A chemical investigation on the kiwi endophytic fungus *Bipolaris* sp. Resulted in the isolation of eight new terpenoids (1–8) and five known analogues (9–13). Compounds 1–5 are novel sativene sesquiterpenoids containing three additional skeletal carbons, while compounds 4 and 5 are rare dimers. Compounds 6–8 and 13 are sesterterpenoids that have been identified from this species for the first time. Compounds 4 and 5 showed antibacterial activity against kiwifruit canker pathogen *Pseudomonas syringae pv. Actinidiae* (Psa) with MIC values of 32 and 64 μg/ml, respectively.

### Keywords
endophytic fungus, *Bipolaris* sp., sesquiterpenoids, sesterterpenoids, antibacterial activity

### 1 Introduction

Kiwifruit is an important global food source produced at a scale of 4 million tons per year (Richardson et al., 2018; Dolly et al., 2021). However, the kiwi plant (*Actinidia chinensis* Planch.) is severely attacked by canker caused by the pathogenic bacterium *Pseudomonas syringae pv. Actinidiae* (Psa) (Renzi et al., 2012; Scortichini et al., 2012). As one of the major countries in the kiwifruit industry, China’s kiwifruit has also suffered extensive damage from canker disease, causing huge economic losses (Serizawa et al., 1989; McCann et al., 2017; Vanneste, 2017). Traditional Psa inhibitors such as copper-based preparations and streptomycin are not friendly to the environment and even cause drug resistance (Bardas et al., 2010; Colombi et al., 2017; Scortichini, 2018; Wicaksono et al., 2018). Therefore, the development of new antibacterial agents is highly desirable.

Endophytes and hosts have formed a close interrelationship in the long-term evolution process, making endophytes an excellent resource for the production of natural antibacterial ingredients (Kusari et al., 2012; Gouda et al., 2016; Gupta et al., 2020). Our strategy intends to explore the active substances against Psa from the
metabolites of the endophytic bacteria of the kiwifruit itself. Some progress has been made in our previous research. For example, 3-decalinoyltetramic acids and cytochalasins from the kiwifruit endophytic fungus *Zopfiella* sp. Showed anti-Psa activity (Yi et al., 2021; Zhang et al., 2021), while imidazole alkaloids from *Fusarium tricinctum* were characterized as anti-Psa agents (Ma et al., 2022). *Bipolaris* sp. is also an endophytic fungus that was characterized from health kiwi plant. Our previous chemical investigation on this fungus yielded a series of sesquiterpenoids (bipolarisorokins A–I) and xanthones with anti-Psa properties from the liquid fermented extract (Yu et al., 2022). In order to search for more anti-Psa agents from this fungus, we further investigated the fermentation products from the culture grown on rice medium. As a result, eight new terpenoids including five sesquiterpenoids (1–5) and three new sesterterpenoids (6–8), as well as five known analogues (9–13), have been obtained (Figure 1). Their structures have been identified by extensive spectroscopic methods, as well as quantum chemical calculations. All compounds were evaluated for their anti-Psa activity. Herein, the isolation, structure elucidation and anti-Psa activity of these isolates are reported.
2 Experimental section

2.1 General experimental procedures

Optical rotations were measured with an Autopol IV polarimeter (Rudolph, Hackettstown, NJ, United States). UV spectra were obtained using a double beam spectrophotometer UH5300 (Hitachi High-Technologies, Tokyo, Japan). 1D and 2D NMR spectra were run on a Bruker Avance III 600 MHz spectrometer with TMS as an internal standard. Chemical shifts (δ) were expressed in ppm with references to the solvent signals. High resolution electrospray ionization mass spectra (HR-ESIMS) were recorded on a LC-MS system consisting of a Q Exactive™ Orbitrap mass spectrometer with an HRESI ion source (ThermoFisher Scientific, Bremen, Germany) used in ultra-high-resolution mode (140,000 at m/z 200) and a UPLC system (Dionex UltiMate 3000 RSLC, ThermoFisher Scientific, Bremen, Germany). Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), RP-18 gel (20–45 μm, Fuji Silysia Chemical Ltd., Kasugai, Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden). Medium-pressure liquid chromatography (MPLC) was performed on a Büchi Sepacore System equipped with a pump manager C-615, pump modules C-605, and a fraction collector C-660 (Büchi Labortechnik AG, Flawil, Switzerland). Preparative high-performance liquid chromatography (prep-HPLC) was performed on an Agilent 1,260 liquid chromatography system equipped with Zorbax SB-C18 columns (5 μm, 9.4 mm × 150 mm, or 21.2 mm × 150 mm) and a DAD detector. Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), and spots were visualized by heating silica gel plates sprayed with 10% H2SO4 in EtOH.

2.2 Fungal material

The fungus Bipolaris sp. was isolated from fresh and healthy stems of kiwifruit plants (Actinidia chinensis Planch, Actinidiaceae), which were collected from the Cangxi county of the Sichuan Province (GPS: N 31°12′, E 105°76′) in July 2018. Each fungus was obtained simultaneously from at least three different healthy tissues. The strain was identified as one species.

### Table 1

| No. | 1<sup>a</sup> | 2<sup>b</sup> | 3<sup>b</sup> |
|-----|---------------|---------------|---------------|
|     | δ<sub>C</sub>, type | δ<sub>H</sub> (J in Hz) | δ<sub>C</sub>, type | δ<sub>H</sub> (J in Hz) | δ<sub>C</sub>, type | δ<sub>H</sub> (J in Hz) |
| 1   | 137.8, C      | 128.2, C      | 138.8, C      |               |               |               |
| 2   | 165.9, C      | 160.0, C      | 169.1, C      |               |               |               |
| 3   | 52.4, C       | 52.8, C       | 53.6, C       |               |               |               |
| 4a  | 33.8, CH<sub>2</sub> | 1.43, d (5.9) | 34.5, CH<sub>2</sub> | 1.33, ddd (13.2, 11.9, 5.8) | 34.6, CH<sub>2</sub> | 1.51, d (13.3, 6.3) |
| 4b  | 1.35, d (12.4, 5.9) | 1.44, m |               |               | 1.42, td (12.8, 5.9) |               |
| 5a  | 25.4, CH<sub>2</sub> | 1.75, dd (11.7, 6.6) | 26.4, CH<sub>2</sub> | 1.76, dt (13.6, 5.2) | 26.4, CH<sub>2</sub> | 1.78, m |
| 5b  | 0.87, m       |               |               | 1.00, overlap  | 0.92, dd (10.3, 6.4) |               |
| 6   | 44.4, CH      | 1.02, overlap | 46.2, CH      | 1.06, m       | 45.8, CH      | 1.09, m  |
| 7   | 44.9, CH      | 3.00, br s    | 48.3, CH      | 2.93, br s    | 46.2, CH      | 2.96, br s |
| 8   | 19.4, CH<sub>2</sub> | 0.93, s | 20.3, CH<sub>2</sub> | 0.92, s | 19.6, CH<sub>2</sub> | 0.98, s |
| 9   | 31.7, CH      | 1.03, overlap | 32.8, CH      | 1.18, m       | 32.7, CH      | 1.01, overlap |
| 10  | 20.8, CH<sub>2</sub> | 0.75, d (6.0) | 21.2, CH<sub>2</sub> | 0.81, d (6.6) | 21.1, CH<sub>2</sub> | 0.78, d (6.2) |
| 11  | 21.8, CH<sub>2</sub> | 1.04, overlap | 22.1, CH<sub>2</sub> | 1.00, overlap  | 22.1, CH<sub>2</sub> | 1.03, d (6.2) |
| 12  | 11.0, CH<sub>2</sub> | 2.02, s | 12.6, CH<sub>2</sub> | 2.01, s | 10.9, CH<sub>2</sub> | 2.07, s |
| 13  | 63.5, CH      | 2.06, d (9.5) | 65.5, CH      | 2.06, d (9.3) | 64.7, CH      | 2.14, d (9.2) |
| 14  | 134.5, CH     | 5.68, ddd (15.4, 9.5, 1.4) | 135.2, CH     | 5.79, ddd (15.4, 9.3, 1.4) | 134.8, CH     | 5.69, ddd (15.5, 9.3, 1.4) |
| 15  | 188.2, CH     | 10.04, s     | 169.9, C      |               | 190.1, CH     | 10.03, s  |
| 16  | 127.5, CH     | 5.51, d (15.3, 5.8) | 129.8, CH     | 5.62, d (15.3, 6.1) | 129.8, CH     | 5.60, d (15.4, 5.9) |
| 17  | 71.5, CH      | 4.58, d (5.8, 1.4) | 73.2, CH      | 4.52, d (6.1, 1.4) | 72.6, CH      | 4.52, d (5.9, 1.4) |
| 18  | 174.2, C      |               | 177.0, C      |               | 176.3, C      |               |
| 19  | 53.0, CH<sub>2</sub> | 3.77, s | 49.8, CH<sub>2</sub> | 3.35, s |               |               |

<sup>a</sup>Measured in CDCl<sub>3</sub>.  
<sup>b</sup>Measured in CD<sub>3</sub>OD.
of the genus *Bipolaris* by observing the morphological characteristics and analysis of the internal transcribed spacer (ITS) regions. A living culture (internal number HFG-20180727-HJ32) has been deposited at the School of Pharmaceutical Sciences, South-Central Minzu University, China.

### 2.3 Fermentation, extraction, and isolation

The fungus *Bipolaris* sp. was cultured on potato dextrose agar (PDA) medium for 7 days, which was used as “seed” to incubate in rice medium. The 500 ml Erlenmeyer flasks containing 100 g rice and 80 ml distilled water in each were sterilized at 120°C for 15 min. Then the pieces of *Bipolaris* sp. PDA medium was inoculated into Erlenmeyer flasks. A total of a hundred 500 ml Erlenmeyer flasks were incubated statically in dark place at 25°C for 28 days.

The cultures of *Bipolaris* sp. were extracted with 5 L methanol four times, and the total residue was obtained by reduced pressure evaporation. Then, the remaining aqueous phase was further extracted four times by EtOAc to afford a crude extract (450 g). The latter was subjected to silica gel CC (200–300 mesh) eluted with a gradient of CHCl3-MeOH (from 1:0 to 0:1, v/v) to afford five fractions, A–E. Fraction B was separated by CC over silica gel with a gradient elution of the CHCl3-MeOH system (from 15:1 to 0:1, v/v) and was separated by HPLC with MeCN-H2O (2:1:79, v/v/v) to obtain 6 (4.3 mg, retention time \( t_R = 263.2 \) min), and 13 (5.2 mg, \( t_R = 29.4 \) min). Fraction B3 was subjected to Sephadex LH-20 (MeOH) and then further repeatedly purified by semipreparative HPLC with MeCN-H2O (32:68, v/v, 3.0 ml/min) to afford 11 (86.2 mg, retention time \( t_R = 24.2 \) min) and 12 (94.3 mg, \( t_R = 27.2 \) min). Fraction B4 was purified using semipreparative HPLC with MeCN-H2O (20:80, v/v, 4.0 ml/min) to afford 10 (7.8 mg, \( t_R = 16.8 \) min) and 9 (9.6 mg, \( t_R = 20.6 \) min). Fraction C was purified by semipreparative HPLC with MeCN-H2O (26:74, v/v, 4.0 ml/min) to afford 7 (4.8 mg, \( t_R = 24.6 \) min) and 8 (7.3 mg, \( t_R = 27.5 \) min). Fraction D was separated by CC over silica gel with a gradient elution of PE-acetone (from 50:1 to 0:1, v/v), and then was purified by semipreparative HPLC with MeCN-H2O (18:82, v/v, 4.0 ml/min) to obtain 1 (6.4 mg, \( t_R = 29.6 \) min), 2 (3.8 mg, \( t_R = 24.3 \) min), and 3 (7.6 mg, \( t_R = 18.5 \) min). Fraction E was purified using Sephadex LH-20 eluted with MeOH and was further separated using semipreparative HPLC with MeOH-H2O (78:22, v/v, 3.0 ml/min) to afford 4 (8.7 mg, \( t_R = 38.2 \) min) and 5 (12.8 mg, \( t_R = 34.3 \) min).

Bipolarisisorokin J (1): colorless oil; \([\alpha]_{D}^{20} = 99.8 \) (c 0.05, MeOH); UV (MeOH) \( \lambda_{\text{max}} \) (log ε) 260 (3.86); \(^1\)H NMR (600 MHz, CD3OD) and \(^{13}\)C NMR (150 MHz, CD3OD) data, see Table 1; positive ion HR-ESI-MS m/z 343.18790, [M + Na]+, (calculated for C33H49O5Na+, 343.18790).

Bipolarisisorokin K (2): colorless oil; \([\alpha]_{D}^{20} = 58.9 \) (c 0.05, MeOH); UV (MeOH) \( \lambda_{\text{max}} \) (log ε) 245 (3.82); \(^1\)H NMR (600 MHz, CD3OD) and \(^{13}\)C NMR (150 MHz, CD3OD) data, see Table 1; positive ion HR-ESI-MS m/z 359.18268, [M + Na]+, (calculated for C33H49O6Na+, 359.18290).

Bipolarisisorokin L (3): colorless oil; \([\alpha]_{D}^{20} = 83.3 \) (c 0.05, MeOH); UV (MeOH) \( \lambda_{\text{max}} \) (log ε) 265 (3.78); \(^1\)H NMR (600 MHz, CD3OD) and \(^{13}\)C NMR (150 MHz, CD3OD) data, see Table 1; positive ion HR-ESI-MS m/z 370.19046, [M + H]+, (calculated for C33H48O5Na+, 370.19039).

Bipolarisisorokin M (4): colorless oil; \([\alpha]_{D}^{20} = 48.9 \) (c 0.05, MeOH); UV (MeOH) \( \lambda_{\text{max}} \) (log ε) 265 (4.15); \(^1\)H NMR (600 MHz, CDCl3) and \(^{13}\)C NMR (150 MHz, CDCl3) data, see Table 2; positive ion HR-ESI-MS m/z 525.35706, [M + H]+, (calculated for C33H48O6Na+, 525.35745).

Bipolarisisorokin N (5): colorless oil; \([\alpha]_{D}^{20} = 45.6 \) (c 0.05, MeOH); UV (MeOH) \( \lambda_{\text{max}} \) (log ε) 255 (4.07); \(^1\)H NMR (600 MHz, CDCl3) and \(^{13}\)C NMR (150 MHz, CDCl3) data, see Table 2; positive ion HR-ESI-MS m/z 541.35254, [M + H]+, (calculated for C33H48O6Na+, 541.35237).

Bipolariterpene A (6): colorless oil; \([\alpha]_{D}^{20} = 25.3 \) (c 0.05, MeOH); UV (MeOH) \( \lambda_{\text{max}} \) (log ε) 260 (3.96); \(^1\)H NMR (600 MHz, CD3OD) and \(^{13}\)C NMR (150 MHz, CD3OD) data, see Table 3; positive ion HR-ESI-MS m/z 483.27176, [M + Na]+, (calculated for C33H40NaO5, 483.27171).

Bipolariterpene B (7): colorless oil; \([\alpha]_{D}^{20} = 28.9 \) (c 0.04, MeOH); UV (MeOH) \( \lambda_{\text{max}} \) (log ε) 265 (3.92); \(^1\)H NMR (600 MHz, CD3OD) and \(^{13}\)C NMR (150 MHz, CD3OD) data, see Table 3; positive ion HR-ESI-MS m/z 441.26044, [M + Na]+, (calculated for C33H38NaO5, 441.26115).

Bipolariterpene C (8): colorless oil; \([\alpha]_{D}^{20} = +26.3 \) (c 0.035, MeOH); UV (MeOH) \( \lambda_{\text{max}} \) (log ε) 205 (4.05); \(^1\)H NMR (600 MHz, CDCl3) and \(^{13}\)C NMR (150 MHz, CDCl3) data, see Table 3; positive ion HR-ESI-MS m/z 461.28955, [M + H]+, (calculated for C32H41O5, 461.28977).

### 2.4 Preparation of (S)-MTPA and (R)-MTPA esters of 1

The samples of 1 (1.5 mg each) were dissolved in pyridine (500 μl), and added with DMAP (2 mg) and (R)- or (S)-MTPA-Cl (10 μl) to the solution. The reaction was stirred at room temperature for 12 h. The productions were individually purified by semipreparative HPLC and eluted with MeCN-H2O (78:22, v/v, 4.0 ml/min) to obtain the (S)-MTPA ester 1a (1.0 mg, \( t_R = 14.0 \) min) and (R)-MTPA ester 1b (0.8 mg, \( t_R = 14.0 \) min), respectively.

(S)-MTPA ester 1a. \(^1\)H NMR (600 MHz, CDCl3): 1.44 (1H, d, \( J = 6.9 \) Hz, H-4a), 1.35 (1H, m, H-4b), 1.75 (1H, dd, \( J = 12.0, 6.7 \) Hz, H-5a), 0.88 (1H, m, H-5b), 1.06 (1H, overlap, H-6), 3.00 (1H, br s, H-7), 0.92 (3H, s, H-8), 1.03 (1H, overlap, H-9), 0.76 (3H, d, \( J = 5.8 \) Hz, H-10), 1.05 (3H, overlap, H-11), 2.01 (3H, s, J = 6.9 Hz).
Table 2 $^1$H and $^{13}$C NMR data for 4 and 5 in CDCl$_3$.

| No. | $\delta_C$ type | $\delta_H$ (J in Hz) | $\delta_C$ type | $\delta_H$ (J in Hz) |
|-----|----------------|----------------------|----------------|----------------------|
| 1   | 137.7, C       |                      | 137.7, C       |                      |
| 2   | 165.9, C       |                      | 165.9, C       |                      |
| 3   | 52.3, C        |                      | 52.3, C        |                      |
| 4a  | 33.8, CH$_2$   | 1.36, td (12.8, 5.8) | 33.8, CH$_2$   | 1.37, d (5.6)        |
| 4b  |                | 1.45, dd (13.3, 6.3) |                | 1.45, dd (13.3, 6.3) |
| 5a  | 25.4, CH$_2$   | 0.86, m              | 25.4, CH$_2$   | 0.88, m              |
| 5b  |                | 1.74, overlap         |                | 1.75, overlap         |
| 6   | 44.5, CH       | 1.05, overlap         | 44.5, CH       | 1.04, overlap         |
| 7   | 31.7, CH$_3$   | 1.03, overlap         | 31.7, CH$_3$   | 1.02, overlap         |
| 8   | 19.6, CH$_3$   | 0.95, s               | 19.6, CH$_3$   | 0.95, s               |
| 9   | 188.2, CH      | 10.05, s              | 188.2, CH      | 10.05, s              |
| 10  | 127.2, CH      | 5.52, dd (15.3, 5.5) | 127.4, CH      | 5.53, dd (15.3, 5.6) |
| 11  | 31.7, CH$_3$   | 4.56, d (5.5)         | 31.7, CH$_3$   | 4.58, d (5.4)         |
| 12  | 137.3, C       |                      | 137.3, C       |                      |
| 2'  | 165.1, C       |                      | 161.7, C       |                      |
| 3'  | 51.0, C        |                      | 50.8, C        |                      |
| 4'  | 34.0, CH$_2$   | 1.42, overlap         | 33.8, CH$_2$   | 1.34, dd (13.0, 5.6) |
| 5'a | 25.2, CH$_2$   | 0.89, overlap         | 25.0, CH$_2$   | 0.99, overlap         |
| 5'b |                | 1.77, overlap         |                | 1.76, overlap         |
| 6'  | 44.9, CH       | 1.01, overlap         | 45.2, CH       | 0.99, overlap         |
| 7'  | 41.7, CH$_3$   | 3.09, br s            | 43.6, CH$_3$   | 3.06, br s            |
| 8'  | 187.3, CH$_3$  | 1.04, s               | 19.2, CH$_3$   | 0.99, s               |
| 9'  | 31.7, CH$_3$   | 1.03, m               | 12.2, CH$_3$   | 1.22, m               |
| 10' | 34.0, CH$_2$   | 0.86, overlap         | 21.0, CH$_2$   | 0.8, d (6.5)          |
| 11' | 21.8, CH$_3$   | 1.05, overlap         | 21.8, CH$_3$   | 1.04, overlap         |
| 12' | 11.0, CH$_2$   | 2.02, s               | 12.8, CH$_2$   | 2.03, s               |
| 13' | 57.7, CH$_3$   | 1.81, dd (8.7, 5.9)   | 58.2, CH$_3$   | 1.76, overlap         |
| 14'a| 66.0, CH$_2$   | 3.80, dd (10.9, 8.9)  | 66.3, CH$_2$   | 3.91, m               |
| 14'b|                | 4.25, dd (11.0, 5.8)  |                | 4.27, dd (10.8, 5.6)  |
| 15' | 188.1, CH      | 10.05, s              |                | 171.3, C              |

H-12), 2.09 (1H, d, $J = 9.4$ Hz, H-13), 5.79 (1H, dd, $J = 15.3$, 9.4 Hz, H-14), 10.05 (1H, s, H-15), 5.62 (1H, dd, $J = 15.3$, 7.4 Hz, H-16), 5.55 (1H, d, $J = 7.5$ Hz, H-17), 3.74 (3H, s, H-19); positive ion HR-ESI-MS $m/z$ 537.24640, [M + H]$^+$, (calculated for C$_{30}$H$_{40}$F$_3$NaO$_6$, 537.24585).

(R)-MTPA ester (1b). $^1$H NMR (600 MHz, CDCl$_3$): 1.43 (1H, m, H-4a), 1.34 (1H, m, H-4b), 1.74 (1H, m, H-5a), 0.83 (1H, m, H-5b), 1.01 (1H, overlap, H-6), 2.93 (1H, br s, H-7), 0.86 (3H, s, H-8), 1.00 (1H, overlap, H-9), 0.75 (3H, d, $J = 5.3$ Hz, H-10), 1.02 (3H, overlap, H-11), 1.98 (3H, s, H-12), 2.04 (1H, d, $J = 9.4$ Hz, H-13), 5.67 (1H, m, H-14), 10.02 (1H, s, H-15), 5.56 (1H, overlap, H-16), 5.57 (1H, overlap, H-17), 3.77 (3H, s, H-19); positive ion HR-ESI-MS $m/z$ 559.22748, [M + Na]$^+$, (calculated for C$_{29}$H$_{35}$F$_3$NaO$_6$, 559.22779).

2.5 NMR calculations

The NMR calculations were carried out using the Gaussian 16 software package (Frisch et al., 2010). Systematic conformational analyses were performed via SYBYL-X 2.1 using the MMFF94 molecular mechanics force field calculation with 10 kcal/mol of cutoff energy (Hehre, 2003; Shao et al., 2006). The optimization and frequency of conformers were calculated on the B3LYP/6-31G(d) level in the Gaussian 16 program package. All the optimized conformers in an energy window of 5 kcal/mol (with no imaginary frequency) were subjected to gauge-independent atomic orbital (GIAO) calculations of their $^{13}$C NMR chemical shifts, using density functional theory (DFT) at the mPW1PW91/6-311+G (dp) level with the PCM model. The calculated NMR data of these conformers were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy. The $^{13}$C NMR chemical shifts for TMS were also calculated by the same procedures and used as the reference. After the calculation, the experimental and calculated data were evaluated by the improved probability DP4$^+$ method (Grimblat et al., 2015).

2.6 Antibacterial activity assay

All compounds were evaluated for their antibacterial activity against Pseudomonas syringae pv. Actinidiae. The antibacterial assay was conducted by the previously described method (Yu et al., 2022). The sample to be tested was added into a 96-well culture plate, and the final compound concentration range from 4 to 256 µg/ml. Bacteria liquid was added to each well until the final concentration is $5 \times 10^7$ CFU/ml. It was then incubated at 27°C for 24 h, and the minimum inhibitory concentration (MIC, with an inhibition rate of ≥90%) was determined by the microplate reader at OD$_{600}$ nm. The medium blank control was used in the experiment. Streptomycin was used as the positive control.

3 Results and Discussion

3.1 Structure characterizations

Bipolarisorokin J 1) was isolated as a colorless oil. The molecular formula was determined as C$_{19}$H$_{28}$O$_4$ with six
degrees of unsaturation based on the HRESIMS data (measured at m/z 343.1879 [M + Na]+, calcd for C19H28NaO4 + 343.18798).

The 13C NMR data of 1 displayed 19 carbon signals, which were assigned as five methyls, two methylenes, eight methines, and four quaternary carbons in association with the HSQC data (Table 1). The 1H NMR data of 1 showed five methyl signals at δH 0.93 (3H, s, H-8), 0.75 (3H, d, J = 6.0 Hz, H-10), 1.04 (3H, overlap, H-11), 2.02 (3H, s, H-12), and 3.77 (3H, s, H-19), two olefinic protons at δH 5.68 (1H, ddd, J = 15.4, 9.5, 1.4 Hz, H-14) and 5.51 (1H, dd, J = 15.3, 5.8 Hz, H-16), and an aldehyde proton at δH 10.04 (H, s, H-15) (Table 1). The characteristic signals of 1D NMR, together with the data of analogues from the same origin, suggested that 1 was most likely a seco-sativene type sesquiterpenoid derivative. According to 1H−1H COSY spectrum, two structural fragments were deduced as shown with bold lines in Figure 2.

TABLE 3 1H and 13C NMR data for 6–8 (δ in ppm).

| no. | δC, type | δH (J in Hz) | δC, type | δH (J in Hz) | δC, type | δH (J in Hz) |
|-----|----------|--------------|----------|--------------|----------|--------------|
| 1   | 50.2, C  |              | 49.8, C  |              | 49.0, C  |              |
| 2a  | 40.5, CH3| 1.69, overlap| 39.9, CH3| 1.94, m      | 39.3, CH3| 2.23, dd (14.8, 8.0) |
| 2b  |          | 2.35, m      | 120.9, CH3| 5.26, m      | 119.6, CH3| 5.12, m      |
| 3   | 123.3, CH| 5.33, overlap| 130.4, C |              | 139.2, C |              |
| 4   | 138.6, C | 2.31, m      | 42.9, CH3| 2.78, m      | 35.3, CH3| 2.29, dd (14.5, 7.2) |
| 5a  | 41.3, CH3| 2.06, m      |          |              | 30.1, CH3| 1.81, m      |
| 5b  | 24.3, CH3| 2.44, overlap| 126.1, CH| 5.75, m      | 1.60, m  |              |
| 6a  | 128.7, CH| 5.35, overlap| 138.0, CH| 5.48, d (15.5)| 75.7, CH| 3.38, dd (8.1, 3.7) |
| 6b  | 137.6, C | 74.3, C      |          |              | 74.8, C  |              |
| 7   | 31.4, CH3| 2.42, overlap; 1.77, m | 39.0, CH3| 1.74, m      | 35.3, CH3| 1.65, overlap |
| 8   | 130.0, CH| 5.39, d (5.4) | 127.4, CH| 5.20, d (8.1) | 126.2, CH| 5.43, d (5.3) |
| 9a  | 30.2, CH3| 2.44, overlap| 30.0, CH3| 2.34, d (15.7)| 29.6, CH3| 2.36, d (16.1) |
| 9b  | 1.95, d (17.9, 9.4) | 1.87, m |          |              | 1.93, m  |              |
| 10a | 50.7, CH3| 2.72, d (10.6)| 50.9, CH3| 2.50, d (9.5) | 49.8, CH3| 2.54, d (8.7) |
| 10b |          |              | 152.2, C |              | 147.4, C |              |
| 11  | 149.9, C |              | 148.8, C |              | 146.6, C |              |
| 12  | 137.6, C |              | 210.1, C |              | 207.8, C |              |
| 13  | 16.8, CH3| 0.96, s      | 16.5, CH3| 0.99, s      | 16.0, CH3| 1.00, s      |
| 14  | 15.5, CH3| 1.66, s      | 18.0, CH3| 1.67, s      | 17.8, CH3| 1.63, s      |
| 15  | 59.5, CH2 | 4.20, d (12.0)| 30.4, CH3| 1.26, s      | 24.6, CH3| 1.24, s      |
| 16  |          | 4.08, d (12.0)|          |              |          |              |
| 17  | 10.5, CH3| 1.57, s      | 11.4, CH3| 1.56, s      | 12.3, CH3| 1.62, s      |
| 18  | 35.2, CH | 2.81, q (7.1) | 38.8, CH | 2.59, q (6.9)| 34.1, CH | 2.77, q (7.2)|
| 19  | 67.6, CH3| 4.31, m      | 65.8, CH3| 3.82, m      | 66.5, CH3| 4.27, dd (10.5, 7.8) |
| 20  |          | 4.26, m      | 3.68, dd (10.4, 6.5) | 4.22, dd (10.6, 7.0) |
| 21a | 14.7, CH3| 1.30, d (7.0) | 14.6, CH3| 1.24, d (7.1) | 14.7, CH3| 1.31, d (7.1) |
| 21b |          | 2.00, s      |          |              | 21.0, CH3| 2.02, s      |
| 22  | 172.7, C |              | 171.1, C |              |          |              |
| 23  | 20.8, CH3|              |          |              |          |              |

*a Measured in CD3OD.  
b Measured in CDCl3.
correlations from $\delta^{H}$ 2.02 (3H, s, Me-12) to $\delta^{C}$ 165.9 (s, C-2), 52.4 (s, C-3) and $\delta^{C}$ 137.8 (s, C-1), from $\delta^{H}$ 0.93 (3H, s, Me-8) to C-2, C-3, 33.8 (t, C-4) and 63.5 (d, C-13), from $\delta^{H}$ 3.00 (1H, br s, H-7) to C-1, C-13, and $\delta^{C}$ 134.5 (d, C-14) established a seco-sativene type sesquiterpene backbone. In addition, one aldehyde group was connected to C-1, which was deduced from HMBC correlations from $\delta^{H}$ 10.04 (H, s, H-15) to $\delta^{C}$ 44.9 (d, C-7) and C-1. Furthermore, the HMBC correlations from $\delta^{H}$ 3.77 (3H, s, Me-19) and 4.58 (1H, dd, $J$ = 5.8, 1.4 Hz, H-17) to $\delta^{C}$ 174.2 (s, C-18) suggested that the connections among C-17, C-18 and C-19. The planar structure of 1 was thus deduced as shown in Figure 2, resembling bipolarisorokin G (10) (Yu et al., 2022). The ROESY correlations (Figure 3) of H-13/H-8, H-13/H-6 and H-12/H-14 revealed that H-3, H-6, H-7 and H-8, were co-facial and assigned to be $\beta$-oriented. Correlations between H-13 and H-16, as well as large coupling constants ($J$ = 15.4 Hz), confirmed the double bonds (C-14 and C-16) to be $E$-geometry. However, the geometry of H-17 cannot be determined by using the NOESY correlation. Regarding the same origin of 1 and 10, the absolute configuration of 1 thus was suggested to be the same as that of 10, except for C-17. However, the stereo-chemistry at C-17 was determined using a modified Mosher’s method (Hoye et al., 2007).

**FIGURE 2**
Key $^1$H-$^1$H COSY and HMBC correlations for 1, 4, 6, and 8.

**FIGURE 3**
Key ROESY correlations for 1, 4, 6, 7, and 8.
The observed differences of chemical shifts ($\Delta \delta = \delta_S - \delta_R$) (Figure 4) indicated that the C-17 absolute configuration is $R$. Consequently, the absolute configuration of 1 was assigned as $3R$, $6R$, $7S$, $13S$, $17R$.

Bipolarisorokin K (2), a colorless oil, was assigned the molecular formula of C$_{19}$H$_{28}$O$_5$ with six degrees of unsaturation based on HRESIMS data (measured at m/z 359.18268 [M + Na]$^+$, calcd for C$_{19}$H$_{28}$NaO$_4$$^+$ 359.18290). The $^1$H and $^{13}$C NMR data of 2 (Table 1) are closely similar to those of 1. The significant difference was the presence of a carboxyl group at C-15 ($\delta_{C}$ 169.9, s) in 2, instead of the aldehyde group in 1. This deduction was identified by the HMBC corrections from $\delta_{H}$ 2.93 (1H, br s, H-7) to $\delta_{C}$ 128.2 (s, C-1), $\delta_{C}$ 160.0 (s, C-2) and C-15, together with its HRESIMS data. Moreover, the absolute configuration of 2 was suggested to be the same with that of 1 based on the nearly identical NMR data, the biosynthetic pathway, and the consistent experimental ECD data of these two compounds (Figure 5).

Bipolarisorokin L (3) was obtained as a colorless oil. Its molecular formula was determined to be C$_{19}$H$_{28}$O$_5$ based on HRESIMS data (measured at m/z 307.19046 [M + H]$^+$, calcd for C$_{19}$H$_{28}$O$_5$$^+$ 307.19039). Comparing its 1D and 2D NMR data with those of 1 indicated that they shared almost the same chemical construction. However, the major difference was that the methyl ester group in 1 was replaced by a carboxyl at C-18 in 3. The loss of a methoxy signal in the $^{13}$C NMR spectrum, the HMBC correlation from $\delta_{H}$ 4.52 (1H, dd, J = 5.9 Hz, 1.4 Hz, H-17) to $\delta_{C}$ 176.3 (s, C-18), and the mass data analysis confirmed the above deduction. The relative configurations of 3 should be in agreement with the configuration of 1 based on the nearly identical NMR data. Finally, the experimental ECD curve of 3 matched well with that of 1 (Figure 5), suggesting that the absolute configuration of 3 was identical to that of 1.

Bipolarisorokin M (4) was obtained as a colorless oil. Its molecular formula of C$_{33}$H$_{48}$O$_5$, together with ten degrees of unsaturation, were established by its HRESIMS data (measured at m/z 525.35706 [M + H]$^+$, calcd for C$_{33}$H$_{49}$O$_5$$^+$ 525.35745). The $^1$H NMR data of 4 displayed signals for two olefinic protons, eight methyl groups, and two protons of aldehyde group (Table 2). The $^{13}$C NMR and DEPT data of 4 exhibited 33 carbon resonances, including eight methyls, five methenes (one oxygenated), twelve methines (one oxygenated, two olefinic and two aldehyde carbons), seven nonprotonated carbons (four olefinic and one ester carbonyl) (Table 2). After literature investigations, the aforementioned NMR data indicated that compound 4 should comprise of two different seco-sativene sesquiterpenoid units. Interpretation of the $^1$H−$^1$H COSY spectrum of 4 revealed the presence of four discrete proton−proton spin systems as shown with bold lines in Figure 2. Further analysis of its HMBC spectra demonstrated the existence of two building blocks of units A and B, which were highly similar to 3 and 11, respectively. The above deduction was confirmed by the HMBC correlations as shown in Figure 2, together with comparison of the $^1$H and $^{13}$C NMR spectroscopic data. Meanwhile, the key HMBC correlation from $\delta_{H}$ 3.80 (1H, dd, J = 10.9 Hz, 8.9 Hz, H-14$^a$) and 4.25 (1H, dd, J =
11.0 Hz, 5.8 Hz, H-14′) to δC 173.8 (s, C-18), along with analysis of the HRESIMS data, suggested the connection by an ester bond between units A and B. Therefore, considering similar NMR data and coupling constants, as well as their concurrent biogenetic relationship, the absolute configurations of 4 should agree with those of 3 and 11, respectively. Finally, the structure of 4 was established as depicted in Figure 1.

Bipolarisorokin N (5) was also isolated as a colorless oil. Its molecular formula was established as C_{33}H_{48}O_{6} based on the HRESIMS ion peak at m/z 541.35254 [M + H]+ (calcd for C_{33}H_{49}O_{6}+ 541.35237), corresponding to ten degrees of unsaturation. The 1D NMR data of 5 closely resembled those of 4 (Table 2), except for the obviously shifted signal of C-15′ (−16.8 ppm) and the absence of aldehyde hydrogen proton signal at H-15′. The HMBC correlations from δH 3.06 (1H, br s, H-7′) to δC 126.0 (s, C-1′) and 171.3 (s, C-15′), as well as the HRESIMS data analysis, led to the location of a carboxyl group (C-15′) at C-1′. Furthermore, the similar ROESY data and experimental ECD curves of 4–5 (Figure 5) suggested that they shared the same absolute configuration. Therefore, the structure of 5 was finally established as shown in Figure 1.

Bipolariterpene A (6) was assigned a molecular formula of C_{27}H_{40}O_{6} with eight degrees of unsaturation based on its HRESIMS data (measured at m/z 483.27176 [M + Na]+, calcd for C_{27}H_{40}NaO_{6}+ 483.27171). The 1H and 13C NMR data (Table 3) showed 27 carbon resonances comprising five methyls (δC 16.8, 15.5, 10.5, 14.7, and 20.8); eight methylenes including six aliphatic ones (δC 40.5, 41.3, 24.3, 31.4, 31.0, and 30.2) and two oxygenated ones at δC 59.5 and 67.6; six methines including two aliphatic ones at δC 50.7 and 35.2, three olefinic ones (δC 123.3, δC 128.7 and δC 130.0), and an oxygenated one at δC 77.1; eight non-protonated carbons with a aliphatic one at δC 50.2, five olefinic ones (δC 138.6, δC 137.6 × 2, δC 149.9, δC 149.5), a carbonyl one at δC 209.9, and an ester carbonyl one at δC 172.7. The general features of its NMR data closely resembled those of the co-isolated known bicyclic sesterterpene fusaproliferin (13) (Nihashi et al., 2002; Gao et al., 2020). The major difference was that an additional hydroxy group was substituted at C-21 in 6, which could be fully established through the HMBC corrections from δH 4.20 (1H, d, J = 12.0 Hz, H-21a) and 4.08 (1H, d, J = 12.0 Hz, H-21b) to δC 128.7 (d, C-7), 137.6 (s, C-8) and 31.4 (t, C-9). The ROESY correlations between Me-20/H-2b, H-3/H-5b, H-
21a/H-6a, H-7/H-9a, H-11/H-13, Me-22/H-14b indicated that the configurations of C-3/C-4, C-7/C-8, and C-12/C-13 double bonds were assigned as E, Z, and E, respectively. Furthermore, as indicated by its ROESY spectrum, the helminthosporic acid (9), bipolarisorokin G (10), helminthosporol (11), helminthospor acid (12) and fusaproliferin (13), by comparing the spectral data with those reported in the literature (Osterhage et al., 2002; Abdel-Latef et al., 2013; Liu et al., 2013; Yu et al., 2022). In this study,

| Compd | Psa |
|-------|-----|
| 1     | 128 |
| 2     | 128 |
| 3     | 128 |
| 4     | 32  |
| 5     | 64  |
| 6     | NA  |
| 7     | NA  |
| 8     | 256 |
| 9     | 256 |
| 10    | 128 |
| 11    | 256 |
| 12    | NA  |
| 13    | NA  |

Strptomycin* 8

*Positive control.

**Table 4 Inhibitory effects of the isolates against Psa (MIC, μg/mL).**
compounds 1 and 2 were isolated as methyl esters, which could be derived from the separation process since methanol was used as the solvent. To verify whether these compounds are of natural origin, we analyzed the ethanol extract of the fermentation broth of the fungus by HPLC (see Supporting Information). As a result, all compounds could be confirmed their natural attributes.

Structurally, compound 9 possessed two additional skeletal carbons, while compounds 1–5 and 10 possessed three additional skeletal carbons, which might be derived from acetyl-CoA or acetoacetyl-CoA. Compounds 11 and 12 were isolated as major components, which were most probably employed as the original precursor to assemble the above compounds. The hydroxyl group at C-14 in 11 was oxidized to produce an aldehyde product, which then underwent aldol condensation with the acetyl-CoA to give 9. Similarly, the aldehyde product combined an acetoacetyl-CoA to give compounds 1–3 and 10. Finally, additional esterification happened between 3 and 11 or 12 led to the formation of 4 or 5, respectively Scheme 1.

3.2 Anti-Psa activity

All compounds (1–13) were evaluated for their anti-Psa activity by using the method as described previously (Yu et al., 2022). Streptomycin was used as the positive control. As a result, compounds 4 and 5 showed certain inhibitory activity, with MICs of 32 and 64 μg/ml, respectively. Additionally, compounds 1–3, and 10 showed weak activity, with MICs of 128 μg/ml (Table 4). The results demonstrated that the additional skeletal carbons of seco-sativene sesquiterpenoids may be vital for Psa inhibitory activity.

4 Conclusion

In conclusion, eight new terpenoids (1–8), along with five known analogues (9–13) was identified from the culture medium of an endophyte Bipolaris sp, a fungus isolated from fresh and healthy stems of kiwifruit plants. Compounds 1–3, together with the known compound 10, represented novel structures of seco-sativene sesquiterpenoids possessing three additional skeletal carbons, which were only found in this fungus. In addition, compounds 4 and 5 were rare seco-sativene/seco-sativene adducts. In anti-Psa activity assay, compounds 4 and 5 displayed certain inhibitory activity against Psa. This study, together with our previous work (Yu et al., 2022), further supported that it is an effective approach to search for anti-Psa agents from endophytic fungi of kiwi plant itself. The endophyte Bipolaris sp. Could be a potential antibacterial strain, while its sativene sesquiterpene products could be potential anti-Psa agents.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

J-JY: methodology, data curation, writing—original draft preparation. W-KW: methodology, data curation. YZ: data curation, methodology. RC: writing—review and editing. JH: conceptualization, funding acquisition. J-KL: funding acquisition. TF: conceptualization, project administration, funding acquisition, writing—review and editing.

Funding

This work was financially supported by the National Natural Science Foundation of China (22177139, 21961142008) and the Fundamental Research Funds for the Central Universities, South-Central Minzu University (CZP21001).

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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Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2022.990734/full#supplementary-material
Measurements and absolute configuration of (-)-terpestacin and (-)-fusaproliferin: Clarification of optical rotational measurements and absolute configurational assignments establishes a homochiral structural series. J. Am. Chem. Soc. 124, 4239–4232. doi:10.1021/ ja010072l

Nishashi, Y., Lim, C.-H., Tanaka, C., Miyagawa, H., and Ueno, T. (2002). Phytoxotoxic sesterterpenes, 11-epiterpestacin, from Bipolaris sorokiniana NSDR-011. Biosci. Biotechnol. Biochem. 66 (3), 683–688. doi:10.1271/bbb.66.683

Osterhage, C., König, G. M., Holler, U., and Wright, A. D. (2002). Rare sesquiterpenoids from the algalicus fungus Drechslera dematioides. J. Nat. Prod. 65 (3), 306–313. doi:10.1021/np010092l

Renzi, M., Copini, P., Taddei, A. R., Rossetti, A., Gallipoli, L., Mazzaglia, A., et al. (2012). Bacterial canker on kiwifruit in Italy: Anatomical changes in the wood and in the primary infection sites. Phytopathology 102 (9), 827–840. doi:10.1094/phoyo- 02-12-0019-r

Richardson, D. P., Ansell, J., and Drummond, L. N. (2018). The nutritional and health attributes of kiwifruit. A review. Eur. J. Nutr. 57 (8), 2659–2676. doi:10.1007/ s00394-018-16Z7-r

Santini, A., Riteni, A., Fogliano, V., Randazzo, G., Mannina, L., Logrieco, A., et al. (1996). Structure and absolute stereochemistry of fusaproliferin, a toxic metabolite from Fusarium proliferatum. J. Nat. Prod. 59 (2), 109–112. doi:10.1021/np960023k

Scortichini, M. (2018). Aspects still to solve for the management of kiwifruit bacterial canker caused by Pseudomonas syringae pv. actinidiae biovar 3. Eur. J. Hortic. Sci. 83 (4), 205–211. doi:10.17660/EJH2018/834.1

Scortichini, M., Marcellotto, S., Ferrante, P., Petriccione, M., and Firaoro, G. (2012). Pseudomonas syringae pv. actinidiae: A re-emerging, multi-faceted, pandemic pathogen. Mol. Plant Pathol. 13 (7), 631–640. doi:10.1111/j.1364-3703.2012.00788.x

Serizawa, S., Ichikawa, T., Takikawa, Y., Tsyuma, S., and Goto, M. (1989). Occurrence of bacterial canker of kiwifruit in Japan description of symptoms, isolation of the pathogen and screening of bactericides. Jpn. J. Phytopathol. 55 (4), 427–436. doi:10.3186/jjphytopath.55.427

Shao, Y., Molnar, L. F., Jung, Y., Kusmann, J., Ochsenfeld, C., Brown, S. T., et al. (2006). Advances in methods and algorithms in a modern quantum chemistry program package. Phys. Chem. Chem. Phys. 8 (27), 3172–3191. doi:10.1039/ b51914A

Vanneste, J. L. (2017). “The scientific, economic, and social impacts of the New Zealand outbreak of bacterial canker of kiwifruit (Pseudomonas syringae pv. actinidiae),” in Annu. Rev. Phytopathol. Palo Alto: Annual Reviews. Editors J. E. Leach and S. E. Lindow, 377–399.

Wicaksono, A. W., Jones, E. E., Casonato, S., Monk, J., and Ridgway, H. J. (2018). Biological control of Pseudomonas syringae pv. actinidiae (Psa), the causal agent of bacterial canker of kiwifruit, using endophytic bacteria recovered from a medicinal plant. Biol. Control 116, 103–112. doi:10.1016/j.biocontrol.2017.03.003

Yu, J. W., He, J. S., Shen, L. T., Liu, J. K., Wang, G. K., and Feng, T. (2021). 3-Decalinoyltetramic acids from kiwi-associated fungus Zopfiella sp. and their antibacterial activity against Pseudomonas syringae. RSC Adv. 11, 18827–18831. doi:10.1039/D1RA01220F

Yu, J. J., Jin, Y. Y., Huang, S. S., and He, J. (2022). Sesquiterpenoids and xanthones from the kiwifruit-associated fungus Bipolaris sp. and their anti-pathogenic microorganism activity. J. Fungi (Basel). 8 (1), 9. doi:10.3939/jfd0010009

Zhang, J. T., He, J., Li, Z. H., Feng, T., and Liu, J. K. (2021). Zopfiellasins A–D, two pairs of epimeric cytochalasins from kiwi-associated fungus Zopfiella sp. and their antibacterial assessment. Molecules 26 (18), 5611. doi:10.3390/ molecules26185611