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ORIGINAL ARTICLE

Macrolides of the bafilomycin family produced by *Streptomyces* sp. CS

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Five new 16-membered macrolides (1–5), belonging to the bafilomycin subfamily, were isolated from the fermentation broth of *Streptomyces* sp. CS, a commensal microbe of *Maytenus hookeri*. Their structures including relative configurations were elucidated based on NMR data and analysis of single crystal X-ray diffraction data. The cytotoxicities of compounds 1–5 on the MDA-MB-435 cell line were analyzed by the MTT method and the IC_{50} values were found to be 4.2, 4.5, 5.5, 3.8 and 11.4 μM, respectively.

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Keywords: bafilomycin; cytotoxicity; macrolide; *Streptomyces* sp. CS

INTRODUCTION

The bafilomycins,[1] including hygrolidins,[2] form a family of 16-membered ring macrolide antibiotics. Bafilomycins A_1, A_2, B_1, B_2, C_1 and C_2 were first isolated from cultures of *Streptomyces griseus*.[3,4] They are potent vacuolar H^+-ATPase inhibitors that exhibit broad antibacterial and antifungal activity.[5] Hygrolidins were first reported as antifungal agents,[6] and then reported to inhibit the growth of *src*- and *ras*-transformed cells without effects on untransformed cells.[7] These studies suggested that this family of 16-membered macrolide antibiotics have the potential to be drug candidates.

Previously, 24-demethyl-bafilomycin C_1 was isolated from the strain *Streptomyces* sp. CS as a new member of the bafilomycin subfamily.[8] During the subsequent research, two more new bafilomycin derivatives, namely, 24-demethyl-bafilomycin A_2 and its dehydration product, were isolated from the same strain.[9] These three compounds also showed strong antifungal and antitumor activities,[8,9] and this encouraged us to search for further members of this subfamily in the same strain and led to the isolation of five new bafilomycin derivatives **1–5**. Here, we describe the fermentation of the producing strain, the isolation, structure elucidation and biological characterization of these five new macrolides.

RESULT

Structure determination

Compound **1**, colorless crystal, was determined to have the molecular formula $C_{34}H_{56}O_9$ based on the HRESIMS and NMR data. Inspection of the NMR data (proton, carbon, DEPT, HSQC and HMBC) revealed a bafilomycin-type 16-membered macrolide (Tables 1 and 2). The ^{13}C-NMR and DEPT spectra of **1** showed 34 carbon signals for seven methyl, three methoxyl, three methylene, sixteen methine and five quaternary carbon atoms. On the basis of HMBC correlations, particularly, those of seven methyl carbons with the adjacent carbons, and ^{1}H-^{1}H COSY spectra, compound **1** was determined to be 24-demethyl-bafilomycin A_1[4] (Figure 1).

Compound **2**, colorless crystal, was determined to have the molecular formula $C_{35}H_{58}O_9$ based on the HRESIMS and NMR data (Tables 1 and 2), and showed similar patterns as compound **1** in the ^{1}H and ^{13}C NMR spectra except for one methoxyl (δ H 3.29 (s, 3H) and δ C 56.2(q)) connected to C-21 according to the HSQC and HMBC experiments, determining the structure of **2** to be 21-*O*-methyl-24-demethyl-bafilomycin A_1 (Figure 1). The X-ray diffraction experiment of a crystal from Me_2CO–H_2O further confirmed the structure and determined the relative configurations of compound **2** (Figure 2) (CCDC 707561).

X-ray crystal structure analysis of compound 2: Colorless needle crystal of $C_{35}H_{58}O_9$, M=622.4, orthorhombic, space group P 21 21 2, a=18.779 (4) Å, b=23.935 (5) Å, c=9.0455 (16) Å, V=4065.6 (13) Å^3, Z=1, D_c=1.042 g cm^{-3}, F (000)=1388, T=293 (2) K. Oxford Gemini S Ultra single crystal diffraction, graphite monochromator, λ(Mo–Kα)=0.71073 Å, μ=0.09 mm^{-1}, size 0.35×0.22×0.8 mm^3, a total of 13798 reflections, of which 6624 were independent ($R_{(int)}$=18.2%), were yielded and collected $4.05<\theta<25.0^\circ$, $-22<h<22$, $-28<k<27$, $-10<l<10$. The structure was solved by direct methods (SHELXS–97, Göttingen, Germany) and refined by full-matrix least squares on F^2 techniques using 415 parameters. Refinement converged at $R_1(F)$=0.105, $wR_2(F^2$, all data)=0.2475, S=0.778, min/max height in the final ΔF map −0.323/0.894 e Å^{-3}. Crystallographic data (excluding structure factors) for structure **2** in this paper have been

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Table 1 The ^{1}H NMR spectroscopic data of 1–5

Number	***1***[a,b]	***2***[a,b]	***3***[a,b]	***4***[a,b]	***5***[a,b]
3	6.70 (s)	6.70 (s)	6.70 (s)	6.75 (s)	6.66 (s)
5	5.97 (d, 9)	5.97 (d, 9)	5.96 (d, 9)	6.01 (d, 9)	5.95 (d, 9)
6	2.57 (br t, 7.2)	2.56 (br t, 7.2)	2.56 (br t, 7.2)	2.51 (br t, 7.2)	2.56 (br t, 7.2)
7	3.32 (br d, 6.6)	3.32 (br d, 6.6)	3.33 (m)	3.45 (br d, 6.6)	3.31 (br d, 6.6)
8	1.88 (m)	1.89 (m)	1.90 (m)	1.84 (m)	1.87 (m)
9	2.07 (m)	2.06 (m)	2.06 (m)	2.42 (m), 1.86 (m)	2.01 (m)
11	5.82 (d, 10.2)	5.80 (d, 10.2)	5.82 (d, 10.2)	5.90 (d, 10.8)	5.82 (d, 10.8)
12	6.69 (dd, 10.2, 15)	6.69 (dd, 10.2, 15)	6.68 (dd, 10.2, 15)	6.51 (dd, 10.8, 15)	6.65 (dd, 10.8,15)
13	5.17 (dd, 9, 15)	5.17 (dd, 9, 15)	5.19 (dd, 9, 15)	5.42 (dd, 6, 15)	5.17 (dd, 10.8,15)
14	4.08 (t, 9)	4.08 (t, 9)	4.05 (t, 9)	3.94 (t, 4.2)	4.05 (dd, 7.2,16.2)
15	4.99 (d, 7.8)	4.99 (d, 7.8)	5.12 (d, 7.8)	5.94 (dd, 3, 8.4)	5.15 (dd, 9,15)
16	1.79 (q, 7.2)	1.82 (q, 7.2)	2.02 (m)	2.88 (dd, 7.2, 16.8)	2.04 (m)
17	4.21 (dd, 2.1, 10.2)	4.20 (dd, 2.1, 10.2)	3.56 (dd, 4.8, 10.2)	5.80 (d, 10.8)	3.82 (m)
18	2.17 (dq, 2.1, 7.8)	2.18 (dq, 2.1, 7.8)	2.07 (dq, 2.1, 7.8)	—	3.00 (dq, 2.4, 7.2)
20	1.15 (m)	2.42 (m), 0.98 (m)	2.45 (m), 1.33 (m)	2.44 (m), 1.04(m)	6.29 (d, 16.2)
21	3.53 (m)	3.18 (m)	3.10 (m)	3.19 (m)	6.91 (dd, 8.4, 16.2)
22	2.20 (m)	1.19 (m)	1.22 (m)	1.26 (m)	2.42 (m)
23	3.49 (m)	3.51 (m)	3.14 (m)	3.21 (m)	3.42 (m)
24	1.68 (m), 1.31 (m)	1.68 (m), 1.30 (m)	1.71 (m), 1.67 (m)	1.80 (m), 1.49 (m)	1.44 (m), 1.39 (m)
25	0.88 (t, 7.2, 3H)	0.86 (t, 7.2, 3H)	0.96 (t, 7.2, 3H)	1.05 (t, 6.6, 3H)	0.93 (t, 7.2, 3H)
CH_3O-2	3.64 (s, 3H)	3.63 (s, 3H)	3.63 (s, 3H)	3.65 (s, 3H)	3.64 (s, 3H)
4a	1.98 (s, 3H)	1.98 (s, 3H)	1.99 (s, 3H)	1.96 (s, 3H)	1.97 (s, 3H)
6a	1.06 (d, 7.2, 3H)	1.06 (d, 7.2, 3H)	1.06 (d, 7.2, 3H)	1.06 (d, 7.2, 3H)	1.08 (d, 7.2, 3H)
8a	0.93 (d, 7.2, 3H)	0.93 (d, 7.2, 3H)	0.94 (d, 7.2, 3H)	1.00 (d, 7.2, 3H)	0.94 (d, 7.2, 3H)
10a	1.93 (s, 3H)	1.93 (s, 3H)	1.92 (s, 3H)	1.70 (s, 3H)	1.89 (s, 3H)
CH_3O-14	3.24 (s, 3H)	3.24 (s, 3H)	3.24 (s, 3H)	3.23 (s, 3H)	3.22 (s, 3H)
16a	0.87 (d, 7.8, 3H)	0.87 (d, 7.2, 3H)	0.95 (d, 7.2, 3H)	1.02 (d, 7.2, 3H)	0.99 (d, 7.2, 3H)
18a	1.01 (d, 7.2, 3H)	1.03 (d, 7.8, 3H)	0.98 (d, 7.8, 3H)	1.70 (s, 3H)	1.10 (d, 7.8, 3H)
22a	0.94 (d, 7.2, 3H)	0.90 (d, 7.2, 3H)	0.90 (d, 7.2, 3H)	0.94 (d, 7.2, 3H)	1.06 (d, 7.2, 3H)
CH_3O-19	—	—	3.04 (s, 3H)	3.00 (s, 3H)	—
CH_3O-21	—	3.29 (s, 3H)	3.27 (s, 3H)	3.29 (s, 3H)	—

[a]^{1}H and ^{13}C NMR spectra were obtained at 600 and 150 MHz, respectively, and recorded in acetone-D_6 at room temperature.
[b]Coupling constants are presented in hertz. Unless otherwise indicated, all proton signals integrate to ^{1}H.

deposited with the Cambridge Crystallographic Data Centre as Supplementary publication number CCDC 707561. Copies of the data can be obtained free of charge on application to CCDC.

Compound **3**, a white amorphous powder, was determined to have the molecular formula $C_{36}H_{60}O_9$ based on the HRESIMS and NMR data (Tables 1 and 2), and showed similar patterns as compound **2** in the ^{1}H and ^{13}C NMR spectra except for one methoxyl (δ H 3.04 (s, 3H) and δ C 46.7(q)) connected to C-19 according to the HSQC and HMBC experiments. Thus, the structure of **3** was determined to be 19,21-di-*O*-methyl-24-demethyl-bafilomycin A_1 (Figure 1).

Compound **4**, a white amorphous powder, was determined to have the molecular formula $C_{36}H_{58}O_8$ based on the HRESIMS and NMR data (Tables 1 and 2), and showed similar patterns as compound **3** in the ^{1}H and ^{13}C NMR spectra except for the signals for H-17, C-17 and C-18. The protons at δ 5.80 (H-17) with the carbon signals at δ 129.6 (C-17) and 136.7 (C-18) indicated the presence of carbon–carbon double bond between C-17 and C-18 and determined the ^{13}C assignments for this moiety with the aid of HMBC and HMQC experiments. Thus, the structure of **4** was determined to be 17,18-dehydro-19,21-di-*O*-methyl-24-demethyl-bafilomycin A_1 (Figure 1).

Compound **5**, a colorless amorphous powder, was determined to have the molecular formula $C_{34}H_{54}O_8$ based on the HRESIMS and NMR data. Inspection of the NMR data (proton, carbon, DEPT, HMQC and HMBC) (Tables 1 and 2) and comparison with compound **1** revealed a bafilomycin-type 16-membered macrolide ring **5a** (Figure 3). The structure of fragment **5b** was determined based on the ^{1}H–^{13}C long-range correlations of the methyl protons at δ 0.86 (H-16a) with the carbons at δ 76.7 (C-15), 39.6 (C-16) and 72.9 (C-17), and the methyl protons at δ 0.97 (H-18A) with the carbons at δ 72.9 (C-17), 46.9 (C-18) and 202.3 (C-19). The structure of fragment **5c** was determined based on the ^{1}H-^{13}C long-range correlations of the methyl protons at δ 0.95 (H-22a) with the carbons at δ 149.3 (C-21), 43.3 (C-22) and 76.4(C-23), and the methyl protons at δ 0.93 (H-25) with the carbons at δ 76.4 (C-23) and 28.6 (C-24). A *trans*-substituted double bond between C-20 and C21 was determined on the basis of two coupled doublets at δ 6.29 (d, J=16.2 Hz, H-20) and 6.91 (dd, J=16.2, 8.4 Hz, H-21) by the ^{1}H NMR spectra of **5**. Thus, the linkage of the fragments **5b** and **5c** were revealed by the ^{1}H-^{13}C long-range correlation of H-20 with C-19 and C-22, H-21 with C-19, C-22, C-23 and C-22a. C-19 was determined to be ketone carbonyl based on its chemical shift at δ 202.3. On comparison with literature data for bafilomycin D, compound **5** was found to be identical except for the lack of a methyl substitute on C-24.[10] Therefore, compound **5** was determined to be 24-demethyl-bafilomycin D.

Cytotoxicities

In our research, the cytotoxic activities of compounds **1–5** against the MDA-MB-435 cell line were tested by the MTT method. These

Table 2 The ^{13}C NMR spectroscopic data of 1–5

Number	*1*[a]	*2*[a]	*3*[a]	*4*[a]	*5*[a]
1	167.7s	167.7s	167.0s	164.3s	166.5s
2	142.0s	142.0s	142.2s	142.4s	142.3s
3	134.0d	134.0d	133.5d	133.0d	133.0d
4	132.8s	132.7s	132.6s	131.6s	132.6s
5	145.6d	145.6d	145.2d	143.5d	144.7d
6	38.0d	38.0d	38.1d	39.4d	38.2d
7	80.5d	80.2d	80.5d	80.5d	80.5d
8	41.7d	41.7d	41.4d	38.9d	41.2d
9	42.3t	42.3t	42.3t	42.6t	42.3t
10	144.8s	144.8s	144.4s	141.8s	144.1s
11	125.3d	125.2d	125.3d	125.6d	125.4d
12	134.4d	134.4d	134.0d	131.4d	133.8d
13	127.1d	127.0d	127.3d	125.7d	127.0d
14	83.4d	83.3d	84.0d	83.5d	84.1d
15	77.4d	77.4d	77.5d	79.1d	76.9d
16	42.9d	42.9d	39.9d	35.4d	39.6d
17	71.5d	71.5d	70.4d	129.6d	72.9d
18	38.4d	38.4d	39.1d	136.7s	46.9d
19	99.8s	99.9d	104.0s	101.3s	202.3s
20	44.5t	39.5t	35.2t	39.5t	129.4d
21	70.3d	80.1d	80.1d	79.8d	149.3d
22	44.7d	42.6d	41.1d	42.0d	43.3d
23	74.8d	74.7d	75.5d	75.8d	76.4d
24	26.5t	26.5t	26.1t	26.5t	28.6t
25	10.8q	10.7q	9.6q	10.3q	10.7q
CH_3O-2	60.1q	60.0q	60.2q	60.1q	60.0q
4a	14.1q	14.1q	14.1q	13.8q	14.1q
6a	17.7q	17.7q	17.8q	17.8q	16.7q
8a	22.3q	22.3q	22.5q	24.5q	22.6q
10a	20.3q	20.3q	20.1q	19.1q	19.9q
CH_3O-14	55.7q	55.7q	55.7q	56.2q	55.8q
16a	10.3q	10.2q	11.1q	17.2q	11.1q
18a	7.39q	7.4q	7.9q	12.5q	9.5q
22a	13.2q	13.1q	13.0q	13.1q	18.0q
CH_3O-19	—	—	46.7q	48.7q	—
CH_3O-21	—	56.2q	56.3q	56.3q	—

[a]The spectra were obtained at 150 MHz, and recorded in acetone-D_6 at room temperature.

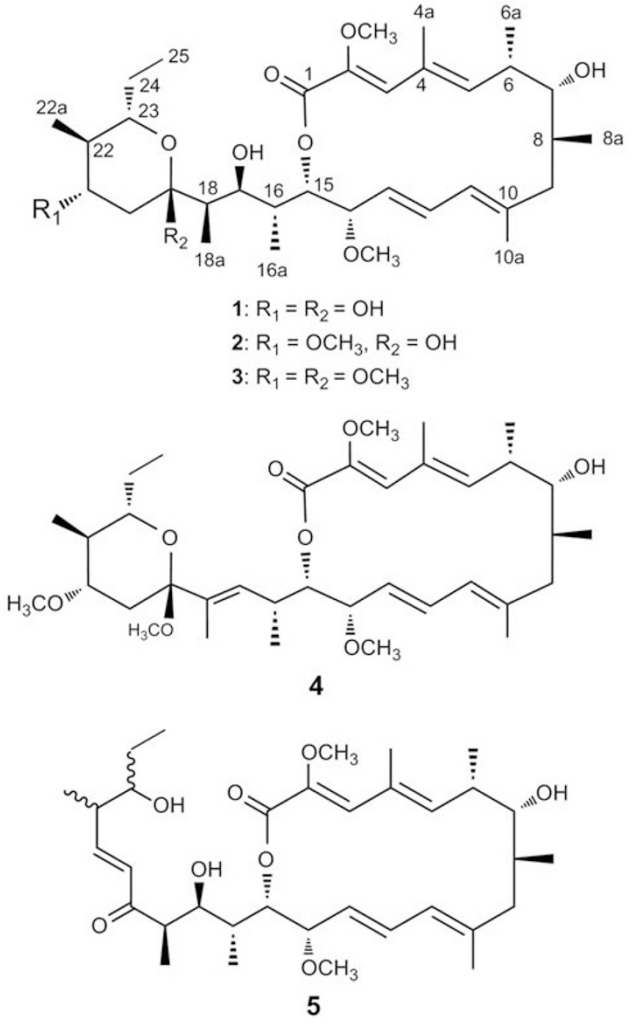

Figure 1 Structures of compounds **1**–**5**.

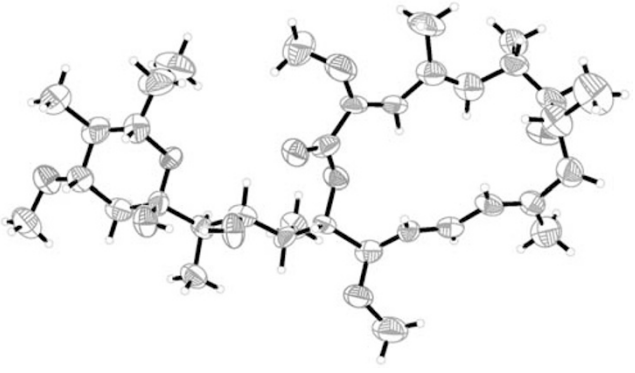

Figure 2 Crystal structure of compound **2**.

compounds showed different cytotoxic activity against MDA-MB-435 cell line *in vitro*. The IC_{50} values of compounds **1**–**5** on MDA-MB-435 cell line are 4.2, 4.5, 5.5, 3.8 and 11.4 μM, respectively.

DISCUSSION

The structures of compounds **1**, **2** and **3** share the same carbon skeleton but differ in the degree of *O*-methylation at C-19 and C-21 (Figure 1). Compound **4** has the same carbon skeleton and *O*-methylation as compound **3** but is dehydrated between C-17 and C-18. Compound **5** was the open chain ketone derivative of the compound **1**. The stereochemistry of bafilomycin A_1, initially assigned on the basis of a molecular modeling analysis of NMR data,[11] was subsequently verified by X-ray crystallography.[12,13] In our research, we determined the stereochemistry of compound **2** (21-*O*-methyl-24-demethyl-bafilomycin A_1) (Figure 2) by X-ray diffraction analysis. Our X-ray diffraction analysis showed that the relative configurations of 24-demethyl-bafilomycin and bafilomycin are the same.

24-Demethyl-bafilomycin C1 and 24-demethyl-bafilomycin A2 almost show the same cytotoxic activities in previous studies.[9] In our research the IC_{50} values of compounds **1**–**4** are nearly on the same level, the inhibitory potency of compound **5** is the weakest. These distinction may be caused by the different structures in six-membered hemiketal ring.[14]

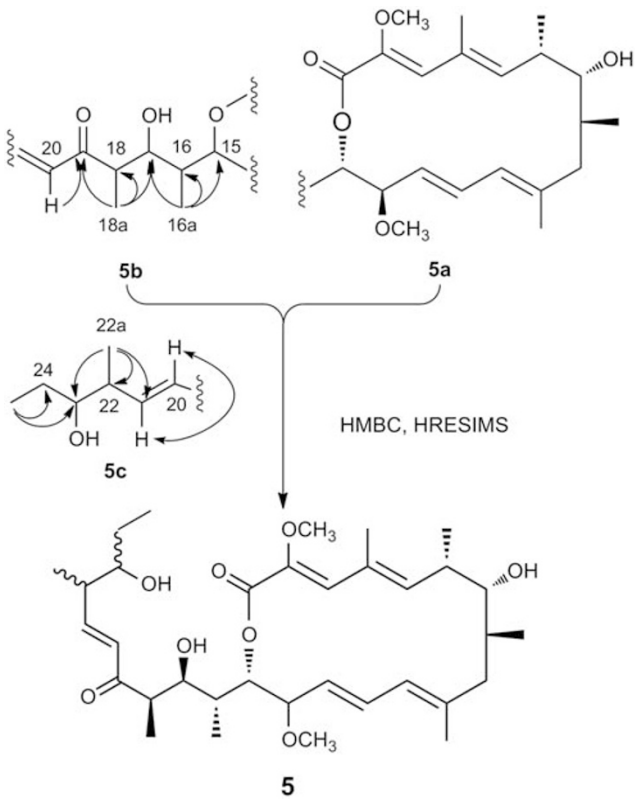

Figure 3 Structural fragments and the conformation of compound **5**.

EXPERIMENTAL PROCEDURE

General

Column chromatography: Silica gel (200–300, and 80–100 mesh; Qingdao Marine Chemical Factory, Qingdao, PR China), silica gel GF_{254} (Merck, Darmstadt, Germany), RP-18 (40–63 μm; Merck) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used. *TLC*: Precoated silica gel GF_{254} plates (0.20–0.25 mm; Qingdao Marine Chemical Factory). Fermentor (model: GUJS 50-200, Zhenjiang East Biotech Equipment and Technology, Zhenjiang, PR China). *UV spectra*: Unico single-beam 210A spectral photometer (Unico, Dayton, NJ, USA); 190–1100 nm, in EtOH. Optical rotations were obtained on a Perkin-Elmer (Perkin-Elmer, Waltham, MA, USA) 341 polarimeter with EtOH as solvent. The IR spectra were measured in KBr on a Nicolet FT-IR 360 in cm^{-1} (Nicolet, Pittsfield, MA, USA). *^{1}H- and ^{13}C-NMR spectra*: Bruker AV-600 spectrometer (Bruker Biospin GmbH, Karlsruhe, Germany), at 600/150 MHz, rep., in D_6-acetone; in p.p.m. rel. to Me_4Si, *J* in Hz. *HRESI–MS*: BRUKER ESI-Bio-Q-TOF mass spectrometer (Bruker, Bremen, Germany); in *m/z*.

Isolation and fermentation of strain *Streptomyces* sp. CS

The strain *Streptomyce* sp. CS was isolated from the callus of *Maytenus hookeri*.[8] A stock of *Streptomyces* sp. CS was cultured on ISP2 agar plates (the medium contained yeast extract 4 g, malt extract 10 g, glucose 4 g and agar 20 g, in 1.0 l tap water; pH 7.2–7.4) at 28 °C for 7 days and a single colony was inoculated to 500 ml Erlenmeyer flasks containing 50 ml ISP2 broth as a seed medium, and then scaled up to 1000 ml Erlenmeyer flasks containing 300 ml ISP2 broth. After 48 h on a rotary shaker (180 r.p.m., 28 °C), 3 l of the precultures were fermented in a 50-l fermentor (240 r.p.m., 28 °C) containing 30 l of sterilized ISP2 broth and 80 ml defoamer GPE-1 (MW: 3000–3600). After 72 h of fermentation, the precultures (30 l) were transferred to a 200-l fermenter containing 120 l of sterilized Waksman synthetic medium (the medium contained glycerol 30 g, $K_2HPO_4.3H_2O$ 1 g, $MgSO_4.7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4.7H_2O$ 0.01 g and $NaNO_3$ 2 g in 1.0 l tap water; pH 7.2–7.4) and 100 ml defoamer GPE-1. Fermentation was carried out at 28 °C for 1 week with aeration (10 l min^{-1}) under constant agitation (300 r.p.m.).

Extraction and isolation

After filtration of the harvested culture of strain *Streptomyces sp.* CS, the mycelium (2.2 kg wet weight) was extracted with Me_2CO (20 l). The extracted solution was collected and evaporated to dryness *in vacuo* to afford 158 g syrupy crude extract. The crude extract (158 g) was dissolved in EtOAc, and then mixed with equal volume distilled water to fully distribute the water-solubility portion and EtOAc solubility portion. The EtOAc portion was collected and evaporated to oil (60 g). The extract (60 g) was subjected to column chromatography (column dimensions: 25×1500 mm, 120 g Sephadex LH-20; MeOH) in six 10 g portions to yield a total of 13 g of bafilomycin fractions without defoamer. This fraction (13 g) was subjected to medium-performance liquid chromatography (column dimensions: 36×230 mm, 130 g, RP-18) and eluted with Me_2CO–H_2O, 3:7, 1:1 and 7:3 (v/v), respectively, to yield four fractions (I, II (3.1 g), III (1.3 g) and IV). Fraction II was subjected to medium-performance liquid chromatography (130 g, RP-18), and eluted with MeOH–H_2O, 13:7 and 7:3 (v/v), to yield four fractions (IIa (130 mg), IIb (1.07 g), IIc (1.3 g) and IId (430 mg)). Fraction IIa (130 mg) was subjected to column chromatography (silica gel; petroleum ether—EtOAc 5:1 and 3:1 (v/v)) to afford **5** (19 mg). Fraction IIb (1.07 g) was subjected to column chromatography (silica gel; petroleum ether—EtOAc 10:1, 5:1, 3:1 and 1:1 (v/v)) to afford **5** (448 mg). Fraction IIc (1.3 g) together with III (1.3 g) was subjected to column chromatography (silica gel; petroleum ether—EtOAc 10:1, 5:1, 2:1 and 1:1 (v/v)) to afford **2** (480 mg) and **1** (668 mg). Fraction IId (430 mg) was subjected to column chromatography (silica gel; petroleum ether—EtOAc 15:1, 10:1 and 5:1 (v/v)) to afford **4** (18 mg), **3** (69 mg) and **2** (38 mg).

Physicochemical properties

1: Colorless needle crystal; m.p. 103.3–105.2 °C; $[\alpha]_D^{20}$ −10.9° (*c* 2.2, EtOH); UV λ_{max}(EtOH) (log ε) nm 241 (2.565), 283 (1.412); IR V_{max} (cm^{-1}) (KBr) 3348, 2922, 2869, 1681, 1445, 1358, 1242, 1098, 1043; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRESIMS *m/z* 631.4303 $[M+Na]^+$ (calcd for $C_{34}H_{56}O_9Na$, 631.3817).

2: Colorless needle crystal; m.p.: 105.3–107.8 °C; $[\alpha]_D^{20}$ −10.3° (*c* 3.5, EtOH); UV λ_{max}(EtOH) (log ε) nm 238 (3.201), 286 (1.931); IR V_{max} cm^{-1} (KBr) 3380, 2925, 2867, 2813, 1680, 1445, 1355, 1241, 1090 ; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRESIMS *m/z* 645.4459 $[M+Na]^+$ (calcd for $C_{35}H_{58}O_9Na$, 645.3973).

3: White powder; $[\alpha]_D^{20}$ +36.9° (*c* 1.3, EtOH); UV λ_{max}(EtOH) (log ε) nm 241 (1.438), 280 (0.828); IR V_{max} cm^{-1} (KBr) 3427, 2958, 2926, 2863, 1642, 1452, 1381, 1157, 1087; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRESIMS *m/z* 659.4739 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_9Na$, 659.4130).

4: White amorphous powder $[\alpha]_D^{20}$ −18° (*c* 4.0, EtOH); UV λ_{max}(EtOH) (log ε) nm 245 (3.666); IR V_{max} cm^{-1} (KBr) 3387, 2960, 2916, 2849, 1712, 1452, 1380, 1238, 1095; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRESIMS *m/z* 641.4192 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_8Na$, 641.4024).

5: White amorphous powder $[\alpha]_D^{20}$ −8° (*c* 1.0, EtOH); UV λ_{max}(EtOH) (log ε) nm 238 (1.306), 280 (0.568); UV (EtOH) λ_{max}(ε) nm 3390, 2924, 1691, 1678, 1461, 1442, 1243, 1097, 1032; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRESIMS *m/z* 613.4237 $[M+Na]^+$ (calcd for $C_{34}H_{54}O_8Na$, 613.3711).

Biological assays

Cytotoxicities of compounds **1–5** were investigated using the human cancer cell line MD-MAB-435, following the MTT standards[15] and *cis*-platin (DDP) was used as a positive control in this experiment.

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