



Streptovertimycins A–H, new fasamycin-type antibiotics produced by a soil-derived *Streptomyces morookaense* strain

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Abstract

Eight new fasamycin-type polyketides, streptovertimycins A–H (1–8), were isolated from soil-derived *Streptomyces morookaense* SC1169 cultivated on wheat grains. Their structures were established by extensive spectroscopic analysis and theoretical computations of ECD spectra. Compounds 1–8 have a fasamycin-type pentacyclic structure featuring a 15-*O*-methyl group. They exhibited potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE) with MIC values in the range of 0.63–5.0 µg/ml. The activity profile provided new insights into the structure–activity relationships of fasamycin-type antibiotics.

Introduction

Fasamycins and congeners are a class of rare aromatic polyketides possessing a partially reduced 1-phenyltetracene pentacyclic core. The first disclosed members of this class of natural products are the *Streptomyces* sp. KB-3346-5 derived naphthacemycins, including three (A–C) different series of 17 compounds [1–3], which were originally termed as “KB-3346-5 substances” in a Japanese patent [3].

Fasamycins A and B were obtained by the heterologous expression of a clone derived from environmental DNA isolated from soil [4, 5]. Other congeners so far reported are all produced by *Streptomyces formicae* isolated from an African fungus-growing plant–ant species, which include fasamycins C–F, formicamycins A–M [6, 7], and formicapyridines A–I [7]. Most of this class of compounds, except the pyridine-containing formicapyridines, were shown to exhibit potent activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) [1–6] and vancomycin-resistant *Enterococcus faecalis* (VRE) [4–6]. Fasamycins A and B were found to inhibit the FabF enzyme associated with the biosynthesis of type II fatty acids (FASII) in bacteria [5]. This mode of action differs from those of antibiotics currently in clinical use [5]. In addition, some naphthacemycins were reported as novel inhibitors of Poly (ADP-ribose) polymerase-1 (PARP1) [8]. The fascinating biological profile of fasamycins and congeners has attracted organic synthetic and medicinal chemists to embark on their synthesis. The total synthesis of fasamycin A and naphthacemycin A₀ have been achieved and the structure–activity relationship (SAR) of the analogues were discussed [9, 10].

Streptomyces morookaense (formerly *Streptoverticillium morookaense*) SC1169 is an actinomycete isolated from soil. Its culture extract was previously found to be active against the economically important fungal phytopathogen *Peronosphythora litchii* and two new active alkaloids were isolated from the extract [11]. During our ongoing search for antibacterial natural products from microorganisms

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[12–16], the culture extract of this strain was found to be also active against *S. aureus* and MRSA. Thus, the strain SC1169 was reinvestigated by focusing on metabolites responsible for the antibacterial activity, leading to the isolation of eight new fasamycin congeners, streptovertimycins A–H (**1–8**), from wheat-grown cultures. We report here the isolation, structure elucidation, and antibacterial activity of these compounds.

Results and discussion

S. morookaense SC1169 was cultivated on wheat grains. The culture was extracted with 95% EtOH and the resulting crude extract was successively partitioned with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc-soluble fraction was subjected to silica gel column chromatography (CC) followed by ODS CC, Sephadex LH-20 CC, and preparative HPLC, to afford the compounds **1–8** (Fig. 1).

Streptovertimycin A (**1**) was obtained as a yellow powder. Its molecular formula was determined as C₂₉H₂₅ClO₇ based on the HR-ESIMS and ¹³C NMR data. The ¹H NMR spectrum (Table 1) exhibited signals for two sets of *meta*-coupled aromatic protons [δ_{H} 6.46 and 6.43 (d, $J = 2.4$ Hz, H-2 and H-4); 6.87 and 7.12 (br s, H-24 and H-22)] indicating the presence of two tetrasubstituted aromatic rings. The spectrum also displayed signals for two isolated aromatic protons [δ_{H} 6.72 and 7.31 (s, H-16 and H-20)], two methoxys [δ_{H} 3.84 (3-OCH₃) and 4.04 (15-OCH₃)], three tertiary methyls [δ_{H} 1.96 (H₃-25), 1.72 (H₃-27), and 1.76 (H₃-27)], and two hydrogen-bonded phenolic hydroxys [δ_{H} 14.34 (9-OH) and 13.50 (13-OH)]. The ¹³C NMR spectrum (Table 2) showed signals for 11 hydrogen-bearing carbons corresponding to the above non-exchangeable protons as indicated by the HSQC spectrum. Other ¹³C NMR resonances included 16 aromatic quaternary carbons, a conjugated carbonyl carbon [δ_{C} 190.2 (C-11)], and an aliphatic quaternary carbon [δ_{C} 38.8 (C-18)]. The connectivities were determined from extensive HMBC correlations (Fig. 2). H–C long range correlations from H-2 to C-1 (δ_{C} 137.6), C-3 (δ_{C} 159.6), C-4 (δ_{C} 98.3), and C-6 (δ_{C} 122.5), from H-4 to C-5 (δ_{C} 152.9), C-2 (δ_{C} 107.7), C-6, and C-3, from H₃-25 (δ_{H} 1.96) to C-1, C-2 and C-6, and from 3-OCH₃

protons to C-3 established a 3,4-disubstituted 1-methoxy-3-methylbenzene ring (A); correlations from H-22 to C-21 (δ_{C} 141.8), C-23 (δ_{C} 157.6), C-24 (δ_{C} 121.9), and C-8 (δ_{C} 117.3) and from H-24 to C-7 (δ_{C} 137.6), C-22 (δ_{C} 110.7), C-23, and C-8 constructed a 1,2,3,5-tetrasubstituted benzene ring (B); and the H-24/C-6 correlation revealed a biaryl linkage between C-6 of ring A and C-7 of ring B. The presence of a 1,8-dihydroxy-10,10-dimethylantracene fused with ring B via C-8/C-21 was indicated by HMBC correlations from H-20 to C-10 (δ_{C} 107.4), C-18 (δ_{C} 38.8), C-11 (δ_{C} 190.2), C-8, and C-22, from H-16 to C-14 (δ_{C} 109.5), C-15 (δ_{C} 161.4), C-12 (δ_{C} 107.9), C-17 (δ_{C} 151.8), C-11, and C-18, from H₃-26 (δ_{H} 1.72) and H₃-27 (δ_{H} 1.76) to C-18, C-19, and C-17, from 9-OH to C-9 (δ_{C} 166.0), C-10, and C-8, and from 13-OH to C-13 (δ_{C} 160.0), C-12, and C-14 (Fig. 2). The additional HMBC correlation of the OCH₃ protons at δ_{H} 4.04 with C-15 located the OCH₃ group at C-15. The low field shifts of C-5 (δ_{C} 152.9) and C-23 (δ_{C} 157.6) and the high field shift of C-14 (δ_{C} 109.5), in combination with the molecular formula, suggested the presence of hydroxyl groups at C-5 and C-23 and the chlorine at C-14. Thus, compound **1** was characterized to be a fasamycin-type metabolite with the structure as shown in Fig. 1.

Once the structure of **1** was established, analysis of UV absorptions (λ_{max} 251, 289, 351, and 425 nm), ESIMS data, and NMR data (Tables 1 and 2) readily found that compounds **2–8** are also fasamycin-type polyketides with structures containing more chlorine atoms than that of **1**. Structural variations among these compounds are the position and number of *O*-methyl groups and chlorine atoms in rings A and B.

Streptovertimycins B (**2**) and C (**3**) were both determined to have a molecular formula of C₂₉H₂₄Cl₂O₇, with an additional chlorine atom relative to that of **1**. The ¹H NMR data of **2** and **3** (Tables 1 and 2) were closely similar to those of **1** except that one of two sets *meta*-coupled aromatic protons appeared as a proton singlet, suggesting that one of aromatic protons in ring A or B of **1** is replaced by the second chlorine in **2** and **3**. Comparison of ¹³C NMR spectrum of **2** with that of **1** readily found that **2** contains an additional aromatic quaternary carbon [δ_{C} 113.9 (C-22)], which was assigned to the second chlorinated carbon on the basis of significantly upfield (by 4.0–5.0 ppm) shifts of C-20, C-21, and C-23 relative to those in **1** as well as the presence of an HMBC correlation of this carbon with the proton singlet [δ_{H} 7.10 (H-24)], which further correlated with C-8 (δ_{C} 117.9) and C-6 (δ_{C} 122.0). The second chlorinated carbon in **3** was located at C-2 (δ_{C} 114.5) as indicated by HMBC correlations of this carbon with H-4 (δ_{H} 5.52) and H₃-25 (δ_{H} 2.04) together with the upfield ¹³C NMR shifts of C-1, C-3, C-5, and C-25 relative to those in **1** and **2** (Table 2). Thus,

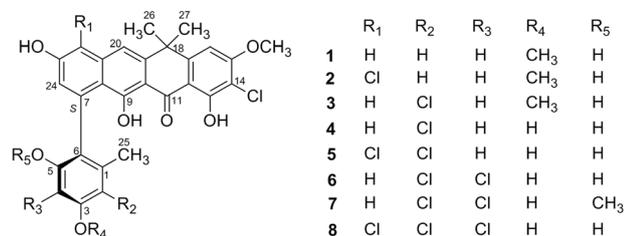


Fig. 1 Structures of streptovertimycins A–H (**1–8**)

Table 1 ^1H NMR (500 MHz) data for streptovertimycins A–H (**1–8**) in CDCl_3^a

Position	1	2	3	4	5	6	7	8
2	6.46 (1H, d, 2.4)	6.49 (1H, d, 2.4)						
4	6.43 (1H, d, 2.4)	6.44 (1H, d, 2.4)	6.52 (1H, s)	6.60 (1H, s)	6.59 (1H, s)			
16	6.72 (1H, s)	6.78 (1H, s)	6.72 (1H, s)	6.75 (1H, s)	6.77 (1H, s)	6.73 (1H, s)	6.72 (1H, s)	6.78 (1H, s)
20	7.31 (1H, s)	7.80 (1H, s)	7.32 (1H, s)	7.32 (1H, s)	7.81 (1H, s)	7.32 (1H, s)	7.30 (1H, s)	7.78 (1H, s)
22	7.12 (1H, br s)		7.17 (1H, d, 2.4)	7.18 (1H, d, 2.4)		7.13 (1H, d, 2.4)	7.14 (1H, br s)	
24	6.87 (1H, br s)	7.10 (1H, s)	6.88 (1H, d, 2.4)	6.89 (1H, d, 2.4)	7.06 (1H, s)	6.80 (1H, d, 2.4)	6.80 (1H, br s)	6.99 (1H, s)
25	1.96 (3H, s)	2.00 (3H, s)	2.04 (3H, s)	2.05 (3H, s)	2.05 (3H, s)	2.05 (3H, s)	2.05 (3H, s)	2.05 (3H, s)
26	1.72 (3H, s)	1.81 (3H, s)	1.74 (3H, s)	1.75 (3H, s)	1.81 (3H, s)	1.73 (3H, s)	1.73 (3H, s)	1.78 (3H, s)
27	1.76 (3H, s)	1.84 (3H, s)	1.77 (3H, s)	1.77 (3H, s)	1.84 (3H, s)	1.77 (3H, s)	1.77 (3H, s)	1.82 (3H, s)
3-OMe	3.84 (3H, s)	3.87 (3H, s)	3.94 (3H, s)					
5-OMe							4.00 (3H, s)	
15-OMe	4.04 (3H, s)	4.08 (3H, s)	4.04 (3H, s)	4.04 (3H, s)	4.08 (3H, s)	4.04 (3H, s)	4.04 (3H, s)	4.06 (3H, s)
9-OH	14.34 (1H, s)	14.38 (1H, s)	14.31 (1H, s)	14.34 (1H, s)	14.34 (1H, s)	14.29 (1H, s)	14.25 (1H, s)	14.31 (1H, s)
13-OH	13.50 (1H, s)	13.40 (1H, s)	13.46 (1H, s)	13.48 (1H, s)	13.33 (1H, s)	13.47 (1H, s)	13.50 (1H, s)	13.36 (1H, s)

^a δ_{H} in ppm (Int, mult, J in Hz)

compounds **2** and **3** were characterized as the 22- and 2-chlorinated derivatives of streptovertimycin A, respectively. Streptovertimycin D (**4**) is also a dichlorinated metabolite. It has a molecular formula containing a CH_2 unit less than that of **2** and **3**. Its 1D NMR spectra were almost identical to those of **3** (Tables 1 and 2). Significant differences found were that the resonances for 3-OMe (δ_{H} 3.94, δ_{C} 56.3) in **3** was absent in **4** and chemical shifts of some carbons in ring A were upfield (C-2, C-3) or downfield (C-4) relative to those in **3**. Accordingly, **4** was found to be a 3-*O*-demethylated analogue of **3**.

The molecular formulae of streptovertimycins E (**5**) and F (**6**) were both determined as $\text{C}_{28}\text{H}_{21}\text{Cl}_3\text{O}_7$, requiring an additional chlorine atom compared with that of **4**. The 1D NMR spectra of **5** and **6** were both closely similar to those of **4** (Tables 1 and 2). Obvious differences observed between the spectra of **5** and **4** were in the signals attributable to ring B and vicinal positions, including H-20, H-24, and C-19–C-24, which instead, were almost identical with those in **2** (Tables 1 and 2), indicating that C-22 is also chlorinated in **5**. The 1D NMR data of **6** differed from those of **4** in the absence of the signals for C-4 methine and the presence of the carbon signal for an additional quaternary carbon (δ_{C} 105.1), indicating that C-4 is the third chlorinated position in **6**. The molecular formula of streptovertimycin G (**7**) was determined to be $\text{C}_{29}\text{H}_{23}\text{Cl}_3\text{O}_7$, with one more CH_2 unit than that of **6**. Analysis of the 1D NMR data of **7** revealed a structure similar to that of **6**, except for the presence of an additional OMe group (δ_{H} 4.00, δ_{C} 60.8). The attachment of this group at C-5 was indicated by the HMBC correlation of the OMe protons with C-5 (δ_{C} 151.2). Therefore, **7** was a 5-*O*-methylated analogue of **6**.

Streptovertimycin H (**8**) gave a molecular formula of $\text{C}_{28}\text{H}_{20}\text{Cl}_4\text{O}_7$, indicating the presence of an additional

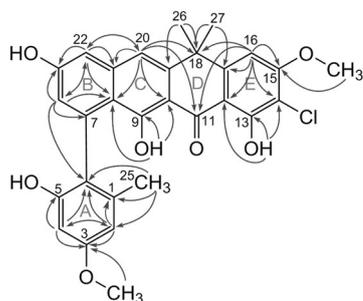
chlorine atom compared with that of **5** and **6**. The 1D NMR data of **8** were comparable to those of both **5** and **6**, in which the resonances for the carbons of ring A of **8** were almost identical to those of **6** (Table 2), whereas the proton and carbon resonances for ring B and vicinal positions (C-19 and CH-20) were closely similar to those of **5** (Tables 1 and 2), indicating that **8** bears the same ring A as that in **6** and ring B as that in **5**. Thus, **8** was characterized as a 22-chlorinated analogue of **6**.

Compounds **1–8** exhibited optical activity with various $[\alpha]_{\text{D}}$ values due to the presence of biaryl axial chirality [6]. The configurations of **1**, **3**, and **6** were solved by theoretical computations of electronic circular dichroism (ECD) spectra. As can be seen in Fig. 3, the calculated ECD spectra for (*S*)-atropisomers of **1**, **3**, and **6** were all in good fit with the measured spectra, whereas the theoretical spectra of (*R*)-isomers were obvious mirror images of the experimental spectra, which designated these compounds as *S*. Other compounds (**2**, **4**, **5**, **7**, and **8**) were also assigned to this configuration based on the similarity of their measured ECD spectra with those of **1**, **3**, and **6** (Fig. 3). It is interesting that compounds **1–8** and the previously reported congeners of known stereochemistry [6, 7] are all (*S*)-atropisomers, though they are from different microbial sources. This configuration is presumable to be a common characteristic in stereochemistry of this class of natural products.

The isolated metabolites were evaluated for their antibacterial activity against a panel of pathogenic bacteria including four Gram-positive bacteria, *S. aureus* ATCC6548 (MSSA), methicillin-resistant *S. aureus* 11646 (MRSA), *E. faecium* (VSE), and vancomycin-resistance *E. faecium* (VRE), and two Gram negative bacteria, *Escherichia coli* ATCC8739 and *Shigella dysenteriae* CMCC51252. Compounds **1–8** exhibited remarkable

Table 2 ^{13}C (125 MHz) chemical shifts (δ_{C} in ppm) for streptovertimycins A–H (1–8) in CDCl_3

Position	1	2	3	4	5	6	7	8
1	137.7	137.4	135.8	134.5	134.4	133.3	134.2	133.1
2	107.7	107.8	114.5	112.4	112.6	112.8	120.5	112.7
3	159.6	159.6	155.1	151.1	151.4	147.6	147.4	147.3
4	98.3	98.2	97.5	100.3	100.6	105.1	112.5	105.0
5	152.9	152.6	150.0	151.3	151.5	151.9	151.2	152.6
6	122.5	122.0	123.2	123.5	123.0	123.7	127.6	123.2
7	137.6	136.0	136.8	136.5	135.5	137.3	137.2	135.8
8	117.3	117.9	117.2	117.1	117.9	117.1	116.6	117.5
9	166.0	165.6	165.7	165.7	165.3	165.6	165.5	165.3
10	107.4	107.7	107.6	107.3	107.7	107.5	107.2	107.6
11	190.2	190.2	190.2	190.2	190.3	190.4	190.3	190.2
12	107.9	107.7	107.5	107.8	107.9	107.9	107.3	107.9
13	160.0	159.9	160.0	159.9	160.0	160.0	159.8	159.9
14	109.5	109.3	109.6	109.4	109.3	109.5	109.4	109.3
15	161.4	161.3	161.3	161.0	161.5	161.2	160.9	161.3
16	100.8	100.8	101.0	100.7	101.1	100.9	100.8	100.9
17	151.8	151.7	151.8	151.7	151.7	151.8	151.7	151.8
18	38.8	39.2	39.3	38.9	39.5	39.1	39.1	39.4
19	145.7	147.4	146.1	146.0	147.6	145.9	145.6	147.4
20	115.2	111.2	115.2	115.0	111.5	115.2	115.2	111.3
21	141.8	136.8	141.4	141.4	136.8	141.3	141.2	136.8
22	110.7	113.9	111.2	110.8	114.2	110.7	110.6	113.8
23	157.6	152.7	157.7	157.4	152.7	157.0	157.6	152.7
24	121.9	120.8	122.1	121.8	121.0	121.1	120.7	120.1
25	20.7	20.6	18.3	18.3	18.3	18.1	18.2	18.1
26	34.7	34.9	34.4	34.6	35.0	34.8	34.6	35.0
27	34.2	34.3	34.3	34.2	34.5	34.2	34.1	34.5
3-OMe	55.3	55.3	56.3					
5-OMe							60.8	
15-OMe	56.5	56.4	56.6	56.4	56.7	56.6	56.4	56.5

**Fig. 2** Key HMBC correlations (arrows) of **1**

antibacterial activity toward Gram-positive bacteria MSSA, MRSA, VSE, and VRE with minimal inhibitory concentration (MIC) values in the range of $0.63\text{--}5.0\ \mu\text{g ml}^{-1}$, but were inactive ($\text{MIC} > 50\ \mu\text{g ml}^{-1}$) against Gram

negative bacteria *E. coli* and *S. dysenteriae*. The results were consistent with previous findings for fasamycins, nephthacemycins, and formicamycins [1–6]. As can be seen in Table 3, compounds **4**, **5/6**, and **8**, containing two, three, and four chlorine atoms, respectively, showed a successive drop in the activity, suggesting that more chlorine atoms could not enhance their antibacterial potency. Compounds **1–3** in comparison with compound **4** supported that a free OH group at C-3 is a favoured structural feature for the activity. Furthermore, the 5-*O*-methyl bearing **7** displayed a 2–4 fold activity superior to the 5-OH containing **6**, indicating that methylation of 5-OH could enhance the potency.

In conclusion, the present study discovered eight new fasamycin-type polyketides, streptovertimycins A–H (**1–8**), and demonstrated that *S. morookaense* SC1169 is a new producer of this class of promising antibacterial substances. Compounds **1–8**, with structures distinct from those of reported congeners, also showed strong activity against MRSA and VRE. Their activity profile provided new insights into SARs of fasamycins and congeners, which would be useful clues to structure optimization of this type of antibiotics.

Material and methods

General experimental procedure

Optical rotations were measured in MeOH on a Perkin-Elmer 343 spectropolarimeter. UV spectra and CD spectra were obtained simultaneously on a Chirascan CD spectrometer (Applied Photophysics Ltd., England) with MeOH as solvent. 1D NMR, and 2D NMR experiments were performed on a Bruker Avance III 500 MHz spectrometer. HR-ESIMS data were collected on a Bruker maXis Q-TOF mass spectrometer. Preparative and semipreparative HPLC were performed on a Shimadzu LC-6AD pump and a Shimadzu RID-10A refractive index detector with a Shimadzu Shim-packed Pro-ODS column ($20 \times 250\ \text{mm}$) and a Waters Nova-Pak[®] HR C-18 column ($7.8 \times 300\ \text{mm}$). Silica gel 60 (100–200 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), YMC ODS (75 μm , YMC Co. Ltd., Kyoto, Japan) and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden) were used in CC. Analytical TLC was performed on HSGF254 silica gel plates (0.2 mm, Yantai Jianguyou Silica Gel Development Co. Ltd., Yantai, China); spots were visualized by spraying with 10% H_2SO_4 solution followed by heating.

Producing actinomycete and fermentation

The actinomycete, *Streptomyces morookaense* (formerly *Streptoverticillium morookaense*) SC1169 as previously

Fig. 3 Comparison between the M06/TZVP/PCM calculated and measured ECD spectra of compounds **1**, **3** and **6** and comparison of the measured ECD spectra of **1–8**

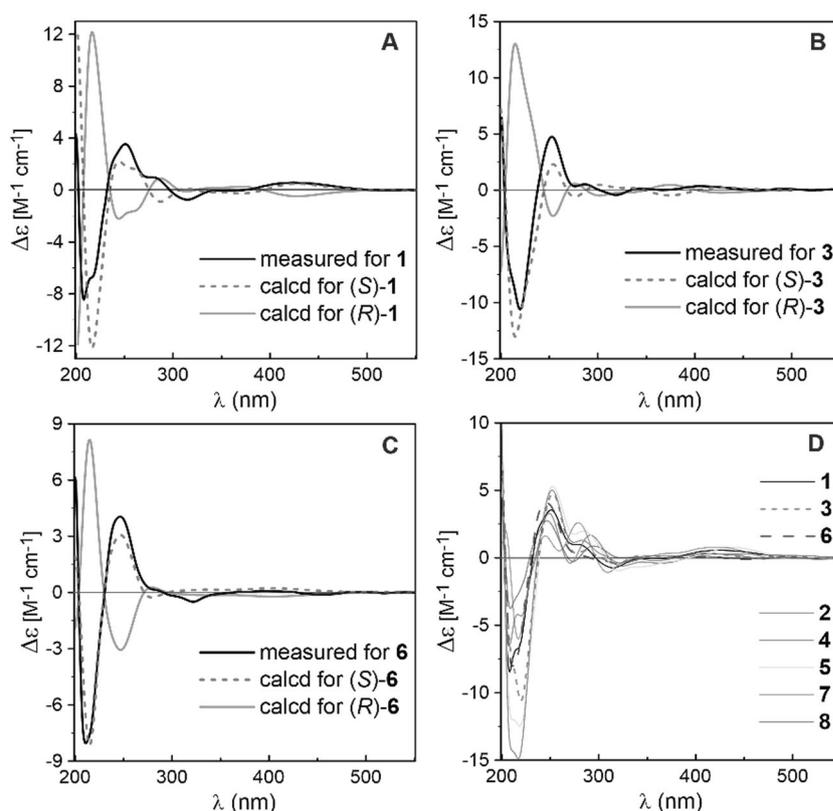


Table 3 Antibacterial activity (MIC, $\mu\text{g ml}^{-1}$) of compounds **1–8**

Compound	MRSA	MSSA	VRE	VSE
1	2.5	2.5	2.5	2.5
2	2.5	2.5	2.5	2.5
3	2.5	2.5	2.5	2.5
4	1.25	1.25	1.25	1.25
5	2.5	2.5	2.5	2.5
6	2.5	2.5	2.5	2.5
7	0.63	0.63	1.25	1.25
8	5.0	5.0	5.0	5.0
Vancomycin	0.63	0.63	>40	2.5
Kanamycin	>40	1.25	NT	NT

NT not tested

described [11], was isolated from a soil sample collected in the Dinghu Mountain Biosphere Reserve, Guangdong, People's Republic of China. The culture plugs of SC1169 grown on PDA medium for 10 days were inoculated into twenty five 500 ml Erlenmeyer flasks containing 100 ml of YMG medium (glucose 0.4%, malt extract 1.0%, yeast extract 0.4%, pH 5.5) and shaken on a rotatory (150 rpm) at 28 °C for 72 h. Ten millilitres of culture broth was transferred into each of two hundred 500 ml flasks containing 60 ml of YMG medium and 60 g of wheat grains, and the incubation was performed in the dark at 28 °C for 30 days.

Extraction and isolation

The solid fermentation cultures were extracted with 95% EtOH three times. The resultant crude extract was dissolved into water and partitioned sequentially with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc-soluble fraction (67.5 g) was separated by a silica gel column using CHCl_3 -MeOH gradient of increasing polarity (100:0–70:30) for elution to afford 16 fractions (Fr. 1–16). Fr.7 was further fractionated by ODS column eluting with MeOH– H_2O (10:90–80:20) to yield ten pooled subfractions (Frs. 7A–7J). Fr. 7H was subjected to preparative HPLC purification, using 72% aqueous MeOH as mobile phase with a flow rate of 5 ml min^{-1} to yield compound **1** (3.0 mg, $t_R = 90.5 \text{ min}$) and **6** (4.0 mg, $t_R = 98.5 \text{ min}$). Fr. 7G was further separated by Sephadex LH-20, followed by semipreparative HPLC (2.5 ml min^{-1}) using 56% aqueous CH_3CN to give compound **3** (1.4 mg, $t_R = 47.5 \text{ min}$), **4** (2.6 mg, $t_R = 52.2 \text{ min}$), and **5** (5.1 mg, $t_R = 46.0 \text{ min}$). Fr. 7I was subjected to semipreparative HPLC (2.5 ml min^{-1}) using 56% aqueous CH_3CN to yield compound **2** (4 mg, $t_R = 71.0 \text{ min}$) and **8** (2.6 mg, $t_R = 76.0 \text{ min}$). Further purification of Fr. 7J on semipreparative HPLC (2.5 ml min^{-1}) using 75% aqueous MeOH afforded **7** (5 mg, $t_R = 34.5 \text{ min}$).

Streptovertimycin A (**1**): yellow powder; $[\alpha]_D^{20} +56.5$ (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (3.82), 250 (3.38), 289 (3.18), 353 (3.12), 429 (3.16) nm; CD (MeOH)

$\Delta\epsilon$ 209 (−8.4), 241 (+3.6), 308 (−1.4), 426 (+0.7); ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS m/z 519.1225 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{29}\text{H}_{24}\text{ClO}_7$, 519.1216).

Streptovertimycin B (2): yellow powder; $[\alpha]_{\text{D}}^{20} +5.0$ (c 0.1, MeOH); UV (MeOH) λ_{max} ($\log \epsilon$) 203 (3.77), 249 (3.32), 290 (3.18), 354 (3.08), 428 (3.10) nm; CD (MeOH) $\Delta\epsilon$ 207 (−6.7), 246 (+3.4), 290 (+2.0), 321 (−1.2), 421 (+0.4); ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS m/z 553.0841 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{29}\text{H}_{23}\text{Cl}_2\text{O}_7$, 553.0826).

Streptovertimycin C (3): yellow powder; $[\alpha]_{\text{D}}^{20} +4.0$ (c 0.1, MeOH); UV (MeOH) λ_{max} ($\log \epsilon$) 204 (3.81), 250 (3.35), 291 (3.18), 355 (3.07), 426 (3.11) nm; CD (MeOH) $\Delta\epsilon$ 225 (−10.8), 246 (+4.8), 416 (+0.4); ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS m/z 553.0837 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{29}\text{H}_{23}\text{Cl}_2\text{O}_7$, 553.0826).

Streptovertimycin D (4): yellow powder; $[\alpha]_{\text{D}}^{20} -11.0$ (c 0.1, MeOH); UV (MeOH) λ_{max} ($\log \epsilon$) 203 (3.87), 250 (3.43), 291 (3.25), 353 (3.17), 427 (3.22) nm; CD (MeOH) $\Delta\epsilon$ 214 (−15.0), 245 (+5.1), 312 (−0.8), 411 (+0.4); ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS m/z 539.0691 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{28}\text{H}_{21}\text{Cl}_2\text{O}_7$, 539.0670).

Streptovertimycin E (5): yellow powder; $[\alpha]_{\text{D}}^{20} +43.5$ (c 0.2, MeOH); UV (MeOH) λ_{max} ($\log \epsilon$) 203 (3.85), 252 (3.49), 292 (3.29), 351 (3.18), 425 (3.23) nm; CD (MeOH) $\Delta\epsilon$ 214 (−12.4), 252 (+5.0), 316 (−1.6), 440 (+1.0); ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS m/z 573.0293 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{28}\text{H}_{20}\text{Cl}_3\text{O}_7$, 573.0280).

Streptovertimycin F (6): yellow powder; $[\alpha]_{\text{D}}^{20} +43.0$ (c 0.1, MeOH); UV (MeOH) λ_{max} ($\log \epsilon$) 205 (3.86), 250 (3.37), 291 (3.23), 353 (3.16), 425 (3.19) nm; CD (MeOH) $\Delta\epsilon$ 210 (−8.3), 240 (+3.7), 312 (−1.3); ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS m/z 573.0302 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{28}\text{H}_{20}\text{Cl}_3\text{O}_7$, 573.0280).

Streptovertimycin G (7): yellow powder; $[\alpha]_{\text{D}}^{20} +63.5$ (c 0.2, MeOH); UV (MeOH) λ_{max} ($\log \epsilon$) 205 (3.90), 252 (3.41), 292 (3.21), 354 (3.15), 426 (3.20) nm; CD (MeOH) $\Delta\epsilon$ 218 (−6.2), 240 (+1.5), 281 (+2.6), 423 (+1.1); ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS m/z 587.0445 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{29}\text{H}_{22}\text{Cl}_3\text{O}_7$, 587.0437).

Streptovertimycin H (8): yellow powder; $[\alpha]_{\text{D}}^{20} +18.0$ (c 0.1, MeOH); UV (MeOH) λ_{max} ($\log \epsilon$) 204 (3.62), 251 (3.27), 290 (3.07), 351 (2.94), 423 (2.94) nm; CD (MeOH) $\Delta\epsilon$ 210 (−3.7), 250 (+2.6), 293 (+1.3); ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS m/z 606.9901 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{28}\text{H}_{19}\text{Cl}_4\text{O}_7$, 606.9890).

Evaluation of antibacterial activity

Four Gram-positive bacterial strains, *S. aureus* (ATCC 6538), MRSA (no. 11646), VSE, and VRE, and two Gram negative bacterial strain, *E. coli* (ATCC 8739) and

S. dysenteriae (CMCC 51252), were used for antibacterial evaluation. The reference strains, MSSA, *E. coli*, and *S. dysenteriae*, were purchased from Microbial Culture Collection Center of Guangdong Institute of Microbiology (Guangzhou, China). VRE (no. 151458137) and VSE (no. 160119481), the clinical isolates from abdomen of patients, were obtained from The First Affiliate Hospital, Sun Yat-sen University. MRSA (no. 11646) was kindly provided by the State Key Laboratory of Respiratory Disease, First Affiliated Hospital of Guangzhou Medical University (Guangzhou, China). The activity was tested by microplate Alamar Blue assay (MABA) as previously described [12] with brain heart infusion medium for VRE and VSE, and Mueller-Hinton broth medium for other strains. The strains were incubated in medium on a rotary shaker at 150 rpm in 28 °C for 12 h. The bacterial suspensions of the strain were quantified with a hemocytometer and adjusted with medium to 1×10^5 CFU ml^{-1} for the MABA. Tested compounds were twofold diluted with the DMSO to give serial concentrations. One hundred microlitres of bacterial suspension of each strain containing Alamar Blue (8%, v/v) and the compound solution (4%, v/v) was added into a 96-well microtiter plate in triplicate. The final concentrations of tested compounds were 10, 5, 2.5, 1.25, 0.63, and 0.31 $\mu\text{g ml}^{-1}$. Vancomycin and kanamycin were used as positive control. Negative control wells were added with DMSO instead of the test compound, and blank control wells contained Alamar Blue but without bacterial suspension. The plates were incubated in the dark at 28 °C for 6–8 h. The final concentration of the well, which was closest to the red one and remained blue, was deemed as the MIC.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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