Bioactive Cytochalasins from *Aspergillus flavipes*, an Endophytic Fungus Associated with the Mangrove Plant *Acanthus ilicifolius*

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Five new cytochalasins, $Z_{16}-Z_{20}$ (**1**–**5**), and three known ones, **6**–**8**, were isolated from *Aspergillus flavipes*, an endophytic fungus associated with *Acanthus ilicifolius*. Their structures were elucidated on the basis of comprehensive spectral analysis. Cytochalasin Z_{17} (**2**) and rosellichalasin (**8**) showed cytotoxic activities against A-549 cell lines with IC_{50} values of 5.6 and 7.9 μM, respectively.

Introduction. – The cytochalasins are a group of secondary fungal metabolites that have attracted a great deal of attention for their unusual structure features and a wide range of biological activities including inhibition of HIV-1 protease 2, as well as antibiotic and antitumour activities [1]. Most recently, several new cytotoxic cytochalasins had also been isolated by our group [2][3]. In our continual effort, a fungal strain *Penicillium flavipes* from mangrove plant was proved to exhibit cytotoxic activity, its bioactive AcOEt extract was chromatographed over a silica gel column and by HPLC to give five new cytochalasins **1**–**5** and three known ones **6**–**8** (*Fig. 1*). Their structures were established by detailed analysis of NMR spectra, such as ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY spectra, and also by comparison with the NMR data of previously reported cytochalasins. Meanwhile, their cytotoxic activities against four cancer cell lines were evaluated.

Results and Discussion – *Structural Elucidations.* Cytochalasin Z_{16} (**1**) was isolated as a white powder. The molecular formula was established as $C_{28}H_{33}NO_5$ by HR-ESI-MS (*m/z* 464.2432, $[M+H]^+$; calc. for $C_{28}H_{34}NO_5^+$: 464.2437). The IR spectrum had bands due to NH and/or OH (3244 cm^{-1}), and an amide CO group (1710 cm^{-1}). The ^{1}H-NMR spectrum (*Table 1*) showed the presence of an amide NH (δ(H) 5.52), three Me *doublet*s (δ(H) 1.21, 1.30, and 1.26), a Me *singlet* (δ(H) 1.78), and ten CH and/or CH_2 groups. Five olefinic H-atoms (δ(H) 5.37, 5.46, 5.67, 5.62, and 6.42) and a phenyl ring (δ(H) 7.16–7.33) were also observed. The ^{13}C-NMR and DEPT spectra of **1** (*Table 2*) revealed the presence of four Me, two CH_2, and 16 CH groups, as well as six quaternary C-atoms, including a carbonate CO (δ(C) 149.8), an amide CO (δ(C) 170.1), and a ketone CO group (δ(C) 212.7). Interpretation of the 1D-NMR spectra led to the conclusion that, with respect to that of **6** [2], **1** had the same 10-phenyl-perhydroisoindol-1-one skeleton, except for the absence of the 6,7-epoxy group being

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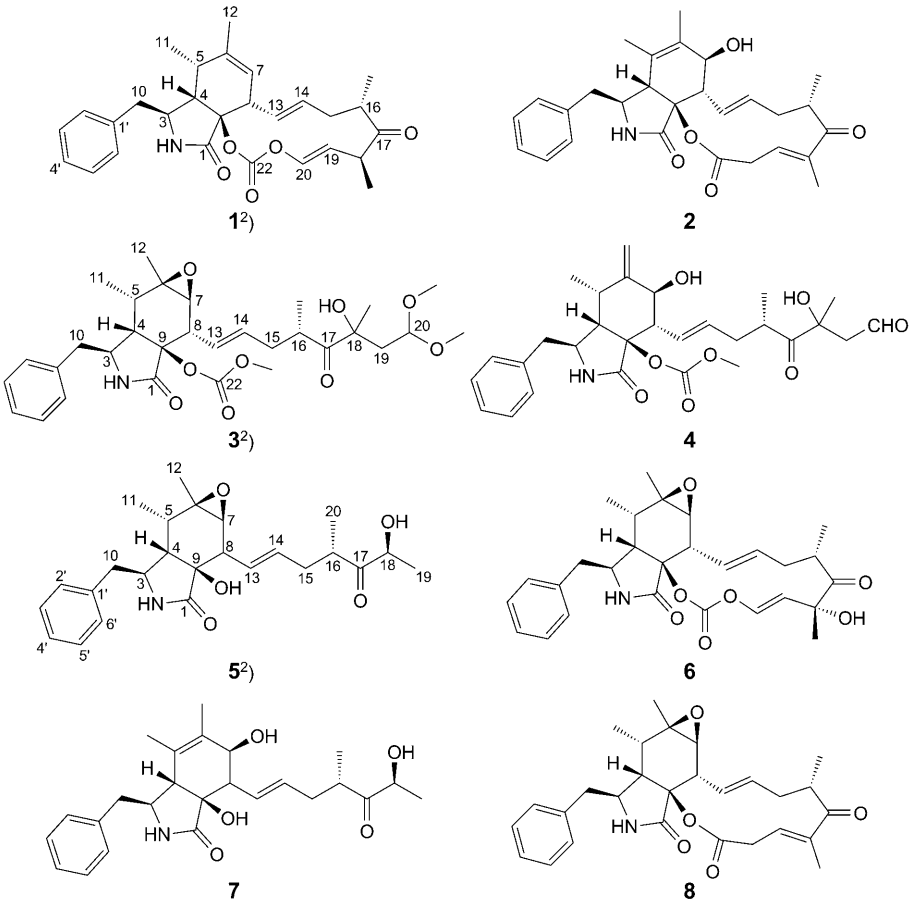

Fig. 1. *Structures of compounds* **1**–**8**

replaced by a C=C bond, which was proved by a broad typical olefinic H-atom at δ(H) 5.37, and by a C=C bond at δ(C) 122.7 and 140.8. Furthermore, the Me *singlet* (Me–C(18)) of **6** was changed to a *doublet* (δ(H) 1.21) in **1**, and the signal of the quaternary C-atom (C(18)) was shifted upfield and changed to a CH C-atom (δ(C) 40.3), which indicated that the HO–C(18)[2]) group was missing in **1**. Therefore, compound **1** was deduced to have the structure as shown in *Fig. 1*.

Cytochalasin Z_{17} (**2**) was also isolated as a white powder. The molecular formula was $C_{28}H_{33}NO_5$, as determined by HR-ESI-MS (*m*/*z* 464.2450, $[M+H]^+$; calc. for $C_{28}H_{34}NO_5^+$: 464.2437). The 1D-NMR spectra of **2** also showed the same 10-phenylperhydroisoindol-1-one skeleton, which was very similar to that of rosellichalasin (**8**) [4], with the exception that one more C=C bond (δ(C) 124.2 and 134.1) was observed. Meanwhile, the secondary OH-bearing C(7)[2]) was shifted downfield ($\Delta\delta$

[2]) Arbitrary atom numbering. For systematic names, see *Exper. Part*.

Table 1. *^{1}H-NMR Data for Compounds* **1**–**5**[2]). At 600 MHz with Me_4Si as internal standard.

Position	**1**[a])	**2**[b])	**3**[b])	**4**[a])	**5**[a])
H–N(2)	5.52 (br. *s*)	8.35 (br. *s*)	8.52 (br. *s*)	5.67 (br. *s*)	6.17 (br. *s*)
3	3.03–3.06 (*m*)	3.39 (*dd*, *J* = 9.5, 4.8)	3.66–3.68 (*m*)	3.29–3.32 (*m*)	3.52–3.56 (*m*)
4	2.90–2.93 (*m*)	3.28 (br. *s*)	2.45–2.47 (*m*)	2.71 (*dd*, *J* = 4.8, 4.8)	2.39 (*dd*, *J* = 7.2, 11.4)
5	2.67–2.72 (*m*)		1.84–1.89 (*m*)	3.10–3.12 (*m*)	2.46–2.49 (*m*)
7	5.37 (br. *s*)	3.59–3.61 (*m*)	2.67 (*d*, *J* = 5.1)	3.91 (*d*, *J* = 10.6)	2.97 (*d*, *J* = 5.4)
8	2.90–2.93 (*m*)	2.91–2.94 (*m*)	2.81 (*dd*, *J* = 8.4, 5.2)	2.87 (*dd*, *J* = 10.2, 9.1)	2.78–2.84 (*m*)
10	3.05–3.08 (*m*), 2.90–2.92 (*m*)	2.93–2.95 (*m*), 2.69 (*dd*, *J* = 12.8, 9.9)	2.87–2.90 (*m*), 2.62–2.66 (*m*)	2.90–2.96 (*m*)	3.00 (*dd*, *J* = 4.8, 16.2), 2.58 (*dd*, *J* = 11.4, 16.2)
11	1.26 (*d*, *J* = 7.3)	1.18 (*s*)	0.61 (*d*, *J* = 7.3)	1.21 (*d*, *J* = 6.7)	1.07 (*d*, *J* = 8.4)
12	1.78 (br. *s*)	1.51 (*s*)	1.14 (*s*)	5.49 (br. *s*), 5.20 (br. *s*)	1.34 (*s*)
13	5.67 (*dd*, *J* = 15.4, 9.9)	5.81 (*dd*, *J* = 15.4, 9.8)	6.04 (*dd*, *J* = 15.8, 8.4)	5.58 (*dd*, *J* = 15.0, 9.2)	5.35 (*dd*, *J* = 11.4, 18.0)
14	5.46 (*ddd*, *J* = 14.6, 9.9, 4.4)	5.23 (*ddd*, *J* = 15.0, 11.0, 3.7)	5.28–5.36 (*m*)	5.64 (*dd*, *J* = 8.1, 6.2)	5.60 (*ddd*, *J* = 18.0, 11.4, 6.0)
15	2.69–2.71 (*m*), 2.12 (*ddd*, *J* = 14.0, 9.9, 6.2)	2.19–2.21 (*m*), 1.70–1.72 (*m*)	2.33–2.38 (*m*), 1.92–1.97 (*m*)	2.38–2.42 (*m*), 2.16–2.21 (*m*)	2.28–2.34 (*m*), 2.14–2.18 (*m*)
16	2.60–2.63 (*m*)	3.45–3.48 (*m*)	3.21–3.24 (*m*)	3.35–3.39 (*m*)	2.78–2.84 (*m*)
Me–C(16)	1.30 (*d*, *J* = 7.3)	0.99 (*d*, *J* = 6.3)	0.88 (*d*, *J* = 7.0)	1.11 (*d*, *J* = 6.7)	–
17	–	–	–	–	–
18	3.36 (*dq*, *J* = 8.1, 6.6)	–	–	–	4.20 (*q*, *J* = 7.2)
Me–C(18)	1.21 (*d*, *J* = 7.0)	1.71 (*s*)	1.17 (*s*)	1.33 (*s*)	–
19	5.62 (*dd*, *J* = 12.4, 8.4)	5.48 (*dd*, *J* = 10.3, 6.3)	2.03 (*dd*, *J* = 13.9, 7.3), 1.65 (*dd*, *J* = 12.6, 3.7)	3.07 (*d*, *J* = 17.1), 2.62 (*d*, *J* = 17.1, 1.1)	1.27 (*d*, *J* = 8.4)
20	6.42 (*d*, *J* = 12.2)	3.57 (*m*), 2.97 (*m*)	4.54 (*dd*, *J* = 7.3, 3.7)	9.71 (br. *s*)	1.15 (*d*, *J* = 9.0)
2′	7.17 (*d*, *J* = 7.0)	7.16 (*d*, *J* = 7.3)	7.16 (*d*, *J* = 7.0)	7.20 (*d*, *J* = 7.0)	7.20 (*d*, *J* = 7.0)
3′	7.31 (*dd*, *J* = 7.2, 7.2)	7.33 (*dd*, *J* = 7.7, 7.3)	7.31 (*dd*, *J* = 7.7, 7.3)	7.33 (*dd*, *J* = 7.3, 7.3)	7.33 (*dd*, *J* = 7.3, 7.3)
4′	7.24 (*dd*, *J* = 7.3, 7.3)	7.23 (*dd*, *J* = 7.3, 7.3)	7.23 (*dd*, *J* = 7.3, 7.3)	7.26 (*dd*, *J* = 7.3, 7.3)	7.26 (*dd*, *J* = 7.3, 7.3)
5′	7.17 (*d*, *J* = 7.0)	7.16 (*d*, *J* = 7.3)	7.16 (*d*, *J* = 7.0)	7.20 (*d*, *J* = 7.0)	7.20 (*d*, *J* = 7.0)
6′	7.31 (*dd*, *J* = 7.2, 7.2)	7.33 (*dd*, *J* = 7.7, 7.3)	7.31 (*dd*, *J* = 7.7, 7.3)	7.33 (*dd*, *J* = 7.3, 7.3)	7.33 (*dd*, *J* = 7.3, 7.3)
2 MeO–C(20)	–	–	3.17 (*s*), 3.08 (*s*)	–	–
MeO–C(22)	–	–	3.70 (*s*)	3.80 (*s*)	–

[a]) Measured in $CDCl_3$. [b]) Measured in (D_6)DMSO.

Table 2. *^{13}C-NMR Data for Compounds* **1**–**5**[2]). At 150 MHz with Me_4Si as internal standard.

Position	**1**[a])	**2**[b])	**3**[b])	**4**[a])	**5**[a])
1	170.1 (*s*)	171.0 (*s*)	170.2 (*s*)	170.6 (*s*)	174.6 (*s*)
3	56.2 (*d*)	58.2 (*d*)	52.8 (*d*)	53.6 (*d*)	54.5 (*d*)
4	50.8 (*d*)	47.4 (*d*)	46.9 (*d*)	49.2 (*d*)	49.4 (*d*)
5	34.5 (*d*)	124.2 (*s*)	35.7 (*d*)	31.3 (*d*)	32.4 (*d*)
6	140.8 (*s*)	134.1 (*s*)	57.1 (*s*)	148.3 (*s*)	60.5 (*s*)
7	122.7 (*d*)	69.0 (*d*)	60.0 (*d*)	68.8 (*d*)	61.2 (*d*)
8	47.7 (*d*)	48.8 (*d*)	45.7 (*d*)	50.6 (*d*)	47.8 (*d*)
9	89.0 (*s*)	84.2 (*s*)	85.2 (*s*)	84.3 (*s*)	77.8 (*s*)
10	43.3 (*t*)	42.7 (*t*)	43.2 (*t*)	43.6 (*t*)	44.7 (*t*)
11	14.3 (*q*)	17.0 (*q*)	12.4 (*q*)	15.5 (*q*)	14.6 (*q*)
12	20.1 (*q*)	14.5 (*q*)	19.3 (*q*)	113.7 (*t*)	21.5 (*q*)
13	129.4 (*d*)	127.6 (*d*)	127.7 (*d*)	127.5 (*d*)	127.1 (*d*)
14	131.8 (*d*)	133.4 (*d*)	130.8 (*d*)	133.4 (*d*)	133.5 (*d*)
15	34.5 (*t*)	40.2 (*t*)	35.4 (*t*)	36.5 (*t*)	38.1 (*t*)
16	46.0 (*d*)	39.1 (*d*)	39.1 (*d*)	38.8 (*d*)	41.1 (*d*)
Me–C(16)	16.3 (*q*)	17.2 (*q*)	15.0 (*q*)	17.4 (*q*)	–
17	212.7 (*s*)	205.4 (*s*)	217.1 (*s*)	216.7 (*s*)	216.5 (*s*)
18	40.3 (*d*)	141.6 (*s*)	76.8 (*s*)	77.9 (*s*)	73.2 (*d*)
Me–C(18)	17.4 (*q*)	12.6 (*q*)	27.1 (*q*)	24.8 (*q*)	–
19	116.5 (*d*)	133.0 (*d*)	43.2 (*t*)	51.5 (*t*)	18.9 (*q*)
20	140.2 (*d*)	37.2 (*t*)	101.0 (*d*)	202.2 (*d*)	17.2 (*q*)
21	–	168.8 (*s*)	–	–	–
22	149.8 (*s*)	–	153.2 (*s*)	153.8 (*s*)	–
1′	137.8 (*s*)	137.6 (*s*)	137.0 (*s*)	137.5 (*s*)	137.3 (*s*)
2′	129.0 (*d*)	128.5 (*d*)	128.4 (*d*)	129.0 (*d*)	128.9 (*d*)
3′	129.0 (*d*)	129.4 (*d*)	129.7 (*d*)	129.0 (*d*)	129.1 (*d*)
4′	127.0 (*d*)	126.6 (*d*)	126.6 (*d*)	127.1 (*d*)	126.9 (*d*)
5′	129.0 (*d*)	128.5 (*d*)	128.4 (*d*)	129.0 (*d*)	128.9 (*d*)
6′	129.0 (*d*)	129.4 (*d*)	129.7 (*d*)	129.0 (*d*)	129.1 (*d*)
2 *Me*O–C(20)	–	–	52.3 (*q*), 52.2 (*q*)	–	–
*Me*O–C(22)	–	–	54.7 (*q*)	54.9 (*q*)	–

[a]) Measured in $CDCl_3$. [b]) Measured in (D_6)DMSO.

8.7 ppm) to 69.0 ppm. Those findings indicated a C(5)=C(6) bond, and that the 6,7-epoxy ring was replaced by a HO–C(7) moiety in **2**.

Cytochalasin Z_{18} (**3**) was obtained as a colorless oil. The molecular formula was established as $C_{31}H_{43}NO_9$ by HR-ESI-MS (*m/z* 596.2894, $[M+Na]^+$; calc. for $C_{31}H_{43}NNaO_9^+$: 596.2836). Compared to the two cytochalasins **1** and **2** described above, compound **3** contained three more MeO groups (δ(H) 3.17, δ(C) 52.3; δ(H) 3.08, δ(C) 52.2; and δ(H) 3.70, δ(C) 54.9). Others signals observed in the 1D-NMR spectra of **3** were very similar to those of previously reported cytochalasin E [2], except that the vinyl ether moiety (δ(H) 5.61, δ(C) 120.3; δ(H) 6.45, δ(C) 142.0) in cytochalasin E was changed to a CH_2 group (δ(H) 2.03 (*dd*), 1.65 (*dd*), δ(C) 43.2) and a $(MeO)_2CH$ group (δ(H) 4.54 (*dd*), δ(C) 101.0). The HMBC correlations (*Fig. 2*) from MeO–C(20)[2]) to C(20) and from MeO–C(22) to C(22) indicated that **3** was a ring-opened derivative of cytochalasin E, with a Me ester at C(22) and a $(MeO)_2$CH(20)

group. The structure of **3** was confirmed by the ^{1}H,^{1}H-COSY and additional HMBC correlations (*Fig. 2*).

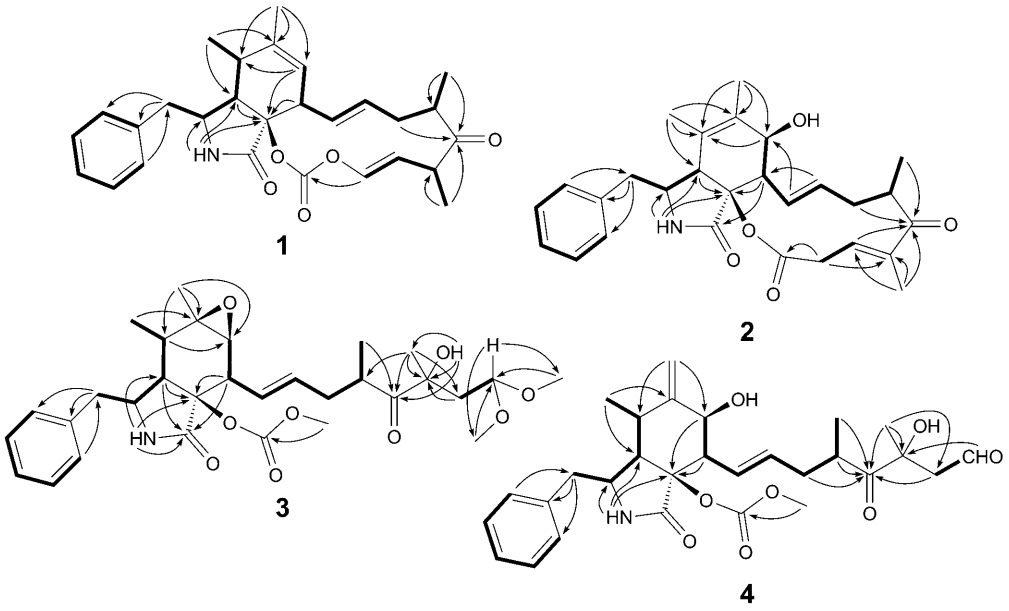

Fig. 2. *^{1}H,^{1}H-COSY and key HMBC correlations of compounds* **1**–**4**

Cytochalasin Z_{19} (**4**) was obtained as a colorless oil. The molecular formula was established as $C_{29}H_{37}NO_8$ by HR-ESI-MS (*m/z* 528.2685, $[M+H]^+$; calc. for $C_{29}H_{38}NO_8^+$: 528.2597). Inspection of the 1D-NMR spectra revealed, with respect to those of **3**, the absence of the two MeO groups of the acetal moiety, whereas a broad signal for an aldehyde group was present at δ(H) 9.71 and δ(C) 202.2. The CH_2 group (δ(H) 3.07 (*dd*), 2.62 (*dd*)) was shifted downfield by *ca.* 1.0 ppm due to the inductive effect of the aldehyde group. Furthermore, an exocyclic olefinic CH_2 group (δ(H) 5.20, 5.49 and δ(C) 148.3, 113.7) and a downfield shifted O-bearing CH group (δ(H) 3.91 and δ(C) 68.8; C(7)[2])) indicated that the epoxy group was opened, and a new exocyclic olefinic CH_2 was formed at C(6) in **4**. This was further confirmed by the similar chemical shifts of C(6), C(7), and C(12) as those recorded for the same C-atoms in cytochalasin Z_7 [2]. All these findings indicated that **4** had a structure as shown in *Fig. 1*.

Two open-chain cytochalasins were also obtained from the AcOEt extract of this strain, such as cytochalasin Z_{20} (**5**) and **7** [3]. In the HR-ESI-MS of **5**, a *pseudo*-molecular mass of 428.2480 for $[M+H]^+$ was observed, indicating the molecular formula of $C_{25}H_{33}NO_5$ equal to that of **7**. The ^{1}H- and ^{13}C-NMR signals of **5** were very similar to those of **7** [3]. However, the signals of the C(5)=C(6)[2]) bond were not observed, and the signal for C(6), which in **7** was found at δ(C) 129.4 for a quaternary olefinic C-atom, was shifted upfield in **5** to δ(C) 60.5. Moreover, a very significative upfield shift ($\Delta\delta$ 10.8) was noticed for C(7), which appeared at δ(C) 61.2. The chemical shifts of C(6) and C(7) in **5** were very similar to those recorded in **3**, which indicated

that **5** had the same epoxy ring located at C(6) and C(7). So, the structure of **5** was suggested as the 6,7-epoxy derivative of **7**.

A series of NOESY experiments on these cytochalasins, **1**–**5**, suggested the same relative configuration of the 10-phenylsubstituted perhydroisoindol-1-one skeleton as that of previous reported ones. It is noteworthy that in all cytochalasins isolated thus far the configuration of the cyclohexane and isoindole moieties are the same [3][5]. Compound **5** was tentatively regarded having the same absolute configuration about their open chains as **7**, which was further confirmed by the comparable NMR data (*Tables 1* and *2*) for their open 8-carbon chains. Differently from the previously reported cytochalasins Z_{10}–Z_{15} [2][3], compounds **3** and **4** were in a class of compounds containing an eight-membered open chain. This type of cytochalasins has been obtained by methanolysis of cytochalasin E [6]. In this article, it is the first report of eight-membered open chain cytochalasins as natural products.

Biological Screening. Compounds **1**–**5** and **8** were evaluated for their cytotoxic activities against the HL-60, A-549, BEL-7402, and P388 cell lines. Vp-16 (etoposide) was used as the positive control with IC_{50} values of 0.04, 1.03, 0.63, and 0.05 μM against HL-60, BEL-7402, A-549, and P388 cells, respectively. Compounds **1** and **4** showed moderate cytotoxic activities against A-549 cell lines with IC_{50} values of 19.5 and 17.4 μM, respectively. Compounds **2** and **8** showed more obvious cytotoxic activities against A-549 cell lines with IC_{50} values of 5.6 and 7.9 μM, respectively.

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Experimental Part

General. HPLC was performed on an *ODS* column (*YMC*-pack *ODS*-A, 10 × 250 mm, 5 μm) at a flow rate of 4 ml/min. UV Spectra: *Beckman DU-640* spectrophotometer; λ_{max} (log ε) in nm. Optical rotations: *Jasco P-1020* digital polarimeter. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *JEOL JNM-ECP600* spectrometer; chemical shifts δ in ppm rel. to Me_4Si, coupling constants *J* in Hz. ESI-MS: *Q-Tof Ultima Global GAA076-LC* mass spectrometer; in *m*/*z*.

Fungal Material. A sample of *Aspergillus flavipes* was isolated from the inner bark of *Acanthus ilicifolius* collected in Dongzhai Gang, P. R. China. The strain was identified by the China Center for Type Culture Collection (CCTCC).

Fermentation, Extraction, and Purification. The fungus was incubated at 28° under shaking conditions for 7 d in 50 500-ml conical flasks containing the liquid medium (200 ml/flask) composed of glucose (10 g/l), maltose (20 g/l), mannitol (20 g/l), monosodium glutamate (10 g/l), KH_2PO_4 (0.5 g/l), $MgSO_4 \cdot 7\ H_2O$ (0.3 g/l), corn steep liquor (1 g/l), yeast extract (3 g/l), and seawater after adjusting its pH to 7.0. The fermented whole broth (10 l) was filtered through cheesecloth to separate into supernatant and mycelia. The former was concentrated under reduced pressure to *ca.* a quarter of the original volume, and then extracted three times with AcOEt to give an AcOEt soln., while the latter was extracted three times with acetone. The acetone soln. was concentrated under reduced pressure to afford an aq. soln. The aq. soln. was extracted three times with AcOEt to give another AcOEt soln. Both AcOEt solns. were combined and concentrated under reduced pressure to give a crude extract (14.0 g).

The crude extract (14.0 g) was separated into 16 fractions (*Frs. 1–16*) on a silica gel (SiO_2) column using gradient elution of $CHCl_3$/MeOH. *Fr. 6*, eluted with $CHCl_3$/MeOH 95 : 5 (1.2 g), was purified into twelve subfractions (*Frs. 6.1–6.12*) by reversed-phase column using stable elution of H_2O/MeOH 3 : 7. *Fr. 6.5* was further purified by extensive HPLC (60% MeOH, 4.0 ml/min), yielding compounds **6** (5.0 mg) and **7** (10 mg). *Fr. 6.8* was further purified by extensive HPLC (65% MeOH, 4.0 ml/min) to

yield compounds **8** (14 mg), and **3** (10 mg). *Fr. 6.11* was further purified by HPLC (70% MeOH, 4.0 ml/min) to yield compounds **1** (14 mg) and **2** (8.0 mg). *Fr. 6.12* was further purified HPLC (80% MeOH, 1.0 ml/min) to yield compounds **4** (25 mg) and **5** (4.5 mg).

Cytochalasin Z_{16} (=*(4*E*,6*S**,8*S**,10*E*,11a*S**,14*S**,14a*S**,15*S**,17a*R**)-15-Benzyl-9,11a,14,14a,15,16-hexahydro-6,8,13,14-tetramethyl[1,3]dioxacyclotridecino[4,5-*d*]isoindole-2,7,17(6*H*,8*H*)-trione*; **1**). White powder (MeOH). $[\alpha]_D^{25} = +36.2$ ($c = 0.1$, MeOH). UV (MeOH): 223 (2.42), 247 (2.11). IR (KBr): 3244, 2937, 1710, 1444, 1294, 1276. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 464.2432 ($[M+H]^+$, $C_{28}H_{34}NO_5^+$; calc. 464.2437).

Cytochalasin Z_{17} (=*(4*E*,7*S**,9*E*,10a*S**,11*S**,13a*S**,14*S**,16a*S**)-14-Benzyl-8,10a,11,13a,14,15-hexahydro-11-hydroxy-5,7,12,13-tetramethyl-2*H*-oxacyclododecino[2,3-*d*]isoindole-2,6,16(3*H*,7*H*)-trione*; **2**). White powder (MeOH). $[\alpha]_D^{25} = +40.8$ ($c = 0.1$, MeOH). UV (MeOH): 223 (2.89), 258 (2.09). IR (KBr): 3323, 2975, 1712, 1434, 1300, 1255. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 464.2450 ($[M+H]^+$, $C_{28}H_{34}NO_5^+$; calc. 464.2437).

Cytochalasin Z_{18} (=*(1a*S**,2*S**,2a*S**,5*S**,5a*S**,6*S**,6a*R**)-5-Benzyloctahydro-2-[(1*E*,4*S*)-6-hydroxy-8,8-dimethoxy-4,6-dimethyl-5-oxooct-1-en-1-yl]-6,6a-dimethyl-3-oxo-2a*H*-oxireno[f]isoindol-2a-yl Methyl Carbonate*; **3**). Colorless oil (MeOH). $[\alpha]_D^{25} = +18.8$ ($c = 0.1$, MeOH). UV (MeOH): 223 (2.14), 244 (0.80). IR (KBr): 3398, 2988, 1691, 1442, 1200. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 596.2894 ($[M+Na]^+$, $C_{31}H_{43}NNaO_9^+$; calc. 596.2836).

Cytochalasin Z_{19} (=*(1*S**,3a*S**,4*S**,5*S**,7*S**,7a*S**)-1-Benzyloctahydro-5-hydroxy-4-[(1*E*,4*S*)-6-hydroxy-4,6-dimethyl-5,8-dioxooct-1-en-1-yl]-7-methyl-6-methylidene-3-oxo-3a*H*-isoindol-3a-yl Methyl Carbonate*; **4**). Colorless oil (MeOH). $[\alpha]_D^{25} = +28.1$ ($c = 0.1$, MeOH). UV (MeOH): 222 (2.32), 258 (0.55). IR (KBr): 3389, 2989, 1690, 1453, 1205. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 528.2685 ($[M+H]^+$, $C_{29}H_{38}NO_8^+$; calc. 528.2597).

Cytochalasin Z_{20} (=*(1a*S*,2a*S*,5*S*,5a*S*,6*S*,6a*R*)-5-Benzylhexahydro-2a-hydroxy-2-[(1*E*,4*S*,6*S*)-6-hydroxy-4-methyl-5-oxohept-1-enyl]-6,6a-dimethyl-3*H*-oxireno[f]isoindol-3-one*; **5**). Colorless oil (MeOH). $[\alpha]_D^{25} = +74.0$ ($c = 0.1$, MeOH). UV (MeOH): 225 (3.20), 258 (1.02). IR (KBr): 3344, 2924, 1697, 1458, 1384, 1075, 973. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*). HR-ESI-MS: 428.2480 ($[M+H]^+$, $C_{25}H_{34}NO_5^+$; calc. 428.2437).

Biological Assays. The cytotoxic activity was evaluated by the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method using A-549, BEL-7402, P388, and HL-60 cell lines. The cell lines were grown in *RPMI-1640* supplemented with 10% FBS (= fetal bovine serum) under a humidified atmosphere of 5% CO_2 and 95% air at 37°. 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 conditions. The test compound soln. (2 µl in DMSO) at different concentrations was added to each well and further incubated for 72 h under the same conditions. Then, 20 µl of the MTT soln. (5 mg/ml in RPMI-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.

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