Cytoglobosins A−G, Cytochalasans from a Marine-Derived Endophytic Fungus, *Chaetomium globosum* QEN-14

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Cytoglobosins A−G (**1**−**7**), seven new cytochalasan derivatives, along with two structurally related known compounds, isochaetoglobosin D (**8**) and chaetoglobosin F_{ex} (**9**), were isolated and identified from the cultures of *Chaetomium globosum* QEN-14, an endophytic fungus derived from the marine green alga *Ulva pertusa*. The structures of the new natural products as well as their relative configurations were elucidated on the basis of 1D and 2D NMR spectra (COSY, HSQC, HMBC, and NOESY) and HRESIMS data. Cytoglobosins C and D (**3** and **4**) displayed cytotoxic activity against the A-549 tumor cell line.

Cytochalasans are a large group of fungal alkaloids with a wide range of biological activities targeting cytoskeletal processes.[1] Some of them also possess phytotoxic, cytotoxic, and antibiotic activities.[1–5] Chaetoglobosins are cytochalasans with the phenyl group in the molecules being replaced by an indolyl group.[1–5] The fungal genus *Chaetomium*, which includes both marine- and terrestrial-derived species, was revealed to be a rich source of chaetoglobosins, and to date around 40 chaetoglobosins and analogues have been isolated and identified from the genus.

In our continuing investigation on the bioactive secondary metabolites from the marine algal-derived endophytic fungi,[6–11] seven new cytochalasan derivatives of the chaetoglobosin class, namely, cytoglobosins A−G (**1**−**7**), together with two structurally related known compounds, isochaetoglobosin D (**8**) and chaetoglobosin F_{ex} (**9**), were isolated and identified from *Chaetomium globosum* QEN-14, an endophytic fungus derived from the marine green alga *Ulva pertusa*. The structures of these metabolites were established by extensive analyses of spectroscopic data including 2D NMR experiments (^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY). The cytotoxic activities against P388, A549, and KB cell lines were tested. Cytoglobosins C (**3**) and D (**4**) showed moderate activity against the A-549 cell line. This paper describes the isolation and structure elucidation of these compounds.

The mycelia and culture broth of *C. globosum* were separated by filtration and then exhaustively extracted with MeOH and EtOAc, respectively. The combined extracts were further purified by a combination of column chromatography (CC) including silica gel, reversed-phase silica gel C_{18}, and Sephadex LH-20 to yield nine cytochalasan derivatives (**1**−**9**) as homogeneous compounds.

Cytoglobosin A (**1**) was obtained as a colorless, amorphous powder, and its molecular formula $C_{32}H_{36}N_2O_5$ was determined by positive HRESIMS data. Detailed analysis of the 1D and 2D NMR spectroscopic data revealed that **1** had a similar structure to chaetoglobosin U (**10**), a cytochalasan-based alkaloid characterized from an endophytic strain of *C. globosum*, residing inside the stem of the healthy plant *Imperata cylindrica*.[12] The main differences between the two compounds are at positions C-6, C-7, and C-12, with the methyl group (C-12) and the C-6/C-7 epoxy group in chaetoglobosin U (**10**) being replaced by the exocyclic olefin $\Delta^{6(12)}$ and the hydroxy substituent at C-7 in **1**. This deviation can be established by comparison of their NMR spectroscopic data. The carbon signals at δ_C 19.6 (CH_3, C-12) for the methyl group and at δ_C 57.5 (C, C-6) and 60.9 (CH, C-7) for the C6/C7 epoxy group in the ^{13}C NMR spectrum of **10** were replaced by two olefinic carbon signals at δ_C 112.2 (CH_2, C-12) and 150.7 (C, C-6) and one oxymethine signal at δ_C 70.5 (CH, C-7), respectively, in **1** (Table 1). In addition, the proton signals at δ_H 1.12 (s, H-12) and 2.86 (d, J = 5.6 Hz, H-7) in the ^{1}H NMR spectrum of **10** were replaced by exocyclic olefinic methylene signals at δ_H 4.86 (s, H-12a) and 5.07 (s, H-12b) and one oxymethine signal at δ_H 3.85 (dd, J = 6.6, 9.5 Hz, H-7) in **1**. The observed HMBC correlations (Figure 1) from H-12 and H-8 to C-7 and from H-11 to C-6 supported the above deduction.

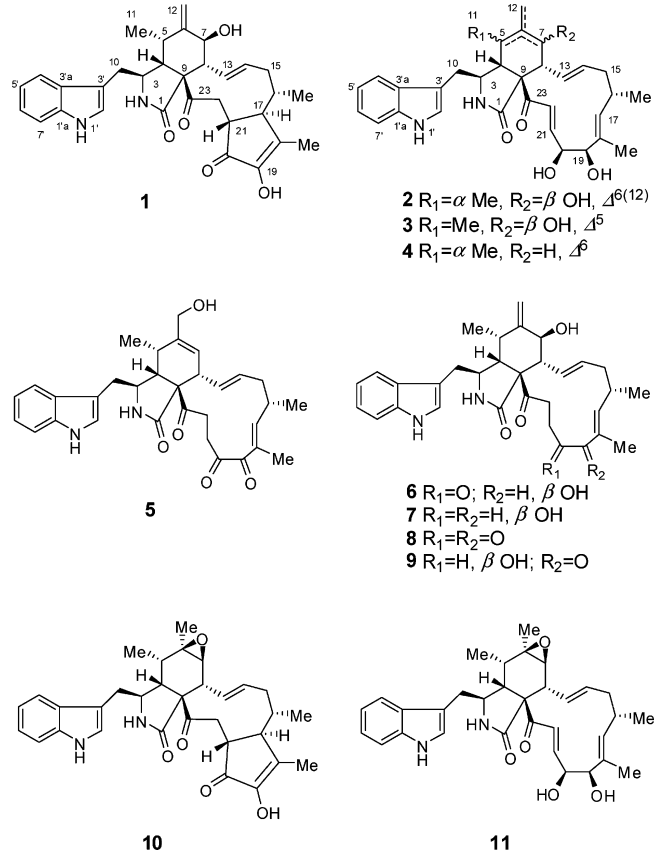

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Table 1. ^{13}C NMR Data for Compounds **1**−**7** in DMSO-d_6 (125 MHz)

no.	1	2	3	4	5	6	7
1	173.6, C	172.8, C	173.2, C	173.3, C	174.0, C	173.0, C	173.2, C
3	51.4, CH	51.5, CH	57.5, CH	52.9, CH	53.8, CH	51.7, CH	51.4, CH
4	46.9, CH	45.9, CH	47.7, CH	48.7, CH	49.6, CH	45.9, CH	46.1, CH
5	31.4, CH	30.8, CH	125.0, C	34.1, CH	34.1, CH	31.5, CH	32.0, CH
6	150.7, C	150.8, C	134.5, C	138.4, C	142.2, C	150.9, C	151.0, C
7	70.5, CH	70.5, CH	67.5, CH	126.0, CH	125.4, CH	69.8, CH	70.2, CH
8	50.9, CH	47.0, CH	49.9, CH	45.4, CH	45.3, CH	48.5, CH	49.8, CH
9	65.3, C	61.0, C	60.5, C	66.1, C	65.2, C	62.0, C	61.6, C
10	32.3, CH_2	32.5, CH_2	31.3, CH_2	33.4, CH_2	30.8, CH_2	33.4, CH_2	31.3, CH_2
11	12.5, CH_3	12.4, CH_3	14.5, CH_3	13.3, CH_3	12.0, CH_3	13.0, CH_3	13.1, CH_3
12	112.2, CH_2	112.0, CH_2	16.7, CH_3	21.3, CH_3	61.4, CH_2	111.9, CH_2	111.7, CH_2
13	130.8, CH	126.3, CH	127.1, CH	128.8, CH	129.0, CH	127.6, CH	130.4, CH
14	132.4, CH	134.5, CH	135.0, CH	132.0, CH	131.3, CH	132.5, CH	130.7, CH
15	43.5, CH_2	39.0, CH_2	39.0, CH_2	41.2, CH_2	39.0, CH_2	41.1, CH_2	41.9, CH_2
16	41.7, CH	31.8, CH	31.7, CH	31.5, CH	32.6, CH	31.7, CH	30.5, CH
17	52.3, CH	131.5, CH	131.6, CH	131.8, CH	154.8, CH	137.2, CH	133.4, CH
18	146.6, C	133.7, C	133.9, C	136.1, C	130.1, C	131.6, C	134.0, C
19	149.3, C	78.2, CH	78.3, CH	78.1, CH	195.7, C	81.6, CH	77.3, CH
20	202.7, C	75.8, CH	75.5, CH	75.3, CH	204.9, C	209.5, C	71.0, CH
21	50.6, CH	147.2, CH	147.9, CH	145.6, CH	31.9, CH_2	31.5, CH_2	26.6, CH_2
22	41.7, CH_2	123.2, CH_2	122.8, CH	125.0, CH	36.6, CH_2	34.2, CH_2	35.9, CH_2
23	212.0, C	197.6, C	198.3, C	198.3, C	208.3, C	208.5 C	210.1, C
16-Me	20.9, CH_3	21.3, CH_3	21.2, CH_3	19.3, CH_3	19.0, CH_3	21.1, CH_3	21.1, CH_3
18-Me	16.4, CH_3	13.5, CH_3	13.5, CH_3	12.8, CH_3	10.2, CH_3	10.6, CH_3	12.3, CH_3
1′a	136.0, C	135.1, C	136.1, C	133.9, C	135.8, C	135.9, C	136.0, C
2′	124.0, CH	123.4, CH	123.2, CH	123.7, CH	125.2, CH	124.6, CH	125.0, CH
3′	109.0, C	109.9, C	110.2, C	109.6, C	108.4, C	108.8, C	108.4, C
3′a	127.4, C	127.3, C	125.9, C	127.5, C	127.9, C	128.8, C	127.7, C
4′	117.9, CH	117.9, CH	118.0, CH	118.0, CH	118.5, CH	118.1, CH	118.3, CH
5′	118.3, CH	118.3, CH	118.3, CH	118.3, CH	118.6, CH	118.4, CH	118.5, CH
6′	120.6, CH	120.8, CH	120.9, CH	120.7, CH	120.8, CH	120.6, CH	120.6, CH
7′	111.4, CH	111.3, CH	110.3, CH	111.3, CH	111.2, CH	111.3, CH	111.0, CH

Chaetoglobosins usually possess 32 carbon atoms in the molecules including an indolyl moiety. A survey of the literature revealed that changes among the structures of the chaetoglobosins mainly occur in positions C-5, C-6, C-7, C-12, C-19, C-20, C-21, and C-22, while the other positions are fixed in most cases.[1–5,12,13] The relative configuration of **1** was established by comparing the NMR data with those reported for chaetoglobosin F_{ex} (**9**)[13] (Table S4 in the Supporting Information) and chaetoglobosin U (**10**)[12] as well as by the NOESY spectroscopic data. In the ^1H NMR spectrum, the large coupling constant ($J = 9.5$ Hz) for H-7 and H-8 suggested the axial−axial orientation of the proton pair. The *E*-geometry of the Δ^{13}-double bond was deduced from the large coupling constant observed for H-13 and H-14 ($J = 15.4$ Hz). In the ^{13}C NMR spectrum of **1**, the similar chemical shifts of carbons C-16, C-17, and C-21 to those of **10**[12] suggested that the carbons C-16, C-17, and C-21 in **1** might share the same configuration. Similarly, the chemical shifts of carbons C-3, C-4, C-5, C-7, and C-8 in the ^{13}C NMR spectrum of **1** were very similar to those in the ^{13}C NMR spectrum of **9** (Table S4). In the NOESY experiment, the observed correlations from H-7 to H-13, from H-4 to H-8, and from H-16 to H-21 (Figure S1 in the Supporting Information) confirmed the relative configuration of **1**, which was compatible with that offered by molecular modeling (Chem 3D, Figure S1).

The structure elucidation of cytoglobosins B−G (**2**−**7**) was straightforward due to their close relationships with isochaetoglobosin D (**8**),[2] chaetoglobosin F_{ex} (**9**),[13] and 20-dihydrochaetoglobosin A (**11**).[13] The Δ^{13}-double bonds in **2**−**7** and Δ^{21}-double bonds in **2**−**4** were deduced to have the *E*-geometry on the basis of the large coupling constants observed, as shown in Table 2.

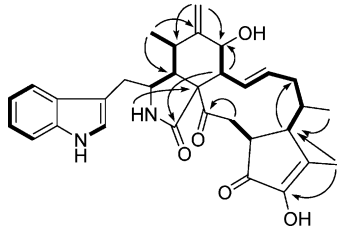

Figure 1. Key COSY (bold lines) and HMBC (arrows) correlations of **1**.

The molecular formula of cytoglobosin B (**2**) was determined to be $C_{32}H_{38}N_2O_5$ by HRESIMS data. The general features of its NMR spectroscopic data (Tables 1 and 2) closely resembled those of 20-dihydrochaetoglobosin A (**11**), a cytochalasan derivative identified from *C. subaffine*.[13] However, the signals for the C-12 methyl and C6/C7 epoxy groups in **11** were missing in the spectra of **2**. Instead carbon signals resonating at δ_C 112.0 (CH_2, C-12) and 150.8 (C, C-6) for the exocyclic olefin $\Delta^{6(12)}$ and at δ_C 70.5 (CH, C-7) for the C-7 oxymethine group were observed in the ^{13}C NMR spectrum of **2**. Moreover, two olefinic protons at δ_H 4.96 (s, H-12a) and 4.78 (s, H-12b) and an allylic oxymethine proton at δ_H 3.67 (dd, $J = 6.7$, 9.5 Hz, H-7) were detected in the ^1H NMR spectrm of **2**. These observations indicated that **2** was an exoallyl alcohol isomer of **11**. The observed $^1H-^1H$ COSY correlation from H-7 to H-8 as well as HMBC correlations from H-11 to C-4, C-5, and C-6, from H-12 to C-6 and C-7, and from H-4 to C-6 confirmed the above deduction.

The configuration of **2** was deduced by analysis of its proton coupling constants and by comparison of the ^{13}C NMR data with those of **11**[13] as well as by a NOESY experiment. The large coupling constant ($J = 9.5$ Hz) for H-7 and H-8 suggested the axial−axial orientation for both protons. In the ^{13}C NMR spectrum of **2**, the chemical shifts of carbons C-13 through C-23 were very similar to those of **11**, indicating that the carbons C-16, C-19, and C-20 in **2** shared the same configuration as in **11**. The observed NOESY correlations (Figure S2 in the Supporting Information) from H-19 to H-17 and H-20 and from H-17 to 16-Me suggested that H-19, H-20, and 16-Me shared an α orientation, while the NOE correlations from H-4 to H-8, H-10, and H-22 and from H-11 to H-3 confirmed the configurations at C-3, C-4, C-5, C-7, C-8, and C-9. Similar to compound **1**, a computer modeled 3D structure of

Table 2. ^{1}H NMR Data for Compounds **1**−**7** in DMSO-d_6 (500 MHz, J in Hz)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**
2	8.11, s	7.86, s	8.31, s	7.83, s	8.12, s	8.04, s	8.12, s
3	3.25, m	3.27, m	3.33, m	3.30, m	3.42, br s	3.25, m	3.25, m
4	2.60, br d (5.8)	2.35, m	2.74, br s	2.58, dd (2.0, 5.8)	2.32, m	2.29, dd (2.4, 5.3)	2.39, dd (2.8, 5.2)
5	2.62, m	2.71, m		2.36, m	2.33, m	2.67, m	2.64, m
7	3.85, dd (6.6, 9.5)	3.67, dd (6.7, 9.5)	3.65, dd (7.4, 9.4)	5.30, br s	5.46, br s	3.62, dd (6.6, 8.1)	3.60, br d (9.9)
8	2.53, d (9.5)	2.64, dd (9.5, 9.4)	2.21, m	2.77, br d (10.5)	2.88, br s	2.53, d (9.7)	2.32, m
10	2.50, m	2.42, m	2.72, m	2.69, dd (5.6, 14.3)	2.93, dd (4.1, 15.0)	2.09, m	2.81, dd (4.6, 14.6)
	2.65, m	2.49, m	2.47, m	2.53, dd (7.5, 14.3)	2.69, dd (3.4, 15.0)		2.52, dd (3.33, 14.6)
11	0.67, d (6.5)	0.46, d (6.6)	1.54, s	0.71, d (7.2)	1.18, d (6.5)	0.77, d (6.7)	0.95, d (6.7)
12	5.07, s	4.96, s	1.13, s	1.62, s	3.94, br s	4.88, s	4.93, s
	4.86, s	4.78, s				5.01, s	5.11, s
13	5.91, dd (15.4, 9.5)	5.91, dd (15.1, 9.4)	5.84, dd (11.4, 15.3)	6.09, dd (10.5, 15.3)	5.87, dd (10.6, 14.3)	5.78, dd (9.7, 15.2)	5.66, dd (9.4, 14.8)
14	5.04, m	5.02, m	5.00, m	5.04, ddd (2.6, 9.8, 15.1)	4.90, ddd (2.3, 11.0, 14.3)	4.98, ddd (2.9, 10.7, 15.2)	4.74, m
15	2.17, m	2.22, m	2.24, m	2.17, m	2.28, m	2.17, m	2.06, m
	1.81, m	1.63, m	1.63, m	1.72, m	1.77, m	1.76, m	1.70, m
16	1.46, m	2.70, m	2.53, m	2.27, m	2.67, m	2.42, m	2.22, m
17	2.13, br d (6.2)	4.62, d (10.0)	4.65, d (10.0)	4.72, d (8.8)	5.87, d (9.2)	5.22, d (9.0)	4.78, m
19		4.03, br s	4.05, br s	3.97, dd (4.0, 7.4)		4.32, d (4.3)	3.39, br s
20		4.48, br s	4.52, br s	4.30, dd (4.3, 7.4)			3.20, br s
21	2.07, br d (4.9)	6.74, dd (3.4, 15.2)	6.83, dd (3.3, 15.1)	6.55, dd (4.0, 15.5)	2.38, m	2.67, m	0.98, m
					1.58, m	1.54, m	0.78, m
22	3.00, dd (6.8, 15.1)	6.54, dd (1.9, 15.2)	6.57, dd (1.9, 15.1)	6.51, d (15.5)	2.39, m	2.68, m	2.33, m
	1.31, dd (2.4, 15.1)				1.05, m	1.64, m	0.61, m
16-Me	0.93, d (7.1)	0.87, d (6.4)	0.89, d (6.5)	0.88, d (6.7)	0.92, d (9.1)	0.92, d (6.5)	0.85, d (6.7)
18-Me	1.91, s	1.66, s	1.65, s	1.57, s	1.70, s	1.33, s	1.50, s
1′	10.85, br s	10.86, br s	10.87, br s	10.83, br s	10.96, br s	10.91, br s	10.89, br s
2′	7.05, d (2.0)	7.03, s	7.10, d (0.87)	7.07, d (2.2)	7.16, d (2.1)	7.10, d (2.2)	7.04, d (2.2)
4′	7.42, d (7.9)	7.36, d (7.8)	7.41, d (7.9)	7.42 d (7.6)	7.57, d (7.9)	7.45, d (7.9)	7.50, d (7.9)
5′	6.95, m	6.95, m	7.00, m	6.97, m	7.00, m	6.95, m	6.95, m
6′	7.00, m	7.03, m	7.06, m	7.02, m	7.07, m	7.00, m	7.01, m
7′	7.28, d (8.0)	7.32, d (8.1)	7.33, d (8.1)	7.30, d (8.1)	7.33, d (8.1)	7.28, d (8.0)	7.28, d (8.0)
7-OH	4.87, d (6.6)	4.72, d (6.4)	4.52, br s			4.74, d (6.5)	4.70, br s
12-OH					4.73, t (5.01)		
19-OH		5.02, br s	5.02, d (4.5)	4.94, d (4.4)		4.90, d (4.3)	4.63, br s
20-OH		5.01, br s	5.10, d (4.3)	4.96, d (4.5)			4.06, br s

2 (Figure S2) also supported the configuration of **2** assigned by the NOESY spectrum.

The ^{1}H and ^{13}C NMR and MS data revealed that **3** is an isomer of **2**. However, the C6(12) double bond in **2** was replaced by the C5 double bond in **3**, and this was indicated by the fact that the proton and carbon signals at δ_H 4.96 (s, H-12a) and 4.78 (s, H-12b) and at δ_C 30.8 (CH, C-5), 150.8 (C, C-6), and 112.0 (CH_2, C-12) in **2** were replaced by signals at δ_H 1.13 (s, H-12) and at δ_C 125.0 (C, C-5), 134.5 (C, C-6), and 16.7 (CH_3, C-12) in **3**, respectively. The chemical shifts for the other protons and carbons of **3** were nearly identical to those of **2**. Further analysis of the 2D NMR spectra (^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY) confirmed the structure of **3**.

Cytoglobosin D (**4**) had the molecular formula $C_{32}H_{38}N_2O_4$ as determined by the HRESIMS data. Exhaustive interpretation of the ^{1}H and ^{13}C NMR data of **4** indicated that this compound had the same molecular skeleton as that of **3**, except that the hydroxy group at C-7 was missing and the tetrasubstituted Δ^5 double bond in **3** was replaced by a trisubstituted Δ^6 double bond in **4**. The proton and carbon resonances at δ_H 5.30 (br s, H-7) and 1.62 (s, H-12) and at δ_C 138.4 (C, C-6), 126.0 (CH, C-7), and 21.3 (CH_3, C-12) in **4** supported the above deduction. The observed ^{1}H−^{1}H COSY correlations from H-7 to H-8 and from H-5 to H-11 as well as HMBC correlations from H-11 to C-4, C-5, and C-6, from H-12 to C-6 and C-7, and from H-4 to C-6 established the structure of compound **4**.

The molecular formula of cytoglobosin E (**5**) was determined as $C_{32}H_{36}N_2O_5$ by the positive HRESIMS data. Comparison of the 1D NMR spectroscopic data of **5** (Tables 1 and 2) with those of isochaetoglobosin D (**8**) (Table S4 in the Supporting Information) suggested that the structures of these two compounds were very similar. The only significant differences between the ^{13}C NMR spectra were the allylic oxymethine carbon signal at δ_C 68.6 (CH, C-7) and the exocyclic olefinic carbon signals at δ_C 150.8 (C, C-6) and 112.4 (CH_2, C-12) in **8** (Table S4) were replaced by the olefinic carbon signals at δ_C 125.4 (CH, C-7) and 142.2 (C, C-6) and by the oxymethylene carbon signal at δ_C 61.4 (CH_2, C-12), respectively. These differences indicated that the hydroxy group at C-7 and the exocyclic double bond at C6(12) of **8** were missing in **5**. Instead, the hydroxy group at C-12 and the double bond at C6(7) were present in **5**. The observed ^{1}H−^{1}H COSY correlations from H-7 to H-8 and from H-8 to H-13, along with the HMBC correlation from H-11 to C-6, supported this assumption. The remaining NMR data for **5** and **8** were nearly identical, which suggested that the shared structural features including the configurations of the two compounds were the same.

Cytoglobosin F (**6**) was assigned the molecular formula $C_{32}H_{38}N_2O_5$ on the basis of HRESIMS data. Analysis of the ^{1}H and ^{13}C NMR spectra showed that the structure of **6** was also similar to that of **8**.[2] The main difference between the two compounds was at position C-19, with the ketone group in **8** being replaced by an oxymethine in **6**. The carbon signal at δ_C 196.0 (C, C-19) for the ketone group in the ^{13}C NMR of **8** was replaced by one oxymethine at δ_C 81.6 (CH, C-19) in **6**, and there is also an appearance of an oxymethine proton signal at δ_H 4.32 (d, J = 4.3 Hz, H-19). The ^{1}H−^{1}H COSY correlation from H-19 to 19-OH and HMBC correlations from H-17 to C-19 and from 18-Me to C-19, C-17, and C-18 established the planar structure of **6**. The similarity of the ^{13}C NMR data of stereogenic centers between **6** and **8** implied that the two compounds possessed the same configuration. Meanwhile, the correlations from H-17 to H-19, from H-4 to H-5 and H-8, and from H-7 to H-13 in the NOESY spectrum established the relative configuration of **6**.

The molecular formula of cytoglobosin G (**7**) was assigned as $C_{32}H_{40}N_2O_5$ by positive HRESIMS data. The main difference between the structures of **7** and **6** was at C-20. The carbon signal at δ_C 209.5 (C, C-20) for the ketone group in the ^{13}C NMR of **6** was replaced by an oxymethine carbon signal at δ_C 71.0 (CH, C-20) in **7**. Accordingly, there is an appearance of an oxymethine proton signal at δ_H 3.20 (br s, H-20) in the ^{1}H NMR spectrum of **7**. In the NOESY spectrum, the observed correlations from 20-OH to 19-OH, from H-19 to H-17, from H-5 to H-4 and H-8, and from H-7 to H-13 confirmed the configuration of **7**.

In addition to cytoglobosins A−G (**1**−**7**), two structurally related known compounds, isochaetoglobosin D (**8**)[2,14] and chaetoglobosin F_{ex} (**9**),[13] were also identified in this study. Isochaetoglobosin D (**8**) was first obtained from chaetoglobosin D by treatment with triethylamine[2] and was later isolated as a natural product from a strain of *C. globosum*, isolated from Thai soil.[14] Only the ^{1}H NMR data in pyridine were previously reported.[2] The complete ^{1}H and ^{13}C NMR data were assigned as listed in Table S4.

Cytoglobosins A−E and G (**1**−**5** and **7**) were evaluated for their cytotoxic abilities against P388, A549, and KB cell lines. Cytoglobosins C (**3**) and D (**4**) displayed activity toward the A-549 cell line (IC_{50} 2.26, 2.55 μM). Cytoglobosins A (**1**), B (**2**), E (**5**), and G (**7**) were found to be inactive (IC_{50} >10 μM).

Experimental Section

General Experimental Procedures. Procedures were the same as recently reported.[11]

Fungal Material. The endophytic fungus *Chaetomium globosum* QEN-14 was isolated from the fresh tissue of the surface-sterilized marine green alga *Ulva pertusa* that was collected at the Qingdao coastline, China, by using the procedures provided in an earlier report.[6,11] Fungal identification was carried out using a molecular biological protocol by DNA amplification and sequencing of the ITS region as described previously.[6] The sequence data derived from the fungal strain have been submitted to and deposited at GenBank under accession no. EU001331. A BLAST search result showed that the sequence was the most similar (98%) to the sequence of *C. globosum* (compared to gi 38503532 gb AY429056.1). The strain is preserved at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences.

Fermentation, Extraction, and Isolation. For chemical investigations, the fungal strain was static cultivated in liquid potato-dextrose broth medium (500 mL seawater, 20 g glucose, 5 g peptone, 3 g yeast extract, and distilled water adding up to 1000 mL, pH 6.0, liquid medium/flask = 300 mL) in 1 L Erlenmeyer flasks for 30 days at room temperature.

The fermented whole broth (300 mL × 100 flasks) was filtered through cheesecloth to separate the culture broth and mycelia. The former was extracted three times with EtOAc (150 mL each flask) to give an extract, while the latter was homogenized and extracted three times with MeOH (100 mL each flask) to give another extract. Because the TLC and HPLC profiles of the two extracts were nearly identical, they were combined before further separation. The combined extracts (45 g) were subjected to column chromatography over silica gel eluting with different solvents of increasing polarity (from petroleum ether to MeOH) to yield 14 fractions (Frs. 1−14) on the basis of TLC analysis. Fr. 12 was chromatographed on a silica gel column eluted with a $CHCl_3$−MeOH gradient (50:1 to 10:1) to afford eight subfractions (Fr. 12-1 to 12-8). Fr. 12-5 was further separated by CC on Sephadex LH-20 ($CHCl_3$−MeOH, 1:1) and on reversed-phase silica gel C_{18} (MeOH−H_2O, 4:1) to afford compounds **1** (14.1 mg), **6** (9.4 mg), **8** (24.2 mg), and **9** (23.2 mg). Fr. 12-6 was also purified by CC on Sephadex LH-20 ($CHCl_3$−MeOH, 1:1) and on reversed-phase silica gel C_{18} (MeOH−H_2O, 4:1) to afford compounds **2** (5.2 mg), **3** (7.4 mg), **4** (3.0 mg), **5** (4.0 mg), and **7** (6.2 mg).

Cytoglobosin A (1): colorless, amorphous powder (DMSO); $[\alpha]_D^{25}$ +5 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 268 (3.23), 239 (3.23), 221 (3.75), 207 (3.58), 203 (3.56) nm; IR (KBr) ν_{max} 3416, 1699, 1651, 1540, 1447, 1367, 1102, 1023, 983 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 551 $[M + Na]^+$, 567 $[M + K]^+$; HRESIMS, *m/z* 551.2507 $[M + Na]^+$ (calcd for $C_{32}H_{36}N_2O_5Na^+$, 551.2521).

Cytoglobosin B (2): colorless, amorphous powder (DMSO); $[\alpha]_D^{25}$ +11 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 290 (3.23), 281 (3.30), 222 (4.13), 203 (4.05) nm; IR (KBr) ν_{max} 3390, 2952, 1686, 1632, 1447, 1437, 1116, 1016, 970 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 553 $[M + Na]^+$, 569 $[M + K]^+$; HRESIMS *m/z* 553.2668 $[M + Na]^+$ (calcd for $C_{32}H_{38}N_2O_5Na^+$, 553.2678).

Cytoglobosin C (3): colorless, amorphous powder (DMSO); $[\alpha]_D^{25}$ +36 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 289 (4.29), 261 (3.45), 254 (3.46), 223 (4.18), 206 (3.86) nm; IR (KBr) ν_{max} 3376, 1692, 1613, 1447, 1261, 1122, 1029, 970 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 553 $[M + Na]^+$, 569 $[M + K]^+$; HRESIMS, *m/z* 553.2659 $[M + Na]^+$ (calcd for $C_{32}H_{38}N_2O_5Na^+$, 553.2678).

Cytoglobosin D (4): colorless, amorphous powder (DMSO); $[\alpha]_D^{25}$ −34 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 290 (3.30), 279 (3.39), 222 (4.17), 203 (4.08) nm; IR (KBr) ν_{max} 3443, 3403, 1672, 1454, 1427, 1328, 1235, 1149, 1096, 963 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 537 $[M + Na]^+$, 553 $[M + K]^+$; HRESIMS *m/z* 537.2731 $[M + Na]^+$ (calcd for $C_{32}H_{38}N_2O_4Na^+$, 537.2729).

Cytoglobosin E (5): colorless, amorphous powder (DMSO); $[\alpha]_D^{25}$ −3 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 290 (4.40), 279 (3.47), 220 (4.26), 204 (4.16) nm; IR (KBr) ν_{max} 3371, 3271, 1689, 1631, 1456, 1433, 1337, 1234, 1153, 1049, 974, 849 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 551 $[M + Na]^+$; HRESIMS *m/z* 551.2517 $[M + Na]^+$ (calcd for $C_{32}H_{36}N_2O_5Na^+$, 551.2521).

Cytoglobosin F (6): colorless, amorphous powder (DMSO); $[\alpha]_D^{25}$ −73 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 282 (2.87), 271 (2.86), 257 (2.81), 219 (3.66) nm; IR (KBr) ν_{max} 3431, 1647, 1633, 1554, 1449, 1255, 1180 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 553 $[M + Na]^+$, 569 $[M + K]^+$; HRESIMS *m/z* 553.2661 $[M + Na]^+$ (calcd for $C_{32}H_{38}N_2O_5Na^+$, 553.2678).

Cytoglobosin G (7): colorless, amorphous powder (DMSO); $[\alpha]_D^{25}$ +4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 290 (3.39), 274 (3.44), 221 (4.17), 204 (4.14) nm; IR (KBr) ν_{max} 3396, 1679, 1616, 1447, 1334, 1023, 960 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 555 $[M + Na]^+$, 571 $[M + K]^+$; HRESIMS *m/z* 555.2832 $[M + Na]^+$ (calcd for $C_{32}H_{40}N_2O_5Na^+$, 555.2834).

Cytotoxicity Assay. The cytotoxic activities against P388, A549, and KB cell lines were determined as previously reported.[15]

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Supporting Information Available: Fully assigned NMR data of compounds **1**−**9** (Tables S1−S4), key NOESY correlations and 3D computer modeling of compounds **1** and **2** (Figures S1 and S2), the plausible rearrangement from **8** to **1** (Scheme S1), and selected NMR spectra of compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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