Novel Antimicrobial Compounds as Ophiobolin-Type Sesterterpenes and Pimarane-Type Diterpene From *Bipolaris* Species TJ403-B1

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Six previously undescribed ophiobolin-type sesterterpenes, namely, bipolatoxins A–F (1–6); and one previously undescribed pimarane-type diterpene, namely, 1β-hydroxy momilactone A (7); together with three known compounds, namely, 25-hydroxyophiobolin I (8), ophiobolin I (9), and ophiobolin A lactone (10); were isolated and identified from the endophytic fungus *Bipolaris* species TJ403-B1. Their structures with absolute configurations were elucidated on the basis of extensive spectroscopic analyses (including 1D and 2D nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectroscopy data), single-crystal X-ray diffraction analyses, and comparison of experimental circular dichroism data. All compounds (except for 5) were evaluated for antimicrobial potential, which indicated that bipolatoxin D (4) showed significant inhibitory activity against *Enterococcus faecalis* with a minimum inhibitory concentration (MIC) value of 8 µg/mL, and ophiobolin A lactone (10) showed significant inhibitory activity against *Acinetobacter baumannii* and *E. faecalis* with MIC values of 8 and 8 µg/mL, respectively.

**Keywords:** *Bipolaris* species TJ403-B1, ophiobolin-type sesterterpenes, pimarane-type diterpene, structure elucidation, antimicrobial activity

INTRODUCTION

Microbial natural products and their derivatives have been an important source of new antibiotics required for the treatment of infectious diseases (Wright, 2017). Since the first antibiotic, penicillin, was discovered from the fungus *Penicillium notatum* in 1928 (Wright, 2017), multiple classes of anti-infectives have been isolated from a variety of fungi, such as gliotoxin, beauvericin, and roquefortine C (Jakubczyk and Dussart, 2020). However, the rapid evolution of antimicrobial resistance in both hospital and community settings is decreasing the efficacy of our current therapies and causing a serious global public health crisis (Baker, 2015; Brown and Wright, 2016). One strategy to combat antimicrobial resistance is to discover and develop novel antimicrobial drugs that are not subject to existing resistance mechanisms (Demicic et al., 2013; Ziemert et al., 2016). Fungi-derived natural products hold great promise in the search for new therapies.
Ophiobolins, which represent a minor group of sesterterpenes featuring a tricyclic (5-8-5 fused) or tetracyclic (5-8-5-5 spirofused) skeleton, are reported to show a broad spectrum of inhibitory activities against nematodes, HMG-CoA reductase, fungi, and bacteria; cytotoxic activity against multiple cancer cells; and anti-inflammatory activity against lipopolysaccharide-induced nitric oxide production (Wang et al., 2013; Liu et al., 2019c), and this member of natural products is widely discovered in the species of Bipolaris. As a part of our ongoing program for exploring new antimicrobial agents from fungi, the fungus Bipolaris species T403-B1 attracted our attention and was systematically studied (Liu et al., 2019b,d). Following a further chemical investigation on the EtOAc extract of the title fungus, six previously undescribed ophiobolin-type sesterterpenes, namely, bipolatoxins A–F (1–6); and one previously undescribed pimaran-type diterpene, namely, 1β-hydroxy momilactone A (7); together with three known compounds (8–10); were isolated and identified. Herein, the detailed isolation, structure identification, and antimicrobial activity of these compounds (Figure 1) are described.

MATERIALS AND METHODS

General Experimental Procedures

An X-5 microscopic melting point apparatus (Beijing Tech, Beijing, China) was used, and the reported melting points were uncorrected. Optical rotations were measured in MeOH on a Perkin-Elmer 341 polarimeter (PerkinElmer, Waltham, MA, United States). Infrared (IR) spectra were acquired on a Bruker Vertex 70 FT-IR instrument (Bruker, Karlsruhe, Germany). Circular dichroism (CD) data were collected from a JASCO J-810 spectrometer (JASCO Co., Salt Lake City, UT, United States). Circular dichroism (CD) data were measured on a Bruker AM-400, a DRX-600, and a Bruker AM-800 instrument, and the 1H and 13C NMR chemical shifts were referenced to the solvent impurity peaks for methanol-d4 (δH 3.31 and δC 49.0) and acetone-d6 (δH 2.05 and δC 206.3). High-resolution electrospray ionization mass spectroscopy (HRESIMS) was performed on a Thermo Fisher LC-LTQ-Orbitrap XL spectrometer (Thermo Fisher, Palo Alto, CA, United States).

Fungus Material

The fungal strain in our project was obtained from the leaves of wheat, which was collected from Wuhan City of Hubei Province, China, in May 2016. Sequence data for this fungal strain have been submitted to the DDBJ/EMBL/GenBank under accession no. MH545913. A voucher sample has been preserved in the culture collection center of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China).

Cultivation, Extraction, and Isolation

The fungal strain was cultured on potato dextrose agar at 28 °C for 5 days to prepare the seed cultures. Then, the agar plugs were inoculated into 450 Erlenmeyer flasks (1 L), previously sterilized by autoclaving, each containing 250 g rice and 250 mL distilled water. All flasks were incubated at 28 °C for 28 days. The fermented rice substrate was extracted five times in 95% aqueous EtOH at room temperature, and the solvent was evaporated under vacuum to afford a residue. The residue was suspended in H2O and successively partitioned with EtOAc to yield a total extract.

The EtOAc extract (300 g) was subjected to RP-C18 silica gel CC with a stepwise gradient of MeOH–H2O (20, 40, 60, 80, and 100%) to afford five major fractions, A–E. Fraction C (40 g) was applied to a silica gel column eluted with petroleum ether–EtOAc (10:0 to 0:1, vol/vol) to furnish eight main fractions, C1–C8. Fraction C4 (11.3 g) was chromatographed on Sephadex LH-20 (CH3CN–H2O, 1:1, vol/vol) and further purified on an RP-C18 silica gel column eluted with MeOH–H2O (40:60 to 60:40, vol/vol) to obtain fractions C4.1–C4.12. Fraction C4.5 (3.0 g) was applied to silica gel CC eluted with stepwise CH2Cl2–MeOH (1:0–100:1, vol/vol) to afford nine fractions (C4.5.1–C4.5.9). Fraction C4.5.2 was repeatedly separated via Sephadex LH-20 (CH2Cl2–MeOH, 1:1, vol/vol) and then separated by semipreparative HPLC (CH3CN–H2O, 73:27, vol/vol, 3.0 mL/min) to afford compound 10 (tf 34.2 min, 11.2 mg). Purification of fraction C4.5.3 by semipreparative HPLC (MeOH–H2O, 80:20, vol/vol, 3.0 mL/min) afforded compound 6 (tf 16.2 min, 4.1 mg). Purification of fraction C4.5.4 by semipreparative HPLC (CH3CN–H2O, 70:30, vol/vol, 3.0 mL/min) afforded compound 7 (tf 11.2 min, 3.2 mg). Fraction C4.5.6 was repeatedly separated via Sephadex LH-20 (CH2Cl2–MeOH, 1:1, vol/vol) and then separated by semipreparative HPLC (CH3CN–H2O, 75:25, vol/vol, 3.0 mL/min) to afford compound 4 (tf 31.2 min, 2.2 mg). Fraction C4.5.7 was repeatedly separated via Sephadex LH-20 (CH2Cl2–MeOH, 1:1, vol/vol) and then purified by semipreparative HPLC (MeOH–H2O, 75:25, vol/vol, 3.0 mL/min) to afford compound 5 (tf 30.9 min, 1.0 mg). Fraction C4.5.9 was repeatedly separated via Sephadex LH-20 (CH2Cl2–MeOH, 1:1, vol/vol) and then purified by semipreparative HPLC (CH3CN–H2O, 40:60, vol/vol, 3.0 mL/min) to afford compound 3 (tf 34.9 min, 4.8 mg). Fraction C4.7 (500 mg) was applied to silica gel CC eluted with stepwise CH2Cl2–MeOH (1:0–60:1, vol/vol) to afford six fractions (C4.7.1–C4.7.6). Purification of fraction C4.7.3 by semipreparative HPLC (MeOH–H2O, 77:23, vol/vol, 3.0 mL/min) afforded compound 1 (tf 30.9 min, 6.3 mg). Purification of fraction C4.7.4 by semipreparative HPLC (MeOH–H2O, 83:17, vol/vol, 3.0 mL/min) afforded compound 2 (tf 31.0 min, 4.5 mg). Fraction C5 (4.1 g) was separated on Sephadex LH-20 CC (CH2Cl2–MeOH, 1:1, vol/vol) and further

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purified by RP-C18 silica gel CC (MeOH–H2O, 40:60 to 60:40, vol/vol) to afford three main fractions, C5.1–C5.3. Compound 8 (tR 34.8 min, 10.6 mg) was purified by semipreparative HPLC (MeOH–H2O, 71:29, vol/vol, 3.0 mL/min) from fraction C5.2. Fraction C6 (2.2 g) was chromatographed on Sephadex LH-20 (CH2Cl2–MeOH, 1:1, vol/vol) and further purified by semipreparative HPLC (MeOH–H2O, 70:30, vol/vol, 3.0 mL/min) to afford compound 9 (tR 39.3 min, 4.5 mg).

Bipolatoxin A (1). Colorless needle crystals; [α]D25 +70 (c 0.10, MeOH); UV (MeOH) λmax (log ε) = 202 (4.11), 229 (4.07) nm; ECD (c 0.18, MeOH) = Δε219 +3.07, Δε313 −0.70; IR νmax = 3,428, 2,935, 1,682, 1,622, 1,459, 1,379, 1,317, 1,227, 1,186, 1,028, 855 cm−1; HRESIMS m/z 407.2570 [M+Na]+ (calcd for C25H36O3Na+, 407.2557); 1H and 13C NMR data, see Tables 1 and 3.

Bipolatoxin B (2). Colorless oil; [α]D25 +65 (c 0.10, MeOH); UV (MeOH) λmax (log ε) = 202 (4.09), 229 (4.02) nm; ECD (c 0.18, MeOH) = Δε218 +1.82, Δε314 −1.14; IR νmax = 3,430, 2,935, 1,684, 1,621, 1,458, 1,378, 1,315, 1,257, 1,171, 1,145, 1,075, 1,027, 977, 857, 815, 616 cm−1; HRESIMS m/z 423.2882 [M+Na]+ (calcd for C26H38O3Na+, 423.2870); 1H and 13C NMR data, see Tables 1 and 3.

Bipolatoxin C (3). Colorless oil; [α]D25 +3 (c 0.10, MeOH); UV (MeOH) λmax (log ε) = 203 (4.14), 229 (3.93) nm; ECD (c 0.18, MeOH) = Δε206 −15.45, Δε222 +1.85, Δε308 −2.30; IR νmax = 3,403, 2,955, 2,933, 2,876, 1,627, 1,516, 1,453, 1,382, 1,343, 1,271, 1,232, 1,172, 1,028, 962, 927, 869, 825, 626 cm−1; HRESIMS m/z 439.2453 [M+Na]+ (calcd for C25H36O3Na+, 439.2450); 1H and 13C NMR data, see Tables 2 and 3.
| No.  | $^{1}$H NMR assignments for compounds 1–4 (δ in ppm and J in Hz). |
|------|---------------------------------------------------------------|
|     | $^{1}$a,b                                                    |
| 1   | 1.17 t (13.6); 2.10 m                                        |
| 2   | 2.95 d (13.0)                                                |
| 3   | 5.92 t (1.5)                                                 |
| 4   |                                                            |
| 5   |                                                            |
| 6   | 3.61 d (3.3)                                                 |
| 7   | 5.73 m                                                      |
| 8   | 2.00 m; 2.39 d (18.7)                                        |
| 9   | 2.71 ddd (3.4, 9.9, 14.1)                                    |
| 10  | 1.45 m; 1.51 m                                              |
| 11  | 1.32 m; 1.52 m                                              |
| 12  | 1.84 m                                                      |
| 13  | 1.55 m                                                      |
| 14  | 1.71 ddd (6.3, 8.8, 14.9); 2.20 m                           |
| 15  | 5.60 m                                                      |
| 16  | 5.59 s                                                      |
| 17  | 2.09 s                                                      |
| 18  | 3.90 d (12.2); 4.30 m                                        |
| 19  | 1.07 s                                                      |
| 20  | 0.90 d (6.6)                                                |
| 21  | 1.26 s                                                      |
| 22  | 1.26 s                                                      |
| 23  | OMe                                                         |

$^{a}$Recorded at 400 MHz in methanol-$d_4$. $^{b}$“m” means overlapped or multiplet with other signals. $^{c}$Recorded at 600 MHz in methanol-$d_4$.

| No.  | $^{1}$H NMR assignments for compounds 5–7 (δ in ppm and J in Hz). |
|------|------------------------------------------------------------------|
| 1    | 1.59 m; 1.99 m                                                  |
| 2    | 2.04 dddd (4.3, 9.6, 12.5)                                      |
| 3    | 1.89 m; 2.21 d (14.8)                                          |
| 4    | 5.00 dd (5.7, 7.1)                                             |
| 5    | 3.75 ddd (2.5, 7.2, 9.6)                                       |
| 6    | 6.88 dt (2.5, 8.4)                                             |
| 7    | 2.16 dddd (8.0, 11.3, 13.1); 2.54 dd (8.9, 13.0)               |
| 8    | 2.28 m                                                         |
| 9    | 1.47 m; 2.03 m                                                 |
| 10   | 2.43 m                                                        |
| 11   | 5.31 m                                                        |
| 12   | 2.24 m                                                        |
| 13   | 1.25 m; 1.52 m                                                |
| 14   | 1.99 m                                                        |
| 15   |                                                     |
| 16   | 4.23 m                                                        |
| 17   | 5.51 m                                                        |
| 18   | 2.24 m                                                        |
| 19   | 1.26 s                                                        |
| 20   | 0.94 s                                                        |
| 21   | 1.07 d (6.7)                                                   |

$^{a}$Recorded at 800 MHz in methanol-$d_4$. $^{b}$“m” means overlapped or multiplet with other signals. $^{c}$Recorded at 400 MHz in methanol-$d_4$. $^{d}$Recorded at 400 MHz in acetone-$d_6$.
TABLE 3 | ¹³C NMR assignments for compounds 1–7.

| No. | ¹a | ²a | ³a | ⁴b | ⁵c | ⁶a | ⁷d |
|-----|----|----|----|----|----|----|----|
| 1   | 47.5 CH₂ 47.5 CH₂ | 47.5 CH₂ | 44.4 CH₂ | 32.3 CH₂ | 131.3 CH | 68.2 CH |
| 2   | 52.4 CH 52.3 CH | 53.1 CH | 53.4 CH | 53.6 CH | 156.4 C | 45.8 CH |
| 3   | 183.1 C | 183.1 C | 183.5 C | 80.9 C | 80.9 C | 40.9 CH | 204.4 C |
| 4   | 130.6 CH | 130.6 CH | 130.9 CH | 47.2 CH₀ | 46.9 CH₂ | 34.1 CH | 54.6 C |
| 5   | 212.0 C 212.0 C | 212.1 C | 84.0 CH | 84.0 CH | 30.4 CH₂ | 44.8 CH |
| 6   | 53.7 CH 53.7 CH | 53.8 CH | 45.8 CH | 46.0 CH | 41.8 CH | 73.8 CH |
| 7   | 135.1 C | 135.2 C | 135.9 C | 132.9 C | 133.8 C | 141.9 C | 115.1 CH |
| 8   | 130.7 CH | 130.6 CH | 129.7 CH | 141.4 CH | 140.3 CH | 139.2 CH | 148.8 C |
| 9   | 30.4 CH₂ 30.4 CH₂ | 31.6 CH₂ | 25.6 CH₂ | 24.5 CH₂ | 24.3 CH₂ | 48.0 CH |
| 10  | 44.3 CH 44.3 CH | 52.1 CH | 55.7 CH | 50.0 CH | 47.3 CH | 38.4 C |
| 11  | 45.9 C 45.9 C | 46.2 C | 44.9 C | 54.2 C | 50.5 C | 24.0 CH |
| 12  | 45.6 CH₂ 45.6 CH₂ | 51.3 CH₂ | 37.6 CH₀ | 84.6 CH | 80.3 CH | 38.0 CH₂ |
| 13  | 27.9 CH₂ 27.9 CH₂ | 121.1 CH | 23.9 CH₂ | 125.9 CH | 31.3 CH | 41.0 C |
| 14  | 52.0 CH 52.1 CH | 151.2 C | 46.4 CH | 151.4 C | 41.4 CH | 48.2 CH |
| 15  | 33.6 CH 33.5 CH | 33.3 CH | 34.9 CH | 32.7 CH | 33.8 CH | 150.4 CH |
| 16  | 41.1 CH⁻ 41.3 CH | 39.0 CH₂ | 41.1 CH₂ | 36.6 CH₂ | 37.1 CH₂ | 110.2 CH₂ |
| 17  | 126.3 CH | 130.4 CH | 125.5 CH | 127.0 CH | 26.3 CH₂ | 27.8 CH₂ | 22.3 CH₂ |
| 18  | 140.6 CH 137.4 CH | 140.9 CH | 140.4 CH | 125.5 CH | 143.6 CH | 21.2 CH₂ |
| 19  | 71.1 C | 78.4 C | 71.1 C | 71.1 C | 192.6 C | 129.2 C | 174.7 C |
| 20  | 17.5 CH₃ 17.5 CH₃ | 17.5 CH₃ | 25.5 CH₃ | 25.5 CH₃ | 22.8 CH₃ | 15.2 CH₃ |
| 21  | 67.0 CH₃ 67.0 CH₃ | 66.5 CH₂ | 174.5 CH | 174.4 CH | 174.4 C | 173.4 C |
| 22  | 23.4 CH₃ 23.4 CH₃ | 22.6 CH₃ | 18.9 CH₃ | 12.5 CH₃ | 14.6 CH₃ | 17.0 CH₃ |
| 23  | 19.0 CH₃ 19.1 CH₃ | 18.7 CH₃ | 17.3 CH₃ | 18.9 CH₃ | 17.0 CH₃ |
| 24  | 30.0 CH₃ 26.2 CH₃ | 30.0 CH₃ | 30.0 CH₃ | 17.8 CH₃ | 12.5 CH₃ |
| 25  | 30.1 CH₃ 26.3 CH₃ | 30.1 CH₃ | 30.0 CH₃ | 25.9 CH₃ | 172.1 C |

OMe 50.5 CH₃

¹a Recorded at 100 MHz in methanol-d₄. ²a Recorded at 150 MHz in methanol-d₄. ³a Recorded at 200 MHz in methanol-d₄. ⁴a Recorded at 100 MHz in acetone-d₆.

X-Ray Crystal Structure Analysis

The suitable crystals of compounds 1, 4, and 8 were acquired from MeOH–H₂O (20:1, vol/vol) at room temperature. The intensity data were recorded on an XtaLAB PRO MM007HF diffractometer (Cu Kα). Using Olex2 (Dolomanov et al., 2009), the structures were solved via direct methods with SHELXL-2014/7 (Sheldrick, 2008). Refinements were executed by the SHELXL-2014/7 refinement package via means of full-matrix least squares on F², and the anisotropic displacement parameters were applied for all the non-hydrogen atoms. All the hydrogen atoms were placed on the calculated positions and refined by a riding model. The crystallographic data for these structures were deposited in the Cambridge Crystallographic Data Center (CCDC 1971181 for 1, CCDC 1913829 for 4, and CCDC 1913832 for 8). Copies of the data can be obtained free of charge on application to CCDC (Cambridge, United Kingdom; e-mail: deposit@ccdc.cam.ac.uk).

Crystallographic data for compound 1: C₂₃H₃₈O₅, M = 386.55, orthorhombic, a = 5.9212(10) Å, b = 15.6798(10) Å, c = 26.2277(2) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 2435.07(5) Å³, T = 100(1) K, space group P2₁2₁2₁, Z = 4, μ(Cu Kα) = 0.523 mm⁻¹, 24,079 reflections measured, 4,861 independent reflections (Rint = 0.0218). The final R1 values were 0.0293 [I > 2σ(I)]. The final wR(F²) values were 0.0772 [I > 2σ(I)]. The final R1 values were 0.0296 (all data). The final wR(F²) values were 0.0774 (all data). The goodness of fit on F² was 1.032. Flack parameter = 0.08(3) (Supplementary Data Sheets S1 and S4).

Crystallographic data for compound 4: C₂₅H₃₆O₄·2H₂O, M = 438.58, monoclinic, a = 14.63410(10) Å, b = 6.02160(10) Å, c = 14.70210(10) Å, α = 90.00°, β = 111.0790(10)°, γ = 90.00°, V = 1,208.87(2) Å³, T = 100.00(1) K, space group P2₁₁, Z = 2, μ(Cu Kα) = 0.678 mm⁻¹, 25,163 reflections measured, 4,543 independent reflections (Rint = 0.0536). The final R1 values were 0.0337 [I > 2σ(I)]. The final wR(F²) values were 0.0879 [I > 2σ(I)]. The final R1 values were 0.0352 (all data). The final wR(F²) values were 0.0886 (all data). The goodness of fit on F² was 1.072. Flack parameter = 0.05(10) (Supplementary Data Sheets S2 and S4).

Crystallographic data for compound 8: C₂₃H₃₆O₄, M = 400.54, monoclinic, a = 11.94134(9) Å, b = 6.14791(4) Å, c = 15.49296(12) Å, α = 90.00°, β = 101.4154(7)°, γ = 90.00°, V = 1114.904(15) Å³, T = 100(2) K, space group P2₁, Z = 2, μ(Cu Kα) = 0.626 mm⁻¹.
23,169 reflections measured, 4,453 independent reflections ($R_{int} = 0.0341$). The final $R_1$ values were 0.0291 [$I > 2\sigma (I)$]. The final $wR(F^2)$ values were 0.0765 [$I > 2\sigma (I)$]. The final $R_1$ values were 0.0301 (all data). The final $wR(F^2)$ values were 0.0772 (all data). The goodness of fit on $F^2$ was 1.026. Flack parameter = −0.16(6) (Supplementary Data Sheets S3, S4).

**Antimicrobial Assay**

**Biological Assay Protocols**

The test strains were acquired from the ATCC: ESBL-producing *Escherichia coli* (ATCC 35218), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 15542), *Klebsiella pneumoniae* (ATCC 700603), methicillin-resistant *Staphylococcus aureus* (ATCC 43300), *Enterococcus faecalis* (ATCC 29212), and *Candida albicans* (ATCC 10231). The reference compounds for the tests were recommended by the National Committee for Clinical Laboratory Standards (Liu et al., 2019a): vancomycin (Sigma, cat #1019508), ceftriaxone (Sigma, cat #1098184), and amikacin (Sigma, St. Louis, United States; cat #861987), along with three known compounds (*Vancomycin* (C-21, δC 67.0) in 1; and (b) the disappearance of a hydroxylated methylene group (C-18) in ophiobolin Q and the double bond migrated from C-16/C-17 to C-17/C-18 in 1. These conclusions were further supported by the $^1H$–$^1H$ COSY correlations (Figure 2) of H-15 (δH 5.55)/H-16 (δH 1.71 and 2.20)/H-17 (δH 5.60)/H-18 (δH 5.59) and HMBC correlations from H-23 (δH 0.90) to C-14 (δC 52.0), C-15 (δC 33.6), and C-16 (δC 41.1) and from H-24 (δH 1.26) to C-18 (δC 140.6), C-19 (δC 71.1), and C-25 (δC 30.1). The NOESY cross-peaks (Figure 3) of H-6 (δH 3.61)/H-10 (δH 2.71)/H-14 (δH 1.84) and H-2 (δH 2.95)/H-17 (δH 1.07) indicated that H-6, H-10, and H-14 were all on the same face with α orientations, whereas H-2 and H-32 were β-oriented. However, for the configuration of C-15, it could not be defined by analysis of the NOE signals. Fortunately, a suitable crystal of 1 was obtained in MeOH–H2O (20:1, vol/vol) at room temperature and then subjected to a single-crystal X-ray diffraction experiment with Cu Kα radiation [Figure 4, Flack parameter = 0.08(3)], which enabled us to establish its absolute configuration as 2S, 6R, 10S, 11R, 14R, and 15S, along with an $E$ geometry of the Δ17,18 double bond. Accordingly, the structure of 1 was defined and named bipolatoxin A. Remarkably, our study further supports that during the cyclization of all ophiobolins AcOS favors a 1,5-H shift (C-8–C-15) to display a sodium adduct ion at m/z 423.2828 ([M+Na]$^+$, calcd for 423.2870). The 1D NMR data (Tables 1 and 3) showed close similarities to those of 1, except for the C-19 hydroxy group in 1 being replaced by a methoxy group (δC 50.5) in 2, as supported by the key HMBC correlation (Figure 2) from δH 3.14 (3H, s, OMe–19) to C-19 (δC 76.4). The close resemblance of the NOESY data (Supplementary Data Sheet S5) and experimental CD curves (Figure 5) of 1 and 2 suggested their identical absolute configuration. Accordingly, the structure of 2 was defined and named bipolatoxin B.

Compound 2 was obtained as a colorless oil, which had a molecular formula of C26H40O3, according to its HRESIMS data at m/z 423.2828 ([M+Na]$^+$, calcd for 423.2870). The 1D NMR data (Tables 1 and 3) showed close similarities to those of 1, except for the C-19 hydroxy group in 1 being replaced by a methoxy group (δC 50.5) in 2, as supported by the key HMBC correlation (Figure 2) from δH 3.14 (3H, s, OMe–19) to C-19 (δC 76.4). The close resemblance of the NOESY data (Supplementary Data Sheet S5) and experimental CD curves (Figure 5) of 1 and 2 suggested their identical absolute configuration. Accordingly, the structure of 2 was defined and named bipolatoxin B.

Compound 3 was similarly purified as a colorless oil. The HRESIMS analysis of 3 displayed a sodium adduct ion at m/z 407.2550 [M+Na]$^+$ (calcd for 407.2557), suggesting a molecular formula of C22H36O3. Subsequent comparison of the $^1H$ and $^{13}C$ NMR data (Tables 1 and 3) of 3 with those of 1 suggested that 3 contained an additional double bond (δC 121.1 and 151.2). Further analyses of the HMBC data (Figure 2) of 3
revealed that the double bond was located between C-13 and C-14, as supported by the correlations from H₃-23 (δH 1.09) to C-14 (δC 151.2) and from H-13 (δH 5.32) to C-10 (δC 52.1) and C-11 (δC 46.2). The stereochemical configuration of 3 was established to be the same as 1 by their closely resembled NOE data (Supplementary Data Sheet S5) as well as shared biogenesis. Accordingly, the structure of 3 was defined and named bipolatoxin C.

The HRESIMS ion at m/z 425.2653 ([M+Na]+, calcd for 425.2662), together with the ¹³C NMR and DEPT data for 4, revealed its molecular formula to be C₂₅H₃₈O₄, requiring an index of hydrogen deficiency of seven. The ¹H and ¹³C NMR data (Tables 1 and 3) of 4 closely resembled those of ophiobolin X (Zhu et al., 2018), indicating that both compounds shared the same A/B/C rings and corresponding substituents, with the only difference being on the side chain that the conjugated double bonds (C-16/C-17 and C-18/C-19) were replaced by an sp³ methylene (δC 41.1, C-16), a double bond between C-17 (δC 127.0) and C-18 (δC 140.4), and a hydroxylated quaternary carbon (δC 71.1, C-19). These conclusions were further confirmed by the ¹H–¹H COSY correlations of H-14/H-15 (H₃-23)/H₂-16/H-17/H-18 and an obvious HMBC correlation from H₃-24 to C-19 (Figure 2). The ROESY correlations (Figure 3) of H-5 (δH 4.99)/H-6 (δH 3.72)/H-2 (δH 2.05) and H₃-20 (δH 1.21)/H-2 (δH 2.05)/H₃-22 (δH 0.99)/H₃-23 (δH 0.84) indicated that H-10 and H-14 were α-oriented, whereas H-2, H-5, H-6, H₃-20, H₃-22, and H₃-23 were all β-oriented. After repeated recrystallization in MeOH–H₂O (20:1, vol/vol)
at room temperature, 4 furnished a crystal suitable for X-ray diffraction analysis (Figure 4). The Flack parameter of 0.05(10) allowed an unambiguous assignment of the complete absolute configurations of all chiral centers as 2S, 3R, 5S, 6S, 10S, 11R, 14R, and 15S, as well as an E geometry of the Δ 17,18 double bond. Accordingly, the structure of 4 was defined and named bipolatoxin D.

Compound 5 gave a molecular formula of C25H36O4, as assigned by a sodium adduct ion at m/z 423.2500 ([M+Na]+, calcd for 423.2506) in the HRESIMS analysis. Comparison of its 1D NMR data (Tables 2 and 3) with those of 4 indicated that the double bond from C-17/C-18 in 4 migrated to C-18/C-19 in 5, and an additional double bond (C-13, δC 125.9; C-14, δC 151.4) and an oxygenated methine (δC/H 84.6/4.23, C-12) were present, as supported by the 1H–1H COSY cross-peaks (Figure 2) of H-12 (δH 4.23)/H-13 (δH 5.31), and H2-17 (δH 1.99)/H-18 (δH 5.15), together with HMBC correlations of H2-22 (δH 0.94) with C-12 (δC 84.6) and of H-18 with C-24 (δC 17.8) and C-25 (δC 25.9). Based on the ROESY correlation (Figure 3) between H-10 (δH 2.28) and H-12, the hydroxy group at C-12 was determined to be β-oriented. The similar ROESY data (Figure 3) and experimental CD curves (Figure 5) of 4 and 5 indicated that both compounds shared the identical absolute configuration. Accordingly, the structure of 5 was defined and named bipolatoxin E.
Compound 6, obtained as a colorless oil, gave a molecular formula of C_{25}H_{36}O_{3} via its (+)-HRESIMS data (m/z 439.2453, [M+Na]^{+}, calc'd for 439.2455). The $^1$H and $^{13}$C NMR data (Tables 2 and 3) of 6 were similar to those of 21-dehydroophiobolin U (Zhu et al., 2018), differing in that a ketone carbonyl, a double bond, and an aldehyde carbon signals were absent, and two carboxy groups ($\delta_{\text{C}}$ 172.1 and 173.4) and an additional oxygenated methine ($\delta_{\text{C}}$ 80.3/3.73) were present in 6. The HMBC correlations (Figure 2) of H-6 ($\delta_{\text{H}}$ 3.83) and H-8 ($\delta_{\text{H}}$ 6.41) with C-21 ($\delta_{\text{C}}$ 173.4) and of H-24 ($\delta_{\text{H}}$ 1.82) and H-18 ($\delta_{\text{H}}$ 6.77) with C-25 ($\delta_{\text{C}}$ 172.1) suggested that two carboxy groups should be located at C-21 and C-25, respectively. The HMBC correlations from H-3 to C-1 ($\delta_{\text{C}}$ 33.8) and C-16 ($\delta_{\text{C}}$ 41.4), together with the $^1$H–$^1$H COSY cross-peaks of H-14 ($\delta_{\text{H}}$ 2.25)/H-15 ($\delta_{\text{H}}$ 1.30 and 1.41)/H-17 ($\delta_{\text{H}}$ 2.25)/H-18 ($\delta_{\text{H}}$ 6.77), suggested that a C-16–C-17 double bond in 21-dehydroophiobolin U was reduced in 6. The HMBC correlations from H-6 to C-2 ($\delta_{\text{C}}$ 155.4) and C-7 ($\delta_{\text{C}}$ 141.9) and from H-1 to C-6 ($\delta_{\text{C}}$ 61.8), C-10 ($\delta_{\text{C}}$ 47.3), and C-11 ($\delta_{\text{C}}$ 50.5) indicated that the double bond from C-2/C-6 in 21-dehydroophiobolin U migrated to C-1/C-2 in 6. The $^1$H–$^1$H COSY correlations of H-12 ($\delta_{\text{H}}$ 3.73)/H-13 ($\delta_{\text{H}}$ 1.41 and 1.72)/H-14 suggested that an oxygenated methane at $\delta_{\text{C}}$ 80.3/3.73 was located at C-12. The NOESY correlations (Figure 3) of H-6/H-32 ($\delta_{\text{H}}$ 1.08), H-12/H-10 ($\delta_{\text{H}}$ 1.90)/H-14, and H-3 ($\delta_{\text{H}}$ 2.47)/H-1 ($\delta_{\text{H}}$ 5.66) indicated that the HO-12 group and H-6 were $\beta$-oriented, whereas H-10, H-14 and H-20 were $\alpha$-oriented. Accordingly, the structure of 6 was defined and named bipolatoxin F.

Compound 7 was isolated as a colorless oil, with the molecular formula of C_{20}H_{35}O_{4}, as determined by its HRESIMS data ([M+H]+ ion peak at m/z 331.1913). Its $^1$H and $^{13}$C NMR data (Tables 2 and 3) were similar to those of momilactone A (Germain and Deslouchamps, 2002), with the replacement of an sp^3 methylene in momilactone A by an oxygenated sp^3 methine ($\delta_{\text{C}}$/$\delta_{\text{H}}$ 68.2/4.18) in 7, which was supported by analysis of its 2D NMR data (Figure 2). In the $^1$H–$^1$H COSY spectrum, the correlation of H-1 ($\delta_{\text{H}}$ 4.18)/H-2 ($\delta_{\text{H}}$ 2.41 and 2.86), together with HMBC correlations from H-5 ($\delta_{\text{H}}$ 2.52) and H-32 ($\delta_{\text{H}}$ 0.93) to C-1 ($\delta_{\text{C}}$ 68.2), suggested that a hydroxylated methine was located at C-1. In the NOESY spectrum (Figure 3), the cross-peaks of H-1/H-5/H-18 suggested that the OH-1 group should be $\beta$-oriented. Accordingly, the structure of 7 was established as shown and named 1$\beta$-hydroxy momilactone A.

The three known compounds were identified as 25-hydroxyophiobolin I (8) (Sugawara et al., 1987), ophiobolin I (9) (Sugawara et al., 1987), and ophiobolin A lactone (10) (Li et al., 1995), by comparison of their NMR data and specific rotations with literature. Remarkably, it is the first time that the absolute structure of compound 8 was defined by the single-crystal X-ray diffraction analysis (Figure 4).

### Biological Evaluation

Because of the limited amount of compound 5, only compounds 1–4 and 6–10 were evaluated for the antimicrobial activity against six drug-resistant microbial pathogens, including ESBL-producing E. coli, A. baumannii, P. aeruginosa, K. pneumoniae, methicillin-resistant S. aureus (MRSA), E. faecalis, and one fungus C. albicans. As shown in Table 4, except for compound 8, all the other test compounds showed antimicrobial activity against certain microbial pathogens (MIC = 8–64 µg/mL), of which compound 4 showed significant inhibitory activity against E. faecalis with an MIC value of 8 µg/mL, and compound 10 showed significant inhibitory activity against A. baumannii and E. faecalis with MIC values of 8 and 8 µg/mL, respectively.

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#### Table 4 | Antimicrobial activity of compounds 1–4 and 6–10.

| Compounds | Gram-negative | Gram-positive | Fungus |
|-----------|---------------|---------------|--------|
|           | Minimum inhibitory concentrations (µg/mL) | MRSA<sup>a</sup> | E. faecalis<sup>b</sup> | C. albicans<sup>c</sup> |
| **ESBL-E. coli<sup>a</sup>** | **A. baumannii<sup>b</sup>** | **P. aeruginosa<sup>c</sup>** | **K. pneumoniae<sup>d</sup>** | **MRSA<sup>e</sup>** | **E. faecalis<sup>f</sup>** | **C. albicans<sup>g</sup>** |
| 1         | ≥100          | ≥100          | 32     | ≥100          | ≥100          | ≥100          | ≥100          |
| 2         | 32            | ≥100          | 100    | ≥100          | 64            | 16            | ≥100          |
| 3         | 32            | ≥100          | 32     | ≥100          | ≥100          | 64            | 8             |
| 4         | ≥100          | ≥100          | 100    | ≥100          | ≥100          | ≥100          | ≥100          |
| 6         | 16            | 32            | 100    | ≥100          | ≥100          | ≥100          | ≥100          |
| 7         | 64            | ≥100          | 100    | ≥100          | ≥100          | ≥100          | ≥100          |
| 8         | ≥100          | ≥100          | 100    | ≥100          | ≥100          | ≥100          | ≥100          |
| 9         | 16            | 32            | ≥100   | 100           | 32            | 8             | ≥100          |
| 10        | 16            | 8             | ≥100   | 32            | 32            | 8             | ≥100          |
| Amikacin  | 4             | 2             | 2      | 2             | 2             | 2             | 2             |
| Ceftriaxone| 8             | 8             | 2      | 2             | 2             | 2             | 2             |
| Vancomycin|               |               |        |               |               | 0.5           | 0.5           |
| Fluconazole|              |               |        |               |               | 1             |               |

<sup>a</sup>ESBL-E. coli = ESBL-producing Escherichia coli ATCC 35218.  
<sup>b</sup>A. baumannii = Acinetobacter baumannii ATCC 19606.  
<sup>c</sup>P. aeruginosa = Pseudomonas aeruginosa ATCC 15542.  
<sup>d</sup>K. pneumoniae = Klebsiella pneumoniae ATCC 700603.  
<sup>e</sup>MRSA = methicillin-resistant Staphylococcus aureus ATCC 43300.  
<sup>f</sup>E. faecalis = Enterococcus faecalis ATCC 29212.  
<sup>g</sup>C. albicans = Candida albicans ATCC 10231.
CONCLUSION

A total of 10 secondary metabolites (1–10), incorporating six new ophiobolin-type sesterterpenes (1–6) and one new pimarane-type diterpene (7), were isolated and identified from the solid cultures of fungus Bipolaris species TJ403-B1. The antimicrobial activity assay revealed that compound 4 showed significant inhibitory activity against E. faecalis with an MIC value of 8 μg/mL, and 10 showed significant inhibitory activity against A.baumannii and E. faecalis with MIC values of 8 and 8 μg/mL, respectively. Our current work not only replenishes new members to ophiobolin-type sesterterpenes, but also furnishes potential antimicrobial lead compounds that are necessary to check further for their synergistic and efflux pump inhibition properties.

DATA AVAILABILITY STATEMENT

The crystallographic data for these structures were deposited in the Cambridge Crystallographic Data Centre (CCDC 1971181 for 1, CCDC 1913829 for 4, and CCDC 1913832 for 8). Direct links: 1. https://www.ccdc.cam.ac.uk/structures/search?access=referee&searchdepnums=1971181&searchauthor=Zhengxi; 2. https://www.ccdc.cam.ac.uk/structures/search?access=referee&searchdepnums=1913829&searchauthor=hu; 3. http://www.ccdc.cam.ac.uk/services/structures?access=referee&searchdepnums=1913832&searchauthor=hu.

AUTHOR CONTRIBUTIONS

LS and ML contributed to the extraction, isolation, identification, and manuscript preparation. YH contributed to the antimicrobial activity test. WA contributed to the fungal isolation and fermentation. HL, SL, CC, and JW advised and assisted Shen’s experiments. ZH guided Shen’s experiments and wrote the manuscript. YZ designed the experiments and revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.00856/full#supplementary-material

DATA SHEET S1 | CheckCIF report of 1.
DATA SHEET S2 | CheckCIF report of 4.
DATA SHEET S3 | CheckCIF report of 8.
DATA SHEET S4 | X-ray crystallographic data (CIFs) of 1, 4, and 8.
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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