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 configurations 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 \( \alpha-(N\text{-formyl})\text{-carboxamide} \) 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 \( \text{C}_{13}\text{H}_{20}\text{N}_{2}\text{O}_{4} \) (five degrees of unsaturation) on the basis of HRESIMS \((m/z\ 269.1497\ [M + H]^+ , \Delta = 0.1\ \text{mmu})\) and NMR data (Table 1). The \( ^1\text{H} \) and \( ^{13}\text{C} \) NMR spectra of 1 showed resonances for two exchangeable protons (\( \delta_{\text{H}} 7.64 \) and 7.34, respectively), one aldehyde proton (\( \delta_{\text{H}} 8.19 \)), four methyl groups including one O-methyl (\( \delta_{\text{H}} 8.19 \)) and one N-methyl (\( \delta_{\text{H}} 2.75 \)), one methylene unit, four olefinic carbons (three of which are protonated), one \( sp^3 \) quaternary carbon, and three carboxylic carbons (\( \delta_{\text{C}} 160.9, 166.8, \) and 170.6, respectively). These data accounted for all the five unsaturations of 1. Interpretation of the \( ^1\text{H}–^1\text{H} \) 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 \( \alpha,\beta\)-unsaturated ketone moiety (C-1–C-3). An HMBC correlation from the O-methyl proton signal H-3 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 H-12 and C-11 established of a prenyl unit. In turn, a correlation from H-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.

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

| Position | \( \delta_{\text{H}} \) (in Hz) | \( \delta_{\text{C}} \) 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\(_2\) | 10, 11, 13 |
| 13       | 1.62, s         | 18.1, CH\(_2\) | 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.

Fig. 1 Structures of metabolites 1–7 isolated from Fusarium sp.
the presence of formamide functionality. The observation was also supported by a $^1J_{CH}$ 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 H$_2$-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 $^3$H–$^1$H 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 4R-1 and 4S-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, and were further compared to the experimental CD curve (Fig. 2). The experimental CD spectrum of 1 matched the ECD spectrum for 4R-1, and was nearly opposite to that for 4S-1, leading to the deduction of the 4R absolute configuration for 1.

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

Table 2 NMR data for 2 (600 MHz, acetone-$d_6$)

| Position | $\delta_H$ (J in Hz) | $\delta_C$, typea | HMBCb |
|----------|----------------------|-------------------|--------|
| 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        | 7.57, br s           | 66.8, qC          | 8      |
| 5        | 156.6, qC            |                   |        |
| 6        | 173.5, qC            |                   |        |
| 7        | 9a 2.53, dd (14.5, 7.3) | 36.1, CH$_2$ | 3, 4, 8, 10, 11 |
| 8        | 2.70, dd (14.5, 7.3) |                   |        |
| 9        | 5.05, t (7.3)        | 116.5, CH         | 4, 9, 12, 13 |
| 10       | 138.2, qC            |                   |        |
| 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
The elemental composition of fusagerin B (2) was deduced to be C_{13}H_{18}N_{2}O_{4} (six degrees of unsaturation) by HRESIMS analysis (m/z 267.1336 [M + H]^+, Δ + 0.3 mmu), two mass units less than that of 1. Analysis of the 1H and 13C 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 H_{3}-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 H_{3}-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 (ε 0.10, MeOH). The absolute configurations of 2a and 2b were deduced by comparison of the experimental and simulated ECD spectra for enantiomers 4R-2 and 4S-2 (Fig. 4). The experimental CD spectra of 2a and 2b matched the calculated ECD curves of 4S-2 and 4R-2, respectively, leading to the deduction of 4S for (−)-fusagerin B (2a), and 4R configuration for (+)-fusagerin B (2b).

Fusagerin C (3) gave a pseudomolecular ion [M + H]^+ peak at m/z 251.1388 (Δ + 0.2 mmu) by HRESIMS, consistent with the molecular formula C_{13}H_{18}N_{2}O_{3} (six degrees of unsaturation). Analysis of its 1H and 13C 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_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_{2}O_{4} on the basis of HRESIMS (m/z 251.1391 [M + H]^+, Δ - 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 ^13C 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 2Z isomer of 3.

Fusagerin E (5) was assigned a molecular formula of C_{13}H_{18}N_{2}O_{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 ^13C NMR chemical shift of C-11 in 5 (δ_C 82.1)
was significantly downfield compared to that in 3 ($\delta_C$ 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, peroxylippidulcine A [18] and bru-
guierin 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 estab-
lished as $\text{C}_{12}\text{H}_{16}\text{N}_{2}\text{O}_2$ (six degrees of unsaturation) on the
basis of HRESIMS ($m/z$ 221.1293 [M – H]$^-$, $\Delta – 0.8$
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 ($\delta_H$/d$C$ 3.77/51.4) in 7 was replaced by a
proton ($\delta_H$ 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 antibac-
terial or antifungal activities against the above-mentioned
organisms (Experimental Section; IC$_{50}$ > 50 $\mu$M). Only
fungerin (7) showed weak antibacterial activity against
Staphylococcus aureus (ATCC 6538) and Streptococcus
pneumoniae (CGMCC 1.1692), with IC$_{50}$ values of 33.8
and 34.5 $\mu$M, respectively, while the positive control
ampicillin showed an IC$_{50}$ value of 0.26 $\mu$M. Compounds
1–6 did not show detectable cytotoxicity against the six cell
lines at a concentration of 20 $\mu$g/mL, whereas compound
7 showed weak cytotoxic effect on the T24 cells, with an
IC$_{50}$ value of 42.2 $\mu$M (the positive control cisplatin
showed an IC$_{50}$ value of 6.71 $\mu$M.

Fusagerin A (1) is the first example of naturally occur-
rizing $\alpha$-(N-formyl)carboxamide, which was only synthe-
sized in situ previously from $\alpha$-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 phar-
macological properties, such as anticonvulsant [23–26],
antitumor [27, 28], anti-HIV [29], antiarrhythmic [30],
antihypertensive [31], hypolipidemic [32], antituberculosis

Table 3 NMR data for 3–6

| Position | 3 | 4 | 5 | 6 |
|----------|---|---|---|---|
|          | $\delta_H$ (J in Hz) | $\delta_H$ (J in Hz) | $\delta_H$ (J in Hz) | $\delta_H$ (J in Hz) |
| 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 |
| 5        | 7.56, s | 140.6, CH | 7.43, s | 138.9, CH | 7.47, s | 139.2, CH | 7.61, s | 139.1, CH |
| 6        | 133.4, qC | 130.8, qC | 131.8, qC | 134.1, qC |
| 7        | 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$_2$ |
| 8        | 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 |
| 9        | 70.9, qC | 70.8, qC | 82.1, qC | 133.0, qC |
| 10       | 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$_3$ |
| 11       | 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$_3$ |
| 12       | 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$_3$ |
| 13       | 3.70, s | 51.4, CH$_3$ | 3.63, s | 51.1, CH$_3$ | 3.80, s | 51.5, CH$_3$ | 10.41, br s | 12.07, s |
| OH-1     | 4.89, s | 4.79, s |
| OH-11$^a$ |  |  |  |  |
| OOH-11$^a$ |  |  |  |  |

$^a$ Recorded at 500 MHz in acetone-$d_6$
$^b$ Recorded at 125 MHz in acetone-$d_6$
$^c$ Recorded at 500 MHz in CDCl$_3$
$^d$ Recorded at 125 MHz in CDCl$_3$
$^e$ Recorded at 400 MHz in DMSO-$d_6$
$^f$ Recorded at 100 MHz in DMSO-$d_6$
$^g$ Recorded at 100 MHz in DMSO-$d_6$
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. \(^1\)H and \(^13\)C NMR data were acquired with Varian Mercury-400, -500, and -600 spectrometers using the solvent signals (acetone-\(d_6\): \(\delta_H = 2.05/\delta_C = 29.8, 206.1\); DMSO-\(d_6\): \(\delta_H = 2.49/\delta_C = 39.5\); CDCl\(_3\): \(\delta_H = 7.26/\delta_C = 76.7\)) 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 cm\(^3\)) 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 H\(_2\)O to give a final spore/cell suspension of \(1 \times 10^6\)/mL.
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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 TLC 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 fusagerin (1: 2.0 mg, tR 15.80 min), D (4: 4.0 mg, tR 16.07 min), and B (2: 3.4 mg, tR 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, tR 29.40 min) and A (1: 3.0 mg, tR 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, tR 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 (6: 4.0 mg, tR 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, tR 24.30 min) and (+)-fusagerin B (2b: 1.0 mg, tR 26.78 min).

3.3.1 Fusagerin A (1)

Pale yellow oil; [α]22D + 5.6 (c 0.2, MeOH); UV (MeOH) λmax (log ε) 210 (2.07), 217 (1.96), 238 (1.73), 285 (1.28) nm; CD (c 3.7 × 10−3 M, MeOH) λmax (Δε) 218 (+7.28), 239 (−2.89), 249 (−1.36), 261 (−2.85), 285 (+1.45); IR (neat) νmax 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) λmax (log ε) 215 (2.62), 237 (1.99) nm; IR (neat) νmax 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; [α]22D + 16.0 (c 0.1, MeOH); CD (c 3.8 × 10−3 M, MeOH) λmax (Δε) 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; [α]22D − 16.0 (c 0.1, MeOH); CD (c 3.8 × 10−3 M, MeOH) λmax (Δε) 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) λmax (log ε) 237 (2.37), 308 (2.39) nm; IR (neat) νmax 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-d6, 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, C-13, H-13 → C-9, C-10, C-11, C-13, C-13, H-14 → C-6, C-8, H-15 → C-1; (DMSO-d6, 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) λmax (log ε) 238 (2.56), 298 (2.41) nm; IR (neat) νmax 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-d6, 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, H-13 → C-9, C-10, C-11, C-13, C-13, H-14 → C-6, C-8, H-15 → C-1; (DMSO-d6, 600 MHz) OH-11 → C-10, C-11, C-12, C-13; HRESIMS m/z 251.1391 (calcd. for C13H19N2O3, 251.1390).

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3.3.7 Fusagerin E (5)

Pale yellow powder; UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 233 (2.31), 293 (2.41) nm; IR (neat) $\nu_{\text{max}}$ 3270 (br), 2973, 1710, 1634, 1515, 1437, 1379, 1262, 1173, 1044 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table 3; HMBC data (acetone-$d_6$, 500 MHz) H-2 $\rightarrow$ C-1, C-3, C-8; 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; H$_3$-12 $\rightarrow$ C-10, C-11, C-13; H$_3$-13 $\rightarrow$ C-10, C-11, C-12; H$_3$-14 $\rightarrow$ C-6, C-8; H$_2$-15 $\rightarrow$ C-1; HRESIMS m/z 267.1339 (calcd. for $\text{C}_{13}\text{H}_{19}\text{N}_{2}\text{O}_4$, 267.1339).

3.3.8 Fusagerin F (6)

Pale yellow powder; UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 236 (1.91), 292 (2.41) nm; IR (neat) $\nu_{\text{max}}$ 3389 (br), 2923, 1688, 1639, 1518, 1415, 1382, 1306, 1274, 1224, 1196, 1148 cm$^{-1}$; $^1$H 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; H$_3$-12 $\rightarrow$ C-10, C-11, C-13; H$_3$-13 $\rightarrow$ C-10, C-11, C-12; H$_3$-14 $\rightarrow$ C-6, C-8; HRESIMS m/z 221.1293 (calcd. for $\text{C}_{13}\text{H}_{19}\text{N}_{2}\text{O}_2$, 221.1285).

3.3.9 Fungerin (7)

$^1$H, $^{13}$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 following the Boltzmann statistics, according to the respective heats of formation. For a better visualization, the rotational strengths were transformed into $\Delta\varepsilon$ values.

3.5 Antimicrobial and Antifungal Bioassays

Antimicrobial and antifungal bioassays were conducted in triplicate by following the National Center for Clinical Laboratory Standards (NCLLS) 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 $10^6$, $10^5$ cells/mL, and $10^4$ 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 IC$_{50}$, 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 % CO$_2$ 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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