Polyketides from the Ascomycete Fungus *Leptosphaeria* sp.

Jie Lin,[†,‡] Shuchun Liu,[†] Bingda Sun,[†] Shubin Niu,[†] Erwei Li,[*,†] Xingzhong Liu,[†] and Yongsheng Che[*,†]

Key Laboratory of Systematic Mycology & Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100190, People's Republic of China, and Graduate School of Chinese Academy of Sciences, Beijing 100039, People's Republic of China

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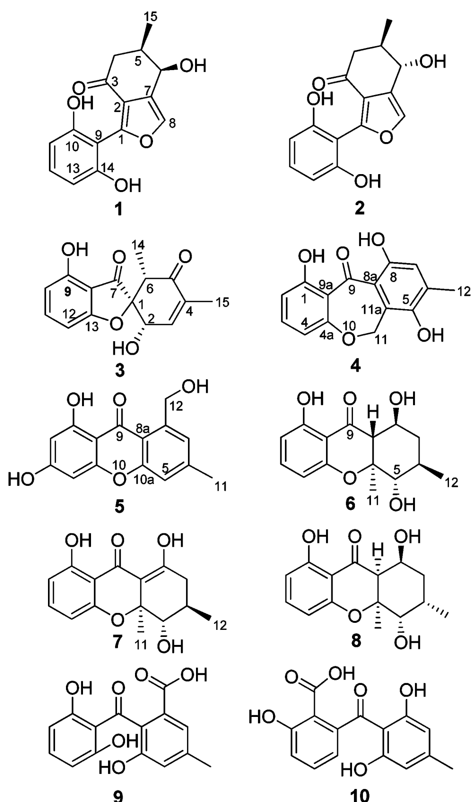
The new polyketides leptosphaerins A−G (**1**−**7**) and the known compounds monodictysin B (**8**), 2-(2,6-dihydroxybenzoyl)-3-hydroxy-5-methylbenzoic acid (**9**), and 2-(2,6-dihydroxy-4-methylbenzoyl)-6-hydroxybenzoic acid (**10**) have been isolated from solid cultures of the ascomycete fungus *Leptosphaeria* sp. The absolute configurations of compounds **1**, **2**, and **6** were assigned using the modified Mosher method, whereas that of C-2 in **3** was determined via the circular dichroism data of the [$Rh_2(OCOCF_3)_4$] complex. Compound **7** showed antifungal activity against *Aspergillus flavus*.

It is generally accepted that fungi thriving in unique and competitive environments are more likely to produce structurally diverse and biologically active secondary metabolites, largely due to the fact that their secondary metabolic capabilities have been influenced by environmental and selection pressures exerted by other organisms.[1−3] On the basis of this consideration, we initiated chemical investigations of *Cordyceps*-colonizing fungi,[4−8] the species that colonize the fruiting body of *Cordyceps sinensis*, which is the combination of the fungus and the dead caterpillars of the moth *Hepialus* spp., primarily found on the Qinghai-Tibetan plateau at altitudes above 3200 m. We also studied those fungi that were isolated from the soil samples in the vicinity of *C. sinensis*. Details of the isolation, structure elucidation, and antifungal activity of the new polyketides **1**−**7** are reported herein.



* To whom correspondence should be addressed. Tel/Fax: +86 10 82618785. E-mail: (Y.C.) cheys@im.ac.cn; (E.L.) liew@im.ac.cn.

† Institute of Microbiology.

‡ Graduate School of Chinese Academy of Sciences.

Results and Discussion

Leptosphaerin A (**1**) was assigned the molecular formula $C_{15}H_{14}O_5$ (nine degrees of unsaturation) on the basis of HRESIMS (*m/z* 297.0735 [M + Na]$^+$; Δ −0.2 mmu) and NMR data (Table 1). The ^{1}H and ^{13}C NMR spectra of **1** showed resonances for three exchangeable protons, one methyl group, one methylene, two methines including one oxymethine, 10 aromatic/olefinic carbons (four of which are oxygenated), and one α,*β*-unsaturated ketone carbon (δ_C 197.2). These data accounted for all the ^{1}H and ^{13}C NMR resonances and required **1** to be a tricyclic compound. The ^{1}H−^{1}H COSY NMR data showed the two isolated spin-systems of C-11−C-13 and C-4−C-6 (including C-15). HMBC correlations from H-5 to C-3 and C-7 and from H-6 to C-2 and C-7 defined the structure of a cyclohexanone ring, whereas those from H-8 to C-1, C-2, and C-7 and from H-6 to C-8 established a furan unit fused to the cyclohexanone ring at C-2/C-7, completing the 6,7-dihydroisobenzofuran-4(5*H*)-one partial structure. A four-bond *W*-type HMBC correlation from H-11 to C-1 revealed the connection of C-1 to C-9.[9] The chemical shifts of C-6 (δ_C 64.2), C-10 (δ_C 158.4), and C-14 (δ_C 158.4), as well as the molecular formula of **1** indicated that C-6, C-10, and C-14 all bear a hydroxy group to complete the gross structure of leptosphaerin A.

The relative configuration of **1** was assigned by analysis of its ^{1}H−^{1}H coupling constants (Table 1). The $J_{4a,5}$ value of 11.0 Hz revealed the pseudoaxial orientations of H-4a and H-5, placing H_3-15 in a pseudoequatorial orientation, whereas the near zero coupling between H-5 and H-6 suggested their *cis* relationship.[10] On the basis of these data, the relative configuration of **1** was assigned as shown.

The absolute configuration of **1** was determined using the modified Mosher method.[11,12] Treatment of **1** with (*S*)- and (*R*)-MTPA Cl afforded the major products *R*- (**1a**) and *S*-MTPA (**1b**) triesters, respectively. The difference in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for the diastereomeric esters **1b** and **1a** was calculated to assign the 6*R* absolute configuration. Therefore, the 5*R* and 6*R* configuration was proposed for **1** on the basis of the Δδ results summarized in Figure S20 (Supporting Information). The CD spectrum of **1** (Figure S15; Supporting Information) showed a positive Cotton effect at 222 (Δε +4.6) nm and a negative one at 292 (Δε −3.8) nm. Comparison of its CD spectrum with those of the known compounds *ent*-dioncophylleine A and monodictyochrome B implied that **1** could be a single diastereomer with *M*-helicity.[13,14] However, this assignment is inconclusive due to the presence of other stereogenic centers in **1**.

Leptosphaerin B (**2**) was assigned the same molecular formula $C_{15}H_{14}O_5$ as **1** by HRESIMS (*m/z* 297.0732 [M + Na]$^+$; Δ +0.1 mmu). Analysis of its ^{1}H and ^{13}C NMR data (Table 1) revealed similar structural features to those of **1**, except that the chemical

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Table 1. NMR Data (500 MHz, Acetone-d_6) of **1** and **2**

	1			**2**	
position	δ_C, mult.	δ_H (J in Hz)	HMBC[a]	δ_C, mult.	δ_H (J in Hz)
1	151.4, qC			151.5, qC	
2	129.9, qC			130.6, qC	
3	197.2, qC			195.5, qC	
4a	43.5, CH_2	2.70, dd (16.9, 11.0)	3, 5, 6, 15	46.0, CH_2	2.67, dd (17.0, 3.8)
4b		2.42, dd (16.9, 3.5)	3, 6, 15		2.42, dd (17.0, 11.0)
5	37.3, CH	2.39, m	3, 7	39.7, CH	2.26, m
6	64.2, CH	4.90, br s	2, 4, 7, 8, 15	68.4, CH	4.60, d (7.5)
7	120.2, qC			120.5, qC	
8	139.0, CH	7.67, br s	1, 2, 7	139.3, CH	7.67, br s
9	109.4, qC			107.6, qC	
10	158.4, qC			158.2, qC	
11	109.2, CH	6.54, d (8.1)	1, 9, 10, 13	108.2, CH	6.53, d (8.2)
12	132.3, CH	7.17, t (8.1)	10, 14	132.0, CH	7.16, t (8.2)
13	109.2, CH	6.54, d (8.1)	9, 11, 14	108.2, CH	6.54, d (8.2)
14	158.4, qC			158.2, qC	
15	16.2, CH_3	1.16, d (6.6)	4, 5, 6, 4b	17.5, CH_3	1.16, d (6.6)
OH-6		4.35, s			4.62, s
OH-10		8.36, s			8.36, s
OH-14		8.36, s			8.36, s

[a] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

Table 2. NMR Data (500 MHz, acetone-d_6) of **3**

position	δ_C, mult.	δ_H (J in Hz)	HMBC[a]
1	95.7, qC		
2	72.5, CH	5.01, d (2.0)	1, 3, 4, 7
3	135.8, CH	6.52, dq (2.0, 1.5)	1, 5, 15
4	142.5, qC		
5	197.0, qC		
6	47.5, CH	3.04, q (7.0)	1, 2, 5, 7, 14
7	199.2, qC		
8	111.9, qC		
9	156.5, qC		
10	108.9, CH	6.45, d (8.0)	7, 8, 9, 12
11	140.5, CH	7.50, dd (8.5, 8.0)	9, 13
12	103.7, CH	6.58, d (8.5)	8, 10, 13
13	172.8, qC		
14	8.2, CH_3	0.90, d (7.0)	1, 5, 6
15	15.5, CH_3	1.81, d (1.5)	3, 4, 5

[a] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

shifts of the C-5 (δ_H/δ_C 2.39/37.3 in **1**; 2.26/39.7 in **2**) and C-6 (δ_H/δ_C 4.90/64.2 in **1**; 4.60/68.4 in **2**) methines were different. Interpretation of its 2D NMR data established the same gross structure, implying that **2** was a stereoisomer of **1**.

The relative configuration of **2** was determined by analysis of its $^1H-^1H$ coupling constants and NOE data (Table 1). A 7.5 Hz coupling constant observed for H-6 in **2**, compared to a near zero value for the same proton in **1**, showed that **2** was different from **1** only in the configuration at C-6. The absolute configuration of **2** was deduced to be 5*R* and 6*S* by analogy to **1** and confirmed using the modified Mosher method as illustrated in Figure S20 (Supporting Information). The CD spectrum of **2** (Figure S16; Supporting Information) showed a negative Cotton effect at 224 ($\Delta\varepsilon$ −2.2) nm, a positive one at 315 ($\Delta\varepsilon$ +0.03), and a negative one at 340 ($\Delta\varepsilon$ −0.09) nm, respectively. These data implied that **2** could be a single diastereomer with *P*-helicity.[13,14] However, this assignment is inconclusive due to different chirality of C-6 in **2**.

Leptosphaerin C (**3**) gave a pseudomolecular ion $[M + Na]^+$ peak at *m/z* 297.0722 (Δ +1.1 mmu) by HRESIMS, consistent with a molecular formula of $C_{15}H_{14}O_5$ (nine degrees of unsaturation). Analysis of its ^1H and ^{13}C NMR data (Table 2) revealed two methyl groups, two methines including one oxymethine, one oxygenated sp^3 quaternary carbon (δ_C 95.7), eight aromatic/olefinic carbons (four of which are protonated), and two α,β-unsaturated ketone carbons (δ_C 197.0 and 199.2, respectively). The $^1H-^1H$ coupling patterns of the three aromatic protons revealed a 1,2,3-trisubstituted aryl ring, which was confirmed by relevant HMBC correlations.

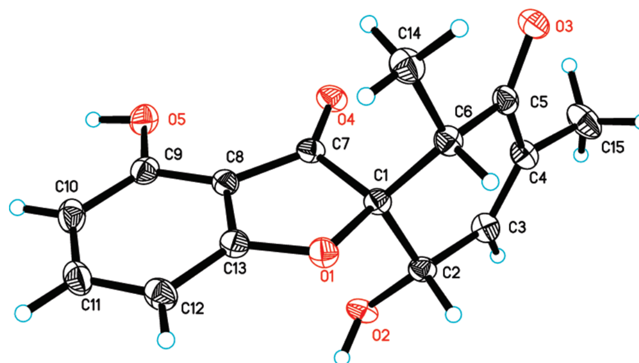


Figure 1. Thermal ellipsoid representation of **3**.

Interpretation of its $^1H-^1H$ COSY NMR data identified two proton spin-systems corresponding to the C-2−C-3 and C-6−C-14 fragments. HMBC correlations from H_3-14 to C-1 and C-5 enabled connection of C-6 to both C-1 and C-5, while those from H_3-15 to C-3, C-4, and C-5 and from H-2 and H-3 to C-1 completed assignment of the cyclohexenone moiety. Correlations from H-2 and H-6 to C-7 and a four-bond *W*-type correlation from H-10 to C-7 located the C-7 ketone carbon between C-1 and C-8. Considering the downfield chemical shift of C-1 (δ_C 95.7) and the unsaturation requirement for **3**, C-1 and C-13 were connected to the same oxygen atom to complete the bisdechlorogeodin skeleton.[15,16] The two exchangeable protons in **3** were assigned by default as the C-2 and C-9 hydroxy groups, respectively. The structure of **3** was confirmed by single-crystal X-ray diffraction analysis (Figure 1).

The absolute configuration of C-1 in **3** was assigned by application of the CD exciton chirality method. The CD spectrum of **3** (Figure S17; Supporting Information) showed a positive Cotton effect at 239 ($\Delta\varepsilon$ +61.0) nm and a negative one at 213 ($\Delta\varepsilon$ −5.2) nm arising from the exciton coupling of the α,β-unsaturated ketone and the benzofuran-3(2*H*)-one moieties. The positive chirality for **3** is opposite to that of the known compound bisdechlorogeodin,[15] suggesting 1*S* absolute configuration. As confirmation, the absolute configuration at C-2 was separately determined on the basis of the circular dichroism of an in situ formed complex with $[Rh_2(OCOCF_3)_4]$,[17] with the inherent contribution subtracted. Upon addition of $[Rh_2(OCOCF_3)_4]$ to a solution of **3** in CH_2Cl_2, a metal complex was generated as an auxiliary chromophore. It has been demonstrated that the sign of the E band (at ca. 350 nm) can be used to correlate the absolute configuration of a secondary alcohol

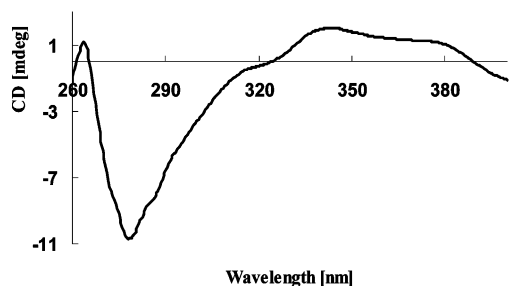


Figure 2. CD spectrum of the Rh complex of **3** with the inherent CD spectrum subtracted.

by applying the bulkiness rule.[17,18] In this case, the Rh complex of **3** displayed a positive E band (Figure 2), correlating with 2*S* absolute configuration. Combining the relative configuration established by X-ray data, the 1*S*, 2*S*, and 6*S* absolute configuration was assigned for **3**.

The elemental composition of leptosphaerin D (**4**) was established as $C_{15}H_{12}O_5$ (10 degrees of unsaturation) by HRESIMS (*m/z* 271.0616 $[M - H]^-$; Δ −0.4 mmu). The ^1H and ^{13}C NMR spectra showed resonances for three exchangeable protons including an intramolecular hydrogen-bonded one at δ_H 13.24, one methyl group, one oxygenated methylene (δ_H/δ_C 5.34/67.4), 12 aromatic carbons (four of which are protonated), and one conjugated ketone carbon (δ_C 198.5). The $^1H-^1H$ coupling patterns for H-2, H-3, and H-4 (Table 3) were indicative of a 1,2,3-trisubstituted aryl ring, as found in **1**−**3**. HMBC correlations from H_3-12 to C-5, C-6, and C-7, from H-7 to C-5 and C-8a, and from H_2-11 to C-5, C-8a, and C-11a established a pentasubstituted aryl ring, with the oxygenated C-11 methylene attached to C-11a. An HMBC cross-peak from H_2-11 to C-4a connected C-4a and C-11 to the same oxygen, whereas the four-bond *W*-type correlations from H-4 and H-7 to the C-9 ketone carbon located C-9 between C-8a and C-9a, completing the dibenzo[*b*,*e*]oxepin-11(6*H*)-one core of **4**. The exchangeable proton at δ_H 13.24 was assigned as the C-1 hydroxy group by HMBC correlations from this proton to C-1, C-2, and C-9a, the one at δ_H 10.48 was assigned as the C-8 hydroxy group on the basis of its correlations to C-7, C-8, and C-8a, and the remaining one at δ_H 7.66 was assigned as the C-5 hydroxy group by default. Therefore, the gross structure of **4** was elucidated as shown.

Leptosphaerin E (**5**) was also assigned the molecular formula $C_{15}H_{12}O_5$ (10 degrees of unsaturation) by HRESIMS (*m/z* 271.0616 $[M - H]^-$; Δ −0.4 mmu). Its UV spectrum showed absorptions at 209, 241, 309, and 351 nm, implying the presence of a xanthone moiety.[19] The $^1H-^1H$ coupling patterns for the four aromatic protons revealed two *m*-substituted aryl rings and were confirmed by relevant HMBC correlations. HMBC cross-peaks from H_3-11 to C-5, C-6, and C-7 and from H_2-12 to C-7, C-8, and C-8a established the connection of C-6 to C-11 and C-8 to C-12, respectively. Correlations from the phenolic proton (δ_H 13.02) to C-1, C-2, and C-9a indicated that C-1 bears a hydroxy group, and the downfield shift for this proton revealed formation of an intramolecular hydrogen bond with the C-9 oxygen ketone functionality, suggesting the connection of C-9 to C-9a. The chemical shifts of C-4a (δ_C 158.3), C-8a (δ_C 116.2), C-9 (δ_C 183.2), and C-9a (δ_C 104.2) indicated that C-8a was connected to C-9, and C-4a and C-8a were both attached to *O*-10 to complete the xanthone skeleton. The remaining two exchangeable protons were assigned as the C-3 and C-12 hydroxy groups, respectively. Therefore, the gross structure of **5** was established as shown.

The molecular formula of leptosphaerin F (**6**) was deduced as $C_{15}H_{18}O_5$ (seven degrees of unsaturation) by HRESIMS (*m/z* 301.1048 $[M + Na]^+$; Δ −0.2 mmu). A molecular formula search identified a tetrahydroxanthone, monodictysin B (**8**),[19] which possesses the same elemental composition as **6** and was also isolated in the current study. Although the NMR spectroscopic data of **6** (Table 3) revealed its structural similarity to **8**, those corresponding to the nonaromatic moiety of **8** were significantly different. Interpretation of the 2D NMR data of **6** established the same gross structure as **8**, indicating their isomeric relationship.

The relative configuration of **6** was assigned by analysis of its $^1H-^1H$ coupling constants and NOE data. The large coupling constants observed for H-5 (11.0 Hz) and H-8a (9.6 Hz) indicated that H-5, H-6, H-8, and H-8a are all pseudoaxially oriented with respect to the cyclohexane-1,4-diol moiety, and these assignments were confirmed by NOE correlations of H-5 with H-8a and H-6 with H-8 (Figure 3). Upon irradiation of H_3-11 in the NOE experiment, enhancements were observed for H-6 and H-8, suggesting the *trans* configuration of H_3-11 and H-8a. These data permitted assignment of the relative configuration for **6**.

The absolute configuration of **6** was determined using the modified Mosher method as illustrated in Figure S21 (Supporting Information). Although **6** has two secondary hydroxy groups (OH-5 and OH-8), the steric hindrance from H_3-11 and H_3-12 resulted in selective derivatization of OH-8. Treatment of **6** with (*S*)-MTPA Cl afforded the *R*-MTPA monoester **6a**, as evidenced by its ^1H NMR spectrum (Figure S22; Supporting Information), whereas derivatization of **6** with (*R*)-MTPA Cl afforded a mixture of the *S*-MTPA monoester **6b** and the diester **6c** in a 2:1 ratio (Figures S23 and S24; Supporting Information), which was purified by RP HPLC to afford the monoester **6b**. The difference in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for **6b** and **6a** was calculated to assign the 8*S* absolute configuration. In addition, the CD spectrum of **6** showed a negative Cotton effect at 307 ($\Delta\varepsilon$ −1.9) and a positive Cotton effect at 366 ($\Delta\varepsilon$ +0.3) nm, respectively, similar to that of (−)-rotoic acid,[20] suggesting the 8a*R* absolute configuration, consistent with that assigned using the modified Mosher method. Considering the relative configuration determined by $^1H-^1H$ coupling constants and NOE data, **6** was assigned the 5*S*, 6*R*, 8*S*, 8a*R*, and 10a*S* absolute configuration.

The molecular formula of leptosphaerin G (**7**) was established as $C_{15}H_{16}O_5$ (eight degrees of unsaturation) by HRESIMS (*m/z* 299.0891 $[M + Na]^+$; Δ −0.1 mmu), two mass units less than that of **6**. Analysis of its ^1H and ^{13}C NMR spectroscopic data (Table 3) revealed similar structural features to **6**, except that the signals of the C-8−C-8a fragment were different. A molecular formula search identified 10-methyl-10-demethoxycarbonylhemisecalonic acid A, a synthetic monomer for the dimeric natural product secalonic acid A,[21] possessing the same gross structure as **7**. Analysis of the $^1H-^1H$ coupling constants and NOESY data of **7** revealed the same relative configuration as the synthetic intermediate. The absolute configuration of C-10a was assigned by analysis of its CD data. The CD spectrum of **7** showed a negative Cotton effect at 337 ($\Delta\varepsilon$ −5.7) nm, similar to that of secalonic acid A,[22] suggesting a 10a*S* configuration. Therefore, **7** was assigned the 5*S*, 6*R*, and 10a*S* absolute configuration, which is the same as 10-methyl-10-demethoxycarbonylhemisecalonic acid A. However, this is the first demonstration of the natural occurrence of **7**.

The known compounds **8**−**10** isolated from the crude extract were identified as monodictysin B, 2-(2,6-dihydroxybenzoyl)-3-hydroxy-5-methylbenzoic acid, and 2-(2,6-dihydroxy-4-methylbenzoyl)-6-hydroxybenzoic acid, respectively, by comparison of their NMR and MS data with those reported.[19,23]

Compounds **1**−**7** were tested for antifungal activity (Table 4). Compound **4** showed antifungal effects against the plant pathogens *Fusarium nivale* (CGMCC 3.4600) and *Piricularia oryzae* (CGMCC 3.3283), with IC_{50} values of 12.5 and 18.1 μM, respectively, with the positive control carbendazim showing IC_{50} values of 2.6 and 5.0 μM, respectively. Compound **7** displayed antifungal activity against *Aspergillus flavus* (CGMCC 3.0951), with an IC_{50} value 14.8 μM, while the positive control amphotericin B showed an IC_{50} value of 3.3 μM.

Table 3. NMR Data (500 MHz, acetone-d_6) of **4**−**7**

	4		**5**		**6**		**7**	
position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	166.5, qC		164.8, qC		161.6, qC		163.1, qC	
2	112.4, CH	6.62, d (8.0)	99.0, CH	6.23, d (2.0)	108.0, CH	6.41, d (8.5)	110.0, CH	6.44, d (7.5)
3	138.6, CH	7.52, t (8.0)	166.4, qC		138.9, CH	7.41, t (8.5)	138.8, CH	7.38, t (7.5)
4	110.4, CH	6.54, d (8.0)	94.3, CH	6.38, d (2.0)	108.0, CH	6.39, d (8.5)	109.0. CH	6.36, d (7.5)
4a	163.4, qC		158.3, qC		159.8, qC		160.0, qC	
5	144.8, qC		117.1, CH	7.25, br s	79.6, CH	3.57, d (11.0)	77.2, CH	3.78, d (10.0)
6	137.8, qC		147.3, qC		32.6, CH	1.62, m	31.3, CH	2.00, m
7a	120.6, CH	6.84, s	124.8, CH	7.49, br s	40.0, CH_2	1.92, dt (12.9, 4.5)	37.1, CH_2	2.61, dd (19.4, 5.2)
7b						1.23, dd (12.9, 13)		2.31, dd (19.4, 10.0)
8	156.0, qC		146.0, qC		66.0, CH	4.07, m	176.1, qC	
8a	121.7, qC		116.2, qC		57.2, CH	2.94, d (9.6)	107.7. qC	
9	198.5, qC		183.2, qC		202.1, qC		189.4, qC	
9a	113.7, qC		104.2, qC		108.4, qC		107.7, qC	
10a			158.3, qC		84.1, qC		82.5, qC	
11	67.4, CH_2	5.34, s	21.9, CH_3	2.49, s	18.3, CH_3	1.25, s	20.5, CH_3	1.44, s
11a	124.7, qC							
12	17.4, CH_3	2.34, s	64.1, CH_2	5.14, d (2.0)	12.0, CH_3	1.06, d (6.5)	18.3, CH_3	1.16, d (6.5)
OH-1		13.24, s		13.02, s				13.82, s
OH-5		7.66, br s				4.64, br s		
OH-8		10.48, s				4.06, br s		11.33, s

Table 4. Antifungal Activities of **1**−**7**

	IC$_{50}$ (μM)		
compound	*F. nivale*	*P. oryzae*	*A. flavus*
1	>150	>150	>150
2	>150	>150	>150
3	144.0 ± 0.7	80.3 ± 0.7	81.4 ± 4.4
4	12.5 ± 1.1	18.1 ± 3.6	>150
5	>150	>150	>150
6	>150	>150	>150
7	>150	>150	14.8 ± 1.9
carbendazim	2.6 ± 0.2	5.0 ± 0.1	
amphotericin B			3.3 ± 0.6

Natural products incorporating the dihydroisobenzofuranone moiety are relatively rare.[10,24,25] Compounds **1** and **2** are structurally related to the known fungal metabolite massarinin B,[10] but differ in having a resorcinol moiety connected to the furan ring rather than a 2,2-dimethylchromene unit. Compound **3** shares the same spiral skeleton as bisdechlorogeodin,[15,16] but differs in having a trisubstituted cyclohex-2-enone joined spirally to the benzofuran-3(2*H*)-one moiety, instead of a disubstituted cyclohexa-2,5-dienone unit. Compound **4** is an analogue of arugosin F with a hydroxy group attached to C-5 rather than to C-11 in the latter.[26] Compound **5** differs from the known 1-hydroxy-6-methyl-8-hydroxymethylxanthone by having one more hydroxy group at C-3,[25] whereas **6** is a C-6 and C-8a stereoisomer of monodictysin B (**8**).[19] Biosynthetic studies of some known analogues of **1**−**7** demonstrated that they originated from the cleavage of an anthraquinone/anthrone precursor.[27,28] The biosyntheses of **1**−**7** could proceed similarly as proposed in Scheme 1.

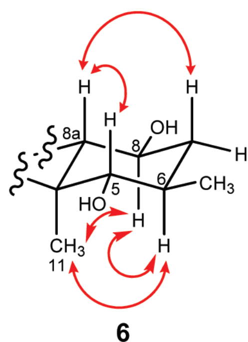
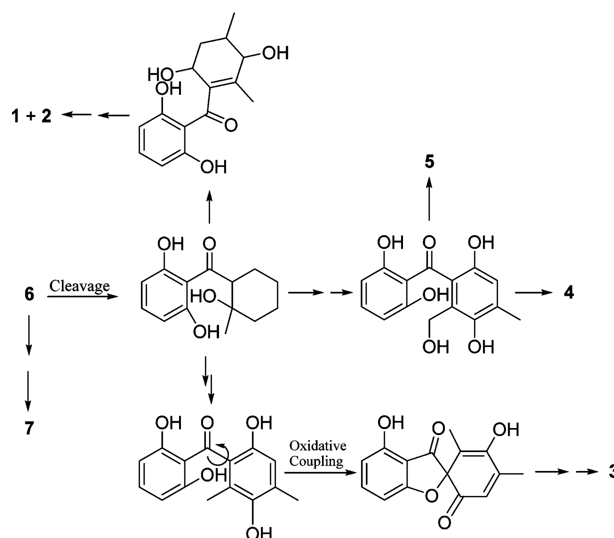


Figure 3. Key NOE correlations for **6**.

Scheme 1. Plausible Biosyntheses of **1**−**7**



Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. CD spectra were recorded on a JASCO J-815 spectropolarimeter using MeOH as solvent. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^{1}H and ^{13}C NMR data were acquired with Varian Mercury 500 and 600 spectrometers using solvent signals (acetone-d_6; δ_H 2.05/δ_C 29.8, 206.1) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer, and HRESIMS data were obtained using Bruker APEX III 7.0T and APEXII FT-ICR spectrometers, respectively.

Fungal Material. The ascomycete fungus *Leptosphaeria* sp. (XZC04-CS-304) was isolated by Dr. Mu Wang from the soil sample on the surface of the fruiting body of *C. sinensis* collected in Linzhi, Tibet, People's Republic of China, in May 2004. A fresh sample of *C. sinensis* was collected, together with the soil partially covered on the surface of its fruiting body, and sealed in a plastic bag. The strain was isolated from the soil suspension in distilled water by the spread-plate technique on a PDA plate with streptomycin. The isolate was identified by one of the authors (B.S.) and assigned the accession number XZC04-CS-304 in X.L.'s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of PDA at 25 °C for 10 days. Agar plugs were used to inoculate Erlenmeyer flasks (250 mL), each containing 50 mL of media (0.4%

glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 rpm for five days. Fermentation was carried out in eight Fernbach flasks (500 mL) each containing 75 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1 × 10^6/mL. Distilled H_2O (100 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days. Its organic solvent extract showed antifungal effects against *Aspergillus flavus* (CGMCC 3.0951) and the plant pathogens *Fusarium nivale* (CGMCC 3.3283) and *Piricularia oryzae* (CGMCC 3.4600).

Extraction and Isolation. The fermented material was extracted repeatedly with EtOAc (4 × 500 mL), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (5.6 g), which was fractionated by silica gel VLC using petroleum ether−EtOAc gradient elution. Fractionation of the extract led to the isolation of seven new polyketides named leptosphaerins A−G (**1**−**7**), along with three known ones, monodictysin B (**8**),[19] 2-(2,6-dihydroxybenzoyl)-3-hydroxy-5-methylbenzoic acid (**9**),[19] and 2-(2,6-dihydroxy-4-methylbenzoyl)-6-hydroxybenzoic acid (**10**).[23] The fraction (119 mg) eluted with 45% EtOAc was separated by Sephadex LH-20 CC eluting with 1:1 CH_2Cl_2−MeOH. The resulting subfractions were combined and further purified by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 22% MeOH in H_2O for 2 min, from 22 to 24% in 30 min; 2 mL/min) to afford **1** (10.7 mg, t_R 17.9 min) and **2** (40.0 mg, t_R 17.5 min). The fraction (90 mg) eluted with 25% EtOAc was purified by RP HPLC (30% MeOH in H_2O for 5 min, from 45 to 80% in 25 min) to afford **3** (2.1 mg, t_R 15.2 min), **6** (28.1 mg, t_R 21.1 min), **7** (2.4 mg, t_R 17.2 min), and **8** (5.1 mg, t_R 20.1 min). The fraction (90 mg) eluted with 50% EtOAc was also separated by RP HPLC (60% MeOH in H_2O for 5 min, from 80 to 90% in 25 min) to afford **4** (28.1 mg, t_R 22.2 min), **5** (3.1 mg, t_R 23.5 min), **9** (3.2 mg, t_R 16.2 min), and **10** (3.0 mg, t_R 17.2 min).

Leptosphaerin A (1): pale yellow solid; $[\alpha]^{25}_D$ −4.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.73), 225 (4.74), 272 (4.57), 318 (4.37), 334 (4.50) nm; CD (*c* 1.0 × 10^{-3} M, MeOH) λ_{max} (Δε) 222 (+4.6), 292 (−3.8); IR (neat) ν_{max} 3283, 2963, 1673, 1592, 1461, 1398, 1308, 1112, 1075 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data see Table 1; NOESY correlations (acetone-d_6, 500 MHz) H-4a ↔ H_3-15; H-4b ↔ H_3-15; H-6 ↔ H_3-15; H_3-15 ↔ H-4a, H-4b, H-6; HRESIMS *m/z* 297.0735 (calcd for $C_{15}H_{14}O_5Na$, 297.0733).

Preparation of (*R*)- (1a) and (*S*)-MTPA (1b) Esters. A sample of **1** (1.0 mg, 0.004 mmol), (*S*)-MTPA Cl (2.0 μL, 0.011 mmol), and pyridine-d_5 (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 24 h. The mixture was evaporated to dryness and purified by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; from 70 to 100% MeOH in 30 min, followed by 100% MeOH for 10 min) to afford the triester **1a** (1.0 mg, t_R 32.3 min): colorless oil; ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.79 (1H, t, *J* = 7.0 Hz, H-12), 7.72 (1H, br s, H-8), 7.42 (1H, d, *J* = 7.0 Hz, H-11), 7.38 (1H, d, *J* = 7.0 Hz, H-13), 5.97 (1H, d, *J* = 3.0 Hz, H-6), 2.33 (1H, dd, *J* = 15.0, 10.0 Hz, H-4a), 2.22 (1H, dd, *J* = 15.0, 3.0 Hz, H-4b), 2.14 (1H, m, H-5), 1.08 (3H, d, *J* = 5.5 Hz, H_3-15).

Another sample of **1** (1.0 mg, 0.004 mmol), (*R*)-MTPA Cl (2.0 μL, 0.011 mmol), and pyridine-d_5 (0.5 mL) were processed as described above for **1a** to afford the triester **1b** (1.1 mg): colorless oil; ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.78 (1H, t, *J* = 7.0 Hz, H-12), 7.72 (1H, br s, H-8), 7.42 (1H, d, *J* = 7.0 Hz, H-11), 7.38 (1H, d, *J* = 7.0 Hz, H-13), 5.97 (1H, d, *J* = 3.0 Hz, H-6), 2.34 (1H, dd, *J* = 15.3, 9.5 Hz, H-4a), 2.23 (1H, dd, *J* = 15.3, 3.3 Hz, H-4b), 2.16 (1H, m, H-5), 1.08 (3H, d, *J* = 5.5 Hz, H_3-15).

Leptosphaerin B (2): pale yellow solid; $[\alpha]^{25}_D$ −31.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.72), 224 (4.73), 272 (4.57), 319 (4.40), 334 (4.53) nm; CD (*c* 1.0 × 10^{-3} M, MeOH) λ_{max} (Δε) 224 (−2.2), 315 (+0.03), 340 (−0.09); IR (neat) ν_{max} 3268, 2963, 1675, 1593, 1462, 1399, 1309, 1112, 1075 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; NOE data (acetone-d_6, 500 MHz) H-4a ↔ H_3-15; H-4b ↔ H-6, H_3-15; H-6 ↔ H-4b, H_3-15; H_3-15 ↔ H-4a, H-4b, H-6; HRESIMS *m/z* 297.0732 (calcd for $C_{15}H_{14}O_5Na$, 297.0733).

Preparation of (*R*)- (2a) and (*S*)-MTPA (2b) Esters. A sample of **2** (1.0 mg, 0.004 mmol), (*S*)-MTPA Cl (2.0 μL, 0.011 mmol), and pyridine-d_5 (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 24 h. The mixture was evaporated to dryness and purified by RP HPLC using the gradient as in the purification of **1a** to afford the triester **2a** (0.7 mg, t_R 32.0 min): colorless oil; ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.78 (1H, t, *J* = 7.0 Hz, H-12), 7.30 (1H, s, H-8), 7.66 (1H, m, H-11), 7.64 (1H, m, H-13), 5.96 (1H, d, *J* = 5.5 Hz, H-6), 2.49 (1H, dd, *J* = 15.0, 3.9 Hz, H-4a), 2.44 (1H, m, H-5), 2.17 (1H, dd, *J* = 15.0, 9.0 Hz, H-4b), 1.02 (3H, d, *J* = 5.6 Hz, H_3-15).

Another sample of **2** (1.0 mg, 0.004 mmol), (*R*)-MTPA Cl (2.0 μL, 0.011 mmol), and pyridine-d_5 (0.5 mL) were processed as described above for **2a** to afford the triester **2b**: colorless oil (1.0 mg); ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.79 (1H, t, *J* = 8.0 Hz, H-12), 7.67 (1H, s, H-8), 7.61 (1H, m, H-11), 7.60 (1H, m, H-13), 5.96 (1H, s, H-6), 2.40 (1H, dd, *J* = 13.5, 3.3 Hz, H-4a), 2.36 (1H, m, H-5), 2.27 (1H, dd, *J* = 13.5, 8.7 Hz, H-4b), 0.92 (3H, d, *J* = 7.0 Hz, H_3-15).

Leptosphaerin C (3): yellow needles (acetone−H_2O); mp 162−165 °C; $[\alpha]^{25}_D$ +81.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 223 (5.67), 271 (5.60), 344 (5.17) nm; CD (*c* 7.4 × 10^{-3} M, MeOH) λ_{max} (Δε) 213 (−5.2), 239 (+61.0), 259 (−5.2), 323 (+54.2); IR (neat) ν_{max} 3386, 3185, 2976, 1702, 1619, 1459, 1335, 1030 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data see Table 2; HREIMS *m/z* 297.0722 (calcd for $C_{15}H_{18}O_5Na$ 297.0733).

X-ray Crystallographic Analysis of Leptosphaerin C (3).[29] Upon crystallization from acetone−H_2O (10:1) using the vapor diffusion method, colorless crystals were obtained for **3**, a crystal (0.27 × 0.13 × 0.11 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a Rigaku RAPID IP diffractometer with graphite-monochromated Mo Kα radiation, λ = 0.71073 Å at 173(2) K. Crystal data: $C_{15}H_{14}O_5$, *M* = 274.26, space group orthorhombic, *P*2(1)2(1)2(1); unit cell dimensions *a* = 5.7411(11) Å, *b* = 9.4947(19) Å, *c* = 24.665(5) Å, *V* = 1344.5(5) Å^3, *Z* = 4, D_{calcd} = 1.355 mg/m^3, μ = 0.102 mm^{-1}, *F*(000) = 576. The structure was solved by direct methods using SHELXL-97[30] and refined by using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were performed using the Siemens Area Detector Absorption Program (SADABS).[31] The 15 405 measurements yielded 1809 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0441 and wR_2 = 0.1084 [$I > 2\sigma(I)$].

C-2 Absolute Configuration of 3.[17,18] According to a published procedure,[17] a sample of **3** (0.5 mg) was dissolved in a dry solution of the stock $[Rh_2(OCOCF_3)_4]$ complex (1.5 mg) in CH_2Cl_2 (200 μL) and was subjected to CD measurements at a concentration of 2.5 mg/mL. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed sign of the E band at ca. 350 nm in the induced CD spectrum was correlated to the absolute configuration of the C-2 secondary alcohol moiety.

Leptosphaerin D (4): yellow powder; UV (MeOH) λ_{max} (log ε) 233 (3.86), 282 (3.92), 355 (3.67) nm; IR (neat) ν_{max} 3415, 1627, 1656, 1462, 1375, 1324, 1218, 1057 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 3; HMBC data (acetone-d_6, 500 MHz) H-2 → C-1, 4, 9a; H-3 → C-1, 4a; H-4 → C-2, 4a, 9, 9a; H-7 → C-5, 8, 8a, 9; H_2-11 → C-4a, 5, 8a, 11a; H_3-12 → C-5, 6, 7; OH-1 → C-1, 2, 3, 9, 9a; OH-8 → C-7, 8, 8a; HRESIMS *m/z* 271.0616 (calcd for $C_{15}H_{11}O_5$, 271.0612).

Leptosphaerin E (5): white powder; UV (MeOH) λ_{max} (log ε) 209 (4.02), 241 (4.16), 309 (3.90), 351 (3.44) nm; IR (neat) ν_{max} 3441, 3212, 2943, 2887, 1619, 1498, 1354, 1195, 1008 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 3; HMBC data (acetone-d_6, 500 MHz) H-2 → C-1, 3, 4, 9a; H-4 → C-2, 3, 4a, 9a; H-5 → C-7, 8a, 10a, 11; H-7 → C-5, 8a, 11, 12; H_3-11 → C-5, 6, 7; H_2-12 → C-7, 8, 8a; OH-1 → C-1, 2, 9a; HRESIMS *m/z* 271.0616 (calcd for $C_{15}H_{11}O_5$, 271.0612).

Leptosphaerin F (6): colorless solid; $[\alpha]^{25}_D$ +44.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.93), 277 (3.78), 353 (3.28) nm; CD (*c* 1.0 × 10^{-4} M, MeOH) λ_{max} (Δε) 222 (+4.9), 307 (−1.9), 366 (+0.3) nm; IR (neat) ν_{max} 3460, 2975, 2920, 1614, 1460, 1358, 1229, 1044 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 3; NOED data (acetone-d_6, 500 MHz) H-5 ↔ H-7b, H-8a, H_3-12; H-6 ↔ H-8, H_3-11; H-7b ↔ H-5, H-8a; H-8 ↔ H-6, H_3-11; H-8a ↔ H-5, H-7b; H_3-11 ↔ H-6, H-8; H_3-12 ↔ H-5; HRESIMS *m/z* 301.1048 (calcd for $C_{15}H_{16}O_4Na$, 301.1046).

Preparation of (*R*)- (6a) and (*S*)-MTPA (6b) Esters. A sample of **6** (1.0 mg, 0.004 mmol), (*S*)-MTPA Cl (2.0 μL, 0.011 mmol), and pyridine-d_5 (0.5 mL) were allowed to react in an NMR tube at ambient

temperature for 24 h. The mixture was evaporated to dryness and purified by RP HPLC (from 70 to 100% MeOH in 30 min, followed by 100% MeOH for 10 min) to afford the monoester **6a** (1.0 mg, t_R 29.4 min): colorless oil; ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.90 (1H, s, OH-1), 7.42 (1H, t, $J = 8.0$ Hz, H-3), 6.44 (1H, d, $J = 8.0$ Hz, H-2), 6.41 (1H, d, $J = 8.0$ Hz, H-4), 5.52 (1H, m, H-8), 4.71 (1H, d, $J = 4.4$, OH-5), 3.77 (1H, dd, $J = 4.4$, 11.0 Hz, H-5), 3.47 (1H, d, $J = 11.0$ Hz, H-8a), 2.25 (1H, dt, $J = 13.0$, 4.5 Hz, H-7a), 1.83 (1H, m, H-6), 1.57 (1H, dd, $J = 13.0$ Hz, H-7b), 1.37 (3H, s, H_3-11), 1.17 (3H, d, $J = 6.5$ Hz, H_3-12).

Another sample of **6** (1.0 mg, 0.004 mmol), (*R*)-MTPA Cl (2.0 μL, 0.011 mmol), and pyridine-d_5 (0.5 mL) were processed as described above for **6a** to afford a mixture of the *S*-MTPA monoester **6b** and the diester **6c** in a 2:1 ratio (Figures S23 and S24; Supporting Information), which was purified by RP HPLC to afford the monoester **6b** (1.0 mg): colorless oil; ^{1}H NMR (acetone-d_6, 500 MHz) δ 11.06 (1H, s, OH-1), 7.45 (1H, t, $J = 8.0$ Hz, H-3), 6.48 (1H, d, $J = 8.0$ Hz, H-2), 6.43 (1H, d, $J = 8.0$ Hz, H-4), 5.25 (1H, m, H-8), 4.69 (1H, d, $J = 4.4$, OH-5), 3.73 (1H, dd, $J = 4.4$, 11.0 Hz, H-5), 3.50 (1H, d, $J = 11.0$ Hz, H-8a), 2.19 (1H, dt, $J = 13.0$, 4.5 Hz, H-7a), 1.80 (1H, m, H-6), 1.38 (3H, s, H_3-11), 1.27 (1H, dd, $J = 13.0$ Hz, H-7b), 1.11 (3H, d, $J = 6.5$ Hz, H_3-12).

Leptosphaerin G (7): colorless solid; $[\alpha]^{25}_D$ −17.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.60), 279 (3.25), 313 (3.51), 346 (3.46) nm; CD (*c* 1.0×10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 220 ($\Delta\varepsilon$ +25.4) nm, 337 ($\Delta\varepsilon$ −5.7); IR (neat) ν_{max} 3509, 2930, 1615, 1584, 1461, 1325, 1226, 1048 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 3; NOESY correlations (acetone-d_6, 500 MHz) H-5 ↔ H-7b, H_3-12; H-6 ↔ H_3-11; H-7b ↔ H-5; H_3-11 ↔ H-6; H_3-12 ↔ H-5; HRESIMS *m/z* 299.0891 (calcd for $C_{15}H_{16}O_4Na$, 299.0890).

Monodictysin B (8): ^{1}H NMR, ^{13}C NMR, and the MS data were consistent with literature values.[19]

2-(2,6-Dihydroxybenzoyl)-3-hydroxy-5-methylbenzoic acid (9): ^{1}H NMR, ^{13}C NMR, and the MS data were consistent with literature values.[19]

2-(2,6-Dihydroxy-4-methylbenzoyl)-6-hydroxybenzoic acid (10): ^{1}H NMR, ^{13}C NMR, and the MS data were consistent with literature values.[23]

Antifungal Assays. Antifungal assays were conducted in triplicate following the National Center for Clinical Laboratory Standards (NCCLS) recommendations.[32] The fungi *Aspergillus flavus* (CGMCC 3.0951), *Piricularia oryzae* (CGMCC 3.3283), and *Fusarium nivale* (CGMCC 3.4600) were obtained from China General Microbial Culture Collection (CGMCC) and were grown on PDA. Targeted fungi (3−4 colonies) were prepared from broth culture (*A. flavus*: 28 °C for 36 h; plant pathogens: 28 °C for 48 h), and the final suspensions contained 10^4 hyphae/mL (in PDB medium). Test samples (4 mg/mL as stock solution in DMSO and serial dilutions) were transferred to a 96-well clear plate in triplicate, and the suspensions of the test organisms were added to each well, achieving a final volume of 200 μL. Alamar blue (10 μL of 10% solution) was added to each well as an indicator, and amphotericin B and carbendazim were used as the positive controls. After incubation (*A. flavus*: 28 °C for 36 h; plant pathogens: 28 °C for 48 h), the fluorescence intensity was measured at Ex/Em = 544/590 nm. The inhibition was calculated and plotted versus test concentrations to afford the IC_{50}.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1**−**7** and CD spectra of **1**−**3**, **6**, and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Gloer, J. B. *Acc. Chem. Res.* **1995**, *28*, 343–350.
(2) Gloer, J. B. Applications of Fungal Ecology in the Search of New Bioactive Natural Products. In *The Mycota IV: Environmental and Microbial Relationships*; Wicklow, D. T., Soderstrom, B., Eds.; Springer-Verlag: Berlin, 1997; pp 249−268.
(3) Meinwald, J.; Eisner, T. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 4539–4540.
(4) Guo, H.; Hu, H.; Liu, S.; Liu, X.; Zhou, Y.; Che, Y. *J. Nat. Prod.* **2007**, *70*, 1519–1521.
(5) Zhang, Y.; Liu, S.; Liu, X.; Che, Y. *J. Nat. Prod.* **2007**, *70*, 1522–1525.
(6) Chen, Y.; Guo, H.; Du, Z.; Liu, X.; Ye, X.; Che, Y. *Cell Prolif.* **2009**, *42*, 838–847.
(7) Zhang, Y.; Liu, S.; Liu, H.; Liu, X.; Che, Y. *J. Nat. Prod.* **2009**, *72*, 1364–1367.
(8) Guo, H.; Sun, B.; Gao, H.; Niu, S.; Liu, X.; Yao, X.; Che, Y. *Eur. J. Org. Chem.* **2009**, 5525–5530.
(9) Huang, X.; He, J.; Niu, X.; Menzel, K.; Dahse, H.; Grabley, S.; Fiedler, H.; Sattler, I.; Hertweck, C. *Angew. Chem., Int. Ed.* **2008**, *47*, 3995–3998.
(10) Oh, H.; Swenson, D. C.; Gloer, J. B.; Carol, A.; Shearer, C. A. *J. Nat. Prod.* **2003**, *66*, 73–79.
(11) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.
(12) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
(13) Bringmann, G.; Dreyer, M.; Kopff, H.; Rischer, H.; Wohlfarth, M.; Hadi, H. A.; Brun, R.; Meimberg, H.; Heubl, G. *J. Nat. Prod.* **2005**, *68*, 686–690.
(14) Pontius, A.; Krick, A.; Mesry, R.; Kehraus, S.; Foegen, S. E.; Müller, M.; Klimo, K.; Gerhäuser, C.; König, G. M. *J. Nat. Prod.* **2008**, *71*, 1793–1799.
(15) Tanaka, Y.; Matsuzaki, K.; Zhong, C. L.; Yoshida, H.; Kawakubo, T.; Masuma, R.; Tanaka, H.; Omura, S. *J. Antibiot.* **1996**, *49*, 1056–1059.
(16) Li, Y.; Sun, B.; Liu, S.; Jiang, L.; Liu, X.; Zhang, H.; Che, Y. *J. Nat. Prod.* **2008**, *71*, 1643–1646.
(17) Frelek, J.; Szczepek, W. J. *Tetrahedron: Asymmetry* **1999**, *10*, 1507–1520.
(18) Gerards, M.; Snatzke, G. *Tetrahedron: Asymmetry* **1990**, *1*, 221–236.
(19) Krick, A.; Kehraus, S.; Gerhäuser, C.; Klimo, K.; Nieger, M.; Maier, A.; Fiebig, H. H.; Atodiresei, I.; Raabe, G.; Fleischhauer, J.; König, G. M. *J. Nat. Prod.* **2007**, *70*, 353–360.
(20) Lawson, M. A.; Kaouadji, M.; Allais, D. P.; Champavier, Y.; Chulia, A. J. *Tetrahedron Lett.* **2006**, *47*, 451–454.
(21) Franck, B.; Stöckigt, J.; Zeidler, U.; Franckowiak, G. *Chem. Ber.* **1973**, *106*, 1198–1220.
(22) Kawahara, N.; Sekita, S.; Satake, M.; Udagawa, S.; Kawai, K. *Chem. Pharm. Bull.* **1994**, *42*, 1720–1723.
(23) Fujimoto, H.; Satoh, Y.; Yamaguchi, K.; Yamazaki, M. *Chem. Pharm. Bull.* **1998**, *46*, 1506–1510.
(24) Kawahara, N.; Nozawa, K.; Nakajima, S.; Kawai, K. *J. Chem. Soc., Perkin Trans. 1* **1988**, 907–911.
(25) Ballantine, J. A.; Ferrito, V.; Hassall, C. H.; Jenkins, M. L. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1825–1830.
(26) Hein, S. M.; Gloer, J. B.; Koster, B.; Malloch, D. *J. Nat. Prod.* **1998**, *61*, 1566–1567.
(27) Turner, W. B.; Aldridge, D. C. *Fungal Metabolites II*; Academic Press: New York, 1983; pp 156−167.
(28) Chexal, K. K.; Holker, J. S. E.; Simpson, T. J. *J. Chem. Soc., Perkin Trans. 1* **1975**, 549–554.
(29) Crystallographic data for **3** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 759772). Copies of the data can be obtained, free of charge, on application to the director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
(30) Sheldrick, G. M. *SHELXL-97, Program for X-ray Crystal Structure Solution and Refinement*; University of Göttingen: Göttingen: Germany, 1997.
(31) Sheldrick, G. M. *SADABS, Program for Empirical Absorption Correction of Area Detector Data*; University of Göttingen: Göttingen: Germany, 1999.
(32) NCCLS document M38-A; NCCLS: Wayne, PA, 2002.

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