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Novel Open-Chain Cytochalsins from the Marine-Derived Fungus *Spicaria elegans*

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Six novel open-chain cytochalasins (**1**−**6**) and one known [12]-cytochalasin (**7**) have been isolated from the fermentation broth of a marine-derived fungus, *Spicaria elegans.* Cytochalasins Z_{10}−Z_{15} (**1**−**6**) are the first reported cytochalasins that contain an open chain to date. The structures of these new compounds were elucidated by spectroscopic methods. The cytotoxic effects on P388, A-549, HL-60, and BEL-7402 cell lines of all compounds were evaluated by the MTT method.

In our effort to search for anticancer compounds,[1–3] a fungal strain identified as *Spicaria elegans* exhibited cytotoxic activity. We have previously reported on the isolation and structural elucidation of three [12]-cytochalasins, Z_7, Z_8, and Z_9, and two [13]-cytochalasins, E and K,[4] from *S. elegans*. Our ongoing investigations of the mass reculture of this species in the same media have led to the isolation of a further six new cytochalasins, Z_{10}−Z_{15} (**1**−**6**), and one known [12]-cytochalasin (**7**). Compounds **1**−**6** (cytochalasins Z_{10}−Z_{15}) are the first ones in a new class of cytochalasins that possess a highly substituted perhydroisoindol-1-one core usually fused with an 11-,[5–8] 13-,[9] 12-,[4,10] or 14-membered[11] macrocyclic ring. These compounds display a wide range of biological activities[9,12,13] and, thus, have great potential in cell biology and medicine. In addition to a highly substituted perhydroisoindol-1-one skeleton, the novel cytochalasins (**1**−**6**) were found to have an open 8-carbon chain system rather than a macrocyclic ring, which was strikingly different from previously reported cytochalasins. Therefore, precise information on the structure and conformation of these novel cytochalasins was important in order to understand their chemical and biological activities. In this paper, we report the isolation, structural elucidation, and cytotoxic activities of six novel open-chain cytochalasins, Z_{10}−Z_{15} (**1**−**6**).

Results and Discussion

The bioactive EtOAc extract of *S. elegans* was chromatographed over a Si gel column and by preparative HPLC to give six new cytochalasins (**1**−**6**) and [12]-cytochalasin (**7**).[10] Their structures were established by detailed analysis of NMR spectra, which included ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY experiments, and also by comparison with the NMR data for the known cytochalasins Z_7, Z_8, Z_9, E, and K we had reported previously.[4]

Cytochalasin Z_{10} (**1**) was a white, amorphous solid. Its HRESIMS gave a $[M + H]^+$ ion peak at *m/z* 430.2596 (calcd for $[M + H]^+$ 430.2593), in agreement with the molecular formula $C_{25}H_{35}NO_5$, 36 mass units smaller than that of cytochalasin Z_7.[4] The IR spectrum showed the presence of hydroxyl and carbonyl groups. Analysis of the 1D NMR data for **1** revealed one carbonyl, three quaternary carbons, 15 methines, three methylenes, and three methyls. Comparison of the ^{1}H and ^{13}C NMR data of **1** with those of cytochalasin Z_7 showed the presence of the same 10-phenyl-substituted perhydroisoindol-1-one skeleton. Compound **1** differed from cytochalasin Z_7 only in the macrocyclic ring, where the α,β-unsaturated ester system [C-19 (157.7, CH), C-20 (123.3, CH), C-21 (167.7, qC)] that is present in the ^{13}C NMR spectrum of cytochalasin Z_7 was missing, and chemical shifts of C-9 (δ_C 79.4 qC) and C-18 (δ_C 68.8 CH) in **1** were upfield and downfield, respectively, which suggested that two hydroxyl groups were located on C-9 and C-18 and the 12-membered macrocyclic ring in cytochalasin Z_7 was changed to an open chain in **1**, which was consistent with the molecular formula. The positions of the substituents in the open chain were confirmed as occurring at C-16 (methyl) and C-17 and C-18 (two hydroxyls) using the HMBC spectrum, which showed correlations from CH_3-19 (1.19 ppm, d) to the two oxygenated methine sp^3 carbons C-17 (78.4 ppm, CH) and C-18 (68.8 ppm, CH) and correlations from CH_3-20 (0.86 ppm, d) to C-15 (38.5 ppm, CH_2), C-16 (35.1 ppm, CH), and C-17. Therefore the structure of the new compound **1** was elucidated.

Cytochalasin Z_{11} (**2**) was an amorphous solid like **1**. Its molecular formula was established as $C_{25}H_{33}NO_5$ by the $[M + H]^+$ ion peak observed at *m/z* 428.2434 (calcd for $[M + H]^+$ 428.2437) in its HRESIMS. Analysis of the 1D NMR spectra of **2** indicated that it had a structure very similar to **1**. The only difference was that the ^{13}C NMR spectrum (Table 1) of **2** contained one more carbonyl carbon at 216.8 ppm (C-17, qC) in place of an oxygenated carbon at 78.4 ppm (C-17, CH) in **1**. This change could be further confirmed by the HMBC correlations from CH_3-19 (1.21 ppm, d) and CH_3-20 (1.01 ppm, d) to the carbonyl carbon at 216.8 ppm (C-17, qC). Thus the structure of **2** was established.

Another metabolite isolated from the same extract is cytochalasin Z_{12} (**3**), obtained as a yellow, amorphous solid. The molecular formula of **3** was also $C_{28}H_{35}NO_5$, as determined by HRESIMS ($[M + Na]^+$ at *m/z* 452.2411, calcd for 452.2413). This compound was isomeric with **1**. Comparison of their ^{1}H and ^{13}C NMR spectra suggested the two exocyclic double-bond resonances at 150.9 ppm (C-6, qC) and 112.8 ppm (C-12, CH_2) in **1** had been replaced by two carbon resonances at 125.0 ppm (C-5, qC) and 129.6 ppm (C-6, qC) attributable to a tetrasubstituted double bond, which were also similar to those observed in the ^{13}C spectrum of cytochalasin K. Furthermore, in the ^{1}H NMR spectrum of **3**, the proton signal at 1.83 ppm due to the C-11 methyl appeared as a singlet, not a doublet. Therefore, as an isomer of **1**, compound **3** was only different in having a tetrasubstituted bond of the perhydroisoindol-1-one core in **1**. This change was also confirmed by 2D NMR correlations (^{1}H−^{1}H COSY correlation of H-8 to H-7).

Cytochalasin Z_{13} (**4**), a colorless oil, was isomeric with **2**, with the same molecular formula $C_{25}H_{33}NO_5$. HRESIMS showed a $[M + H]^+$ ion peak at *m/z* 428.2399 (calcd for 428.2437). Comparison of the 1D NMR spectra of compounds **4** and **2** showed the only difference was also that the exocyclic double bond in **2** [148.1 ppm (C-6, qC,), 112.3 ppm (C-12, CH_2)] was replaced by a tetrasubstituted double bond in **4** [124.9 ppm (C-5, qC), 129.4 ppm (C-6,

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Table 1. ^{1}H NMR Data for Compounds **1**–**6**[a]

H	**1**[b] (*J*/Hz)	**2**[c] (*J*/Hz)	**3**[c] (*J*/Hz)	**4**[c] (*J*/Hz)	**5**[c] (*J*/Hz)	**6**[c] (*J*/Hz)
2		6.49 (br s)	6.59 (br s)	6.66 (br s)	5.49 (br s)	5.90 (1H, s)
3	3.38 (br dd, 12.1, 5.5)	3.32 (m)	3.46 (m)	3.30 (td, 10.6, 2.9)	3.48 (m)	3.41 (1H, m)
4	2.29 (dd, 5.8, 5.5)	2.43 (br d, 7.3)	2.62 (br d, 6.4)	2.60 (br s)	2.65 (br d, 5.2)	2.64 (1H, d, 4.8)
5	3.05 (qd, 7.0, 5.8)	3.28 (m)				
7	3.94 (br d, 7.7)	4.08 (br d, 3.7)	3.80 (br s)	3.71 (br d, 3.7)	3.83 (br s)	3.83 (1H, brs)
8	2.64 (dd, 9.5, 7.7)	2.70 (dd, 10.5, 3.7)	2.88 (dd, 8.4, 2.2)	2.82 (br d, 10.3)	2.85 (br d, 10.0)	2.76 (1H, d, 7.0)
10[a]	2.98 (dd, 13.9, 5.5, 10a)	3.25 (m, 10a)	3.34 (dd, 13.9, 3.4, 10a)	3.42 (br dd, 13.6, 2.6, 10a)	3.37 (dd, 13.6, 2.9, 10a)	3.33 (1H, dd, 13.4, 3.3, Ha)
10[b]	2.74 (dd, 13.9, 6.6, 10b)	2.41 (dd, 13.6, 10.0, 10b)	2.69 (dd, 13.9, 9.8, 10b)	2.56 (ddd, 13.6, 10.6, 2.9, 10b)	2.64 (br d, 13.6, 10b)	2.71 (1H, dd, 13.4, 9.3, Hb)
11	1.02 (d, 7.0)	1.31 (d, 7.0)	1.83 (br s)	1.88 (br s)	1.89 (br s)	1.80 (3H, s)
12	5.19 (br s) *Z* 5.04 (br s) *E*	5.13 (br s) *Z* 5.04 (br s) E	1.85 (br s)	1.90 (br s)	1.87 (br s)	1.86 (3H, s)
13	5.46 (br dd, 15.0, 9.5)	5.03 (br dd, 15.1, 10.5)	5.14 (br dd, 15.0, 8.4)	4.97 (br dd, 15.0, 10.3)	5.28 (br dd, 15.0, 10.0)	5.23 (1H, dd, 14.7, 9.5)
14	5.58 (br dd, 15.0, 7.0)	5.49 (ddd, 15.1, 10.2, 4.4)	5.59 (ddd, 15.0, 8.0, 6.6)	5.49 (ddd, 15.0, 10.2, 4.0)	5.66 (ddd, 15.0, 6.0, 6.0)	5.54 (1H, dd, 14.7, 7.3)
15[a]	2.09 (br dd, 13.5, 6.7, 15a)	2.07 (br d, 13.2, 15a)	1.95 (m, 15a)	2.16 (br d, 13.1, 15a)	2.07 (m, 2H)	1.82 (2H, m)
15[b]	1.97 (br dd, 13.5, 7.0, 15b)	2.19 (br d, 13.2, 15b)	1.91 (m, 15b)	2.06 (br d, 13.1, 15b)		
16	1.88 (qd, 7.0, 3.0)	2.73 (m)	1.65 (br q, 6.6)	2.71 (m)	1.97 (br q, 6.6)	1.99 (1H, m)
17	3.26 (dd, 7.7, 3.0)		3.23 (dd, 44, 5.5)		3.97 (br s)	3.98 (1H, brs)
18	3.62 (dq, 7.7, 6.2)	4.16 (q, 7.3)	3.68 (br q, 6.2)	4.03 (q, 7.3)		
19	1.19 (d, 6.2)	1.21 (d, 7.3)	1.04 (d, 6.2)	1.20 (d, 7.3)	2.06 (s)	2.14 (3H, s)
20	0.86 (d, 7.0)	1.01 (d, 6.6)	0.78 (d, 6.6)	0.99 (d, 6.6)	0.60 (d, 6.6)	0.93 (3H, 6.6)
2′, 6′	7.21 (m)	7.23 (m)	7.17 (m)	7.27 (m)	7.17 (m)	7.17 (2H, d, 7.3)
3′, 5′	7.31 (m)	7.41 (m)	7.30 (m)	7.41 (m)	7.32 (m)	7.32 (2H, t, 7.3)
4′	7.22 (m)	7.30 (m)	7.23 (m)	7.30 (m)	7.28 (m)	7.26 (1H, t, 7.3)

[a] Spectra were recorded at 600 MHz for ^{1}H using TMS as internal standard. [b] Measured in CD_3OD. [c] Measured in $CDCl_3$.

qC)]. Further analysis of the 2D NMR (COSY, HMQC, and HMBC) experiments confirmed the structure as **4**.

Cytochalasin Z_{14} (**5**) was a yellow oil. Its HRESIMS (*m*/*z* 410.2337 [M − H_2O+ H]$^+$ (calcd for $C_{25}H_{32}NO_4$, 410.2331) suggested that it was an isomer of compounds **2** and **4**, with a molecular formula of $C_{25}H_{33}NO_5$. Comparison of **5** and **4** showed **5** contained the same tetrasubstituted double bond at 124.9 ppm (C-5, qC) and 129.9 ppm (C-6, qC). The only difference between the two compounds was the exchange of the position of the carbonyl and the hydroxyl carbons. This change could be explained by the downfield shift of CH_3-19 in **5** at δ_H 2.06 ppm, δ_C 25.1 ppm, compared to CH_3-19 in **4** at δ_H 1.20 ppm, δ_C 18.9 ppm. This structure was also in agreement with the observed significant HMBC correlations from CH_3-20 (0.60 ppm, d) to C-15 (37.3 ppm, CH_2), C-16 (35.5 ppm, CH), and C-17 (77.8 ppm, CH). Thus the structure of **5** was established.

Cytochalasin Z_{15} (**6**) was a yellow oil. Its HRESIMS ([M + H]$^+$ *m*/*z* 428.2451, calcd for $C_{25}H_{34}NO_5$ 428.2437) suggested that it was an isomer of compounds **2**, **4**, and **5**, also with a molecular formula of $C_{25}H_{33}NO_5$. Comparison of **6** and **5**, combined with the HMBC correlations from CH_3-20 (0.93 ppm, s) to C-15 (33.6 ppm, CH_2), C-16 (35.8 ppm, CH), and C-17 (80.8 ppm, CH), showed these two compounds had the same planar structure. The only difference of **6** from **5** was the downshift of C-17 (δ_H 3.99 ppm, δ_C 80.8 ppm compared to δ_H 3.97 ppm, δ_C 77.8 ppm in **5**) and the downshift of CH_3-20 in **6** (δ_H 0.93 ppm, δ_C 17.1 ppm compared to δ_H 0.60 ppm, δ_C 12.7 ppm in **5**), suggesting that the configuration of C-17 and CH_3-20 in **6** was different from that in **5**.

Previous studies have suggested that the essential elements of most cytochalasins' skeleton have the same stereochemistry, viz., *cis*-stereochemistry across the 5/6 ring junction and the *trans*-stereochemistry of the macrocyclic ring.[8] A series of NOESY and NOE experiments on these new cytochalasins (**1**−**6**) suggested the same relative stereochemistry of the 10-phenyl-substituted perhydroisoindol-1-one skeleton, as correlations were observed between protons H-3/CH_3-11, H-4/H-8 and 2H-10, H-5/H-8, and H-8/H-14. Correlations between CH_3-11/H-12*E* and H-12*Z*/H-7 were also observed in compounds **1** and **2**. The relative configurations of the three consecutive stereogenic centers (C-16, C-17, and C-18) in the open 8-carbon chain in **1** were first proposed on the basis of the comparison of the ^{13}C NMR chemical shifts of **1** with the published values for synthetic diastereomers (**a**−**d**) sharing common partial structures from C-16 to C-20 with C-17 substitution (Figure 3).[14] Detailed analysis of ^{13}C NMR data of **a**−**d** indicated that the chemical shifts of C-19 and C-20 methyl groups were clearly dependent on the relative configurations of the methyl-hydroxy-hydroxy-substituted stereogenic centers of these compounds. The similarities between the chemical shifts of C-19 and C-20 in compounds **1** and **3** (Table 2) and those in compound **c** suggested that C-19 and C-20 in **1** and **3** had the same relative configuration as that of **c**. Thus, the 8-carbon chain's relative configurations of **1** and **3** were assigned as 16*S**, 17*R**, 18*R**.

Comparison of the 1D NMR spectra (Tables 1 and 2) of **2** with those of **4** showed almost identical ^{13}C NMR chemical shifts for the 8-carbon chains in the two compounds. As a result, the relative configurations of the side chain in **2** should be the same as those in **4**. Comparison of the 1D NMR spectra (Tables 1 and 2) of **5** with those of **6** found that the differences were at C-17 and C-20 (3.97, H-17, 77.8, CH, C-17; 0.60, CH_3-20, 12.7, qC, C-20 in **5**; 3.98, H-17, 80.8, CH, C-17; 0.93, CH_3-20, 17.1, qC, C-20 in **6**). These data implied that the relative orientations of the methyl at C-16 and the hydroxyl group at C-17 in compounds **5** and **6** were different (Figure 1) in these two compounds.

The absolute configuration of **1** was established by a convenient Mosher ester method using the (*S*)- and (*R*)-MTPA esters. Tri-Mosher ester derivatives (**1a** and **1b**) of **1** were prepared (Figure

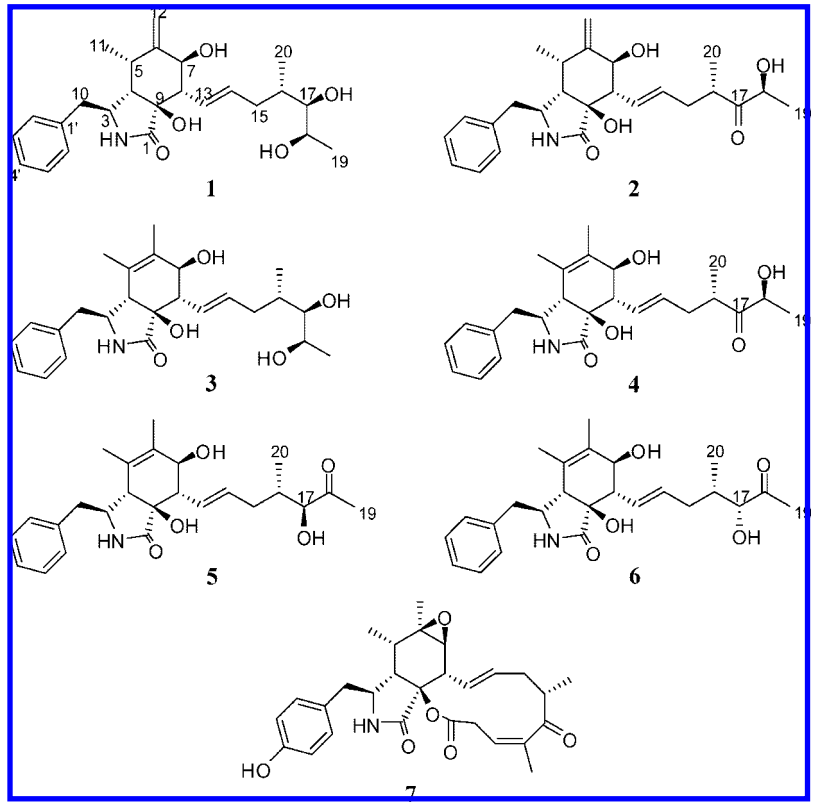

Figure 1. Structures of compounds **1**−**7**.

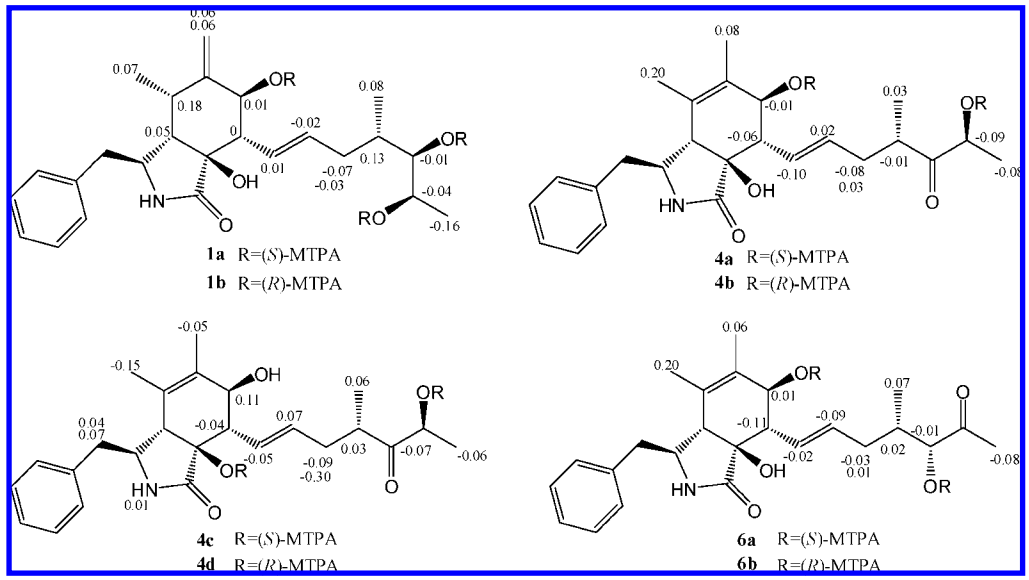

Figure 2. $\Delta\delta$ value [$\Delta\delta$ (in ppm) $= \delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters of compounds **1**, **4**, and **6**.

2), and the positive value of δ^{S-R} for H-11 and H-12 and negative value of δ^{S-R} for H-14 and H-15[15]established the 7*S* configuration, which was consistent with that in cytochalasins Z_7−Z_9.[4] It had been confirmed that the modified Mosher's method was useful for acyclic 1,2-glycols possessing simple alkyl groups,[16a−c] and most recently Riguera's group[16d] has predicted four general patterns for 1,2-diols. The absolute configuration of the vicinal carbinol carbons in **1** was determined by the analysis of the ^{1}H NMR spectrum of the di-MTPA ester of **1** in accordance with method of Riguera. The negative values of δ^{S-R} for H-17 and H-18 indicated 17*R*, 18*R* configurations in **1**.[16d] Taken together, these data established the absolute configurations of compound **1** as 7*S*, 16*S*, 17*R*, 18*R*. Similarly, the 7*S* configurations in both **4** and **6**, the 18*R* configuration in **4**, and the 17*R* configuration in **6** were also established by the Mosher ester method (Figure 2).

Finally, it is noteworthy that in all cytochalasins isolated thus far, the *S* configuration of C-16 in the macrocyclic ring moieties is maintained throughout the series. Open-chain cytochalsins are presumably biosynthesized from those with a 12-membered macrocyclic ring. Thus, we tentatively concluded that all the open-chain cytochalasins have an *S* configuration at C-16. This is also consistent with the 16*S* configuration deduced above in compound **1**. Therefore, the absolute configurations of all six new compounds have been assigned as shown in Figure 1.

Compounds **1**−**7** were evaluated for their cytotoxicities against the P388, A-549, HL-60, and BEL-7402 cell lines by the MTT

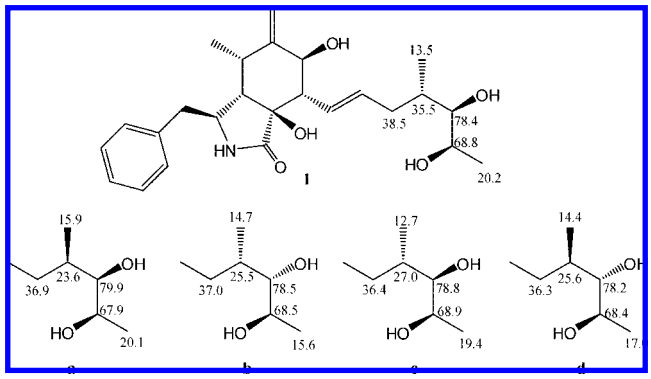

Figure 3. Comparison of ^{13}C NMR chemical shifts (in CD_3OD-d_4) of compound **1** with four synthetic model compounds (**a**−**d**) to establish the relative configurations at C-16, C-17, and C-18 in **1**.

method.[17] Compounds **1** and **2** showed moderate cytotoxicities against the A-549 cell line with IC_{50} values of 9.6 and 4.3 μM, respectively (Table 3).

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. IR spectra were taken on a Nicolet Nexus 470 spectrophotometer in KBr disks. ^1H, ^{13}C NMR and DEPT spectra and 2D NMR were recorded on a JEOL JNM-ECP 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), 10 × 250 mm, 5 μm, 4 mL/min].

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 depository 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 80 × 1000 mL conical flasks containing liquid medium (300 mL/flask) composed of glucose (20 g/L), peptone (5 g/L), malt extract (3 g/L), yeast extract (3 g/L), and seawater after adjusting its pH to 7.0. The fermented whole broth (24 L) was filtered through cheesecloth to separate it 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 EtOAc to give an EtOAc 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 EtOAc to give another EtOAc solution. Both EtOAc solutions were combined and concentrated under reduced pressure to give a crude extract (23.0 g).

Purification. The crude extract (23.0 g) was separated into 15 fractions on a silica gel column using gradient elution of petroleum ether/acetone and TLC monitoring (on silica gel plates with $CHCl_3$/MeOH, 95:5, as eluent). Fraction 12, eluted with petroleum ether/acetone, 5:5 (2.7 g), was purified into eight subfractions by another silica gel column using stable elution of chloroform/MeOH, 9:1. Subfraction 12-1 was further purified by extensive HPLC (60% MeOH, 4.0 mL/min), giving compound **1** (20 mg, t_R 16 min). Subfraction 12-3 was further purified by extensive HPLC (60% MeOH, 4.0 mL/min) to yield compounds **7** (24 mg, t_R 22min) and **4** (30 mg, t_R 30 min). Subfraction 12-4 was further purified by HPLC (60% MeOH, 4.0 mL/min) to yield compounds **2** (40 mg, t_R 11 min), **3** (8 mg, t_R 15 min), and **5** (3 mg, t_R 25 min). Subfraction 12-5 was further purified by HPLC (60% MeOH, 4.0 mL/min) to yield compound **6** (25 mg, t_R 24 min).

Preparation of the (*S*)- and (*R*)-MTPA Ester Derivatives of 1, 4, and 6 and Determination of the Absolute Stereochemistry. Compounds **1** (5.0 mg), **4** (1.0 mg), and **6** (1.0 mg) were separately transferred into a clean reaction bottle and dried completely under vacuum. Deuterated pyridine (0.5 mL) and (*S*)-(+)-*R*-methoxy-*R*-(trifluoromethyl)phenylacetyl chloride (1 equiv) were added into the reaction bottle quickly under a N_2 gas stream and then stirred for 24 h at room temperature. The organic layer was then washed with water, HCl (1 M), water, $NaHCO_3$ (sat), and water, dried (Na_2SO_4), and concentrated under reduced pressure to obtain the ester. Final purification was achieved by HPLC (90% MeOH, 4.0 mL/min): **1a** and **1b**, t_R = 11.7 min; **4a** and **4b**, t_R = 6.9 min; **4c** and **4d**, t_R = 11.4 min; **6a** and **6b**, t_R = 6.0 min. ^1H NMR data of the (*S*)-MTPA ester derivative (**1a**) of **1** (600 MHz, $CDCl_3$): δ 5.63 (1H, brs, 2-NH), 3.33 (1H, m, H-3), 2.39 (1H, dd, J = 3.6, 5.4 Hz, H-4), 3.04 (1H, m, H-5), 5.47 (1H, d, J = 8.8 Hz, H-7), 2.90 (1H, dd, J = 8.8, 9.2 Hz, H-8), 2.93 (1H, dd, J = 4.7, 13.6 Hz, H-10a), 2.74 (1H, dd, J = 8.8, 13.6 Hz, H-10b), 1.05 (3H, d, J = 6.6 Hz, H-11), 5.38 (1H, brs, H-12a), 5.17 (1H, brs, H-12b), 5.73 (1H, dd, J = 9.9, 5.4 Hz, H-13), 5.36 (1H, ddd, J = 7.0, 7.3, 15.0 Hz, H-14), 1.84 (1H, m, H-15a), 1.74 (1H, m, H-15b), 1.73 (1H, m, H-16), 5.15 (1H, dd, J = 4.4, 4.4 Hz, H-17), 5.25 (1H, m, H-18), 1.11 (3H, d, J = 6.2 Hz, H-19), 0.79 (3H, d, J = 6.2 Hz, H-20), 7.12−7.53 (20H, m, Ph-H). ^1H NMR data of the (*R*)-MTPA ester derivative (**1b**) of **1** (600 MHz, $CDCl_3$): δ 5.61 (1H, brs, 2-NH), 3.31 (1H, m, H-3), 2.34 (1H, dd, J = 4.4, 4.4 Hz, H-4), 2.87 (1H, m, H-5), 5.46 (1H, d, J = 7.2 Hz, H-7), 2.89 dd, J = 8.8, 9.2 Hz), 2.94 (1H, dd, J = 4.7, 13.6 Hz, H-10a), 2.70 (1H, dd, J = 8.8, 13.6 Hz, H-10b), 0.99 (3H, d, J = 6.6 Hz, H-11), 5.32 (1H, brs, H-12a), 5.11 (1H, brs, H-12b), 5.72 (1H, dd, J = 9.9, 15.4 Hz, H-13), 5.39 (1H, ddd, J = 7.0, 7.3, 15.0 Hz, H-14), 1.91 (1H, m, H-15a), 1.78 (1H, m, H-15b), 1.60 (1H, m, H-16), 5.15 (1H, dd, J = 4.4, 4.4 Hz, H-17), 5.29 (1H, m, H-18), 1.27 (3H, d, J = 6.2 Hz, H-19), 0.70 (3H, d, J = 6.2 Hz, H-20), 7.16−7.54 (20H, m, Ph-H). ^1H NMR data of the (*S*)-MTPA ester derivative (**4a**) of **4** (600 MHz, $CDCl_3$): δ 5.69 (1H, brs, 2-NH), 3.43 (1H, m, H-3), 2.64 (1H, brs, H-4), 5.55 (1H, d, J = 7.3 Hz, H-7), 2.67 (1H, dd, J = 7.0, 9.5 Hz, H-8), 3.18 (1H, dd, J = 5.5, 13.6 Hz, H-10a), 2.87 (1H, dd, J = 8.8, 13.6 Hz, H-10b), 1.61 (3H, s, H-11), 1.66 (3H, s, H-12), 5.69 (1H, dd, J = 9.9, 15.4 Hz, H-13), 5.54 (1H, ddd, J = 7.0, 7.3, 15.0 Hz, H-14), 2.37 (1H, m, H-15a), 2.05 (1H, m, H-15b), 2.71 (1H, m, H-16), 5.25 (1H, q, J = 7.0 Hz, H-18), 1.41 (3H, d, J = 7.3 Hz, H-19), 1.06 (3H, d, J = 6.6 Hz, H-20), 7.18−7.64 (15H, m, Ph-H). ^1H NMR data of the (*R*)-MTPA ester derivative (**4b**) of **4** (600 MHz, $CDCl_3$): δ 5.82 (1H, brs, 2-NH), 3.44 (1H, m, H-3), 2.65 (1H, brs, H-4), 5.56 (1H, d, J = 7.3 Hz, H-7), 2.73 (1H, m, H-8), 3.16 (1H, dd, J = 5.9, 13.2 Hz, H-10a), 2.92 (1H, dd, J = 8.5, 13.2 Hz, H-10b), 1.41 (1H, s, H-11), 1.58 (1H, s, H-12), 5.79 (1H, dd, J = 9.9, 15.4 Hz, H-13), 5.52 (1H, ddd, J = 7.0, 7.3, 15.0 Hz, H-14), 2.33 (1H, m, H-15a), 2.13 (1H, m, H-15b), 2.72 (1H, m, H-16), 5.34 (1H, q, J = 7.3 Hz, H-18), 1.49 (3H, d, J = 7.0 Hz, H-19), 1.03 (3H, d, J = 6.9 Hz, H-20), 7.18−7.60 (15H, m, Ph-H). ^1H NMR data of the (*S*)-MTPA ester derivative (**4c**) of **4** (600 MHz, $CDCl_3$): δ 5.74 (1H, brs, 2-NH), 3.41 (1H, m, H-3), 3.54 (1H, brs, H-4), 3.61 (1H, brs, H-7), 3.00 (1H, brd, J = 9.1 Hz, H-8), 3.36 (1H, dd, J = 2.5, 13.6 Hz, H-10a), 2.88 (1H, dd, J = 10.6, 13.6 Hz, H-10b), 1.74 (3H, s, H-11), 1.85 (3H, s, H-12), 5.07 (1H, dd, J = 9.5, 15.0 Hz, H-13), 5.56 (1H, ddd, J = 7.7, 7.3, 15.0 Hz, H-14), 2.35 (1H, m, H-15a), 1.93 (1H, m, H-15b), 2.61 (1H, m, H-16), 5.21 (1H, q, J = 7.0 Hz, H-18), 1.35 (3H, d, J = 7.0 Hz, H-19), 0.94 (3H, d, J = 7.0 Hz, H-20), 7.24−7.60 (15H, m, Ph-H). ^1H NMR data of the (*R*)-MTPA ester derivative (**4d**) of **4** (600 MHz, $CDCl_3$): δ 5.73 (1H, brs, 2-NH), 3.44 (1H, m, H-3), 3.45 (1H, brs, H-4), 3.50 (1H, brs, H-7), 3.04 (1H, brd, J = 7.3 Hz, H-8), 3.32 (1H, dd, J = 2.9, 13.6 Hz, H-10a), 2.81 (1H, dd, J = 10.3, 13.6 Hz, H-10b), 1.90 (3H, s, H-11), 1.90 (3H, s, H-12), 5.12 (1H, dd, J = 9.9, 15.4 Hz, H-13), 5.49 (1H, ddd, J = 7.0, 7.3, 15.0 Hz, H-14), 2.65 (1H, m, H-15a), 2.01 (1H, m, H-15b), 2.58 (1H, m, H-16), 5.27 (1H, q, J = 7.0 Hz, H-18), 1.41 (3H, d, J = 7.0 Hz, H-19), 0.88 (3H, d, J = 7.0 Hz, H-20), 7.20−7.61 (15H, m, Ph-H). ^1H NMR data of the (*S*)-MTPA ester derivative (**6a**) of **6** (600 MHz, $CDCl_3$): δ 5.55 (1H, brs, 2-NH), 3.40 (1H, m, H-3), 2.64 (1H, brs, H-4), 5.44 (1H, brs, H-7), 2.67 (1H, dd, J = 5.5, 9.2 Hz, H-8), 3.24 (1H, dd, J = 1.5, 13.6 Hz, H-10a), 2.77 (1H, dd, J = 9.1, 13.6 Hz, H-10b), 1.67 (3H, s, H-11), 1.75 (3H, s, H-12), 5.52 (1H, m, H-13), 5.44 (1H, m, H-14), 1.98 (1H, m, H-15a), 1.81 (1H, m, H-15b), 2.16 (1H, m, H-16), 5.04 (1H, d, J = 3.6 Hz, H-17), 2.11 (3H, s, H-19), 0.89 (3H, d, J = 7.0 Hz, H-20), 7.15−7.58 (15H, m, Ph-H). ^1H NMR data of the (*R*)-MTPA ester derivative (**6b**) of **6** (600 MHz, $CDCl_3$): δ 5.57 (1H, brs, 2-NH), 3.41 (1H, m, H-3), 2.65 (1H, brs, H-4), 5.44 (1H, brs, H-7), 2.78 (1H, brd, J = 5.3, 9.3 Hz, H-8), 3.22 (1H, dd, J

Table 2. ^{13}C NMR Data for Compounds **1**−**6**[a]

C	**1**	**2**	**3** (ppm)	**4**	**5**	**6**
1	177.6 qC	175.0 qC	176.2 qC (177.9)	175.4 qC	175.4 qC	175.7 qC
3	54.3 CH	52.9 CH	57.2 CH (58.8)	57.1 CH	56.9 CH	57.3 CH
4	53.5 CH	53.6 CH	52.5 CH (53.8)	52.6 CH	52.9 CH	52.9 CH
5	31.0 CH	27.8 CH	125.0 qC (127.1)	124.9 qC	124.9 qC	124.8 qC
6	150.9 qC	148.1 qC	129.6 qC (131.4)	129.4 qC	129.9 qC	130.3 qC
7	74.4 CH	76.1 CH	72.8 CH (73.5)	73.0 CH	73.0 CH	72.4 CH
8	54.0 CH	53.2 CH	50.6 CH (52.7)	50.5 CH	51.4 CH	51.1 CH
9	79.4 qC	79.4 qC	78.2 qC (78.8)	78.6 qC	78.2 qC	77.7 qC
10	44.1 CH_2	43.7 CH_2	43.6 CH_2 (44.0)	44.3 CH_2	44.1 CH_2	43.9 CH_2
11	15.1 CH_3	15.9 CH_3	18.4 CH_3 (17.0)	18.7 CH_3	18.6 CH_3	18.3 CH_3
12	112.8 CH_2	112.3 CH_2	17.5 CH_3 (18.3)	18.0 CH_3	17.6 CH_3	17.3 CH_3
13	128.6 CH	128.1 CH	126.5 CH (127.8)	127.8 CH	127.4 CH	127.2 CH
14	135.7 CH	132.1 CH	134.0 CH (135.8)	132.1 CH	134.1 CH	133.9 CH
15	38.5 CH_2	38.4 CH_2	36.7 CH_2 (38.6)	38.6 CH_2	37.3 CH_2	33.6 CH_2
16	35.1 CH	40.9 CH	34.0 CH (35.4)	40.8 CH	35.5 CH	35.8 CH
17	78.4 CH	216.8 qC	76.2 CH (78.8)	216.7 qC	77.8 CH	80.8 CH
18	68.8 CH	73.0 CH	68.1 CH (68.9)	73.2 CH	210.0 qC	210.1 qC
19	20.2 CH_3	18.8 CH_3	17.1 CH_3 (20.2)	18.9 CH_3	25.1 CH_3	25.8 CH_3
20	13.5 CH_3	17.6 CH_3	14.6 CH_3 (13.5)	17.8 CH_3	12.7 CH_3	17.1 CH_3
1′	138.5 qC	137.3 qC	137.0 qC (138.7)	137.3 qC	137.0 qC	137.2 qC
2′, 6′	130.7 CH	129.1 CH	128.8 CH (129.7)	129.3 CH	128.9 CH	129.0 CH
3′, 5′	129.6 CH	128.8 CH	129.0 CH (130.8)	128.8 CH	128.9 CH	128.9 CH
4′	127.8 CH	127.0 CH	126.9 CH (128.1)	127.0 CH	127.2 CH	127.0 CH

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

Table 3. Cytotoxicities of Compounds **1**−**7** in Four Cancer Cell Lines

	cytotoxicity (IC_{50}, μM)			
compd	P388	A-549	HL-6	BEL-7402
1	>100	9.6	69	>100
2	69	4.3	45	>100
3	>100	92	89	>100
4	67	76	80	88
5	>100	>100	>100	>100
6	>100	>100	>100	>100
7	79	96	66	94

= 1.5, 13.6 Hz, H-10a), 2.80 (1H, dd, J = 9.1, 13.6 Hz, H-10b), 1.47 (3H, s, H-11), 1.69 (3H, s, H-12), 5.54 (1H, m, H-13), 5.53 (1H, m, H-14), 2.01 (1H, m, H-15a), 1.80 (1H, m, H-15b), 2.14 (1H, m, H-16), 5.05 (1H, q, J = 3.6 Hz, H-17), 2.20 (3H, s, H-19), 0.82 (3H, d, J = 7.0 Hz, H-20), 7.15−7.68 (15H, m, Ph-H).

Cytochalasin Z_{10} (1): colorless oil (MeOH); $[\alpha]^{25}_D$ +58.0 (*c* 0.080, MeOH); UV (MeOH) λ_{max} (log ε) 225 (3.40), 258 (1.03) nm; IR (KBr) ν_{max} 3344, 2924, 1696 1458, 1374, 1072, 974 cm^{-1}; ^1H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m/z* 430.2596 $[M + H]^+$ (calcd for $C_{25}H_{36}NO_5$, 430.2593).

Cytochalasin Z_{11} (2): colorless oil; $[\alpha]^{25}_D$ +114.8 (*c* 0.080, MeOH); UV (MeOH) λ_{max} (log ε) 222 (2.613), 258 (0.698) nm; IR (KBr) ν_{max} 3368, 2969, 1694 1454, 1015, 697 cm^{-1}; ^1H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m/z* 428.2434 $[M + H]^+$ (calcd for $C_{25}H_{34}NO_5$, 428.2434).

Cytochalasin Z_{12} (3): colorless oil: $[\alpha]^{25}_D$ +43.2 (*c* 0.080, MeOH); UV (MeOH) λ_{max} (log ε) 222 (2.052), 258 (0.555) nm; IR (KBr) ν_{max} 3368, 2965, 1688, 1447, 1082, 983, 758 cm^{-1}; ^1H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m/z* 412.2477 $[M - H_2O + H]^+$ (calcd for $C_{25}H_{34}NO_4$, 412.2488).

Cytochalasin Z_{13} (4): colorless oil; $[\alpha]^{25}_D$ +80.8 (*c* 0.160, MeOH); UV (MeOH) λ_{max} (log ε) 223 (2.879), 258 (1.097) nm; IR (KBr) ν_{max} 3323, 2965, 1712, 1454, 1348, 1155, 976, 764 cm^{-1}; ^1H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m/z* 428.2399 $[M + H]^+$ (calcd for $C_{25}H_{34}NO_5$, 428.2437).

Cytochalasin Z_{14} (5): colorless oil; $[\alpha]^{25}_D$ +8.87 (*c* 0.080, MeOH); UV (MeOH) λ_{max} (log ε) 223 (2.134), 258 (1.003) nm; IR (KBr) ν_{max} 3367, 2988, 1690 1452, 1000, 697 cm^{-1}; ^1H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m/z* 410.2337 $[M - H_2O + H]^+$ (calcd for $C_{25}H_{32}NO_4$, 410.2331).

Cytochalasin Z_{15} (6): colorless oil; $[\alpha]^{25}_D$ +98.0 (*c* 0.086, MeOH); UV (MeOH) λ_{max} (log ε) 222 (2.022), 258 (0.550) nm; IR (KBr) ν_{max} 3367, 2989, 1690 1453, 1005, 697 cm^{-1}; ^1H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m/z* 428.2451 $[M + H]^+$ (calcd for $C_{25}H_{34}NO_5$ 428.2437).

Biological Assays. Active fractions were assayed using the MTT method[15] with the mouse temperature-sensitive $p34^{cdc2}$ mutant cell line tsFT210. Cytotoxic activity was evaluated by the MTT method using P388, A-549, HL60, and BEL-7402 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). An aliquot (200 μL) of these cell suspensions at a density of 5×10^4 cell mL^{-1} was plated in 96-well microtiter plates and incubated for 24 h at the above conditions. Then 2 μL of the test compound solutions (in DMSO) at different concentrations was added to each well and further incubated for 72 h in the same conditions. MTT solution (20 μL of 5 mg/mL in IPMI-1640 medium) was added to each well and incubated for 4 h. Old medium containing MTT (150 μL) was then gently replaced by DMSO and pipetted to dissolve any formazan crystals that had formed. Absorbance was then determined on a Spectra Max Plus plate reader at 570 nm.

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