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Bipolarins A–H, eight new ophiobolin-type sesterterpenes with antimicrobial activity from fungus *Bipolaris* sp. TJ403-B1

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[ABSTRACT] Bipolarins A–H (**1**–**8**), eight new tetracyclic ophiobolin-type sesterterpenes featuring a rare oxaspiro[4.4]nonane moiety, were isolated from cultures of fungus *Bipolaris* sp. TJ403-B1. Their structures and absolute configurations were elucidated by comprehensive spectroscopic analyses, single-crystal X-ray diffraction experiments, electronic circular dichroism and ^{13}C NMR calculations. Additionally, compound **5** exhibited significant selective antimicrobial activity against *Enterococcus faecalis* with an MIC value 8 μg·mL^{-1}.

[KEY WORDS] *Bipolaris* sp.; Ophiobolins; Sesterterpenes; Antimicrobial activity

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Introduction

Sesterterpenes, which are derived from five isoprene units, represent a relatively rare subclass of terpenes. One impressive member of this subclass is ophiobolins, characterized by an unusual 5-8-5 fused tricyclic or 5-8-5-5 spiro-fused tetracyclic skeleton. Since ophiobolin A was firstly identified from *Ophiobolus miyabeanus* in 1958 [1], ophiobolin-type sesterterpenes have received much attention from synthetic and biosynthetic purposes because of their intriguing molecular architectures and broad spectrum of bioactivities [2-3]. For example, ophiobolin A inhibited calmodulin-activated cyclic nucleotide phosphodiesterase [4] and induced rhabdomyosarcoma cancer (RD) cell line apoptosis [5]; ophiobolin K exhibited cytotoxicity against various tumor cell lines [6], including adriamycin-resistant mouse leukemia cells, with IC_{50} values ranging from 0.27 to 0.65 μmol·L^{-1}, which indicated the promising potentials of ophiobolins for biological and pharmaceutical uses. Up to now, more than 50 naturally occurring ophiobolins have been isolated and characterized from fungal genera *Aspergillus* [7], *Emericella* [6], *Drechslera* [8-9], *Ulocladium* [10], and *Bipolaris* [11-13].

In our continuing researches with the aim of discovering new secondary metabolites with diverse structures and bioactivities from fungi [14-17], the cultures of *Bipolaris* sp. TJ403-B1 fermented on rice media were investigated, which led to the isolation of seven ophiobolin-derived sesterterpenes with diverse new skeletons [18]. To discover more structurally intriguing and bioactive natural products from this fungus, we performed a further chemical investigation on the ethyl acetate extract to afford eight new tetracyclic ophiobolin-type sesterterpenes featuring a rare oxaspiro[4.4]nonane moiety, termed as bipolarins A–H (**1**–**8**). In this study, the isolation and structure elucidation as well as the antimicrobial activity against seven microbial pathogens (ESBL-producing *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*,

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Dedicated to Professor SUN Han-Dong on the Occasion of His 80th Birthday

Klebsiella pneumoniae, methicillin-resistant *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans*) of these ophiobolins are described.

Results and Discussion

Compound **1** had the molecular formula $C_{25}H_{34}O_5$, based upon the HRESI-MS analysis as well as the ^{13}C NMR and DEPT data. The 1D NMR data (Tables 1 and 3) of **1** showed close structural resemblances to those of 8-deoxyophiobolin J [19], except for the presence of a ketone carbonyl (δ_C 211.3) and a hydroxylated methylene (δ_C 68.0) in **1**. A hydroxyl group was located at C-25 based on the HMBC correlation (Fig. 2) from H_3-24 (δ_H 1.68) to C-25 (δ_C 68.0) and the NOESY correlation (Fig. 3) between H-18 (δ_H 5.47) and H_2-25 (δ_H 3.93). The HMBC correlation from H_2-21 (δ_H 4.68 and 5.12) to C-8 (δ_C 211.3) suggested that a ketone carbonyl should be located at C-8. The NOESY cross-peaks of H-2 (δ_H 3.06)/H_3-22 (δ_H 0.98)/H-9β (δ_H 2.51)/H-15 (δ_H 2.22)/H-9α (δ_H 2.85) and H-17 (δ_H 4.72)/H_3-24 (δ_H 1.68) indicated that H-2, H-17, and H_3-22 were all β-oriented, while H-15 was α-oriented. However, no useful NOE signals for assigning the configuration of C-10 could be observed. Finally, a crystal of **1** suitable for X-ray diffraction crystallographic analysis was furnished (Fig. 4), and the absolute configuration of **1** was unequivocally confirmed as 2*R*, 10*R*, 11*R*, 14*S*, 15*S*, 17*R* with a Flack parameter of 0.04(3). Accordingly, the structure of **1** was defined and named bipolarin A.

Compound **2** was obtained as a yellow oil, and its molecular formula $C_{25}H_{34}O_5$ was deduced based upon the HRESI-MS analysis and ^{13}C NMR data. The 1D NMR data (Tables 1 and 3) showed close similarities to those of the known compound ophiobolin I [20], except for a methyl group in ophiobolin I being replaced by a carboxyl group (δ_C 174.7) in **2**. The carboxyl group was located at C-25 in **2**, based on the HMBC correlation (Fig. 2) from H_3-24 (δ_H 1.82) to C-25 (δ_C 174.7). Additionally, the C-18/C-19 double bond was determined as having an *E*-geometry, judging from the ROESY correlations (Fig. 3) of H-17 (δ_H 4.72)/H_3-24 (δ_H 1.82) and H-16β (δ_H 1.93)/H-18 (δ_H 6.57). The key ROESY correlations of H-2 (δ_H 2.91)/H_3-22 (δ_H 1.07)/H-15 (δ_H 2.33) and H-6 (δ_H 3.51)/H-10 (δ_H 2.61) suggested that H-6, H-10, and H-15 should be on the same side with α-orientations, while H-2, H-17, and H_3-22 were all β-oriented. Accordingly, the structure of **2** was defined and named bipolarin B.

Table 1 ^{1}H NMR data for compounds 1–4 in CD_3OD (δ in ppm, *J* in Hz)

Position	**1**[a, c]	**2**[b, c]	**3**[a, c]	**4**[a, c]
1	1.12 m; 2.04 dd (1.8, 13.9)	1.33 t (13.1); 2.09 m	1.50 m; 2.12 m	1.67 m; 1.76 m
2	3.06 d (11.9)	2.91 d (12.9)	2.93 m	1.76 m
3	-	-	-	-
4	6.06 m	5.93 s	5.94 m	2.14 d (14.3); 2.28 d (14.3)
5	-	-	-	-
6	-	3.51 d (3.2)	3.66 d (3.7)	3.32 m
7	-	-	-	-
8	-	5.72 d (5.0)	5.94 m	6.97 m
9	2.51 m; 2.85 m	2.11 m; 2.50 d (18.3)	4.20 m	2.29 m; 2.57 m
10	2.51 m	2.61 dd (3.6, 14.6)	2.64 dd (2.7, 11.5)	2.42 dd (2.3, 13.9)
11	-	-	-	-
12	1.47 m; 1.66 m	1.48 m; 1.77 m	1.53 m; 1.73 m	1.45 m; 1.76 m
13	1.60 m; 1.79 m	1.60 dd (5.1, 13.1); 1.77 m	1.57 m; 1.85 m	1.65 m; 1.77 m
14	-	-	-	-
15	2.22 q (7.0)	2.33 q (7.1)	2.88 m	2.27 m
16	1.86 m	1.93 m	1.86 m; 1.93 m	1.84 t (7.1)
17	4.72 m	4.72 q (7.4)	4.60 dt (5.3, 8.2)	4.65 dt (7.0, 8.6)
18	5.47 dd (1.4, 8.5)	6.57 d (8.1)	5.26 dt (1.4, 8.7)	5.19 dt (1.4, 8.5)
19	-	-	-	-
20	2.12 s	2.10 s	2.09 s	1.26 s
21	4.68 d (16.3); 5.12 d (16.3)	3.91 d (12.3); 4.28 d (12.3)	4.00 dd (1.3, 13.2); 4.22 m	-
22	0.98 s	1.07 s	1.07 s	0.86 s
23	1.07 d (6.9)	1.08 d (7.0)	1.12 d (6.9)	1.05 d (6.9)
24	1.68 d (1.4)	1.82 s	1.66 d (1.3)	1.68 d (1.3)
25	3.93 s	-	1.69 d (1.0)	1.71 d (1.4)

[a]Recorded at 400 MHz; [b]Recorded at 600 MHz; [c]"m" means overlapped or multiplet with other signals

Table 2 ^{1}H NMR data of compounds 5–8 in CD_3OD (δ in ppm, *J* in Hz)

Position	**5**[b, d]	**6**[c, d]	**7**[b, d]	**8**[a, d]
1	2.29 d (12.4); 2.64 d (12.4)	2.30 d (12.4); 2.63 d (12.4)	1.32 d (15.0); 1.97 d (15.0)	1.23 m; 2.10 dd (1.7, 13.5)
2	-	-	-	2.94 d (12.1)
3	2.98 m	2.99 t (7.1)	2.47 m	-
4	2.07 d (19.4); 2.72 dd (6.3, 18.7)	2.07 m; 2.72 dd (6.3, 18.7)	1.74 m; 1.87 m	5.86 s
5	-	-	4.55 dt (4.1, 9.2)	-
6	-	-	3.05 d (9.2)	2.15 m
7	-	-	-	1.90 m
8	6.06 t (7.4)	6.07 t (7.9)	6.00 t (8.1)	1.84 m; 1.96 m
9	1.71 m; 2.07 m	1.71 m; 2.06 m	2.09 m; 2.21 m	1.34 m
10	1.70 m	1.70 m	1.68 m	1.95 m
11	-	-	-	-
12	1.54 m; 1.68 m	1.55 t (9.6); 1.68 m	1.39 m; 1.58 m	1.40 m; 1.60 m
13	1.69 m; 2.23 m	1.69 m; 2.24 m	1.51 m; 2.04 m	1.53 m; 1.76 m
14	-	-	-	-
15	2.14 m	2.12 m	2.23 m	2.30 q (6.8)
16	1.76 m; 1.85 m	1.72 m; 1.82 dt (8.7, 12.3)	1.73 m; 1.79 m	1.76 m; 1.84 m
17	4.56 dt (5.8, 8.5)	4.50 m	4.47 dt (6.2, 8.3)	4.64 q (7.4)
18	5.45 dq (1.5, 8.4)	5.17 d (8.4)	5.17 dt (1.4, 8.5)	5.23 dt (1.5, 8.6)
19	-	-	-	-
20	1.24 d (7.2)	1.24 d (7.2)	0.91 d (6.8)	2.14 s
21	4.06 d (12.6); 4.28 d (12.6)	4.07 d (12.6); 4.28 d (12.6)	3.94 d (11.6); 4.24 d (11.6)	3.60 dd (8.7, 10.5); 4.14 dd (5.6, 10.5)
22	0.82 s	0.81 s	0.99 s	1.10 s
23	1.07 d (7.2)	1.06 d (7.2)	1.07 d (7.1)	1.04 d (7.0)
24	1.71 s	1.70 s	1.70 d (1.4)	1.69 d (1.2)
25	3.95 m	1.75 s	1.74 d (1.3)	1.74 d (1.4)

[a]Recorded at 400 MHz; [b]Recorded at 600 MHz; [c]Recorded at 800 MHz; [d]"m" means overlapped or multiplet with other signals

Table 3 ^{13}C NMR data of compounds 1–8 in CD_3OD (δ in ppm)

Position	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[b]	**6**[c]	**7**[b]	**8**[a]
1	49.9 CH_2	48.4 CH_2	49.1 CH_2	44.1 CH_2	41.5 CH_2	41.5 CH_2	48.9 CH_2	49.7 CH_2
2	45.6 CH	52.6 CH	50.4 CH	54.2 CH	183.8 C	183.8 C	84.9 C	47.1 CH
3	179.7 C	183.0 C	183.0 C	80.8 C	40.2 CH	40.2 CH	40.8 CH	184.2 C
4	132.5 CH	130.8 CH	130.8 CH	54.1 CH_2	44.7 CH_2	44.7 CH_2	40.8 CH_2	129.4 CH
5	198.6 C	211.8 C	210.6 C	217.0 C	210.6 C	210.6 C	72.0 CH	214.2 C
6	134.4 C	53.2 CH	52.9 CH	53.8 CH	139.7 C	139.7 C	57.6 CH	57.9 CH
7	139.4 C	135.9 C	139.6 C	130.1 C	135.4 C	135.4 C	138.3 C	40.8 CH
8	211.3 C	129.1 CH	130.2 CH	142.6 CH	133.7 CH	133.8 CH	135.8 CH	26.1 CH_2
9	44.8 CH_2	29.1 CH_2	69.0 CH	29.9 CH_2	22.6 CH_2	22.6 CH_2	23.1 CH_2	21.6 CH_2
10	52.6 CH	54.7 CH	61.8 CH	52.7 CH	61.1 CH	61.1 CH	64.3 CH	53.0 CH
11	43.8 C	43.5 C	42.5 C	42.3 C	41.7 C	41.7 C	44.2 C	42.6 C
12	42.4 CH_2	42.6 CH_2	43.6 CH_2	43.1 CH_2	40.1 CH_2	40.1 CH_2	43.3 CH_2	42.7 CH_2
13	31.1 CH_2	31.6 CH_2	31.4 CH_2	31.7 CH_2	32.9 CH_2	32.9 CH_2	30.7 CH_2	31.9 CH_2
14	97.6 C	99.0 C	99.4 C	97.8 C	95.5 C	95.3 C	97.5 C	98.0 C
15	37.5 CH	36.5 CH	34.8 CH	36.7 CH	38.0 CH	38.0 CH	37.8 CH	36.2 CH
16	42.7 CH_2	42.5 CH_2	43.0 CH_2	42.9 CH_2	44.0 CH_2	44.1 CH_2	43.8 CH_2	43.1 CH_2

Continued

Position	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[b]	**6**[c]	**7**[b]	**8**[a]
17	73.2 CH	73.4 CH	73.2 CH	73.4 CH	72.2 CH	72.5 CH	72.3 CH	73.5 CH
18	127.6 CH	141.3 CH	128.3 CH	128.2 CH	126.5 CH	126.8 CH	127.0 CH	128.0 CH
19	139.0 C	132.4 C	135.4 C	135.9 C	139.5 C	136.9 C	136.7 C	136.0 C
20	17.3 CH_3	17.5 CH_3	17.2 CH_3	25.2 CH_3	19.5 CH_3	19.5 CH_3	13.1 CH_3	17.8 CH_3
21	59.2 CH_2	66.8 CH_2	65.5 CH_2	173.1 C	65.5 CH_2	65.5 CH_2	65.2 CH_2	64.7 CH_2
22	21.2 CH_3	22.6 CH_3	22.5 CH_3	23.2 CH_3	18.6 CH_3	18.6 CH_3	18.1 CH_3	22.4 CH_3
23	16.3 CH_3	16.3 CH_3	16.6 CH_3	16.2 CH_3	18.3 CH_3	18.4 CH_3	18.2 CH_3	16.5 CH_3
24	13.9 CH_3	13.5 CH_3	18.0 CH_3	18.1 CH_3	14.0 CH_3	18.3 CH_3	18.2 CH_3	18.1 CH_3
25	68.0 CH_2	174.7 C	25.9 CH_3	25.9 CH_3	68.0 CH_2	26.0 CH_2	26.0 CH_3	26.0 CH_3

[a]Recorded at 100 MHz in CD_3OD; [b]Recorded at 150 MHz in CD_3OD; [c]Recorded at 200 MHz in CD_3OD

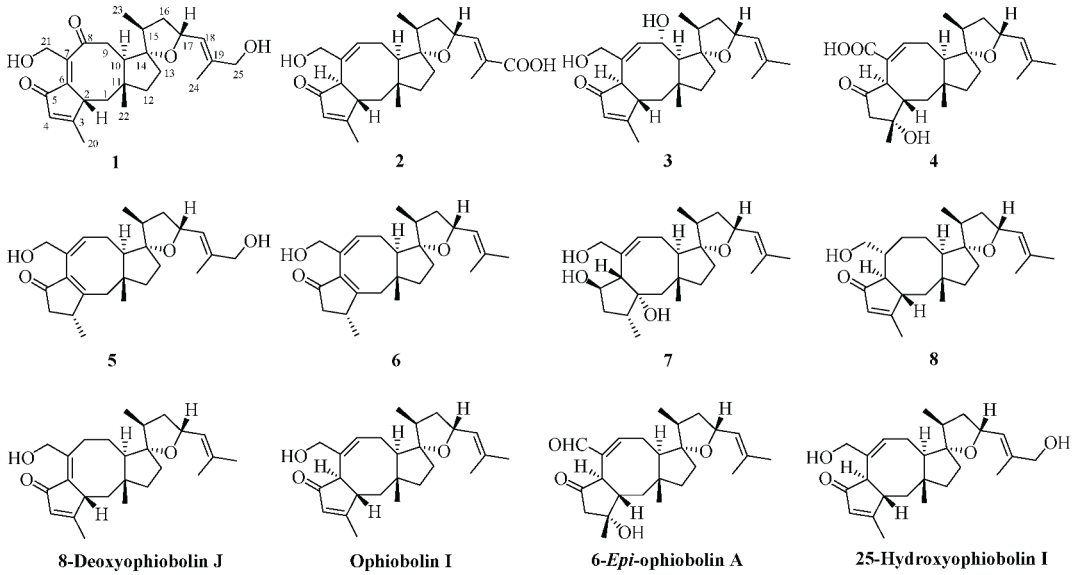

Fig. 1 Chemical structures of new compounds 1–8 and known structures 8-deoxyophiobolin J, ophiobolin I, 6-*epi*-ophiobolin A, and 25-hydroxyophiobolin I

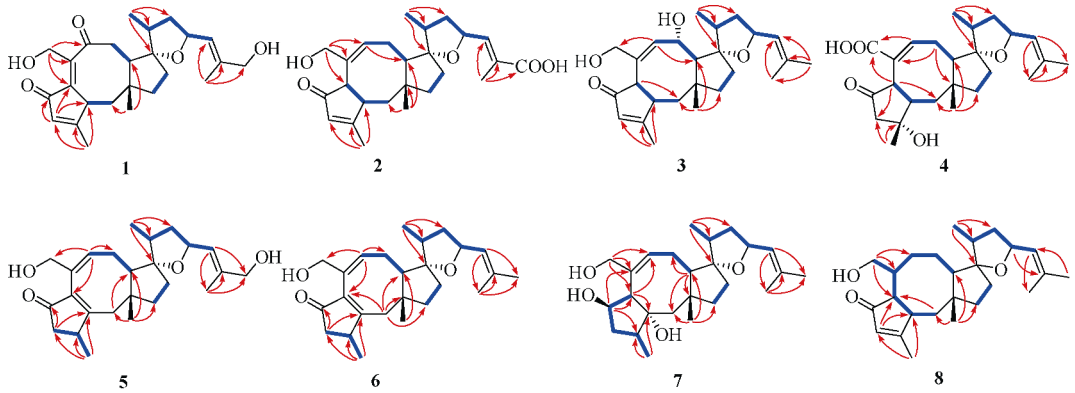

Fig. 2 Selected ^1H–^1H COSY (blue bold lines) and HMBC (red arrows) correlations of 1–8

Compound **3**, obtained as a colorless oil, gave the molecular formula $C_{25}H_{36}O_4$, based on a sodium adduct ion at *m/z* 423.2489 (Calcd. for 423.2506) from the HRESI-MS analysis. Comparison of the ^1H and ^{13}C NMR data (Tables 1 and 3) of **3** with the known compound ophiobolin I [20] revealed that they were structural analogues, with the exception that a methylene group in ophiobolin I was replaced by a hydroxylated methine group (δ_C 69.0, C-9) in **3**. This conclusion was further corroborated by the ^1H–^1H COSY correlations (Fig. 2) of H-8 (δ_H 5.94)/H-9 (δ_H 4.20)/H-10 (δ_H 2.64).

Based upon the NOESY correlation (Fig. 3) between H-9 (δ_H 4.20) and H_3-22 (δ_H 1.07), the HO-9 group was deduced as *α*-oriented. Accordingly, the structure of **3** was defined and named bipolarin C.

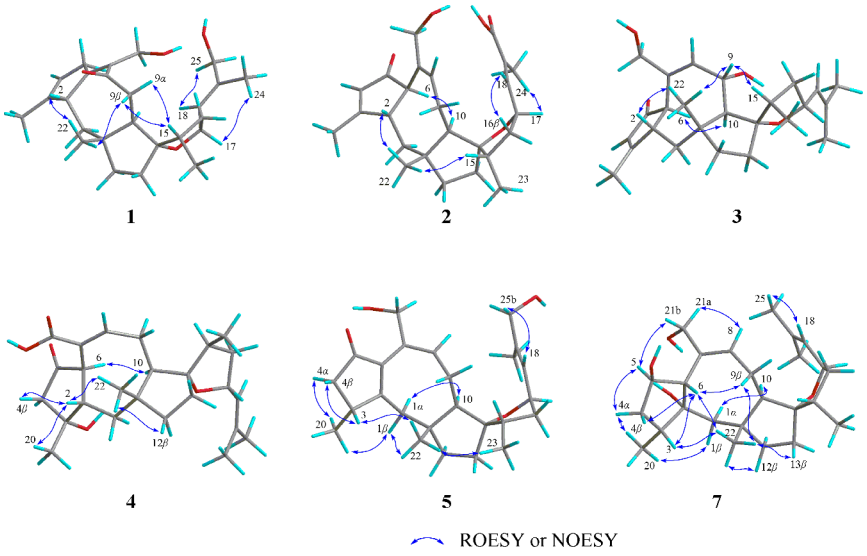

Fig. 3 Selected ROESY/NOESY correlations of 1–5 and 7

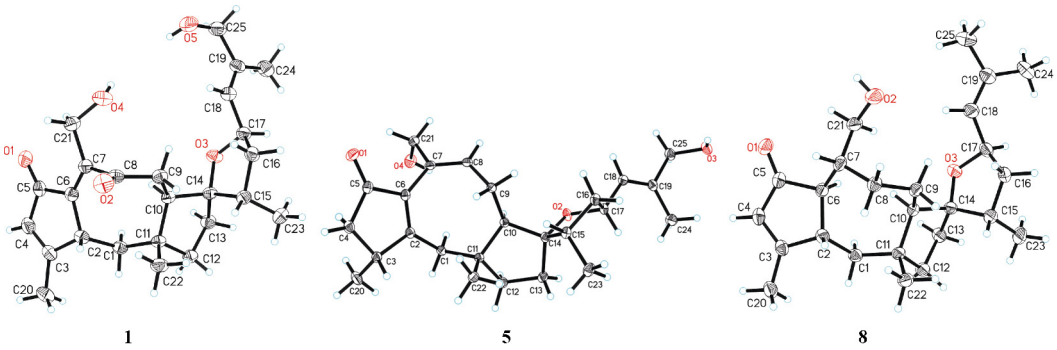

Fig. 4 X-ray crystallographic structures of 1, 5 and 8

Compound **4** was isolated as a colorless oil with a molecular formula of $C_{25}H_{36}O_5$, as established *via* the (+)-HRESI-MS data (*m/z* 439.2456, $[M + Na]^+$, Calcd. for 439.2455). Its 1D NMR data (Tables 1 and 3) were closely related to those of the known compound 6-*epi*-ophiobolin A [20], except that an aldehyde carbonyl was absent and a carboxyl group (δ_C 173.1, C-21) was evident in **4**, as supported by the HMBC correlations (Fig. 2) from H-6 (δ_H 3.32) and H-8 (δ_H 6.97) to C-21 (δ_C 173.1). The similarities of their NOESY data suggested that **4** and 6-*epi*-ophiobolin A possessed identical relative configurations. Accordingly, the structure of **4** was defined and named bipolarin D.

Compound **5** was isolated as colorless crystals, displaying an $[M + Na]^+$ ion at *m/z* 423.2515 (Calcd. for 423.2506) in the HRESI-MS data, which correlated to a molecular formula of $C_{25}H_{36}O_4$. The ^1H, ^{13}C, and DEPT NMR data (Tables 2 and 3) were similar to those of 25-hydroxyophiobolin I [20]. The difference was that the C-3–C-4 double bond of 25-hydroxyophiobolin I flipped to a C-2–C-6 double bond in **5**, as supported by the HMBC correlations (Fig. 2) from H-8 (δ_H 6.06) to C-6 (δ_C 139.7) and from H_3-20 (δ_H 1.24) to C-2 (δ_C 183.8), C-3 (δ_C 40.2), and C-4 (δ_C 44.7). The ROESY correlation (Fig. 3) between H-3 (δ_H 2.98) and H_3-22 (δ_H 0.82) suggested that the H_3-20 was *α*-oriented in **5**. To determine its absolute configuration, the quantum mechanical calculation of electronic circular dichroism (ECD) spectrum of **5** was performed using the time-dependent density functional theory method. The calculated ECD spectrum obtained at the B3LYP/6-31G(d) level was identical with the experimental one (Fig. 5), suggesting the absolute configuration of **5** to be 3*R*, 10*R*, 11*R*, 14*R*, 15*S*, 17*R*. Fortunately, a crystal suitable for X-ray diffraction crystallographic analysis (Fig. 4) of **5** was performed by using Cu K*α* radiation, which further confirmed its absolute configuration with a Flack parameter of 0.02(7). Accordingly, the structure of **5** was defined and named bipolarin E.

Compound **6** was purified as a colorless oil, giving the molecular formula $C_{25}H_{36}O_3$, based upon the ^{13}C NMR and HRESI-MS data (*m/z* 407.2555, $[M + Na]^+$, Calcd. for 407.2557). The 1D NMR data (Tables 2 and 3) showed close

similarities to those of **5**, except for a hydroxylated methylene (δ_C 68.0) in **5** being replaced by methyl at C-25 (δ_C 26.0) in **6**. This conclusion was further corroborated by the HMBC correlations (Fig. 2) from H-18 (δ_H 5.17) and H_3-24 (δ_H 1.70) to C-25 (δ_C 26.0). Similar ROESY data showed that **6** possessed the same relative configuration as that of **5**. Finally, the absolute configuration of **6** (3*R*, 10*R*, 11*R*, 14*R*, 15*S*, 17*R*) was identified by comparison of its experimental electronic circular dichroism (ECD) curve with that of **5** (Fig. 4). Accordingly, the structure of **6** was defined and named bipolarin F.

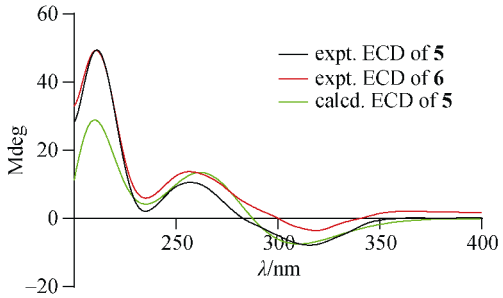

Fig. 5 Experimental ECD spectra of 5–6 and calculated ECD spectrum of 5

The molecular formula of compound **7** was assigned as $C_{25}H_{40}O_4$, based upon the (+)-HRESI-MS sodium adduct ion at *m/z* 427.2807 ([M + Na]$^+$, Calcd. for 427.2819). The 1D and 2D NMR data (Tables 2 and 3) showed that **7** was structurally similar to **6**, with the exception that a double bond and a ketone carbonyl were absent and an additional oxygenated methine ($\delta_{C/H}$ 72.0/4.55, C-5) and an oxygenated nonprotonated carbon signal (δ_C 84.9, C-2) were present in **7**, as inferred by the HMBC correlations (Fig. 2) from H_3-20 (δ_H 0.91) to C-2 (δ_C 84.9) and from H-5 (δ_H 4.55) to C-3 (δ_C 40.8), C-6 (δ_C 57.6), and C-7 (δ_C 138.3). The key ROESY correlations of H_3-22 (δ_H 0.99)/H-3 (δ_H 2.47)/H-6 (δ_H 3.05) confirmed that H_3-20 and HO-2 were *α*-oriented, while H-6 was *β*-oriented. The ROESY correlations (Fig. 3) of H-21b (δ_H 4.24)/H-5 (δ_H 4.55)/H-4*α* (δ_H 1.87)/H_3-20 (δ_H 0.91) indicated that HO-5 group was *β*-oriented. Therefore, the relative structure of **7** was determined. On this basic, the density functional theory (DFT) was used to calculate the ^{13}C NMR chemical shifts of **7** at the B3LYP/6-31G(d) level in MeOH. Accordingly, the correlation coefficient (R^2) for **7** was 0.9981 (Fig. 6), and the corresponding mean absolute error (MAE) and corrected mean absolute error (CMAE) were 1.5 and 1.5 ppm, respectively, which further supported our proposed relative structure. Accordingly, the structure of **7** was defined and named bipolarin G.

Compound **8** was also isolated as colorless crystals, displaying an [M + Na]$^+$ ion at *m/z* 409.2715 (Calcd. for 409.2713) in the HRESI-MS data, which correlated to a molecular formula of $C_{25}H_{38}O_3$. By comparing the NMR data (Tables 2 and 3) of **8** with those of ophiobolin I [20], both compounds were found to have the same carbon backbones. Exhaustive interpretation of the ^{1}H and ^{13}C NMR spectra of **8** revealed that the signals for the double bond at C-7 and C-8 in ophiobolin I were missing, and two sp^3 carbon signals resonating at δ_C 40.8 (CH, C-7) and 26.1 (CH_2, C-8) ppm were observed for **8**. This conclusion was further supported by the HMBC correlations (Fig. 2) from H_2-21 (δ_H 3.60 and 4.14) to C-6 (δ_C 57.9), C-7 (δ_C 40.8), and C-8 (δ_C 26.1). However, no useful NOE signals for assigning the configuration of C-7 could be observed. Finally, a single-crystal X-ray diffraction using Cu K*α* radiation was performed, and the absolute configuration of **8** was unequivocally confirmed as 2*S*, 6*R*, 7*R*, 10*R*, 11*R*, 14*S*, 15*S*, 17*R* with a Flack parameter of 0.09(5) (Fig. 4). Accordingly, the structure of **8** was defined and named bipolarin H.

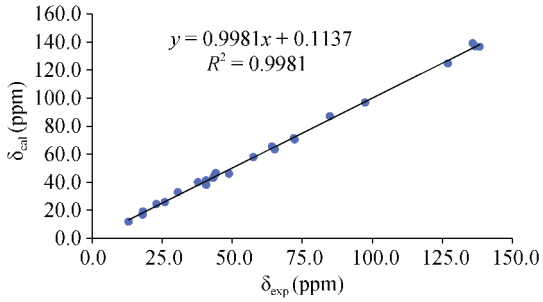

Fig. 6 Linear correlation between the experimental and calculated ^{13}C NMR chemical shifts for 7

In this study, due to the limited amount of **7**, all other compounds **1**–**6** and **8** were evaluated for their antimicrobial activities against seven microbial pathogens including six drug-resistant bacteria (ESBL-producing *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus*, *Enterococcus faecalis*) and one fungus (*Candida albicans*). As a result, compound **5** showed significant inhibitory activity against *Enterococcus faecalis* with an MIC value 8 μg·mL^{-1} (Table 4). Compound **5** further exhibited moderate antimicrobial activity against *Pseudomonas aeruginosa* with an MIC value of 32 μg·mL^{-1}, while compound **6** was active against fungus *Candida albicans* with an MIC value of 32 μg·mL^{-1}. Moreover, compounds **2**, **4** and **6** showed weak inhibitory activity against methicillin-resistant *Staphylococcus aureus* with MIC values of 64 μg·mL^{-1}. This study might provide new chemicals for the exploitation of new antimicrobial agents.

Experimental

General experimental procedures

Optical rotations were measured by using a PerkinElmer 341 instrument. UV and FT-IR spectra were recorded by using a Varian Cary 50 and a Bruker Vertex 70 instrument, respectively. ECD spectra were obtained with a JASCO J-810 spectrometer. High-resolution electrospray ionization mass spectrometry (HRESI-MS) were performed by using the positive ion mode with a Thermo Fisher LC-LTQ-Orbitrap XL

Table 4 ***In vitro*** **antimicrobial activities of compounds 1–6 and 8**

Compounds	MIC (μg·mL^{-1})						
	Gram-negative				Gram-positive		Fungus
	[a]ESBL-*E. coli*	[b]*A. baumannii*	[c]*P. aeruginosa*	[d]*K. pneumoniae*	[e]*S. aureus*	[f]*E. faecalis*	[g]*C. albicans*
1	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100
2	≥ 100	≥ 100	≥ 100	≥ 100	64	≥ 100	≥ 100
3	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	64	≥ 100
4	≥ 100	≥ 100	≥ 100	≥ 100	64	≥ 100	≥ 100
5	≥ 100	≥ 100	32	≥ 100	≥ 100	8	≥ 100
6	≥ 100	≥ 100	≥ 100	≥ 100	64	≥ 100	32
8	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100
Amikacin	4	2	2	8	-	-	-
Ceftriaxone	8	8	2	2	-	-	-
Vancomycin	-	-	-	-	0.5	0.5	-
fluconazole	-	-	-	-	-	-	1

[a]ESBL-*E. coli*: ESBL-producing *Escherichia coli* ATCC 35218; [b]*A. baumannii*: *Acinetobacter baumannii* ATCC 19606; [c]*P. aeruginosa*: *Pseudomonas aeruginosa* ATCC 15542; [d]*K. pneumoniae*: *Klebsiella pneumoniae* ATCC 700603; [e]MRSA: methicillin-resistant *Staphylococcus aureus* ATCC 43300; [f]*E. faecalis*: *Enterococcus faecalis* ATCC 29212; [g]*C. albicans*: *Candida albicans* ATCC 10231

spectrometer. The 1D (^{1}H, ^{13}C, and DEPT) and 2D (HSQC, HMBC, ^{1}H–^{1}H COSY, and ROESY/NOESY) NMR spectra were carried out by using Bruker AM-400, DRX-600, and Bruker AM-800 instruments with tetramethylsilane as an internal standard, and the chemical shifts (δ) were expressed in ppm and referenced to the solvent signals. Column chromatography (CC) was carried out with silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), octadecylsilyl (ODS, 50 μm, YMC Co., Ltd., Tokyo, Japan), and Sephadex LH-20 (40−70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden). Semi-preparative HPLC was performed using an Agilent 1100 liquid chromatograph with a reversed-phase (RP) C_{18} column (5 μm, 10 mm × 250 mm, Welch Materials, Inc.). Thin-layer chromatography (TLC) was performed with silica gel 60 F_{254} (Yantai Chemical Industry Research Institute). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Fungal material

The fungal strain *Bipolaris* sp. TJ403-B1 was isolated from the leaves of wheat, which was collected from Wuhan City of Hubei Province, China, in May 2016. The sequence data for this fungal strain have been submitted to GenBank with accession No. MH545913. The fungal strain was preserved in the culture collection center of Tongji Medical College, Huazhong University of Science and Technology.

Extraction and isolation

The endophytic fungus *Bipolaris* sp. was cultivated on potato dextrose agar (PDA) at 28 °C for 5 days to prepare the seed cultures. Agar plugs were inoculated into 450 × 1 L Erlenmeyer flasks, previously sterilized by autoclaving, each containing 250 g of rice and 250 mL of distilled water. All flasks were incubated at 28 °C for 4 weeks. The fermented rice substrate was extracted for five times with 95% aqueous EtOH at room temperature, and the solvent was evaporated under vacuum to afford a residue. The residue was suspended in water, and then extracted exhaustively with EtOAc and *n*-BuOH, respectively. The EtOAc residue (300 g) was chromatographed by an RP-C_{18} silica gel column with a step-wise gradient of MeOH/H_2O (20 : 80 to 100 : 0) to afford five fractions A−E.

Fraction C (40 g) was subjected to a silica gel column with a stepwise gradient elution of petroleum ether/ethyl acetate (10 : 0 to 0 : 1) to afford eight fractions C1−C8. Fraction C2 (6 g) was subject to Sephadex LH-20 eluted with CH_2Cl_2/MeOH (*V*/*V*, 1 : 1), and further purified by an RP-C_{18} silica gel column (MeOH/H_2O, from 40 : 60 to 60 : 40, *V*/*V*) to obtain fractions C2.1−C2.5. Fraction C2.3 (970 mg) was chromatographed on a silica gel column eluted with CH_2Cl_2/MeOH (1 : 0 to 100 : 1) to afford five fractions C2.3.1−C2.3.5. Fraction C2.3.3 was further subjected to semi-preparative HPLC (CH_3CN/H_2O, 75 : 25, 3.0 mL·min^{-1}) to yield compound **6** (t_R 20.5 min, 4.3 mg). Fraction C2.3.4was further subjected to semi-preparative HPLC (CH_3CN/H_2O, 75 : 25, 3.0 mL·min^{-1}) to yield **8** (t_R 31.2 min, 2.7 mg). Fraction C4 (11.3 g) was chromatographed by Sephadex LH-20 eluted with CH_2Cl_2/MeOH (*V*/*V*, 1 : 1), and further purified by an RP-C_{18} silica gel column with a step-wise gradient of MeOH/H_2O (40 : 60 to 60 : 40) to obtain fractions C4.1−C4.12. Compound **1** (t_R 17.2 min, 25.8 mg) was isolated by semi-preparative HPLC (MeOH/H_2O, 65 : 35, *V*/*V*, 3.0 mL·min^{-1}) from fraction C4.1. Fraction C4.2 (900 mg) was chromatographed on a silica gel column eluted with CH_2Cl_2/MeOH (1 : 0 to 60 : 1) to afford fractions C4.2.1−C4.2.3. Fraction C4.2.3 was further subjected to semi-preparative HPLC (MeOH/H_2O, 70 : 30, 3.0 mL·min^{-1}) to yield compound **2** (t_R 23.2 min, 3.4 mg). Repeated purification of fraction C4.4 (1.1 g) using Sephadex LH-20 (CH_2Cl_2/MeOH, 1 : 1, *V*/*V*),

silica gel CC (CH_2Cl_2/ MeOH, 500 : 1−60 : 1, *V/V*), and semi-preparative HPLC (MeOH/H_2O, 68 : 32, *V/V*, 3.0 mL·min^{-1}) afforded compound **3** (t_R 46.6 min, 4.5 mg). Fraction C4.5 (3.0 g) was subjected to silica gel CC eluted with stepwise CH_2Cl_2/MeOH (1 : 0−100 : 1) to afford nine fractions (C4.5.1−C4.5.9). Repeated purification of fraction C4.5.3 by semi-preparative HPLC using CH_3CN/H_2O (80 : 20, *V/V*, 3.0 mL·min^{-1}) afforded compounds **4** (t_R 13.7 min, 17.3 mg). Fraction C4.5.7 (280 mg) was separated through Sephadex LH-20 eluted with CH_2Cl_2/MeOH (1 : 1, *V/V*) two fractions (C4.5.7a−C4.5.7b). Compound **7** (t_R 30.9 min, 2.1 mg) was purified by semi-preparative HPLC (MeOH/H_2O, 75 : 25, *V/V*, 3.0 mL·min^{-1}) from fraction C4.5.7a. Compound **5** (t_R 26.0 min, 12.6 mg) was purified from fraction C6 (2 g) by using Sephadex LH-20 (1 : 1, *V/V*), silica gel column (CH_2Cl_2/MeOH, 200 : 1 to 20 : 1) and semi-preparative HPLC (CH_3CN/H_2O, 54 : 46, *V/V*, 3.0 mL·min^{-1}).

Bipolarin A (**1**): colorless needle crystals; $[\alpha]_D^{25}$ +56 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.14), 255 (4.03) nm; ECD (*c* 0.18 , MeOH) $\Delta\varepsilon_{236}$ −0.14, $\Delta\varepsilon_{259}$ −4.07, $\Delta\varepsilon_{306}$ +1.09; IR ν_{max} 3431, 2926, 1687, 1620, 1451, 1385, 1316, 1270, 1213, 1073, 677 cm^{-1}; HRESI-MS *m/z* 437.2308 $[M + Na]^+$ (Calcd. for $C_{25}H_{34}O_5Na^+$, 437.2298); ^{1}H and ^{13}C NMR data, see Tables 1 and 3.

Bipolarin B (**2**): yellow oil; $[\alpha]_D^{25}$ +42 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.11), 220 (4.00) nm; ECD (*c* 0.18, MeOH) $\Delta\varepsilon_{209}$ +9.22, $\Delta\varepsilon_{230}$ −0.99, $\Delta\varepsilon_{247}$ +1.32, $\Delta\varepsilon_{318}$ −1.18; IR ν_{max} 3429, 2945, 1680, 1619, 1405, 1384, 1318, 1263, 1206, 1164, 1115, 1072, 1030, 866, 771, 613 cm^{-1}; HRESI-MS *m/z* 453.2009 $[M + K]^+$ (Calcd. for $C_{25}H_{34}O_5K^+$, 453.2038) and 437.2263 $[M + Na]^+$ (Calcd. for $C_{25}H_{34}O_5Na^+$, 437.2298); ^{1}H and ^{13}C NMR data, see Tables 1 and 3.

Bipolarin C (**3**): colorless oil; $[\alpha]_D^{25}$ +41 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.20), 228 (3.99) nm; ECD (*c* 0.18, MeOH) $\Delta\varepsilon_{224}$ −1.82, $\Delta\varepsilon_{243}$ +2.02; IR ν_{max} 3433, 2928, 2875, 1688, 1619, 1451, 1443, 1381, 1315, 1271, 1215, 1114, 1067, 1029, 865, 819, 613 cm^{-1}; HRESI-MS *m/z* 423.2489 $[M + Na]^+$ (Calcd. for $C_{25}H_{36}O_4Na^+$, 423.2506); ^{1}H and ^{13}C NMR data, see Tables 1 and 3.

Bipolarin D (**4**): colorless oil; $[\alpha]_D^{25}$ +25 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.23), 227 (4.08) nm; ECD (*c* 0.18, MeOH) $\Delta\varepsilon_{223}$ +15.03; IR ν_{max} 3434, 2953, 1754, 1673, 1636, 1457, 1416, 1381, 1298, 1231, 1143, 1028, 970, 919, 878, 799, 766, 746 cm^{-1}; HRESI-MS *m/z* 439.2456 $[M + Na]^+$ (Calcd. for $C_{25}H_{36}O_5Na^+$, 439.2455); ^{1}H and ^{13}C NMR data, see Tables 1 and 3.

Bipolarin E (**5**): colorless needle crystals; $[\alpha]_D^{25}$ +14 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.37) nm; ECD (*c* 0.18, MeOH) $\Delta\varepsilon_{211}$ +73.06, $\Delta\varepsilon_{259}$ +15.45, $\Delta\varepsilon_{315}$ −11.56; IR ν_{max} 3407, 2958, 2929, 2869, 1682, 1619, 1453, 1384, 1280, 1024, 650 cm^{-1}; HRESI-MS *m/z* 423.2515 $[M + Na]^+$ (Calcd. for $C_{25}H_{36}O_4Na^+$, 423.2506); ^{1}H and ^{13}C NMR data, see Tables 2 and 3.

Bipolarin F (**6**): colorless oil; $[\alpha]_D^{25}$ +71 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.29) nm; ECD (*c* 0.18, MeOH) $\Delta\varepsilon_{211}$ +34.62, $\Delta\varepsilon_{255}$ +9.56, $\Delta\varepsilon_{319}$ −2.57; IR ν_{max} 3428, 2961, 2928, 2872, 1694, 1620, 1456, 1410, 1383, 1324, 1286, 1231, 1174, 1118, 1060, 1030, 964, 920, 896, 864, 741, 697, 591 cm^{-1}; HRESI-MS *m/z* 407.2555 $[M + Na]^+$ (Calcd. for $C_{25}H_{36}O_3Na^+$, 407.2557); ^{1}H and ^{13}C NMR data, see Tables 2 and 3.

Bipolarin G (**7**): colorless oil; $[\alpha]_D^{25}$ −15 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.17) nm; ECD (*c* 0.18, MeOH) $\Delta\varepsilon_{207}$ +1.60, $\Delta\varepsilon_{237}$ +0.20; IR ν_{max} 3418, 2950, 2925, 2850, 1711, 1609, 1530, 1445, 1412, 1361, 1315, 1235, 1115, 1068, 1028, 821, 771, 730, 516 cm^{-1}; HRESI-MS *m/z* 427.2807 $[M + Na]^+$ (Calcd. for $C_{25}H_{40}O_4Na^+$, 427.2819); ^{1}H and ^{13}C NMR data, see Tables 2 and 3.

Bipolarin H (**8**): $C_{25}H_{38}O_3$; colorless needle crystals; $[\alpha]_D^{25}$ −64 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.99), 231 (3.96) nm; ECD (*c* 0.18, MeOH) $\Delta\varepsilon_{227}$ −6.58, $\Delta\varepsilon_{262}$ −0.61, $\Delta\varepsilon_{315}$ −1.88; IR ν_{max} 3430, 2929, 2875, 2338, 1681, 1623, 1449, 1381, 1315, 1267, 1188, 1040, 822, 668, 608, 569 cm^{-1}; HRESI-MS *m/z* 409.2715 $[M + Na]^+$ (Calcd. for $C_{25}H_{38}O_3Na^+$, 409.2713); ^{1}H and ^{13}C NMR data, see Tables 2 and 3.

X-ray crystal structure analysis

Crystals of compounds **1**, **5** and **8** were obtained from MeOH/H_2O (20 : 1, *V/V*) at 4 °C. The intensity data were collected at 100 K on a XtaLAB PRO MM007HF diffractometer using Cu Kα radiation. Using Olex2 [21], the structures were solved by direct methods with SHELXL-2014/7 [22]. Refinements were performed with SHELXL-2014/7 refinement package *via* means of full-matrix least-squares on F^2, with anisotropic displacement parameters used for all the non-hydrogen atoms. The hydrogen atoms were located at the calculated positions and refined with a riding model. The crystallographic data for these structures have been deposited in the Cambridge Crystallographic Data Centre (CCDC 1913831 for **1**, CCDC 1914339 for **5** and CCDC 1902857 for **8**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB 1EZ, UK [fax: Int. +44-(0)-(1223)-336033; E-mail: deposi@ccdc.cam.ac.uk].

Crystallographic data for compound **1**: $C_{25}H_{34}O_5{\cdot}H_2O$, M = 432.54, orthorhombic, a = 7.73070(10) Å, b = 14.3172(3) Å, c = 20.9791(4) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 2322.01(7) Å^3, T = 295(2) K, space group $P2_12_12_1$, Z = 4, μ(Cu Kα) = 0.705 mm^{-1}, 21012 reflections measured, 4293 independent reflections (R_{int} = 0.0227). The final R_1 values were 0.0287 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0820 ($I > 2\sigma(I)$). The final R_1 values were 0.0288 (all data). The final $wR(F^2)$ values were 0.0821 (all data). The goodness of fit on F^2 was 1.076. Flack parameter = 0.04(3).

Crystallographic data for compound **5**: $C_{25}H_{36}O_4{\cdot}H_2O$, M = 418.55, monoclinic, a = 8.49840(10) Å, b = 13.3122(2) Å, c = 10.80500(10) Å, α = 90.00°, β = 112.9144(14)°, γ = 90.00°, V = 1125.93(3) Å^3, T = 100(1) K, space group $P2_1$, Z = 2, μ(Cu Kα) = 0.674 mm^{-1}, 10360 reflections measured, 4294 independent reflections (R_{int} = 0.0168). The final R_1 values

were 0.0263 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0685 ($I > 2\sigma(I)$). The final R_1 values were 0.0265 (all data). The final $wR(F^2)$ values were 0.0685 (all data). The goodness of fit on F^2 was 1.045. Flack parameter = 0.02(7).

Crystallographic data for compound **8**: $C_{25}H_{38}O_3$, M = 386.55, monoclinic, a = 18.01290(10) Å, b = 9.864 Å, c = 19.64890(10) Å, α = 90.00°, β = 104.63°, γ = 90.00°, V = 3377.84(3) Å^3, T = 100.00(10) K, space group $P2_1$, Z = 6, μ(Cu Kα) = 0.566 mm^{-1}, 87782 reflections measured, 13246 independent reflections (R_{int} = 0.0408). The final R_1 values were 0.0408 ($I > 2\sigma(I)$). The final $wR(F_2)$ values were 0.1099 ($I > 2\sigma(I)$). The final R_1 values were 0.0421 (all data). The final $wR(F_2)$ values were 0.1112 (all data). The goodness of fit on F_2 was 1.025. Flack parameter = 0.09(5).

^{13}C NMR and ECD spectrum calculations

The conformations generated by BALLOON [23-24] were subjected to semiempirical PM3 quantum mechanical geometry optimizations using the Gaussian 09 program (Revision D.01, Gaussian, Inc., Wallingford CT, 2009). Duplicate conformations were identified and removed when the root- mean-square (RMS) distance was less than 0.5 Å for any two geometry-optimized conformations. The remaining conformations were further optimized at the B3LYP/6-31G(d) level in MeOH with the IEFPCM solvation model using Gaussian 09, and the duplicate conformations emerging after these calculations were removed according to the same RMS criteria above. The harmonic vibrational frequencies were calculated to confirm the stability of the final conformers. The electronic circular dichroism (ECD) spectrum and NMR chemical shifts were calculated for each conformer using the TDDFT methodology at the LC-wPBE/6-311++G(d, p)//B3LYP/6-31G(d) level with MeOH as solvent by the IEFPCM solvation model implemented in Gaussian 09 program. The spectra were combined after Boltzmann weighting according to their population contributions.

Biological assay protocols

The test strains were acquired from the American Type Culture Collection (ATCC, Manassas, USA): methicillin-resistant *Staphylococcus aureus* (ATCC 43300), ESBL- producing *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 15542), *Klebsiella pneumoniae* (ATCC 700603), *Acinetobacter baumannii* (ATCC 19606), *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 [25]: Vancomycin (Sigma, cat # 861987); Amikacin (Sigma, cat # 1019508); Ceftriaxone (Sigma, cat # 1098184); Fluconazole (Sigma, cat # 1271700); compounds **1**–**6**, and **8** were ≥ 95% pure (HPLC, wavelength = 210 nm).

Determination of the minimum inhibitory concentrations (MICs)

Determinations of the MICs were conducted according to our previously reported broth microdilution method [26]. In short, the inoculum was standardized to approximately 5 × 10^5 CFU·mL^{-1}. The plates were incubated at 37 °C for 16 h, and the MIC values were recorded as the lowest concentration of antibiotic, at which no visible microbial growths were observed. Each experiment was performed three times.

Statistical analysis

GraphPad Prism 5.0 software (GraphPad, San Diego, USA) was used to carry out Statistical analysis of the data. The data were expressed as the means ± SD. Values were analyzed with SPSS version 12.0 software (Softonic, Barcelona, Spain) by one-way analysis of variance (ANOVA), and $P < 0.05$ was considered statistically significant.

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