10-Phenyl-[12]-cytochalasins Z_7, Z_8, and Z_9 from the Marine-Derived Fungus *Spicaria elegans*

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The three new 10-phenyl-[12]-cytochalasins Z_7, Z_8, and Z_9 (**1**−**3**), together with two known analogues, cytochalasins E (**4**) and K (**5**), were isolated from the marine-derived fungus *Spicaria elegans*. This is only the second report to date that cytochalasins contain a 12-membered macrocyclic ring. The structures of the three new cytochalasins were elucidated by spectroscopic methods, and their absolute configurations were determined for the first time by the modified Mosher's method. All five cytochalasins were evaluated for their cytotoxic effects on P388 and A-549 cell lines by the MTT method.

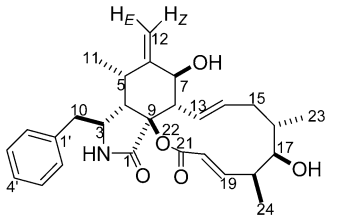
Numerous natural products with novel structures and distinct biological activities have been discovered as the secondary metabolites of marine-derived microbes. In an effort to search for new anticancer compounds, more than 2000 microbial strains isolated from the sediment samples collected from China's Jiaozhou Bay were screened using mammalian tsFT210 cells. Among them, a fungal strain identified as *Spicaria elegans* exhibited cytotoxic activity. The active constituents of this strain were investigated through a bioassay-guided isolation procedure. The investigation resulted in the isolation of three new cytochalasins (**1**−**3**), namely, cytochalasins Z_7, Z_8, and Z_9, together with two known analogues, cytochalasins E and K (**4**, **5**) (Figure 1).

Cytochalasins are fungal metabolites that have been isolated from different genera of fungi such as *Phomopsis*,[1,2] *Chalara*,[3] *Hyposylon*,[4] *Xylaria*,[5,6] *Daldinia*,[7−9] and most recently *Pseudeurotium*[10] and *Phoma exigua*.[11] They have attracted a great deal of attention for their unusual structural features, such as highly substituted perhydroisoindol-1-one fused with an 11- or 13-membered macrocyclic ring, and a wide range of biological activities including inhibition of HIV-1 protease 2[4] as well as antibiotic and antitumor activities.[12,13] Their most unusual property is the ability to cause cells to extrude their nuclei, leading to the formation of nuclei-free cells.[7] To date, more than 80 cytochalasins have been isolated from a range of fungi, and to the best of our knowledge, among them only one cytochalasin with a 12-membered macrocyclic ring has been reported[8] prior to our new cytochalasins (**1**−**3**). In this paper we report the isolation, structure elucidation, and cytotoxic activities of the five cytochalsins (**1**−**5**).

Results and Discussion

The bioactive ethyl acetate extract of the *S. elegans* was chromatographed on silica gel columns and extensive reversed-phase preparative HPLC to give five pure compounds (**1**−**5**). Compounds **4** and **5** were identified as cytochalasin E[14,15] and cytochalasin K,[15] respectively, by comparison of their spectroscopic data with those reported in the literature. Although cytochalasins E and K have previously been isolated, only limited NMR spectral data have been reported. To assist the structure elucidation of cytochalasins Z_7, Z_8, and Z_9 (**1**−**3**) through spectral data comparison, we also made full ^{1}H and ^{13}C NMR assignments for **4** and **5**.

Cytochalasin Z_7 (**1**) was obtained as white needles after recrystallization from MeOH. High-resolution mass measurement on the



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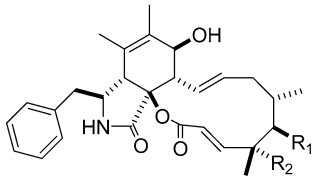
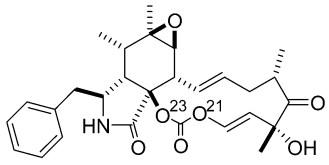


2 R_1=OH, R_2=H

3 R_1=H, R_2=OH



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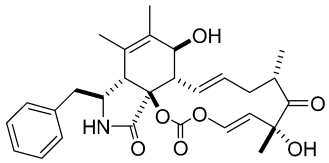

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Figure 1. Structures of compounds **1**−**5**.

M + Na^+ peak (*m*/*z* 488.2427, calcd for $[M + Na]^+$ 488.2413) in ESIMS, in combination with ^{1}H and ^{13}C NMR data (Tables 1 and 2), supported the molecular formula $C_{28}H_{35}NO_5$. The IR spectrum showed the presence of hydroxyl and carbonyl groups. Analysis of the 1D NMR data for **1** revealed two carbonyls, three quarternary carbons, 17 methines, three methylenes, and three methyls.

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Table 1. ^{1}H NMR Data for Compounds **1**−**5**[a]

H (*J*/Hz)	**1**[b]	**2**[b]	**3**[c]	**4**[d]	**5**[b]
2(NH)	9.43 (br s)	9.65 (br s)	7.43 (br s)	6.71 (br s)	9.84 (br s)
3	3.71 (br t, 6.4)	3.91 (br dd, 8.2, 6.9)	3.57 (ddd, 7.7, 5.8, 2.2)	3.77 (br s)	3.84 (br t, 6.9)
4	3.54 (dd, 6.4, 2.8)	4.35 (br s)	3.78 (br s)	3.03 (br s)	4.31 (br s)
5	3.61 (br q, 6.4)			2.28 (br q, 6.5)	
6					
7	4.57 (br d, 10.0)	4.68 (br d, 9.7)	3.93 (dd, 10.2, 6.0)	2.64 (br d, 10.0)	4.51 (br d, 9.6)
8	3.98 (ddd, 10.0, 9.6, 1.9)	3.73 (dd, 10.1, 9.7)	2.99 (dd, 10.2, 10.0)	2.60 (br d, 10.0)	3.48 (dd, 10.0, 9.6)
10a		3.26 (dd, 12.8,6.9)	3.04 (dd, 12.8, 5.8)	2.90 (br d, 13.6)	3.04 (m)
10b	2.93 (m, 2H)	3.19 (dd, 12.8, 8.2)	2.84 (br d, 12.8)	2.71 (dd, 13.6, 7.0)	2.99 (m)
11-Me	0.93 (d, 6.4)	1.45 (br s)	1.28 (br s)	1.09 (d, 6.5)	1.48 (br s)
12-Me	5.51 (br s) *Z* 5.15 (br s) *E*	1.86 (br s)	1.60 (br s)	1.27 (s)	1.85 (br s)
13	6.66 (br dd, 15.1, 9.6)	6.80 (br dd, 15.0, 10.1)	6.10 (ddd, 15.4, 10.0, 2.2)	5.89 (br dd, 15.0, 10.0)	6.91 (dd, 15.1, 10.0)
14	5.51 (br dd, 15.1, 6.9)	5.44 (ddd, 15.0, 10.1, 4.6)	5.10 (ddd, 15.4, 11.4, 4.0)	5.22 (br dd, 15.0, 11.3)	5.51 (ddd, 15.1, 11.0, 3.2)
15a	2.16 (m, 2H)	2.12 (m, 2H)	2.07 (m)	2.64 (m)	2.83 (br dd, 13.7, 11.0)
15b			1.90 (br dd, 14.3, 11.4)	2.15 (br d, 12.4)	2.05 (br d, 13.7)
16	1.70 (m)	1.63 (m)	2.04 (m)	2.93 (m)	3.02 (m)
17	4.11 (m)	4.08 (m)	1.74 (dd, 13.9, 5.2) a 1.65 (dd, 13.9, 2.2) b		
18	2.93 (m)	2.93 (m)			
19	7.59 (br d, 15.5)	7.42 (dd, 15.7, 4.1)	6.94 (br d, 15.9)	5.60 (br d, 11.8)	6.08 (dd, 11.9, 2.3)
20	5.96 (br d, 15.5)	5.95 (dd, 15.7)	5.68 (d, 15.9)	6.45 (d, 11.8)	7.21 (d, 11.9)
23	1.15 (d, 6.9)	1.13 (d, 7.3)	1.00 (d, 7.3)	1.16 (d, 6.2), 24-Me	1.00 (d, 6.9), 24-Me
24	1.17 (dd, 6.9, 2.3)	1.17 (d, 6.8)	1.33 (s)	1.48 (s), 25-Me	1.57 (s), 25-Me
OH			3.50 (d, 6.0), 7-OH	4.41 (br s), 18-OH	
2′,6′	7.25 (m, 2H)	7.30 (m, 2H)	7.23 (m, 2H)	7.16 (m, 2H)	7.25 (m, 2H)
3′,5′	7.26 (m, 2H)	7.36 (m, 2H)	7.33 (m, 2H)	7.34 (m, 2H)	7.26 (m, 2H)
4′	7.21 (m)	7.23 (m)	7.24 (m)	7.27 (m)	7.20 (m)

[a] Spectra were recorded at 600 MHz for ^{1}H using TMS as internal standard. [b] Measured in pyridine-d_5. [c] Measured in acetone- d_6. [d] Measured in $CDCl_3$.

Table 2. ^{13}C NMR Data for Compounds **1**−**5**[a]

C	**1**[b]	**2**[b]	**3**[c]	**4**[d]	**5**[b]
1	172.9 qC	173.4 qC	172.6 qC	170.3 qC	171.0 qC
3	54.1 CH	59.8 CH	59.7 CH	53.6 CH	59.4 CH
4	49.6 CH	50.7 CH	50.3 CH	47.6 CH	48.8 CH
5	32.2 CH	125.6 qC	125.8 qC	35.7 CH	125.2 qC
6	150.2 qC	135.5 qC	134.8 qC	57.3 qC	134.3 qC
7	71.2 CH	70.8 CH	70.9 CH	60.5 CH	70.7 CH
8	50.3 CH	52.0 CH	51.5 CH	45.7 CH	50.7 CH
9	85.6 qC	85.8 qC	85.4 qC	87.1 qC	88.1 qC
10	45.3 CH_2	44.3 CH_2	44.0 CH_2	44.6 CH_2	44.6 CH_2
11	13.9 CH_3	17.6 CH_3	17.5 CH_3	13.1 CH_3	17.7 CH_3
12	113.1 CH_2	15.2 CH_3	14.6 CH_3	19.6 CH_3	14.9 CH_3
13	126.8 CH	127.0 CH	126.6 CH	128.4 CH	131.2 CH
14	135.7 CH	138.1 CH	138.6 CH	131.4 CH	131.7 CH
15	43.7 CH_2	44.0 CH_2	45.5 CH_2	39.1 CH_2	40.0 CH_2
16	33.0 CH	32.7 CH	30.3 CH	40.8 CH	41.2 CH
17	78.2 CH	78.3 CH	54.8 CH_2	211.9 qC	212.2 qC
18	43.9 CH	43.8 CH	72.4 qC	77.2 qC	77.5 qC
19	157.7 CH	157.2 CH	160.1 CH	120.4 CH	121.9 CH
20	123.3 CH	123.3 CH	120.1 CH	142.0 CH	142.8 CH
21	167.7 qC	167.6 CH	167.7 qC		
22				149.3 qC	149.3 qC
23	19.5 CH_3	19.5 CH_3	27.3 CH_3		
24	9.1 CH_3	8.9 CH_3	22.4 CH_3	20.0 CH_3	20.2 CH_3
25				24.3 CH_3	25.1 CH_3
1′	135.8 qC	138.9 qC	138.9 qC	135.8 qC	138.2 qC
2′,6′	130.0 CH	130.0 CH	130.0 CH	129.7 CH	130.0 CH
3′,5′	128.9 CH	128.9 CH	129.3 CH	128.8 CH	128.9 CH
4′	126.8 CH	126.9 CH	127.3 CH	127.2 CH	127.0 CH

[a] Spectra were recorded at 150 MHz for ^{13}C using TMS as internal standard.

Comparison of the ^{1}H and ^{13}C NMR data with those of the known cytochalasin K (**5**) showed the presence of the same 10-phenyl-substituted perhydroisoindol-1-one skeleton, except for the tetra-substituted double bond in **5** being replaced by an exocyclic double bond in **1**. This difference could be confirmed by the ^{1}H NMR spectrum of **1**, which displayed the C-11 methyl as a doublet (0.93 ppm, d, J = 6.4 Hz), instead of the singlet as in **5**. Moreover, HMBC correlations observed from H-12*E* (5.15 ppm) to C-5 (32.2 ppm, d) and C-6 (150.2 ppm, s) and from H-12*Z* (5.51 ppm) to C-6 (150.2 ppm, s) and C-7 (71.2 ppm, d) could further confirm this change. The HMBC spectrum also supported this 10-phenyl-substituted perhydroisoindol-1-one skeleton in **1**, showing correlations from H-3 (3.71 ppm), H-4 (3.54 ppm), and H-8 (3.98 ppm) to C-9 (85.6 ppm, s), from H-3 to C-1 (172.9 ppm, s) and C-1′ (135.8 ppm, s), and from 2H-10 (2.93 ppm) to C-2′, C-6′ (130.0 ppm, d) and C-3 (54.1 ppm, d).

Further comparison of the 1D NMR spectra of **1** with those of **5** showed mainly two changes in **1** in the macrocyclic ring moiety. First, the ^{1}H NMR spectrum of **1** revealed two more proton signals at 4.11 ppm (H-17, a proton on an oxygenated carbon) and 2.93 ppm (H-18). As expected, the ^{13}C NMR revealed the presence of two more methine carbons at 78.2 ppm (C-17, an oxygenated carbon) and 43.9 ppm (C-18) in **1**, as well as its lack of the carbonyl at 212.2 ppm and the oxygenated quaternary carbon at 77.5 ppm observed in **5**. These ^{13}C NMR data suggested that the C-17 carbonyl in **5** was hydrogenated into the C-17 OH group and the C-18 OH group in **5** was absent in **1**. This was confirmed by the HMBC correlations from the Me-23 protons (1.15 ppm) to one methylene carbon (43.7 ppm, t, C-15) and two methine carbons (33.0 ppm, d, C-16; 78.2 ppm, d, C-17) and the correlations from the Me-24 protons (1.17 ppm) to an olefinic carbon (157.7 ppm, d, C-19) and two methine carbons (C-17 and C-18). Moreover, COSY correlations between 2H-15 (2.16 ppm) and H-16 (1.70 ppm), between H-16 and H-17 (4.11 ppm), between H-17 and H-18 (2.93 ppm), and between H-18 and H-19 (7.59 ppm) also supported this change.

Second, the ^{13}C NMR data showed a large downfield shift of the carbonyl in **1** at 167.7 ppm (C-21) compared to 149.3 ppm (C-22) in **5** and showed an upfield shift of C-9 in **1** at 85.2 ppm compared to 88.1 ppm in **5**. This change in the ^{13}C NMR data could be explained by the unique vinyl carbonate moiety [H-19 (6.08

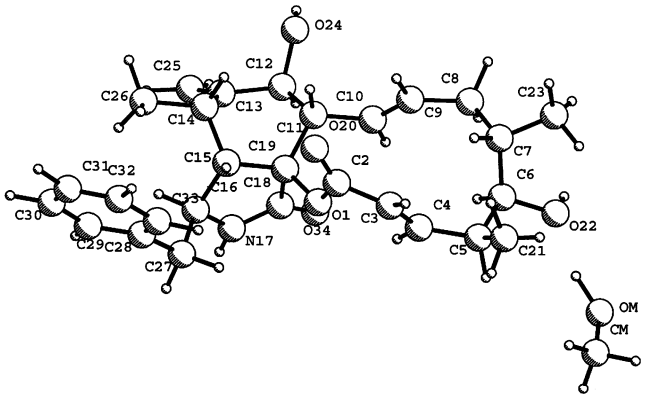

Figure 2. Final X-ray drawing of compound **1**.

ppm, dd, $J = 11.9$, 2.3 Hz), H-20 (7.21 ppm, d, $J = 11.9$ Hz); C-19 (121.9 ppm, d), C-20 (142.8 ppm, d), C-22 (149.3 ppm, s)] in **5** being changed to an α,β-unsaturated ester in **1** [H-19 (7.59 ppm, br d, $J = 15.5$ Hz), H-20 (5.96 ppm, br d, $J = 15.5$ Hz); C-19 (157.7 ppm, d), C-20 (123.3 ppm, d), C-21 (167.7 ppm, s)], thus decreasing the size of the macrocyclic ring in **1** from 13 to 12 atoms, consistent with the previously known 10-phenyl-[12]-cytochalasin found in the fungus *Daldinia* sp.[8] This change could be further supported by the two fewer oxygens in the molecular formula of **1** relative to **5** (one of the two missing oxygens had already been attributed to the lack of the C-18 OH group). The HMBC spectrum also showed correlations between H-19 and H-20 with the C-21 carbonyl, and CH_3-24 with C-17 (78.2 ppm, d), C-18 (43.9 ppm, d), and C-19. On the basis of these multiple lines of evidence, the complete two-dimensional structure of **1** was elucidated.

A series of NOE and NOESY correlations (H-3/CH_3-11, CH_3-11/H-12*E*, H-12*Z*/H-7, H-4/H-8 and 2H-10, H-5/H-8, H-8/H-14, H-14/H-16, and H-16/CH_3-24) and the X-ray diffraction structure of **1** (Figure 2) confirmed its two-dimensional structure and relative configuration and also demonstrated that the conformation of the 10-phenyl-substituted perhydroisoindol-1-one skeleton was in accord with those of other known cytochalasins (the six-membered ring adopts a slightly twisted boat conformation and the five-membered amide ring adopts an envelope conformation). However, the twisted boat conformation of the macrocyclic ring, in which the two *E*-alkenes oriented roughly perpendicular to each other, was slightly different from the previously reported [11]-cytochalasins[9] and [13]-cytochalasins[14] (in which the two *E*-alkenes oriented roughly parallel, while the macrocyclic ring adopted a chairlike conformation).

Cytochalaisin Z_8 (**2**) is a white needle. Its HRESIMS (*m*/*z* 488.2392, calcd for $[M + Na]^+$ 488.2413) suggested that it was an isomer of compound **1**, with a molecular formula of $C_{28}H_{35}NO_5$. The structure of **2** was established by comparing its NMR data with those of **1**. The ^1H and ^{13}C NMR spectra of **2** and **1** were very similar. The two exocyclic double-bond carbon resonances 150.2 ppm (C-6, s) and 113.1 ppm (C-12, t) in **1** had been replaced by two carbon resonances at 125.6 ppm (C-5, s) and 135.5 ppm (C-6, s), attributable to a tetrasubstituted double bond, which were also similar to those observed in the ^{13}C spectrum of **5**. Furthermore, in the ^1H NMR spectrum of **2**, the proton signal at 1.45 ppm due to the C-11 methyl appearing as a singlet was also consistent with the six-membered ring as seen in **5**. Therefore, as an isomer of **1**, compound **2** is only different in having a tetrasubstituted double bond in the six-membered ring, as opposed to an exocyclic double bond in **1**. COSY and HMBC correlations also supported this structure. The relative stereochemistry was determined by the chemical shifts, coupling constants, and NOESY correlations and was found to be the same as that of **1**. Thus, the structure of compound **2** was established.

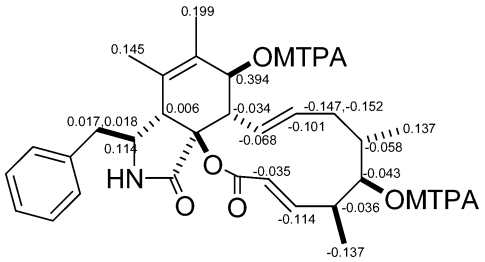

Figure 3. Values of $\Delta\delta_S - \delta_R$ of the MTPA esters of compound **2**.

Cytochalasin Z_9 (**3**) is a white needle crystallized from MeOH. The molecular formula of **3** is also $C_{28}H_{35}NO_5$, as determined by HRESIMS ($[M + Na]^+$ at *m*/*z* 488.2436, calcd for 488.2413). This compound is isomeric with compounds **1** and **2**. Its ^1H and ^{13}C NMR data showed it had the same 10-phenyl-substituted perhydroisoindol-1-one skeleton as **5** and **2**, but the macrocyclic ring was slightly different from the above two cytochalasins. Comparison of the ^{13}C NMR data of **2** and **3** suggested that the differences could be explained by the replacement of an oxygenated methine at C-17 and the tertiary C-18 in **2** by a methylene carbon (54.8 ppm, t, C-17) and an oxygenated quaternary carbon (72.4 ppm, s, C-18) with a methyl at 22.4 ppm (CH_3-24, q) in **3**. This is consistent with CH_3-24 (1.33 ppm) appearing as a singlet in the ^1H NMR spectrum. This structure is also in agreement with the observed COSY and HMBC correlations. The stereochemistries at C-16 and C-18 were determined by the H-14/H-16 and H-16/CH_3-24 NOESY correlations. In addition, comparison with the known 12-membered macrocyclic ring cytochalsin[8] with the same macrocyclic ring moiety as **3** further supported the assigned structure of **3**.

The absolute configuration of **2** was established by a convenient Mosher ester procedure in which the sample was treated with MTPA chlorides in deuterated pyridine directly in NMR tubes.[16] Esterification of **2** with (*S*)- and (*R*)-MTPA-Cl occurred at the C-7 and C-17 hydroxy groups to give the (*R*)- and (*S*)-MTPA esters **2r** and **2s**, respectively. Their ^1H NMR data were assigned on the basis of the COSY correlations. The observed chemical shift differences ($\Delta\delta_S - \delta_R$ Figure 3) suggested that the absolute configuration of C-7 was *S* and that of C-17 was *R*. This assignment was consistent with the relative configurations of C-7 and C-17. Therefore the complete absolute stereochemistry of compound **2** could be assigned as 22-oxa-(12)-cytochalasa-5,13,19-triene-1,21-dione-7,17-dihydroxy-16,18-dimethyl-10-phenyl-(7*S*,13*E*,16*S*,17*R*,18*S*,19*E*) (Figure 1). In the CD spectrum **1** showed a strong splitting Cotton effect at 196 nm ($\Delta\epsilon$ +71.77), 210 nm ($\Delta\epsilon$ −43.43), and 258 nm ($\Delta\epsilon$ +3.74), which was very similar to compound **2** [200 nm ($\Delta\epsilon$ +50.78), 217 nm ($\Delta\epsilon$ −27.95), and 260 nm ($\Delta\epsilon$ +8.42)]. So the absolute stereochemistry of **1** was identical with **2** as 22-oxa-(12)-cytochalasa-6(12),13,19-triene-1,21-dione-7,17-dihydroxy-16,18-dimethyl-10-phenyl-(7*S*,13*E*,16*S*,17*R*,18*S*,19*E*). The absolute stereochemistries of **3**, **4**, and **5** were tentatively assigned as shown on biogenetic grounds by comparison to the co-metabolites **1** and **2**.

Compounds **1**−**5** were evaluated for their cytotoxicities against the P388 and A-549 cell lines by the MTT method.[17] All five evaluated cytochalasins showed cytotoxicity toward both cancer cell lines. Compounds **1**, **2**, **3**, and **5** were slightly active, with IC_{50} values ranging from 8.4 to 99 μM (Table 3). Interestingly, despite its slight structural difference from compound **5** with the presence of an epoxide, compound **4** was almost 3 orders of magnitude more cytotoxic than **5**, with an IC_{50} value of 0.093 μM and 0.0062 μM against P388 and A-549, respectively. This suggests that the epoxide group at C-6/C-7 might be an essential part of the pharmacophore of cytochalsins and the relatively minor structural variations in the macrocyclic ring may not make significant differences to the cytotoxicity. This warrants further investigation of these compounds.

Table 3. Cytotoxicities of Compounds **1**−**5** in Two Cancer Cell Lines

compound	cytotoxicity (IC_{50}, μM)	
	P388 cells	A-549 cells
1	75	8.8
2	56	21
3	99	8.7
4	0.093	0.0062
5	89	8.4

Experimental Section

General Experimental Procedures. Melting points were measured using a Yanaco MP-500D micro-melting point apparatus and were uncorrected. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. CD spectra were obtained on a JASCO J-810 spectropolarimeter. UV spectra were recorded on a Beckmen DU 640 spectrophotometer. IR spectra were taken on a Nicolet NEXUS 470 spectrophotometer in KBr disks. ^{1}H, ^{13}C NMR and DEPT spectra and 2D NMR were recorded on a JEOL Eclips-600 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ values. NOESY experiments were carried out using a mixing time of 0.5 s. 1D NOE spectra were obtained on a Varian INOVA-400 spectrometer. ESIMS was measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semiprepartive HPLC was performed using an ODS column [Shin-pak ODS (H), 20 × 250 mm, 5 μm, 4 mL/min].

X-ray Structure Determination of Cytochalasin Z_7 (1). X-ray diffraction intensity data of **1** were collected on a MAC DIP-2030K diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) by the ω scan technique [scan width 0−180°, $2\theta \leq 50°$]. Altogether 3049 reflections were collected, of which 2950 with $|F|^2 \geq 2\sigma|F|^2$ were observed. The structure was solved by direct methods and refined by block-matrix least-squares procedure to $R_1 = 0.0832$, $wR_2 = 0.1119$. Hydrogen positions were found from difference Fourier maps and geometric calculations. All calculations were carried out on a personal computer using the SHELX-97 program system.

Fungal Material. The fungus *Spicaria elegans* was isolated from the marine sediments collected in Jiaozhou Bay, China. It was preserved in the China Center for Type Culture Collection (patent depositary number: KLA03 CCTCC M 205049). Working stocks were prepared on potato dextrose agar slants stored at 4 °C.

Fermentation and Extraction. The fungus was grown under static conditions at 24 °C for 25 days in 50 1000-mL conical flasks containing the liquid medium (300 mL/flask) composed of glucose (20 g/L), peptone (5 g/L), malt extract (3 g/L), and yeast extract (3 g/L) and seawater after adjusting its pH to 7.0. The fermented whole broth (15 L) was filtered through cheesecloth to separate into supernatant and mycelia. The former was concentrated under reduced pressure to about a quarter of the original volume and then extracted three times with ethyl acetate to give an ethyl acetate solution, while the latter was extracted three times with acetone. The acetone solution was concentrated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with ethyl acetate to give another ethyl acetate solution. Both ethyl acetate solutions were combined and concentrated under reduced pressure to give a crude extract (15.0 g).

Purification. The crude extract (15.0 g) showing strong cytotoxity against tsFT210 cells at 50 μg/mL was separated into 15 fractions on a silica gel column using a step gradient elution of petroleum ether/acetone. The isolation of compounds **1**−**5** was achieved through a bioassay-guided fractionation procedure using the bioassay method described above. The bioactive fraction 11, eluted with 6:4 petroleum ether/acetone (1.6 g), was recrystallized from MeOH, yielding compound **4** as a colorless crystal (80 mg). The bioactive fraction 12, eluted with 5:5 petroleum ether/acetone (2.7 g), was purified into eight subfractions by another silica gel column using an isocratic elution of 95:5 $CHCl_3$/MeOH. Among these eight subfractions the active subfraction 12-1 was further purified by extensive PHPLC (60% MeOH, 4.0 mL/min), to give compound **5** (40 mg, t_R 19 min) and compound **3** (10 mg, t_R 23 min). Active subfraction 12-3 was further purified by extensive PHPLC (60% MeOH, 4.0 mL/min) to yield compound **1** (8 mg, t_R 22 min) and compound **2** (16 mg, t_R 28 min).

Biological Assays. Active fractions were assayed using the MTT method[17] with the mouse temperature-sensitive p34^{cdc2} mutant cell line tsFT210. Cytotoxic activity was evaluated by the MTT method using P388 and A-549 cell lines. The cell lines were grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C (tsFT210 cell line at 32 °C). Those cell suspensions (200 μL) at a density of 5×10^4 cell mL^{-1} were plated in 96-well microtiter plates and incubated for 24 h at the above condition. The test compound solution (2 μL in DMSO) at different concentrations was added to each well and further incubated for 72 h in the same condition. Then 20 μL of the MTT solution (5 mg/mL in IPMI-1640 medium) was added to each well and incubated for 4 h. The old medium containing MTT (150 μL) was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a Spectra Max Plus plate reader at 540 nm.

Preparation of the (*S*)- and (*R*)-MTPA Ester Derivatives of 2 by a Convenient Mosher Ester Procedure. Compound **2** (2.0 mg) was transferred into a clean NMR tube and was dried completely under vacuum. Deuterated pyridine (0.5 mL) and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 μL) were added into the NMR tube quickly under a N_2 gas stream, and then the NMR tube was shaken carefully to mix the sample and the MTPA chloride evenly. The reaction NMR tube was allowed to stand at room temperature, and ^{1}H NMR monitored the reactions at certain intervals. The reaction was found to be complete after 48 h. ^{1}H NMR data of the (*R*)-MTPA ester derivative (**2r**) of **2** were obtained from the reaction NMR tube directly and were assigned on the basis of the COSY correlations. Similarly, another portion of compound **2** (2.0 mg) was reacted in a second NMR tube with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 μL) at room temperature for 72 h using deuterated pyridine (0.5 mL) as the solvent, to afford the (*S*)-MTPA ester derivative (**2s**) of **2**.

Cytochalasin Z_7 (1): colorless needles (MeOH); mp 194−196 °C; $[\alpha]^{25}_D$ +58.3 (*c* 0.080, MeOH); CD (MeOH) λ_{max} ($\Delta\epsilon$) 196 (+71.77), 210 (−43.43), 258 (+3.74) nm; UV (MeOH) λ_{max} (log ϵ) 236 (3.31) nm; IR (KBr) ν_{max} 3358, 2937, 1706, 1447, 1248, 1086, 1042 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m*/*z* 488.2427 [M + Na]$^+$ (calcd for $C_{28}H_{35}NO_5Na$, 488.2413).

Cytochalasin Z_8 (2): colorless needles (MeOH); mp 215−217 °C; $[\alpha]^{25}_D$ +68.2 (*c* 0.085, MeOH); CD (MeOH) λ_{max} ($\Delta\epsilon$) 200 (+50.78), 217 (−27.95), 260 (+8.42) nm; UV (MeOH) λ_{max} (log ϵ) 234 (3.47) nm; IR (KBr) ν_{max} 3342, 2921, 1697, 1458, 1246, 1032 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m*/*z* 488.2392 [M + Na]$^+$ (calcd for $C_{28}H_{35}NO_5Na$, 488.2413).

Cytochalasin Z_9 (3): colorless needles (MeOH); mp 252−254 °C; $[\alpha]^{25}_D$ +70.4 (*c* 0.100, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (3.37) nm; IR (KBr) ν_{max} 3328, 2918, 1699, 1454, 1348, 1247, 1147, 1010 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m*/*z* 488.2436 [M + Na]$^+$ (calcd for $C_{28}H_{35}NO_5Na$, 488.2413).

Cytochalasin E (4): colorless needles (MeOH); mp 200−202 °C; $[\alpha]^{25}_D$ +19.3 (*c* 0.145, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (2.80), 258 (2.49) nm; IR (KBr) ν_{max} 3204, 2932, 1759, 1717, 1454, 1314, 1241 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); ESIMS *m*/*z* 496 [M + H]$^+$.

Cytochalasin K (5): colorless needles (MeOH); mp 241−244 °C; $[\alpha]^{25}_D$ +66.1 (*c* 0.075, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (3.30), 258 (2.50) nm; IR (KBr) ν_{max} 3310, 2919, 1752, 1706, 1454, 1308, 1202 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m*/*z* 496.2353 [M + H]$^+$ (calcd for $C_{28}H_{34}NO_7$, 496.2335).

(*R*)-MTPA ester derivative (2r): ^{1}H NMR (pyridine-d_5, 600 MHz) δ 3.806 (1H, br dd, J = 8.2, 6.6 Hz, H-3), 4.253 (1H, br s, H-4), 6.242 (1H, br d, J = 9.9 Hz, H-7), 3.754 (1H, dd, J = 11.5, 9.9 Hz, H-8), 3.283 (1H, dd, J = 13.0, 6.6 Hz, H-10a), 3.141 (1H, dd, J = 13.0, 8.2 Hz, H-10b), 1.175 (3H, s, CH_3-11), 1.291 (3H, s, CH_3-12), 6.781 (1H, dd, J = 15.3, 11.5 Hz, H-13), 5.267 (1H, ddd, J = 15.3, 11.0, 3.8 Hz, H-14), 2.348 (1H, m, H-15a), 1.961 (1H, br d, J = 11.0 Hz, H-15b), 1.707 (1H, m, H-16), 5.216 (1H, m, H-17), 2.937 (1H, m, H-18), 7.366 (1H, br d, J = 15.9 Hz, H-19), 5.977 (1H, dd, J = 15.9, 1.6 Hz, H-20), 0.746 (3H, d, J = 7.7 Hz, CH_3-23), 1.052 (3H, d, J = 6.6 Hz, CH_3-24).

(*S*)-MTPA ester derivative (2s): ^{1}H NMR (pyridine-d_5, 600 MHz) δ 3.920 (1H, br dd, J = 8.3, 6.6 Hz, H-3), 4.259 (1H, br s, H-4), 6.281 (1H, br d, J = 9.0 Hz, H-7), 3.720 (1H, dd, J = 11.0, 9.0 Hz, H-8), 3.300 (1H, dd, J = 13.0, 6.6 Hz, H-10a), 3.159 (1H, dd, J = 13.0, 8.3 Hz, H-10b), 1.320 (3H, s, CH_3-11), 1.490 (3H, s, CH_3-12), 6.713 (1H,

dd, $J = 15.4$, 11.0 Hz, H-13), 5.166 (1H, br dd, $J = 15.4$, 14.0 Hz, H-14), 2.196 (1H, m, H-15a), 1.814 (1H, br d, $J = 14.0$ Hz, H-15b), 1.649 (1H, m, H-16), 5.173 (1H, m, H-17), 2.901 (1H, m, H-18), 7.252 (1H, br d, $J = 15.9$ Hz, H-19), 5.942 (1H, dd, $J = 15.9$, 1.7 Hz, H-20), 0.883 (3H, d, $J = 7.1$ Hz, CH_3-23), 0.915 (3H, d, $J = 6.6$ Hz, CH_3-24).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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