

Fusagerins A–F, New Alkaloids from the Fungus *Fusarium* sp.

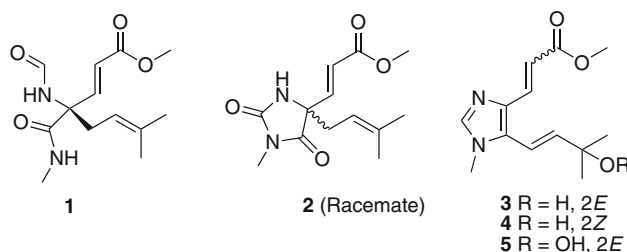


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Abstract Fusagerins A–F (**1–6**), six new alkaloids including a unique one with the rare α -(*N*-formyl)carboxamide moiety (**1**), a hydantoin (imidazolidin-2,4-dione) derivative (**2**), and four fungerin analogues (**3–6**), were isolated from the crude extract of the fungus *Fusarium* sp., together with the known compound fungerin (**7**). Compound **2** was isolated as a racemate and further separated into two enantiomers on a chiral HPLC column. The structures of **1–6** were determined mainly by NMR experiments, and the absolute configuration of **1** and **2** was assigned by electronic circular dichroism (ECD) calculations. Compound **7** showed antibacterial activity against *Staphylococcus aureus* and *Streptococcus pneumoniae*, and weak cytotoxicity against the T24 cells.

Graphical Abstract



Keywords *Fusarium* sp. · Alkaloids · Structure elucidation · Configuration determination

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1 Introduction

Fungi are important sources of bioactive secondary metabolites [1], and those from special and competitive habitats are especially likely to produce structurally diverse and unique natural products due to their highly evolved secondary metabolism [2–4]. Based on this consideration, and our previous success in the discovery of new bioactive secondary metabolites from the of unique niches [5], a special group of fungi which were isolated from the fruiting body and larvae of *Cordyceps sinensis* were chemically investigated, leading to the isolation of a variety of cytotoxic natural products [6–9]. During the course, we also screened the fungal species isolated from the soil samples that were collected on the Qinghai-Tibetan plateau at altitudes above 3200 m, the environment in which *Cordyceps sinensis* typically reside [10–13]. A strain of *Fusarium* sp. was such a fungus, and an EtOAc extract prepared from solid-substrate fermentation products of the fungus was subjected to chemical investigation. Fractionation of the crude extract afforded fusagerins A–F (Fig. 1; 1–6), six new alkaloids including a unique one with the rare α -(*N*-formyl)carboxamide moiety (1), a hydantoin (imidazolidin-2,4-dione) derivative (2), and four fungerin analogues (3–6), along with the known compound fungerin (Fig. 1; 7) [14]. Details of the isolation, structure elucidation, and biological activities of these compounds are reported herein.

2 Results and Discussion

The molecular formula of fusagerin A (1) was established as $C_{13}H_{20}N_2O_4$ (five degrees of unsaturation) on the basis of HRESIMS (m/z 269.1497 [$M + H$] $^+$, $\Delta - 0.1$ mmu) and NMR data (Table 1). The 1H and ^{13}C NMR spectra of 1 showed resonances for two exchangeable protons (δ_H 7.64 and 7.34, respectively), one aldehyde proton (δ_H 8.19), four methyl groups including one *O*-methyl (δ_H 3.71) and one *N*-methyl (δ_H 2.75), one methylene unit, four olefinic carbons (three of which are protonated), one sp^3 quaternary carbon, and three carboxylic carbons (δ_C 160.9, 166.8, and

Table 1 NMR data for 1 (600 MHz, acetone- d_6)

Position	δ_H (J in Hz)	δ_C^a type	HMBC ^b
1		166.8, qC	
2	5.95, d (15.9)	121.2, CH	1, 3, 4
3	7.20, d (15.9)	148.3, CH	1, 2, 4, 8, 9
4		64.0, qC	
5	7.64, br s		
6	8.19, s	160.9, CH	4
7	7.34, br s		
8		170.6, qC	
9a	2.67, dd (14.5, 7.3)	35.2, CH ₂	3, 4, 8, 10, 11
9b	2.99, dd (14.5, 7.3)		
10	5.04, t, (7.3)	118.1, CH	9, 12, 13
11		136.6, qC	
12	1.69, s	26.1, CH ₃	10, 11, 13
13	1.62, s	18.1, CH ₃	10, 11, 12
14	2.75, d (4.6)	26.7, CH ₃	8
15	3.71, s	51.7, CH ₃	1

^a Recorded at 150 MHz

^b HMBC correlations, optimized for 8 Hz, are stated from proton(s) to the indicated carbons

170.6, respectively). These data accounted for all the five unsaturations of 1. Interpretation of the 1H – 1H COSY NMR data of 1 revealed the presence of three isolated spin-systems, which were C-2–C-3, C-9–C-10, and *N*-7–C-14, respectively. In the HMBC spectrum of 1, cross peaks from the H-2 and H-3 olefinic protons to the carboxylic carbon at 166.8 ppm (C-1) established an α,β -unsaturated ketone moiety (C-1–C-3). An HMBC correlation from the *O*-methyl proton signal H₃-15 to the C-1 carboxylic carbon located the *O*-methyl group at C-1. Correlations of the H-10 olefinic proton with C-12 and C-13, and of H₃-12 and H₃-13 with C-10 and C-11 established of a prenyl unit. In turn, a correlation from H₃-14 to the C-8 carboxylic carbon revealed the presence of the C-8/*N*-7 amide bond in the structure. In addition, a one-bond coupling was noted between the C-6 carbonyl resonance and the deshielded H-6 proton resonance in the HSQC spectrum, suggesting

Fig. 1 Structures of metabolites 1–7 isolated from *Fusarium* sp.

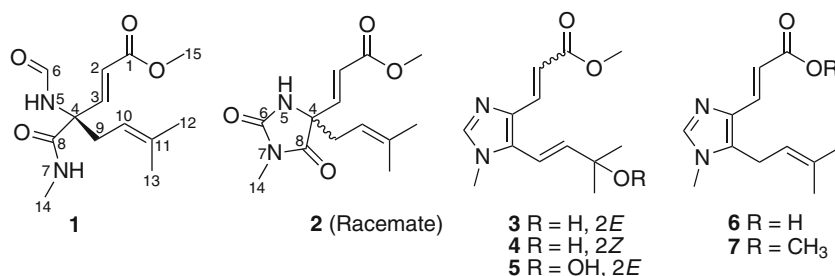
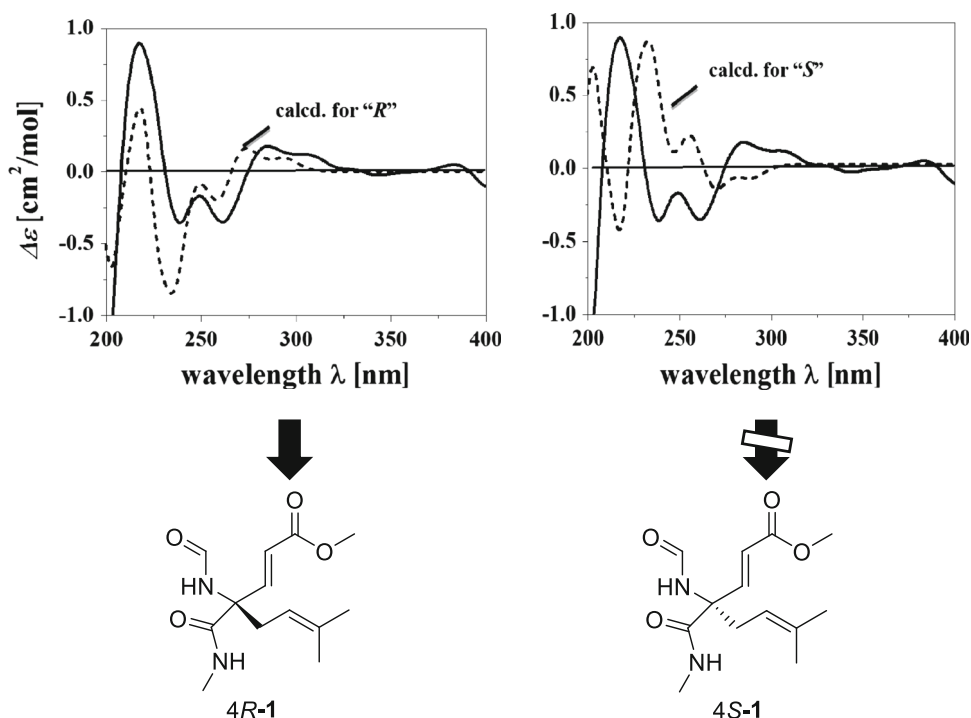


Fig. 2 The experimental CD spectrum of **1** (solid) and the calculated ECD spectra (dash) of two enantiomers 4*R*-**1** and 4*S*-**1**



the presence of formamide functionality. The observation was also supported by a $^1J_{C,H}$ value of 190 Hz measured in the HMBC spectrum, which was consistent with the reported values of 190 and 189 Hz [15, 16]. Key HMBC correlations from H-2, H-3, H-6, and H-10 to C-4, and from H-3 and H₂-9 to C-8 indicated that C-3, N-5, C-8, and C-9 are all connected to C-4. Although no HMBC correlations were observed for the two exchangeable protons, considering the relevant 1H - 1H COSY correlation between H-7 and H-14, as well as the unsaturation requirement for **1**, they were assigned as 5- and 7-NH, respectively. On the basis of these data, the gross structure of fusagerin A was established as shown in Fig. 1.

The C-2/C-3 olefin was assigned the *E*-geometry based on a large coupling constant of 15.9 Hz observed for the corresponding olefinic protons. To determine the absolute configuration for the C-4 stereogenic center in fusagerin A (**1**), the theoretical CD spectra for the two enantiomers 4*R*-**1** and 4*S*-**1** (Fig. 2) were predicted using quantum chemical calculations. Due to the flexible nature of the structure, the conformational species of each enantiomer were defined using the Random Search approach in Sybyl8.0 software. Six conformations were selected from 380 results within a range of 6 kcal/mole above the global minimum. For each conformer, the respective CD spectrum was calculated by TD-B3LYP/6-311++g(2d, p), after optimization using DFT at the B3LYP/6-31G(d) level in Gaussian09 program [17]. The overall calculated CD spectra were then generated according to Boltzmann weighting of each conformer,

Table 2 NMR data for **2** (600 MHz, acetone-*d*₆)

Position	δ_H (J in Hz)	δ_C , type ^a	HMBC ^b
1		166.3, qC	
2	6.13, d (15.8)	122.4, CH	1, 3, 4
3	6.97, d (15.8)	145.8, CH	1, 2, 4, 8, 9
4		66.8, qC	
5	7.57, br s		8
6		156.6, qC	
7			
8		173.5, qC	
9a	2.53, dd (14.5, 7.3)	36.1, CH ₂	3, 4, 8, 10, 11
9b	2.70, dd (14.5, 7.3)		
10	5.05, t (7.3)	116.5, CH	4, 9, 12, 13
11		138.2, qC	
12	1.68, s	26.0, CH ₃	4, 9, 10, 11, 13
13	1.63, s	18.2, CH ₃	4, 9, 10, 11, 12
14	2.89, s	24.6, CH ₃	6, 8
15	3.72, s	52.0, CH ₃	1, 2

^a Recorded at 150 MHz

^b HMBC correlations, optimized for 8 Hz, are stated from proton(s) to the indicated carbons

and were further compared to the experimental CD curve (Fig. 2). The experimental CD spectrum of **1** matched the ECD spectrum for 4*R*-**1**, and was nearly opposite to that for 4*S*-**1**, leading to the deduction of the 4*R* absolute configuration for **1**.

The elemental composition of fusagerin B (**2**) was deduced to be $C_{13}H_{18}N_2O_4$ (six degrees of unsaturation) by HRESIMS analysis (m/z 267.1336 $[M + H]^+$, $\Delta + 0.3$ mmu), two mass units less than that of **1**. Analysis of the 1H and ^{13}C NMR spectroscopic data of **2** (Table 2) revealed the presence of structural features similar to those found in **1**, except that the signals for the H-6 aldehyde proton and the H-7 exchangeable proton in **1** were not observed, and the *N*-methyl proton signal H₃-14 was observed as a singlet instead of a doublet in the 1H NMR spectrum of **2**, implying that the C-6 and C-8 carboxylic carbons are connected to *N*-7 to form a 3-methylimidazolidine-2,4-dione moiety. This postulation was also supported by the HMBC correlations from H₃-14 to C-6 and C-8. On the basis of these data, the planar structure of **2** was established as depicted (Fig. 1).

Despite the presence of a stereogenic center at C-4, the measured optical rotation value of fusagerin B (**2**) was zero, and no Cotton effect was observed in its CD spectrum, suggesting that **2** is a racemate. A portion of **2** was separated using HPLC on a chiral column to afford two enantiomers, (–)-fusagerin B (Figs. 3, **2a**) and (+)-fusagerin B (Figs. 3, **2b**), with the measured specific optical rotation values of -16 and $+16$, respectively (c 0.10, MeOH). The absolute configurations of **2a** and **2b** were deduced by comparison of the experimental and simulated ECD spectra for enantiomers 4*R*-**2** and 4*S*-**2** (Fig. 4). The

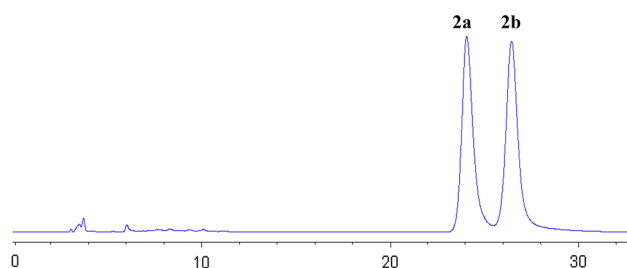
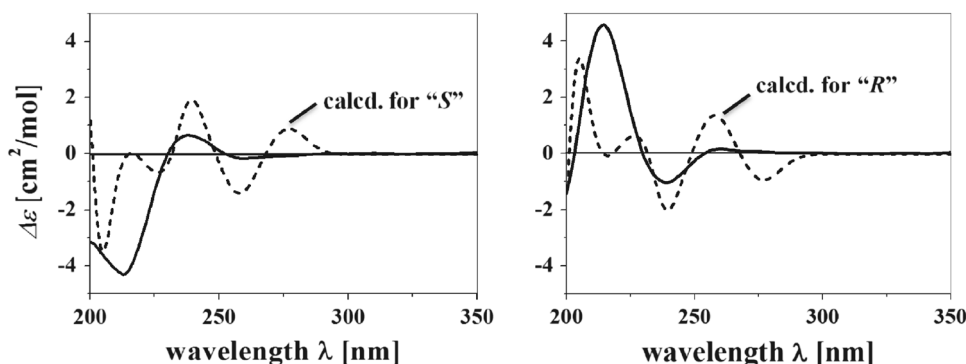


Fig. 3 The HPLC chromatogram of **2** using a Kromasil 5-CelluCoat RP column (4.6×250 mm, $5 \mu m$) eluted with CH_3CN/H_2O (30:70, v/v; flow rate 0.5 mL/min; UV detection at 210 nm)

Fig. 4 The experimental CD spectrum of **2** (solid) and the calculated ECD spectra (dash) of two enantiomers, **2a** (4*S*-**2**) and **2b** (4*R*-**2**)



experimental CD spectra of **2a** and **2b** matched the calculated ECD curves of 4*S*-**2** and 4*R*-**2**, respectively, leading to the deduction of 4*S* for (–)-fusagerin B (**2a**), and 4*R* configuration for (+)-fusagerin B (**2b**).

Fusagerin C (**3**) gave a pseudomolecular ion $[M + H]^+$ peak at m/z 251.1388 ($\Delta + 0.2$ mmu) by HRESIMS, consistent with the molecular formula $C_{13}H_{18}N_2O_3$ (six degrees of unsaturation). Analysis of its 1H and ^{13}C NMR data (Table 3) revealed structural similarity to the co-isolated known compound fungerin (**7**) [14], except that the C-11 olefinic carbon in **7** was reduced to an oxygenated sp^3 quaternary carbon (δ_C 70.8), and the C-9 methylene carbon in **7** was oxidized to an olefinic carbon (δ_H/δ_C 6.66/112.3), which were confirmed by the 1H - 1H COSY NMR correlations of 9-H with 10-H, and HMBC cross peaks from the exchangeable proton OH-11 to C-10, C-12, and C-13. The C-2/C-3 and C-9/C-10 olefins were all assigned the *E*-geometry based on the large coupling constants observed for the relevant olefinic protons (15.3 and 16.2 Hz, respectively). Therefore, the structure of **3** was determined as shown in Fig. 1.

The molecular formula of fusagerin D (**4**) was determined to be $C_{13}H_{18}N_2O_3$ on the basis of HRESIMS (m/z 251.1391 $[M + H]^+$, $\Delta - 0.1$ mmu) and NMR data (Table 3), which is the same as that of **3**, implying isomeric relationship between the two compounds. The 1H and ^{13}C NMR spectra of **4** showed nearly identical resonances to those of **3**, except that the chemical shifts for the C-2/C-3 olefin (δ_H/δ_C 6.52/115.1 and 7.64/136.7 in **3**; 5.74/118.2 and 6.68/129.5 in **4**) were significantly different. Interpretation of the 2D NMR data of **4** established the same planar structure as **3**, and a small coupling constant of 12.1 Hz observed between H-2 and H-3 in **4** compared to 15.3 Hz in **3**, indicating that **4** is the 2*Z* isomer of **3**.

Fusagerin E (**5**) was assigned a molecular formula of $C_{13}H_{18}N_2O_4$ (six degrees of unsaturation) by HRESIMS (m/z 267.1339 $[M + H]^+$, Δ 0 mmu), containing one more oxygen atom than that of **3**. Analysis of its NMR data (Table 3) revealed structural characteristics similar to those of **3**, but the ^{13}C NMR chemical shift of C-11 in **5** (δ_C 82.1)

Table 3 NMR data for **3–6**

Position	3		4		5		6	
	$\delta_{\text{H}}^{\text{a}}$ (J in Hz)	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{a}}$ (J in Hz)	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{c}}$ (J in Hz)	$\delta_{\text{C}}^{\text{d}}$	$\delta_{\text{H}}^{\text{e}}$ (J in Hz)	$\delta_{\text{C}}^{\text{f}}$
1		168.3, qC		168.8, qC		168.4, qC		168.2, qC
2	6.52, d (15.3)	115.1, CH	5.74, d (12.1)	118.2, CH	6.62, d (15.5)	115.9, CH	6.23, d (15.2)	114.0, CH
3	7.64, d (15.3)	136.7, CH	6.68, d (12.1)	129.5, CH	7.59, d (15.5)	135.1, CH	7.47, d (15.2)	135.3, CH
4		135.8, qC		135.9, qC		135.7, qC		133.0, qC
6	7.56, s	140.6, CH	7.43, s	138.9, CH	7.47, s	139.2, CH	7.61, s	139.1, CH
8		133.4, qC		130.8, qC		131.8, qC		134.1, qC
9	6.66, d (16.2)	112.3, CH	6.57, d (16.1)	112.8, CH	6.44, d (16.6)	114.8, CH	3.43, d (7.0)	21.6, CH ₂
10	6.28, d (16.2)	146.2, CH	6.16, d (16.1)	144.8, CH	6.14, d (16.6)	140.9, CH	5.05, t (7.0)	120.0, CH
11		70.9, qC		70.8, qC		82.1, qC		133.0, qC
12	1.40, s	30.3, CH ₃	1.37, s	30.4, CH ₃	1.52, s	24.4, CH ₃	1.68, s	25.3, CH ₃
13	1.40, s	30.3, CH ₃	1.37, s	30.4, CH ₃	1.52, s	24.4, CH ₃	1.73, s	17.7, CH ₃
14	3.70, s	32.7, CH ₃	3.65, s	32.3, CH ₃	3.65, s	32.6, CH ₃	3.53, s	31.2, CH ₃
15	3.70, s	51.4, CH ₃	3.63, s	51.1, CH ₃	3.80, s	51.5, CH ₃		
OH-1							12.07, s	
OH-11 ^g	4.89, s		4.79, s					
OOH-11 ^a					10.41, br s			

^a Recorded at 500 MHz in acetone-*d*₆^b Recorded at 125 MHz in acetone-*d*₆^c Recorded at 500 MHz in CDCl₃^d Recorded at 125 MHz in CDCl₃^e Recorded at 400 MHz in DMSO-*d*₆^f Recorded at 100 MHz in DMSO-*d*₆^g Recorded at 100 MHz in DMSO-*d*₆

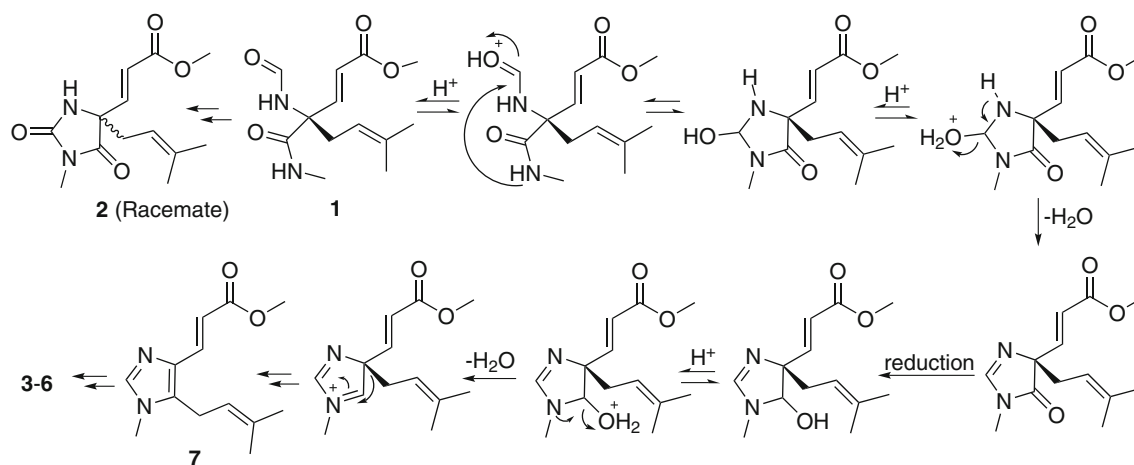
was significantly downfield compared to that in **3** (δ_{C} 70.9), suggesting that the C-11 hydroxy group was oxidized to a peroxy unit. A similar peroxy moiety was also found in the known compounds, peroxyllipidulcine A [18] and bruguierin C [19]. The absolute configuration of **5** was deduced as shown by analogy to **3** and **4**.

The molecular formula of fusagerin F (**6**) was established as C₁₂H₁₆N₂O₂ (six degrees of unsaturation) on the basis of HRESIMS (m/z 221.1293 [M + H]⁺, Δ – 0.8 mmu), which is 14 mass units less than that of the known compound fungerin (**7**) [14]. The NMR data of **6** (Table 3) are nearly identical to those of **7**, except that the C-15 methyl group ($\delta_{\text{H}}/\delta_{\text{C}}$ 3.77/51.4) in **7** was replaced by a proton (δ_{H} 12.07), indicating that **6** bears a free carboxylic acid moiety at C-2.

Compounds **1–7** were tested for their antimicrobial activities against a panel of bacteria and fungi [20, 21]. Compounds **1–6** did not show noticeable in vitro antibacterial or antifungal activities against the above-mentioned organisms (Experimental Section; IC₅₀ > 50 μM). Only fungerin (**7**) showed weak antibacterial activity against *Staphylococcus aureus* (ATCC 6538) and *Streptococcus*

pneumoniae (CGMCC 1.1692), with IC₅₀ values of 33.8 and 34.5 μM , respectively, while the positive control ampicillin showed an IC₅₀ value of 0.26 μM . Compounds **1–7** were also tested for cytotoxicity against a panel of six human tumor cell lines, HeLa (cervical epithelial cells), A549 (lung carcinoma epithelial cells), MCF-7 (breast cancer cells), HCT116 (colon cancer cells), SW480 (colon cancer cells), and T24 (bladder cancer cells). Compounds **1–6** did not show detectable cytotoxicity against the six cell lines at a concentration of 20 $\mu\text{g}/\text{mL}$, whereas compound **7** showed weak cytotoxic effect on the T24 cells, with an IC₅₀ value of 42.2 μM (the positive control cisplatin showed an IC₅₀ value of 6.71 μM).

Fusagerin A (**1**) is the first example of naturally occurring α -(*N*-formyl)carboxamide, which was only synthesized in situ previously from α -aminonitriles using formic-acetic anhydride [22]. Fusagerin B (**2**) is a new member of the extensively studied hydantoin (2,4-imidazolidindione) class of natural products which possess a variety of pharmacological properties, such as anticonvulsant [23–26], antitumor [27, 28], anti-HIV [29], antiarrhythmic [30], antihypertensive [31], hypolipidemic [32], antituberculosis



Scheme 1 Hypothetical biosynthetic pathways for compounds 1–6

[33], and antifungal activities [34]. However, **2** differs from the known precedents by having different substituents on the 2,4-imidazolidindione ring. The only two fungal metabolites incorporating the hydantoin moiety were both isolated from marine-derived fungi [35, 36]. Other examples of this class of natural products were mainly isolated from marine organisms including exiguamine A [37], agesamides A and B [38], naamidines and isonaamidines [39–47], parazoanthines A–E [48], and 5-methoxy-5-(4-methoxyphenyl)-3-methyl-2,4-imidazolidinedione [49]. Compounds **3–6** are closely related to fungerin (**7**) [14], all possessing the imidazole ring. To our knowledge, only three fungerin derivatives have been reported including visoltricin [50], and hydroxyfungerins A and B [51]. Compounds **3–6** differ from the above-mentioned natural products mainly on the identities of the side chains. The plausible biogenetic pathways for compounds **1–6** are illustrated in Scheme 1.

3 Experimental Section

3.1 General Experimental Procedures

Optical rotations were measured on a polAAR3005 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. The CD spectra were recorded on a JASCO J-815 spectropolarimeter. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ¹H and ¹³C NMR data were acquired with Varian Mercury-400, -500, and -600 spectrometers using the solvent signals (acetone-*d*₆: δ_{H} 2.05/ δ_{C} 29.8, 206.1; DMSO-*d*₆: δ_{H} 2.49/ δ_{C} 39.5; CDCl₃: δ_{H} 7.26/ δ_{C} 76.7) as references. The HSQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS and HRESIMS data

were obtained using an Agilent Accurate-Mass-Q-TOF LC/MS 6520 instrument equipped with an electrospray ionization (ESI) source. The fragmentor and capillary voltages were kept at 125 and 3500 V, respectively. Nitrogen was supplied as the nebulizing and drying gas. The temperature of the drying gas was set at 300 °C. The flow rate of the drying gas and the pressure of the nebulizer were 10 L/min and 10 psi, respectively. All MS experiments were performed in positive ion mode. Full-scan spectra were acquired over a scan range of *m/z* 100–1000 at 1.03 spectra/s. HPLC separations were performed on an Agilent 1260 instrument (Agilent, USA) equipped with a variable wavelength UV detector. Chiral HPLC analysis and separation were performed on a Kromasil 5-CelluCoat RP column (4.6 × 250 mm; 5 μm; AkzoNobel).

3.2 Fungal Material

The culture of *Fusarium* sp. was isolated from a soil sample collected on the Qinghai-Tibetan plateau at an altitude of 3800 m, in May, 2008. The isolate was identified based on morphology and sequence (Genbank Accession No. JQ284030) analysis of the ITS region of the rDNA. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. Agar plugs were cut into small pieces (about 0.5 × 0.5 × 0.5 cm³) under aseptic conditions, 15 pieces were used to inoculate in three 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. After sterilization, three flasks of the inoculated media were incubated at 25 °C on a rotary shaker at 170 rpm for 5 days to prepare the seed culture. Spore inoculum was prepared by suspending the seed culture in sterile, distilled H₂O to give a final spore/cell suspension of 1 × 10⁶/mL.

Fermentation was carried out in eight Fernbach flasks (500 mL), each containing 80 g of rice. Distilled H₂O (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi 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.

3.3 Extraction and Isolation

The fermented material was extracted repeatedly with EtOAc (4 × 1.0 L), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (50.0 g), which was fractionated by silica gel VLC using petroleum ether–EtOAc–MeOH gradient elution. The fraction (150 mg) eluted with 10:90 petroleum ether–EtOAc was separated by Sephadex LH-20 column chromatography (CC) eluting with MeOH. The resulting subfractions were combined and further purified by semipreparative RP HPLC (Agilent Zorbax SB-C₁₈ column; 5 μm; 9.4 × 250 mm; 50 % MeOH in H₂O for 30 min; 2 mL/min) to afford fusagerins C (**3**; 2.0 mg, *t_R* 15.80 min), D (**4**; 4.0 mg, *t_R* 16.07 min), and B (**2**; 3.4 mg, *t_R* 28.10 min). The fraction (150 mg) eluted with EtOAc was also separated again by Sephadex LH-20 CC eluting with MeOH. The resulting subfractions were combined and further purified by RP HPLC (20 % MeOH in H₂O for 2 min, followed by 20–100 % over 50 min; 2 mL/min) to afford fusagerins E (**5**; 6.3 mg, *t_R* 29.40 min) and A (**1**; 3.0 mg, *t_R* 32.69 min). The fraction (120 mg) eluted with 99:1 EtOAc–MeOH was further fractionated by Sephadex LH-20 CC eluting with MeOH. The combined subfractions was purified by RP HPLC (65 % MeOH in H₂O for 15 min, followed by 65–100 % over 60 min; 2 mL/min) to afford fungerin (**7**; 74.8 mg, *t_R* 16.32 min). The (140 mg) fraction eluted with MeOH was separated by Sephadex LH-20 CC eluting with MeOH, and the resulting subfractions were purified by RP HPLC (30 % MeOH in H₂O for 10 min, followed by 30–100 % over 20 min; 2 mL/min) to afford fusagerin F (**6**; 4.0 mg, *t_R* 15.24 min). A portion of compound **2** was separated using a Kromasil 5-CelluCoat RP column (30 % CH₃CN in H₂O for 30 min; 0.5 mL/min) to afford (–)-fusagerin B (**2a**; 1.0 mg, *t_R* 24.30 min) and (+)-fusagerin B (**2b**; 1.0 mg, *t_R* 26.78 min).

3.3.1 Fusagerin A (**1**)

Pale yellow oil; $[\alpha]_D^{22} + 5.6$ (*c* 0.2, MeOH); UV (MeOH) λ_{\max} (log ϵ) 210 (2.07), 217 (1.96), 238 (1.73), 285 (1.28) nm; CD (*c* 3.7×10^{-3} M, MeOH) λ_{\max} ($\Delta\epsilon$) 218 (+7.28), 239 (–2.89), 249 (–1.36), 261 (–2.85), 285 (+1.45); IR (neat) ν_{\max} 3325 (br), 2953, 1723, 1666, 1532, 1438, 1384, 1313, 1277, 1200, 1178 cm^{–1}; ¹H, ¹³C NMR, and HMBC

data see Table 1; HRESIMS *m/z* 269.1497 (calcd. for C₁₃H₂₁N₂O₄, 269.1496).

3.3.2 Fusagerin B (**2**)

Colorless oil; UV (MeOH) λ_{\max} (log ϵ) 215 (2.62), 237 (1.99) nm; IR (neat) ν_{\max} 3311 (br), 2929, 1782, 1721, 1658, 1460, 1395, 1317, 1278, 1199, 1176, 1028 cm^{–1}; ¹H, ¹³C NMR, and HMBC data see Table 2. HRESIMS *m/z* 267.1336 (calcd. for C₁₃H₁₉N₂O₄, 267.1339).

3.3.3 (–)-Fusagerin A (**2a**)

Colorless oil; $[\alpha]_D^{22} - 16.0$ (*c* 0.1, MeOH); CD (*c* 3.8×10^{-3} M, MeOH) λ_{\max} ($\Delta\epsilon$) 213 (–4.32), 238 (+0.65), 260 (–0.18); UV, IR, ¹H, ¹³C NMR, HMBC, and HRESIMS data were the same as **2**.

3.3.4 (+)-Fusagerin (**2b**)

Colorless oil; $[\alpha]_D^{22} + 16.0$ (*c* 0.1, MeOH); CD (*c* 3.8×10^{-3} M, MeOH) λ_{\max} ($\Delta\epsilon$) 216 (+4.58), 239 (–1.05), 260 (+0.15); UV, IR, ¹H, ¹³C NMR, HMBC, and HRESIMS data were the same as **2**.

3.3.5 Fusagerin C (**3**)

Colorless oil; UV (MeOH) λ_{\max} (log ϵ) 237 (2.37), 308 (2.39) nm; IR (neat) ν_{\max} 3389 (br), 2972, 1720, 1632, 1509, 1438, 1377, 1302, 1278, 1230, 1197, 1173 cm^{–1}; ¹H and ¹³C NMR data see Table 3; HMBC data (acetone-*d*₆, 500 MHz) H-2 → C-1, C-3, C-4; H-3 → C-1, C-2, C-4, C-8; H-6 → C-4, C-8; H-9 → C-4, C-8, C-10, C-11, C-12, C-13; H-10 → C-8, C-11, C-12, C-13; H₃-12 → C-9, C-10, C-11, C-13; H₃-13 → C-9, C-10, C-11, C-12; H₃-14 → C-6, C-8; H₃-15 → C-1; (DMSO-*d*₆, 600 MHz) OH-11 → C-10, C-11, C-12, C-13; HRESIMS *m/z* 251.1388 (calcd. for C₁₃H₁₉N₂O₃, 251.1390).

3.3.6 Fusagerin D (**4**)

Colorless oil; UV (MeOH) λ_{\max} (log ϵ) 238 (2.56), 298 (2.41) nm; IR (neat) ν_{\max} 3381 (br), 2972, 1708, 1631, 1511, 1436, 1376, 1302, 1264, 1232, 1196, 1172 cm^{–1}; ¹H and ¹³C NMR data see Table 3; HMBC data (acetone-*d*₆, 500 MHz) H-2 → C-1, C-3; H-3 → C-1, C-2, C-4, C-8; H-6 → C-4, C-8; H-9 → C-4, C-8, C-10, C-11, C-12, C-13; H-10 → C-8, C-11, C-12, C-13; H₃-12 → C-9, C-10, C-11, C-13; H₃-13 → C-9, C-10, C-11, C-12; H₃-14 → C-6, C-8; H₃-15 → C-1; (DMSO-*d*₆, 600 MHz) OH-11 → C-10, C-11, C-12, C-13; HRESIMS *m/z* 251.1391 (calcd. for C₁₃H₁₉N₂O₃, 251.1390).

3.3.7 Fusagerin E (5)

Pale yellow powder; UV (MeOH) λ_{\max} (log ϵ) 233 (2.31), 293 (2.41) nm; IR (neat) ν_{\max} 3270 (br), 2973, 1710, 1634, 1515, 1437, 1379, 1262, 1173, 1044 cm^{-1} ; ^1H and ^{13}C NMR data see Table 3; HMBC data (acetone- d_6 , 500 MHz) H-2 \rightarrow C-1, C-4; H-3 \rightarrow C-1, C-2, C-4; H-6 \rightarrow C-4, C-8; H-9 \rightarrow C-4, C-10, C-11; H-10 \rightarrow C-8, C-11, C-12, C-13; H₃-12 \rightarrow C-10, C-11, C-13; H₃-13 \rightarrow C-10, C-11, C-12; H₃-14 \rightarrow C-6, C-8; H₃-15 \rightarrow C-1; HRESIMS m/z 267.1339 (calcd. for C₁₃H₁₉N₂O₄, 267.1339).

3.3.8 Fusagerin F (6)

Pale yellow powder; UV (MeOH) λ_{\max} (log ϵ) 236 (1.91), 292 (2.41) nm; IR (neat) ν_{\max} 3389 (br), 2923, 1688, 1639, 1518, 1415, 1382, 1306, 1274, 1224, 1196, 1148 cm^{-1} ; ^1H and ^{13}C NMR data see Table 3; HMBC data (DMSO- d_6 , 400 MHz) H-2 \rightarrow C-1, C-3, C-4; H-3 \rightarrow C-1, C-2, C-4; H-6 \rightarrow C-4, C-8, C-14; H-9 \rightarrow C-4, C-8, C-10, C-11; 10-H \rightarrow C-8, C-9, C-12, C-13; H₃-12 \rightarrow C-10, C-11, C-13; H₃-13 \rightarrow C-10, C-11, C-12; H₃-14 \rightarrow C-6, C-8; HRESIMS m/z 221.1293 (calcd. for C₁₂H₁₇N₂O₂, 221.1285).

3.3.9 Fungerin (7)

^1H , ^{13}C NMR, and the MS data were consistent with literature values [7].

3.4 Computational Details

Conformational analyses of each enantiomer of **1** and **2** were performed using Random Search method with MMFF94s force field and MMFF94 charges in Sybyl8.0 software. For each conformation of both compounds, TD-B3LYP/6-311++g(2d, p)//B3LYP/6-31G(d) calculations yielded excitations with corresponding oscillator and rotational strength values performed with the program package Gaussian09 [17]. Then the overall CD spectra were summed up following the Boltzmann statistics, according to the respective heats of formation. For a better visualization, the rotational strengths were transformed into $\Delta\epsilon$ values.

3.5 Antimicrobial and Antifungal Bioassays

Antimicrobial and antifungal bioassays were conducted in triplicate by following the National Center for Clinical Laboratory Standards (NCCLS) recommendations [20, 21]. The bacterial strains, *Staphylococcus aureus* (ATCC 6538), *Streptococcus pneumoniae* (CGMCC 1.1692), and *Escherichia coli* (CGMCC 1.2340) were grown on Mueller–Hinton agar, the yeasts, *Candida albicans* (ATCC 10231) and *Geotrichum candidum* (AS2.498), were grown on Sabouraud dextrose agar,

and the fungus, *Aspergillus fumigatus* (ATCC 10894), was grown on potato dextrose agar. Targeted microbes (3–4 colonies) were prepared from broth culture (bacteria: 37 °C for 24 h; fungus: 28 °C for 48 h), and the final spore suspensions of bacteria (in MHB medium), yeasts (in SDB medium), and *Aspergillus fumigatus* (in PDB medium) were 10⁶, 10⁵ cells/mL, and 10⁴ mycelial fragments/mL, respectively. Test samples (10 mg/mL as stock solution in DMSO and serial dilutions) were transferred to 96-well clear plate in triplicate, and the suspension of the test organisms was added to each well achieving a final volume of 200 μL (ampicillin and fluconazole were used as positive controls). After incubation, the absorbance at 595 nm was measured with a microplate reader (TECAN). The inhibition rate was calculated and plotted versus test concentrations to afford the IC₅₀, whereas the MIC was defined as the lowest test concentration that completely inhibited the growth of the test organisms.

3.6 MTS Assay [52]

In a 96-well plate, each well was plated with (2–5) $\times 10^3$ cells (depending on the cell multiplication rate). After cell attachment overnight, the medium was removed, and each well was treated with 100 μL medium containing 0.1 % DMSO, or appropriate concentrations of the test compounds and the positive control cisplatin (100 mM as stock solution of a compound in DMSO and serial dilutions; the test compounds showed good solubility in DMSO and did not precipitate when added to the cells). The plate was incubated for 48 h at 37 °C in a humidified, 5 % CO₂ atmosphere. Proliferation assessed by adding 20 μL of MTS (Promega) to each well in the dark, followed by a 90 min incubation at 37 °C. The assay plate was read at 490 nm using a microplate reader. The assay was run in triplicate.

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Compliance with Ethical Standards

Conflicts of Interest The authors declare no conflict of interest.

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