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# 3-Decalinoyltetramic acids from kiwi-associated fungus *Zopfiella* sp. and their antibacterial activity against *Pseudomonas syringae*†

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Four rare 3-decalinoyltetramic acid derivatives, zofielliamides A–D (1–4), were obtained from cultures of kiwi-associated fungus *Zopfiella* sp. Their structures with absolute configurations were established by extensive spectroscopic methods and single crystal X-ray diffraction. The compounds possessed rare pentacyclic systems that might derive from a polyene precursor via [4 + 2] intramolecular Diels–Alder reactions. Compounds 1, 2, and 4 showed antibacterial activity against plant pathogen *Pseudomonas syringae* with MIC values of 64, 32, and 64  $\mu\text{g mL}^{-1}$ , respectively.

## Introduction

For a long time, natural products have been a good source of leading compounds for drug discovery,<sup>1</sup> while fungal products with unique and diverse scaffolds are extraordinarily valuable sources.<sup>2</sup> It is estimated that there are 2.2 to 3.8 million fungal species.<sup>3</sup> However, the number of described fungal species currently stands at around 120 000.<sup>3</sup> Therefore, fungi are important biological resources and a huge treasure house for mining active natural products. Endophytic fungi, due to the process of co-evolution with the host plants, produce a series of active secondary metabolites. Our recent chemical investigations on endophytic fungi reported a number of bioactive secondary metabolites including terpenoids, cytochalasans, and episidones.<sup>4–7</sup>

*Zopfiella* sp. is an endophytic fungus that was isolated from kiwi plant (*Actinidia chinensis* Planch). A plate face-off method experiment indicated that the fungus *Zopfiella* sp. could inhibit the growth of *Pseudomonas syringae*, a well-known pathogen that causes kiwi fruit canker disease.<sup>8</sup> In order to discover the antibacterial chemical constituent from this fungus, a chemical investigation on the extract of the fermentation in rice medium was carried out. Previously, bisabolane sesquiterpenoids and  $\alpha$ -pyrones were obtained and reported.<sup>8</sup> Currently, four 3-decalinoyltetramic acid derivatives, zofielliamides A–D (1–4, Fig. 1),

were obtained in the study of the same extract. Their structures were established by extensive spectroscopic methods, and their absolute configurations were established by the single crystal X-ray diffraction. All compounds were evaluated for their antibacterial activity against *Pseudomonas syringae*. Herein, the isolation, structural elucidation, and the biological activity of these isolates are discussed.

## Results and discussion

Zofielliamide A (1) was isolated as colorless crystals. Its molecular formula was determined as  $\text{C}_{25}\text{H}_{35}\text{NO}_5$  on the basis of

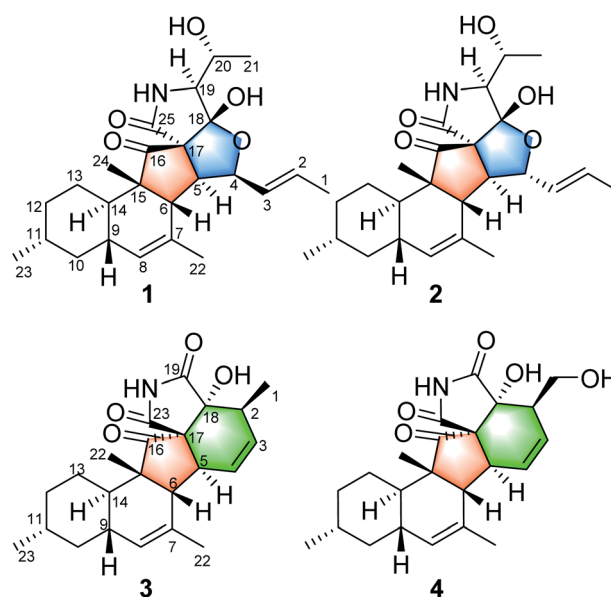


Fig. 1 Compounds 1–4 isolated from *Zopfiella* sp.

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HRESIMS at  $m/z$  452.24051  $[M + Na]^+$  (calcd for  $C_{25}H_{35}NO_5Na^+$ , 452.24074), revealing nine degrees of unsaturation. The IR absorption band at 3367, 1734, 1667, 1448  $cm^{-1}$  revealed the existence of hydroxy, carbonyl, and double bonds. In the  $^1H$  NMR spectrum (Table 1), five methyl signals were readily identified including two singlets at  $\delta_H$  1.63 (3H,  $H_3$ -22), 0.91 (3H,  $H_3$ -24) and three doublets at  $\delta_H$  1.72 (3H, d,  $J$  = 6.5 Hz,  $H_3$ -1), 1.16 (3H, d,  $J$  = 6.5 Hz,  $H_3$ -21), 0.87 (3H, d,  $J$  = 6.6 Hz,  $H_3$ -23). Three signals for heteroatom-containing carbons at  $\delta_H$  4.88 (1H, m, H-4), 4.10 (1H, qd,  $J$  = 6.5, 3.2 Hz, H-20), 3.34 (1H, d,  $J$  = 3.2 Hz, H-19) were also detected. In addition, three olefinic protons at  $\delta_H$  5.66 (1H, dd,  $J$  = 15.2, 6.5 Hz, H-2), 5.90 (1H, dd,  $J$  = 15.2, 9.6 Hz, H-3), 5.18 (1H, br s, H-8) indicated at least two double bonds in the structures. The  $^{13}C$  NMR spectrum afforded 25 carbon resonances that were ascribable for five  $CH_3$ , three  $CH_2$ , eleven CH, and six non-protonated carbons according to the DEPT and HSQC spectra (Table 1). Of them, signals at  $\delta_C$  214.4 (C, C-16) and 173.9 (C, C-25) were assigned to two carbonyl carbons, while signals at  $\delta_C$  131.6 (CH, C-2), 132.5 (CH, C-3), 134.3 (C, C-7), and 128.9 (CH, C-8) should be ascribable for carbons in two double bonds. These data indicated that **1** should have a pentacyclic system. The literature investigation,<sup>9–16</sup> as well as analysis of HMBC and  $^1H$ - $^1H$  COSY data (Fig. 2), suggested that **1** should be a 3-decalinoyltetramic acid derivative related to fusarisetin A, an acinar morphogenesis inhibitor from a soil fungus, *Fusarium* sp. FN080326.<sup>9</sup> The

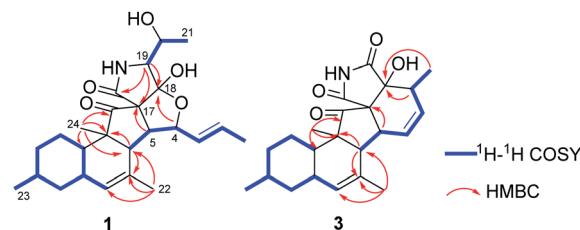


Fig. 2 Key  $^1H$ - $^1H$  COSY and HMBC correlations for **1** and **3**.

significant difference was that three additional skeletal carbons presented in **1**. The  $^1H$ - $^1H$  COSY correlations from H-1 to H-3 and from H-19 to H-21, as well as HMBC correlations from H-1 to C-2 and C-3, and from C-21 to C-20 and C-19, suggested that two additional carbons (C-1 and C-2) linked to C-3 and one additional carbon (C-21) linked to C-20. Detailed analysis of 2D NMR data suggested that the other parts of **1** were the same to those of fusarisetin A.<sup>9</sup> After attempts, a single crystal of **1** was afforded, while the single crystal X-ray diffraction not only confirmed the planar structure of **1** but also determined the absolute configuration of **1** as shown in Fig. 3 (Flack parameter =  $-0.03(5)$ ; CCDC: 2070473†).

Zofielliamide B (**2**) was isolated as white powder. Its molecular formula was determined as  $C_{25}H_{35}NO_5$  by the HRESIMS data at  $m/z$  452.24025  $[M + Na]^+$  (calcd for  $C_{25}H_{35}NO_5Na^+$ ,

Table 1  $^1H$  (600 MHz) and  $^{13}C$  (150 MHz) data for compounds **1**–**4** ( $\delta$  in ppm,  $J$  in Hz)

	<b>1</b> <sup>a</sup>		<b>2</b> <sup>a</sup>		<b>3</b> <sup>b</sup>		<b>4</b> <sup>a</sup>	
Entry	$\delta_H$	$\delta_C$ , type	$\delta_H$	$\delta_C$ , type	$\delta_H$	$\delta_C$ , type	$\delta_H$	$\delta_C$ , type
1	1.72, d (6.5)	17.9, $CH_3$	1.68, d (6.5)	17.7, $CH_3$	1.25, d (7.4)	14.6, $CH_3$	3.96, dd (10.8, 4.6) 3.54, dd (10.8, 8.3)	61.1, $CH_2$
2	5.66, dd (15.2, 6.5)	131.6, CH	5.76, dd (15.2, 6.5)	130.8, CH	2.52, m	41.6, CH	2.44, m	51.2, CH
3	5.90, dd (15.2, 9.6)	132.5, CH	5.40, dd (15.2, 9.6)	132.0, CH	5.59, dd (8.9, 2.7)	132.9, CH	5.84, dd (9.2, 2.9)	129.8, CH
4	4.88, m	86.6, CH	4.83, dd (9.6, 4.2)	89.7, CH	6.49, dd (8.9, 3.4)	134.5, CH	6.52, dd (9.2, 3.5)	136.1, CH
5	3.18, dd (9.4, 9.0)	56.2, CH	2.70, dd (9.7, 4.2)	58.8, CH	2.82, dd (10.6, 3.4)	44.3, CH	2.81, ddd (12.3, 3.5)	45.5, CH
6	2.44, d (9.4)	48.8, CH	2.30, d (9.7)	55.6, CH	3.28, d (10.6)	48.5, CH	3.17, d (12.3)	49.9, CH
7		134.3, C		133.8, C		132.2, C		133.9, C
8	5.18, br s	128.9, CH	5.20, br s	128.2, CH	5.24, br s	127.9, CH	5.26, br s	128.5, CH
9	1.87, m	37.2, CH	1.61, m	37.8, CH	1.89, m	37.2, CH	1.92, m	38.4, CH
10	1.75, m; 0.77, m	43.4, $CH_2$	1.78, m; 0.78, m	43.3, $CH_2$	1.81, m; 0.73, m	42.4, $CH_2$	1.81, m; 0.74, m	43.5, $CH_2$
11	1.40, m	34.0, CH	1.42, m	34.1, CH	1.45, m	32.8, CH	1.44, m	34.1, CH
12	1.65, m; 0.93, m	36.2, $CH_2$	1.65, m; 0.93, m	36.2, $CH_2$	1.75, m; 0.87, m	35.4, $CH_2$	1.71, m; 0.85, m	36.4, $CH_2$
13	1.27, m; 0.99, m;	26.2, $CH_2$	1.30, m; 1.02, m;	26.3, $CH_2$	1.77, m; 1.16, m	25.1, $CH_2$	1.76, m; 1.14, m	26.1, $CH_2$
14	1.76, m	37.6, CH	1.81, m	38.2, CH	1.17, m	40.2, CH	1.26, m	40.6, CH
15		58.8, C		57.7, C		53.8, C		54.7, C
16		214.4, C		214.4, C		212.5, C		211.9, C
17		76.4, C		76.0, C		74.0, C		75.8, C
18		110.7, C		111.4, C		80.2, C		79.8, C
19	3.34, d (3.2)	68.3, CH	3.36, d (3.5)	68.5, CH		175.8, C		179.2, C
20	4.10, qd (6.5, 3.2)	66.3, CH	4.10, qd (6.5, 3.5)	66.5, CH	1.88, s	23.5, $CH_3$	1.88, s	23.7, $CH_3$
21	1.16, d (6.5)	20.4, $CH_3$	1.19, d (6.5)	20.9, $CH_3$	0.88, d (6.2)	22.5, $CH_3$	0.88, d (6.6)	22.8, $CH_3$
22	1.63, s	24.7, $CH_3$	1.72, s	23.6, $CH_3$	1.08, s	16.7, $CH_3$	1.01, s	16.9, $CH_3$
23	0.87, d (6.6)	22.8, $CH_3$	0.88, d (6.6)	22.8, $CH_3$		170.6, C		174.4, C
24	0.91, s	14.3, $CH_3$	0.90, s	14.3, $CH_3$				
25		173.9, C		173.9, C				
18-OH					3.27, s		3.33, s	

<sup>a</sup> Measured in methanol- $d_4$ . <sup>b</sup> Measured in  $CDCl_3$ .



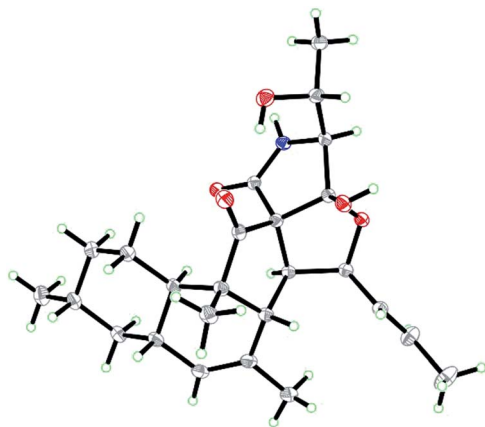


Fig. 3 ORTEP diagram of 1.

452.24074), the same to that of 1. All spectroscopic data of 2 displayed similar patterns to those of 1. Detailed analysis of 2D NMR data suggested that 2 had the same planar structure to that of 1. The ROESY spectrum also display similar patterns to that of 1. However, the correlations of H-4/H-6 and H-6/H-24 suggested that H-4 should be  $\beta$  oriented (Fig. 4), differing from that in 1. The CD spectrum also displayed similar curve to that of 1 (see ESI<sup>†</sup>), revealing the absolute configuration as shown in Fig. 1.

Zofielliamide C (3) was isolated as colorless crystals. The molecular formula was identified as  $C_{23}H_{29}NO_4$  by the HRE-SIMS data at  $m/z$  406.19880  $[M + Na]^+$  (calcd for  $C_{23}H_{29}NO_4Na^+$ , 406.19888). All the spectroscopic data suggested that 3 should also be a 3-decalinoyltetramic acid derivative. A literature investigation, as well as analysis of 2D NMR data, suggested that 3 was similar to that of phomopsichalasin.<sup>17</sup> The significant difference was that the degradation of the *p*-hydroxy-benzyl group at C-19 in 3, which was replaced by a carbonyl carbon at C-19 ( $\delta_C$  175.8, C). In addition, two methyls at C-3 and C-9 in phomopsichalasin were degraded in 3 as supported by the HMBC and  $^1H$ - $^1H$  COSY data (Fig. 2). The single crystal X-ray diffraction supported the planar structure of 3 and determined its absolute configuration as shown in Fig. 5 (Flack parameter = 0.04(9); CCDC: 2070474<sup>†</sup>).

Zofielliamide D (4) was isolated as white powder. The molecular formula was established as  $C_{23}H_{29}NO_5$  according to HRESIMS data at  $m/z$  422.19327  $[M + Na]^+$  (calcd. for  $C_{23}H_{29}NO_5Na^+$ , 422.19379). All spectroscopic data suggested that 4 was similar to 3. A significant modification was that the

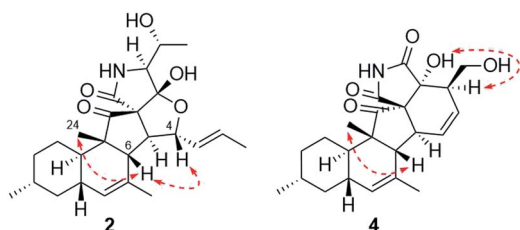


Fig. 4 Key ROESY correlations for 2 and 4.

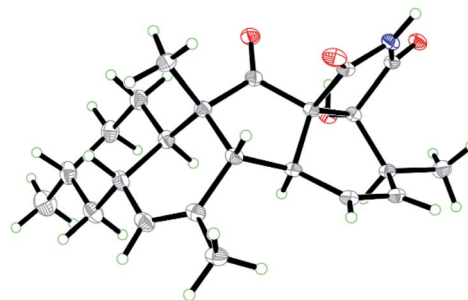


Fig. 5 ORTEP diagram of 3.

methyl of C-1 in 3 was oxidized into a hydroxymethylene in 4. It was supported by the HMBC correlations from  $\delta_H$  3.96 (1H, dd,  $J$  = 10.8, 4.6 Hz, H-1a) and 3.54 (1H, dd,  $J$  = 10.8, 8.3 Hz, H-1b) to  $\delta_C$  51.2 (CH, C-2), 129.8 (CH, C-3), and 79.8 (C, C-18), as well as the  $^1H$ - $^1H$  COSY cross peak between H-1 and H-2. The ROESY data between H-2 and OH-18 suggested that H-2 should be  $\alpha$  oriented (Fig. 4), the same to that of 3. In addition, the similar CD curve of 4 with that of 3 also suggested the absolute configuration of 4 as shown in Fig. 1 (see ESI<sup>†</sup>).

3-Decalinoiltetramic acids are a kind of special tetramic acid derivatives featured with decalin and pyrrolidine-2,4-diketone units. Due to its complex and diverse structure with good biological activity, 3-decalinoiltetramic acids have become a research focus. The structures of compounds 1–4 are intriguing because of the possibility that they might arise *via* a biosynthetic pathway involving a pair of net  $[4 + 2]$  intramolecular Diels–Alder reactions commencing from the polyene precursor 5 (Fig. 6). The *trans*-decalin formation (6) has been demonstrated as catalyzed by Fsa2-family enzymes.<sup>18</sup> A further  $[4 + 2]$  intramolecular Diels–Alder reaction might give the final pentacyclic skeleton of 3 and 4. Compounds 1 and 2 might derive from 6 *via* a Michael addition to give a new carbon bond between C-5 and C-17. Then a nucleophilic addition gave a hemiketal at C-18 (Fig. 6).

Compounds 1–4 were evaluated for their antibacterial activity against *Pseudomonas syringae*. As a result, compounds 1, 2, and 4 showed antibacterial activity with MIC values of 64, 32, and 64  $\mu g mL^{-1}$ , respectively. To the best of our knowledge, this

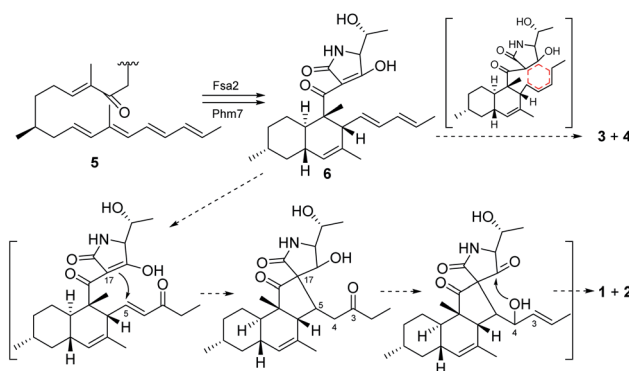


Fig. 6 Proposed biosynthesis for compounds 1–4.



is the first report of the antibacterial activity of 3-decalinoyltetramic acid derivatives against *P. syringae*.

## Conclusions

In summary, four new 3-decalinoyltetramic acid derivatives with rare carbon skeletons have been identified from cultures of the fungus *Zopfiella* sp. Their structures with absolute configurations were established. Compounds **1**, **2**, and **4** exhibited antibacterial activity against plant pathogen *Pseudomonas syringae*.

## Experimental section

### General experimental procedures

Optical rotations were measured on a Rudolph Autopol IV polarimeter. UV spectra were obtained on a UH5300 UV-VIS Double Beam Spectrophotometer. IR spectra were obtained by using a Shimadzu Fourier Transform Infrared spectrometer with KBr pellets. NMR spectra were acquired with a Bruker Avance III 600 instrument. ECD spectra were recorded with an Applied Photophysics Chirascan-Plus spectrometer. High resolution electrospray ionization mass spectra (HRESIMS) were recorded on a LC-MS system consisting of a Q Exactive™ Orbitrap mass spectrometer with an ESI ion source used in ultra-high resolution mode (140 000, at  $m/z$  200) and a Dionex UltiMate 3000 RSLC UPLC system. Silica gel (200–300 mesh and 500–800 mesh), RP-18 gel (40–75  $\mu\text{m}$ ) and Sephadex LH-20 were used for column chromatography. Preparative HPLC was performed on an Agilent 1260 liquid chromatography system with a Zorbax SB-C18 (5  $\mu\text{m}$ ,  $9.4 \times 150$  mm) column and a DAD detector.

### Fungal material and cultivation conditions

The endophytic fungus *Zopfiella* sp. was isolated from healthy tissue of kiwi plant (*Actinidia chinensis* Planch). It was identified as a species of the genera *Zopfiella* by ITS sequencing with an accession number KR154941.1. A further identification on this fungus is ongoing. Culture medium consists of glucose (5%), pork peptone (0.15%), yeast (0.5%),  $\text{KH}_2\text{PO}_4$  (0.05%) and  $\text{MgSO}_4$  (0.05%). Initial pH was adjusted to 6.0, the fermentation was initially implemented on an Erlenmeyer flask for 6 days until the mycelium biomass reached to the maximum. Then it was transferred to rice medium for 24 °C in dark culture for 30 days. Rice medium: 75 g of rice, 75 mL of water, placed in a 250 mL Erlenmeyer flask, sterilized at 121 °C for 15 min, a total of 200 bottles.

### Extraction and isolation

The rice cultural broth (15 kg) was extracted five times with EtOAc. The EtOAc layer was concentrated under reduced pressure to give an oily crude extract (200 g). The extract (200 g) was subjected to column chromatography on silica gel (200–300 mesh) with a gradient of  $\text{CHCl}_3/\text{MeOH}$  (1 : 0, 40 : 1, 20 : 1, 10 : 1, 5 : 1, 2 : 1, 0 : 1, v/v) to obtain seven fractions (A–G). Fraction D (35 g) was subjected to MPLC, eluted with a stepwise gradient of  $\text{MeOH}/\text{H}_2\text{O}$  (10 : 90–100 : 0, v/v) to afford fractions

D1–D15. Fraction D6 (5 g) was fractionated by silica gel column chromatography eluting with a stepwise gradient of petroleum ether/acetone (6 : 1, 5 : 1, 4 : 1, 3 : 1, 2 : 1, 1 : 1, v/v) to yield thirteen subfractions D6-1 to D6-13. Subfraction D6-3 (82 mg) was purified on a preparative  $\text{C}_{18}$  HPLC column ( $\text{MeCN}/\text{H}_2\text{O}$  from 30/70 to 40/60 in 25 min, 4 mL  $\text{min}^{-1}$ ) to yield **1** (6.0 mg, retention time ( $t_R$ ) = 12.1 min) and **2** (3.2 mg,  $t_R$  = 14.4 min). Fraction E (18 g) was subjected to MPLC, eluted with a stepwise gradient of  $\text{MeOH}/\text{H}_2\text{O}$  (10 : 90–100 : 0, v/v) to afford fractions E1–E8. Fraction E3 (390 mg) was isolated by silica gel eluted with petroleum ether/acetone (6 : 1) to give colorless crystals **3** (16 mg) and a mixture. The mixture was purified on a preparative  $\text{C}_{18}$  HPLC column ( $\text{MeCN}/\text{H}_2\text{O}$  from 25/75 to 35/65 in 25 min, 4 mL  $\text{min}^{-1}$ ) to yield **4** (2.8 mg).

**Zofielliamide A.** Colorless crystals, mp: 273–275 °C;  $[\alpha]_D^{20} = +44.4$  ( $c$  0.10, MeOH); ECD (MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ) 224 (+11.2), 298 (+6.8) nm; IR (KBr)  $\nu_{\text{max}}$  3367, 2947, 2833, 1734, 1667, 1448, 1031  $\text{cm}^{-1}$ ;  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  (150 MHz) NMR data, see Table 1; HRESIMS:  $m/z$  452.24051  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{25}\text{H}_{35}\text{NO}_5\text{Na}^+$ , 452.24074).

**Zofielliamide B (2).** White powder;  $[\alpha]_D^{20} = +35.3$  ( $c$  0.15, MeOH); ECD (MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ) 226 (+17.8), 298 (+6.9) nm; IR (KBr) 3400, 2949, 2837, 1731, 1651, 1454, 1018  $\text{cm}^{-1}$ ;  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  (150 MHz) NMR data, see Table 1; HRESIMS:  $m/z$  452.24025  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{25}\text{H}_{35}\text{NO}_5\text{Na}^+$ , 452.24074).

**Zofielliamide C (3).** Colorless crystals, mp: 263–270 °C;  $[\alpha]_D^{20} = +94.5$  ( $c$  0.18, MeOH); ECD (MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ) 204 (+44.2), 232 (+24.8), 317 (+8.1) nm; IR (KBr) 3491, 3232, 2947, 1735, 1699, 1215, 1132, 1091  $\text{cm}^{-1}$ ;  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  (150 MHz) NMR data, see Table 1; HRESIMS:  $m/z$  406.19880  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{23}\text{H}_{29}\text{NO}_4\text{Na}^+$ , 406.19888).

**Zofielliamide D (4).** White powder;  $[\alpha]_D^{20} = +6.84$  ( $c$  0.65, MeOH); ECD (MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ) 202 (+16.3), 233 (+6.8), 317 (+3.5) nm; IR (KBr)  $\nu_{\text{max}}$  3390, 2949, 2835, 1732, 1653, 1452, 1031  $\text{cm}^{-1}$ ;  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  (150 MHz) NMR data, see Table 1; HRESIMS:  $m/z$  422.19327  $[\text{M} + \text{Na}]^+$  (calcd. for  $\text{C}_{23}\text{H}_{29}\text{NO}_5\text{Na}^+$ , 422.19379).

**X-ray crystallographic data for zofielliamide A.** A colorless cube.  $\text{C}_{25}\text{H}_{35}\text{NO}_5$ ,  $M = 429.54$ ,  $a = 17.6760(4)$  Å,  $b = 17.6760(4)$  Å,  $c = 13.1360(4)$  Å,  $\alpha = 90.00^\circ$ ,  $\beta = 90.00^\circ$ ,  $\gamma = 120.00^\circ$ ,  $V = 3554.36(19)$  Å<sup>3</sup>,  $T = 156(2)$  K, space group  $P6_5$ ,  $Z = 6$ ,  $\mu(\text{CuK}\alpha) = 1.54178$  mm<sup>−1</sup>, 83 650 reflections measured, 5041 independent reflections ( $R_{\text{int}} = 0.049$ ). The final  $R_1$  values were 0.0164 ( $I > 2\sigma(I)$ ). The final  $R_1$  values were 0.0285 (all data). The final  $wR(F^2)$  values were 0.0688 (all data). The goodness of fit on  $F^2$  was 1.077. Flack parameter =  $-0.03(5)$ . CCDC: 2070473 (<http://www.ccdc.cam.ac.uk>).†

**X-ray crystallographic data for zofielliamide C (3).** Light colorless BLOCK-like.  $\text{C}_{23}\text{H}_{29}\text{NO}_4$ ,  $M = 383.47$ ,  $a = 6.8544(3)$  Å,  $b = 43.8920(19)$  Å,  $c = 6.7683(3)$  Å,  $\alpha = 90.00^\circ$ ,  $\beta = 90.00^\circ$ ,  $\gamma = 90.00^\circ$ ,  $V = 2036.27(15)$  Å<sup>3</sup>,  $T = 150(2)$  K, space group  $P2_12_12_1$ ,  $Z = 4$ ,  $\mu(\text{CuK}\alpha) = 1.54178$  mm<sup>−1</sup>, 22 455 reflections measured, 4309 independent reflections ( $R_{\text{int}} = 0.0597$ ). The final  $R_1$  values were 0.0503 ( $I > 2\sigma(I)$ ). The final  $R_1$  values were 0.0581 (all data). The final  $wR(F^2)$  values were 0.1593 (all data). The goodness of fit on  $F^2$  was 1.110. Flack parameter = 0.04. CCDC: 2070474 (<http://www.ccdc.cam.ac.uk>).†





### Antibacterial assay

The tested bacterium *Pseudomonas syringae* was donated to Northwest Agricultural & Forest University. It was cultured in Mueller Hinton broth (MHB) (Guangdong Huankai Microbial Sci. & Tech. Co., Ltd.) at 37 °C overnight with shaking (200 rpm). A sample of each culture was then diluted 40-fold in fresh MHB broth and incubated with shaking (200 rpm) at 37 °C for 2.5 h. The resultant mid-log phase cultures were diluted to a concentration of  $5 \times 10^5$  CFU mL<sup>-1</sup>, then 50 mL was added to each well of the compound-containing plates. The minimum inhibition concentration (MIC) was determined by measuring bacterial growth after 24 h on performing 1 : 2 serial dilutions of each compound ranging from 1 to 128 µg mL<sup>-1</sup>. Since there is no effective antibiotic drug on *Pseudomonas syringae*, no positive control was included in this experiment.

### Conflicts of interest

There are no conflicts to declare.

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