) as that of isochaetoglobosin D<sub>b</sub> supported the above conclusion. The results imply that reasonable biosynthetic consideration could complement spectroscopic structural determination, and also support that the <sup>1</sup>H-NMR rule of chaetoglobosins summarized in our previous work can provide help for dereplication and rectification.

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### 1. Introduction

Chaetoglobosins are a large member of mycotoxins originating from a PKS-NRPS hybrid megasynthetase.<sup>1–3</sup> The core skeleton mainly consists of three parts: an indol-3-yl, a perhydro-isoindolone and a macrocyclic ring. Other post-modifications mainly including oxygenation, dehydration, and rearrangement increase the chemical diversity of this group of mycotoxins.<sup>4,5</sup> According to the possible biosynthesis and structural features, 9 sub-types in the perhydro-isoindolone part and 14 sub-types in the macrocycle ring of chaetoglobosins are classified in our previous work.<sup>5</sup> Recently, Qiu *et al.* described two new chaetoglobosin analogues named isochaetoglobosin D<sub>b</sub> and cytoglobosin A<sub>b</sub>, respectively, from an extreme fungus *Chaetomium globosum* SNSHI-5.<sup>6</sup> The authors elucidated the new structures mainly based on NMR spectra. Structural

features of isochaetoglobosin D<sub>b</sub> revealed that this compound is a new sub-type of chaetoglobosins in the macrocycle ring by possessing a unique 3,4-substituted pyrrole ring. Our group have a longstanding interest in the structural elucidation, biosynthesis and biological effects of chaetoglobosins.<sup>5,7,8</sup> The special pyrrole unit in the macrocyclic ring of isochaetoglobosin D<sub>b</sub> is different from any one of 14 sub-types summarized in our previous work,<sup>5</sup> and also the unusual chemical shift values and coupling constants of the 3,4-substituted pyrrole ring in the structure implies incorrect structure determination. Carefully comparing NMR chemical shift values from different analogues, and analyzing coupling constants from different-substituted pyrrole ring also together with considering chaetoglobosin biosynthesis revealed that the structure of isochaetoglobosin D<sub>b</sub> was incorrect and should be revised to be as penochalasin C (Fig. 1). The NMR spectra of penochalasin C was measured in the same solvent (DMSO-*d*<sub>6</sub>) as that of isochaetoglobosin D<sub>b</sub>, which further supported the above conclusion that isochaetoglobosin D<sub>b</sub> and penochalasin C are the same structure. In this report, structural revision from isochaetoglobosin D<sub>b</sub> to penochalasin C is provided based on NMR data analysis and biosynthetic consideration.

### 2. Results and discussion

The structure of isochaetoglobosin D<sub>b</sub> was characterized mainly by NMR spectra including <sup>1</sup>H, <sup>13</sup>C, <sup>1</sup>H-<sup>1</sup>H COSY, HSQC, and HMBC spectrum.<sup>6</sup> In the ESI,† the cross peaks of H-21, H-22 and NH-24 were observed in the <sup>1</sup>H-<sup>1</sup>H COSY spectrum, but in the HMBC spectrum, the cross peaks from H-21, H-22 or NH-24 to

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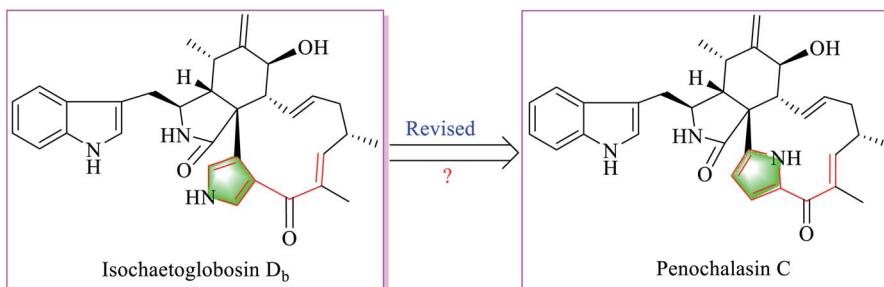


Fig. 1 The structure of isocheatoglobosin D<sub>b</sub> and penochalasin C.

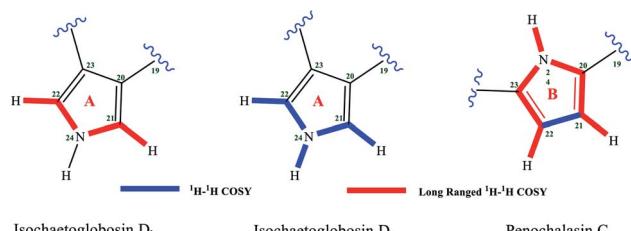


Fig. 2 Two possible fragments A or B in isocheatoglobosin D<sub>b</sub> based on  $^1\text{H}$ - $^1\text{H}$  COSY correlations.

C-20, C-21, C-22 or C-23 were not observed.<sup>5</sup> The *W*-long-ranged correlations in conjugated ring system such as pyrrole ring is often observed in  $^1\text{H}$ - $^1\text{H}$  COSY spectrum, for example, the correlation of H-21 with H-22 in fragment A. Considering no HMBC correlations from H-21, H-22 and NH-24 to any carbons in the structure of isocheatoglobosin D<sub>b</sub> (**1**), actually, the fragment B also conforms to the *W*-long-ranged correlations (of H-22 with NH-24, and of H-21 with NH-24) in the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum. Thus, according to the ESI $^{\dagger}$  provided by authors, there might exist two possible sub-structures about the pyrrole ring: fragments A or B (Fig. 2).

If the fragment A is right, the chemical shift values of  $\beta$ -H/C (H/C-21) on the pyrrole ring are not reasonable compared with compounds possessing similar pyrrole units. There is an  $\alpha$ , $\beta$ -unsaturated ketone group in fragment A, and the  $\beta$ -position is connected with a nitrogen atom, which will lead the chemical shift values of  $\beta$ -H/C to be deshielded. The chemical shift values of  $\beta$ -H/C are 7.40/127.3, and 7.67/123.7 in verrucarin E<sup>9,10</sup> and azamonosporascone<sup>11</sup> with the similar  $\alpha$ , $\beta$ -unsaturated ketone

group, whereas chemical shift values of  $\beta$ -H/C of isocheatoglobosin D<sub>b</sub> were 6.60/113.7.<sup>6</sup> The differences of  $\beta$ -H/C in similar fragment of three compounds are significant, implying that the structure of fragment A in isocheatoglobosin D<sub>b</sub> is not right (Fig. 3).

The  $^{13}\text{C}$ -NMR chemical shift values of fragment A (including C-19) in isocheatoglobosin D<sub>b</sub> (**1**),<sup>6</sup> and fragment B in penochalasins A-C,<sup>12</sup> armochaetoblobsin K-M, and R were compared and analyzed (Fig. 4).<sup>13</sup> Comparison of the  $^{13}\text{C}$  chemical shift values of C-19, C-20, C-21, C-22 and C-23 between isocheatoglobosin D<sub>b</sub>, penochalasins A-C, armochaetoblobsins K-M, and R revealed that the chemical shift values of these carbons were nearly same. They implied that the pyrrole ring (fragment A) in isocheatoglobosin D<sub>b</sub> should be reassigned as fragment B (Table 1).

Coupling constants analysis are also diagnostic about the substitution position on a pyrrole ring. If a pyrrole ring is substituted at C-2 and C-5 such as found in penochalasin C, the coupling constants of H-3/H-4 is, usually, more than 3.0 Hz ( $^3J_{3,4} > 3.0$  Hz); If a pyrrole ring is substituted at C-3 and C-4 such as found in isocheatoglobosin D<sub>b</sub>, the coupling constants of H-2/H-5 (as *W*-long-ranged correlation) is at 2.0–3.0 Hz ( $^4J_{2,5} = 2.0$ –3.0 Hz).<sup>14–19</sup> Analysis of the  $^1\text{H}$  NMR of isocheatoglobosin D<sub>b</sub> revealed that the coupling constants of H-21/H-22 was 3.6 Hz, which did not conform to the rule mentioned-above. On the contrary, the coupling constants of H-21/H-22 in penochalasin C was also 3.6 Hz (Fig. 5). These analyses further supported that the fragment A in isocheatoglobosin D<sub>b</sub> (**1**) should be assigned as fragment B.

Penochalasins A-C were first isolated from a marine alga symbiotic fungus *Penicillium* species in 1995, and other

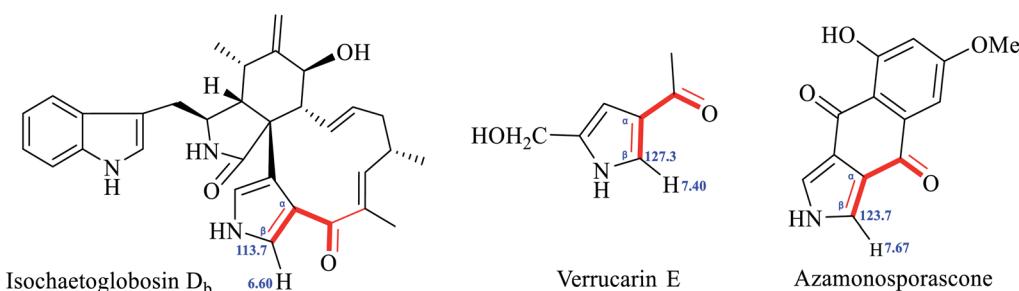


Fig. 3  $\beta$ -H/C chemical shift values of isocheatoglobosin D<sub>b</sub>,<sup>6</sup> verrucarin E<sup>9,10</sup> and azamonosporascone.<sup>11</sup>



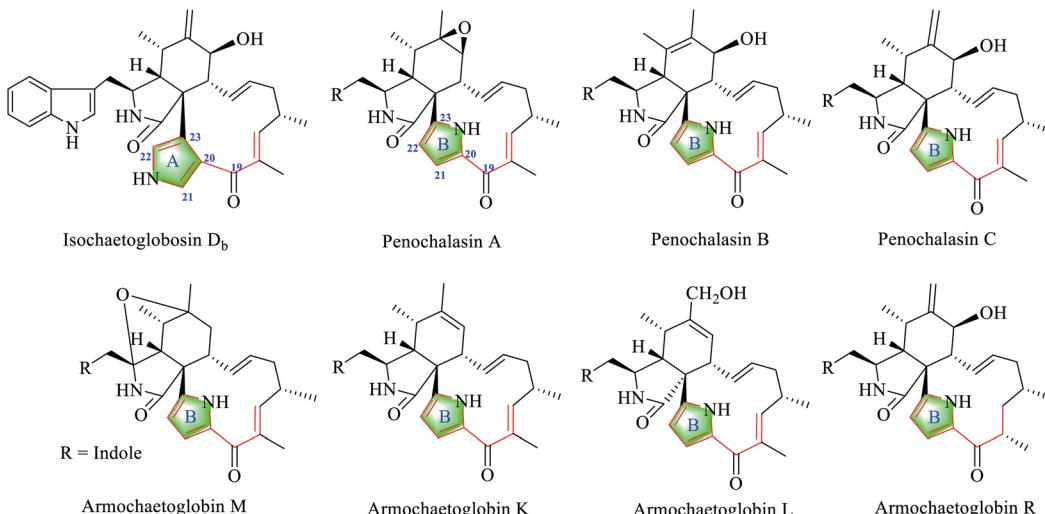


Fig. 4 Structures of isochaetoglobosin D<sub>b</sub>, penochalasins A–C<sup>12</sup>, armochaetoglobins K–M and R.

Table 1 <sup>13</sup>C-NMR data of pyrrole ring in 1<sup>6</sup>, penochalasins A–C<sup>12</sup>, armochaetoglobins K–M and R<sup>20</sup> in DMSO-*d*<sub>6</sub>, CDCl<sub>3</sub> and CD<sub>3</sub>OD

Pos.	Penochalasin A (CDCl <sub>3</sub> )	Penochalasin B (CDCl <sub>3</sub> )	Penochalasin C (CDCl <sub>3</sub> )	Armochaetoglobins K (CD <sub>3</sub> OD)	Armochaetoglobins L (CDCl <sub>3</sub> )	Armochaetoglobins M (CDCl <sub>3</sub> )	Armochaetoglobins R (DMSO- <i>d</i> <sub>6</sub> )
19	189.3	188.47	189.49	188.04	190.5	190.6	187.6
20	130.1	126.79	126.81	126.90	130.4	130.5	129.7
21	113.7	114.92	114.46	115.07	116.4	116.4	114.8
22	108.0	109.48	109.47	109.17	111.3	111.2	108.9
23	140.7	138.90	138.50	139.81	142.3	142.2	137.2

analogues including penochalasins D–H, and chaetoglobosin O were later isolated from the same fungus.<sup>12,15</sup> It was the first report of chaetoglobosin analogues by possessing a unique pyrrole ring in the macrocyclic ring system. From the structural features, the pyrrole ring in penochalasin A (penochalasin B) might be originated from penochalasin E/F (penochalasin H) through the possible intermediate chaetoglobosin C (chaetoglobosin G) by amination and dehydration at C-20, C-21, C-22 and C-23 (Fig. 6).

In 2006, our group isolated five analogues including chaetoglobosins C, E, F, U and penochalasin A from an endophytic fungus *Chaetomium globosum* IFB-E019.<sup>7</sup> Though the structural relationship of these chaetoglobosins were not suggested at that time, the macrocyclic difference in chaetoglobosin C and

chaetoglobosin U, penochalasin A implied that the additional cyclopent-2-en-1-one (C-17, C-18, C-19, C-20 and C-21, Fig. S14†) in chaetoglobosin U might be derived from chaetoglobosin C by the intramolecular Michael-addition reaction at C-17 and C-21, whereas the pyrrole ring in penochalasin A could be biosynthesized from chaetoglobosin C by same reactions as those found in Fig. 6.

Recently, Prof Zhang's group also isolated a series of new pyrrole-based chaetoglobosins armochaetoglobins K–R together with other new analogues from *Chaetomium globosum* (TW1-1).<sup>13,20,21</sup> The authors suggested the possible biosynthetic pathway of pyrrole-based chaetoglobosins according to the structural features. When analyzing the structural characteristics, we found the same biosynthetic relationships of these



Fig. 5 Coupling constants analysis of pyrrole ring in 1 and penochalasin C.



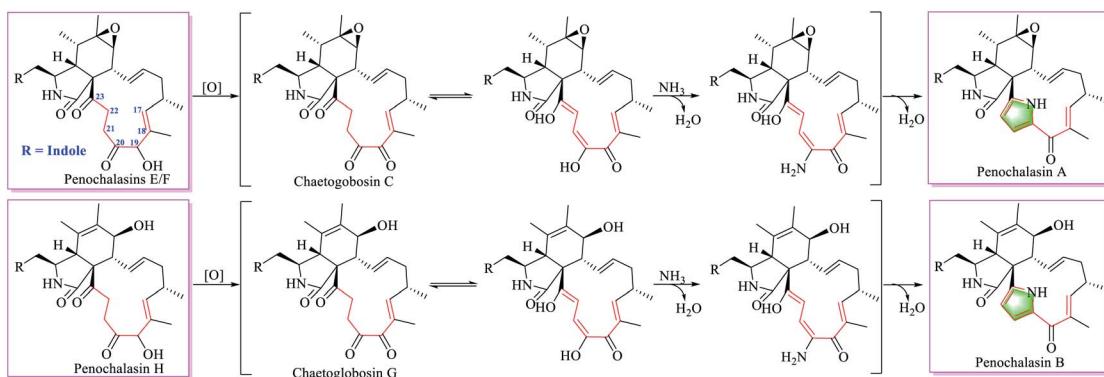


Fig. 6 Putative biosynthetic relationship of chaetoglobosin analogues.

Table 2 NMR data of compounds isochaetoglobosin D<sub>b</sub><sup>6</sup> and penochalasin C<sup>12,22,23</sup> in DMSO-*d*<sub>6</sub> and CDCl<sub>3</sub><sup>a</sup>

Position	Isochaetoglobosin D <sub>b</sub> (DMSO- <i>d</i> <sub>6</sub> )		Penochalasin C (DMSO- <i>d</i> <sub>6</sub> )		Penochalasin C (CDCl <sub>3</sub> )	
	$\delta_{\text{H}}$ , mult ( <i>J</i> in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ , mult ( <i>J</i> in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ , mult ( <i>J</i> in Hz)	$\delta_{\text{C}}$
1		175.3, C		175.2, C		169.87, C
2	8.16, s		8.13, s		5.80, br s	
3	3.30, m	53.4, CH	3.29, m	53.3, CH	3.54, dt (10.2, 4.0)	53.16, CH
4	2.35, m	51.6, CH	2.35, m	51.5, CH	2.75, t (4.0)	53.00, CH
5	2.72, m	31.8, CH	2.72, m	31.8, CH	2.98, qd (6.5, 4.0)	32.32, CH
6		151.6, C		151.5, C		147.92, C
7	3.78, m	69.3, CH	3.78, dd (6.0, 10.2)	69.1, CH	4.02, br d (10.8)	68.62, CH
8	3.19, m	47.9, CH	3.19, t (10.2)	47.9, CH	3.05, t (10.0)	49.82, CH
9		49.5, C		49.4, C		49.82, C
10	2.95, m 2.92, m	33.1, CH <sub>2</sub> 112.2, CH <sub>2</sub>	2.94, m 2.94, m	33.1, CH <sub>2</sub> 112.1, CH <sub>2</sub>	2.98, dd (14.0, 10.2) 3.16, dd (14.0, 4.0)	34.85, CH <sub>2</sub> 114.62, CH <sub>2</sub>
11	0.58, d (6.6)	13.9, CH <sub>3</sub>	0.59, d (6.6)	13.8, CH <sub>3</sub>	1.24, d (6.5)	15.10, CH <sub>3</sub>
12	4.86, s		4.86, s		5.25, s	
	5.16, s		5.16, s		5.48, s	
13	6.16 dd	132.0, CH	6.17 dd (9.6, 15.6)	131.9, CH	6.67, ddd (15.5, 10.0, 1.6)	132.63, CH
14	5.56, m	135.1, CH	5.56, m	135.0, CH	5.82, ddd (15.5, 11.5, 3.2)	138.08, CH
15	1.87, m	41.4, CH <sub>2</sub>	1.87, m	41.3, CH <sub>2</sub>	2.19 dt (15.5, 11.5); 2.61 dddd (13.5, 4.8, 3.2, 1.6)	41.27, CH <sub>2</sub>
	2.43, m		2.43, m			
16	2.76, m	33.3, CH	2.76, m	33.2, CH	2.91, m	34.09, CH
17	5.29, dd (9.6, 1.5)	146.1, CH	5.28, d (9.0)	145.9, CH	5.68, dq (9.4, 18)	142.07, CH
18		135.4, C		135.2, C		135.08, C
19		189.3, C		189.1, C		188.04, C
20		130.1, C		130.0, C		126.90, C
21	6.60, d (3.6)	113.7, CH	6.59, dd (2.4, 3.6)	113.5, CH	7.02, dd (3.9, 2.7)	115.07, CH
22	5.65, t (3.3)	108.0, CH	5.66, dd (2.4, 3.6)	107.9, CH	6.18, dd (3.9, 2.7)	109.17, CH
23		140.7, C		140.6, C		139.81, C
24	10.53, br s		10.52, br s		10.78, br s	
25	0.98, d (6.8)	19.9, CH <sub>3</sub>	0.97, d (6.6)	19.8, CH <sub>3</sub>	1.10, d (7.0)	19.78, CH <sub>3</sub>
26	1.81, s	13.2, CH <sub>3</sub>	1.81, s	13.1, CH <sub>3</sub>	1.95, d (2.0)	13.68, CH <sub>3</sub>
1'	10.91, brs		10.90, s		8.21, br s	
2'	7.16, d (2.2)	124.6, CH	7.15, d (1.8)	124.5, CH	7.09, d (2.3)	122.86, CH
3'		110.2, C		110.1, C		111.47, C
3'a		128.2, C		128.1, C		129.77, C
4'	7.36, d (9.0)	118.4, CH	7.35, d (9.0)	118.3, CH	7.55, dd (8.0, 1.0)	118.44, CH
5'	7.05, t (7.1)	121.3, CH	7.05, t (7.2)	121.2, CH	7.25, td (8.0, 1.0)	122.60, CH
6'	6.94, t (7.7)	118.9, CH	6.94, t (7.8)	118.7, CH	7.15, td (8.0, 1.0)	119.99, CH
7'	7.34, d (9.0)	111.9, CH	7.34, d (9.0)	111.8, CH	7.40, dd (8.0, 1.0)	111.62, CH
7'a		136.6, C		136.4, C		136.51, C
7-OH	4.87, d (5.9)		4.81, d (5.4)		2.00, br s	

<sup>a</sup> There are some signal assignments are corrected for isochaetoglobosin D<sub>b</sub>.

analogues as those found in Fig. 6 and S14.† For example armochaetoglobin X might come from armochaetoglobin U by the intramolecular Michael-addition reaction, which could be originated from isochaetoglobosin J by oxidation and dehydration, whereas the pyrrole ring in armochaetoglobin K might be derived from isochaetoglobosin J by amination and dehydration (Fig. S15†).

Qiu *et al.* reported two new chaetoglobosin analogues isochaetoglobosin D<sub>b</sub> and cytoglobosin A<sub>b</sub> isolated from an extreme fungus *C. globosum* SNSHI-5.<sup>6</sup> Though the authors did not report known analogues or possible intermediates from this fungus, according to the structural characteristics, the possible biosynthetic relationship from these chaetoglobosins were suggested, which possesses the same biosynthetic pathway as those found in Fig. 6, and S14–S16.† Thus, the 3,4-substituted pyrrole in isochaetoglobosin D<sub>b</sub> should be reassigned to be the 2,5-substituted pyrrole in penochalasin C. This result also conforms to the rule summarized in our previous report.

Fortunately, penochalasin C was isolated from an endophytic fungus *C. globosum* in our lab.<sup>22,23</sup> The NMR spectra of penochalasin C were obtained in the same solvent system (DMSO-*d*<sub>6</sub>) as that of isochaetoglobosin D<sub>b</sub> (ESI†). <sup>1</sup>H-<sup>1</sup>H COSY spectrum revealed the correlations H-21, H-22 and 24-NH, and the HMBC correlations from H-21 to C-20, C-22, and C-23, from H-22 to C-20, C-21, and C-23 confirmed a 2,5-substituted pyrrole unit in penochalasin C. The other NMR data including <sup>1</sup>H, and <sup>13</sup>C data of penochalasin C were the same as those of isochaetoglobosin D<sub>b</sub>, which further confirmed the conclusion that penochalasin C and isochaetoglobosin D<sub>b</sub> were the same structure (Table 2).

### 3. Conclusion

In conclusion, isochaetoglobosin D<sub>b</sub> is revised to be penochalasin C based on NMR data analysis including chemical shift value, coupling constants analysis, and biosynthetic consideration. The results in this report not only confirm that reasonable biosynthetic consideration could complement spectroscopic structural determination, but also further confirm that the <sup>1</sup>H-NMR rule chaetoglobosin summarized in our previous work can provide helps for chaetoglobosin dereliction and rectification.

## 4. Experimental section

### 4.1 General experimental procedures

NMR spectra were acquired with a Bruker AVANCE600 spectrometer. HRESIMS were obtained using a TOF-ESI-MS (Bruker UHR-TOF maXis). Preparation of HPLC was conducted on Agilent 1260 and equipped with a DAD detector (G1315D) and a 4.6 mm × 150 mm i.d., 5 μm, C18 column (HITACHI LaChrom, Tokyo, Japan). Silica gel (200–300 mesh) (Qingdao Marine Chemical Plant) and Sephadex LH-20 (40–70 μm) (Pharmacia Biotech AB, Uppsala, Sweden), chromatographically pure methanol was purchased from TEDIA Chemical Reagent Limited Company. (TEDIA, Ohio, America) and deuterated reagent was purchased from Sigma-Aldrich.

### 4.2 Fungal material and fermentation

The strain of *C. globosum* was isolated from *Imperata cylindrical* and identified by Dr Chun-yong Song and deposited in the Institute of Functional Biomolecules of Nanjing University. It was grown on PDA plates at 28 °C for 5 days. Then the fresh mycelium was inoculated into autoclaving sterilized potato liquid media (potato 200 g and glucose 20 g with water 1000 mL) cultivated for 7 days in shaking culture (28 °C, 140 rpm). The resulting seeds were separated 15 mL per bottle and inoculated into autoclaving sterilized solid media (millet 7.5 g, wheat bran 7.5 g, yeast extract 0.5 g, sodium tartrate 0.1 g, sodium glutamate 0.1 g, green grind 0.01 g and corn oil 0.1 mL with water 15 mL in 250 mL flasks) cultivated for 30 days.

### 4.3 Extraction and isolation

After the solid fermentation product was crushed and dried, the culture was extracted with chloroform-methanol (1 : 1) for four times, and the water extract was extracted with ethyl acetate, the solvent was evaporated to afford 26 g of crude extract. The original extract was fractionated on a silica gel column chromatography (CC) using chloroform-methanol gradient elution (100 : 0–0 : 100 v/v, each 1000 mL) to get seven fractions (Fr.1 to Fr.7). Fr.2 (3.8 g) was fractionated on a silica gel CC eluted with chloroform-methanol gradient elution (100 : 0, 100 : 1, 100 : 2, 100 : 4, 100 : 8 and 0 : 100 v/v, each 1000 mL) to get six sub-fractions (Fr.2-1 to Fr.2-6). Fr.2-2 was fractionated on a silica gel CC eluted with chloroform-methanol gradient elution (100 : 0–0 : 100 v/v, each 1000 mL) to get Fr. 2-2-2 and Fr. 2-2-3, Fr. 2-2-3 was eluted by Sephadex LH-20 using chloroform-methanol gradient elution to get Fr.2-2-3-3 (1 : 1 v/v, each 1000 mL). Fr.2-2-3-3 recrystallization mother liquor was purified by HPLC (55% CH<sub>3</sub>OH in H<sub>2</sub>O, v/v, 1.0 mL min<sup>-1</sup>, 254 nm, *t*<sub>R</sub> = 35.1 min) to obtain compound penochalasin C (12 mg).

### Conflicts of interest

There are no conflicts of interest to declare.

### Acknowledgements

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