



Hamuramicins A and B, 22-membered macrolides, produced by an endophytic actinomycete *Allostreptomyces* sp. K12-0794

Takuya Suga^{1,2} · Tōru Kimura² · Yuki Inahashi^{1,2} · Masato Iwatsuki^{1,2} · Kenichi Nonaka^{1,2} · Akira Také¹ · Atsuko Matsumoto^{1,2} · Yōko Takahashi¹ · Satoshi Ōmura¹ · Takuji Nakashima^{1,2}

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Abstract

Two new compounds, designated as hamuramicins A (**1**) and B (**2**), were isolated from the cultured broth of an endophytic actinomycete *Allostreptomyces* sp. K12-0794 by silica gel column chromatography and HPLC. The structures of **1** and **2** were elucidated as 22-membered macrolide containing triene and trienone with an alkyl side chain by spectroscopic analyses including NMR experiments. Both compounds showed growth inhibition activity against *Kocuria rhizophia* and *Xanthomonas oryzae* pv. *oryzae* as well as human cell line toxicity.

Introduction

The genus *Streptomyces* is well-known as an excellent source of useful chemicals produced as secondary metabolites. Non-*Streptomyces* organisms can also be a good source of new compounds, as reported by Tiwari and Gupta [1]. Our group has discovered many important compounds, such as staurosporine [2], the clostomicins [3] and setamycin [4], from non-*Streptomyces* strains. Recently, the mangromicins (genus *Lechevalieria*) [5–7] and sagamilactam (genus *Actinomadura*) [8] were found from non-*Streptomyces* strains by physico-chemical screening by our group [9]. With respect to soil samples used to isolate actinomycetes, the genus *Streptomyces* is predominant, accounting for over 90% of actinomycetes found. We focused on plant roots as a source of actinomycetes

producing new compounds as the frequency of isolating non-*Streptomyces* strains from plant root samples has been seen to be higher than that from soil samples, *Streptomyces* strains isolated from plant root samples estimated to constitute a mere 22.9% [10].

Endophytic actinomycete strains have a capacity to produce a wide variety of biological active compounds [10–12]. The spoxazomicins (genus *Streptosporangium*) [13], trehangelins (genus *Polymorphospora*) [14] and actinoallolides (genus *Actinoallomurus*) [15] were all found from endophytic actinomycetes by our group. This influenced our approach to search for new compounds from the cultured broths of endophytic non-*Streptomyces* strains.

Our screening program has discovered two new 22-membered macrolides, named hamuramicins A (**1**) and B (**2**), from a cultured broth of an endophytic non-*Streptomyces* strain, *Allostreptomyces* sp. K12-0794 (Fig. 1). The strain was isolated from a fern root gathered in Hamura, Tokyo. The strain, K12-0794, was identified as a member of the genus *Allostreptomyces* based on the morphology, chemical compositions and phylogenetic analysis. *Allostreptomyces* was proposed as new genus by Huang et al. in 2017 [16]. This is the first report of the isolation of new secondary metabolites from the cultured broth of the genus *Allostreptomyces*. In this paper, the isolation, physico-chemical properties, structure elucidation and some bioactivities of **1** and **2** are described.

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- ✉ Takuya Suga
di13003@st.kitasato-u.ac.jp
- ✉ Takuji Nakashima
takuji@lisci.kitasato-u.ac.jp

¹ Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Tokyo 108-8641, Japan

² Graduate School of Infection Control Sciences, Kitasato University, 5-9-1 Shirokane, Tokyo 108-8641, Japan

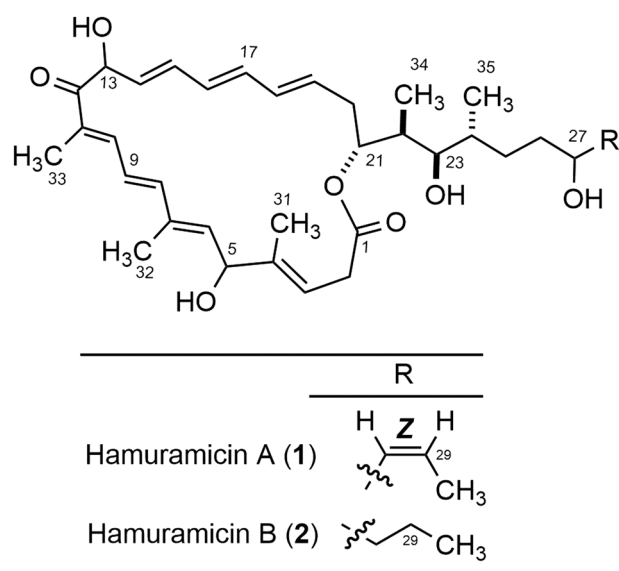


Fig. 1 Structures of hamuramicins A (1) and B (2)

Results and discussion

Isolation of hamuramicins A (1) and B (2)

The procedure for isolation of **1** and **2** is summarized in Supplementary Scheme S1. A culture broth (200 L) was centrifuged at 12,000 rpm and the mycelial cake was mixed with acetone (21 L). After stirring for 3 h, the acetone extract was evaporated under reduced pressure to remove acetone. The aqueous solution was extracted twice by 7 L of ethyl acetate, and the organic layer was partitioned once by 7 L of H₂O. The organic layer was concentrated to dryness in vacuo, and dissolved in a small amount of acetonitrile. The acetonitrile soluble fraction filtered by filter paper was concentrated to dryness in vacuo to yield a crude material (30.6 g). The material was dissolved in a small amount of CHCl₃ and then applied to a silica gel column (50 i.d. × 120 mm; Fuji Silysia Chemical, Aichi, Japan), which was eluted stepwise with 1 L of a mixture of CHCl₃-acetone (1:0, 4:1, 3:1, 2:1, 1:1 and 0:1) in this order, and the fractions were divided into two fractions (each 500 mL). Compounds **1** and **2** were included in four fractions (3:1–1 to 2:1–2). The CHCl₃-acetone 3:1–2 and 2:1–1 fractions (total weight, 1.9 g), which contained **1** and **2** with high purity, were dissolved in a small amount of MeOH and applied to a preparative HPLC (Capcell pak C18 UG-120, 20 i.d. × 250 mm, Shiseido Co., Tokyo, Japan) with 80% of MeOH/H₂O solvent (flow rate, 7.0 mL/minute; detection, UV 210 nm). The peaks with retention times of 16 and 20 min were collected and concentrated in vacuo in dark to dryness to yield hamuramicin A (**1**, 329.3 mg) and hamuramicin B (**2**, 397.9 mg), respectively.

Structure elucidation of hamuramicin A (1)

Compound **1** was obtained as a pale yellow powder ($[\alpha]_D^{23} -140$; *c* 0.1, MeOH) and readily soluble in CHCl₃, CH₂Cl₂, acetonitrile, acetone, MeOH and DMSO. It showed UV absorption (Supplementary Figure S1) maxima at 330 nm (ϵ 19850), 275 nm (ϵ 58210) and 265 nm (ϵ 54310) in MeOH. The IR absorption (Supplementary Figure S2) at 1720 and 3424 cm⁻¹ indicated the presence of ketones and hydroxyl groups, respectively. The molecular formula of **1** was elucidated by HR-ESI-MS to be C₃₅H₅₀O₇ (found *m/z* 605.3450 [M + Na]⁺, calcd. *m/z* 605.3454), requiring 11 degrees of unsaturation. The ¹H and ¹³C NMR spectral data of **1** is listed in Table 1, Supplementary Figures S3 and S4. The ¹³C NMR and HSQC (Supplementary Figure S5) spectra indicated 35 carbons, which were classified into five *sp*² fully substituted carbons including two carbonyl carbons, 13 *sp*² methines, five oxygenated *sp*³ methines, two *sp*³ methines, four methylenes, and six methyls. The ¹H–¹H COSY (Fig. 2a and Supplementary Figure S6) of **1** indicated the assignments from H₂-2 to H-3, from H-5 to H-6, from H-8 to H-10 and from H-13 to H₃-30. The HMBC (Fig. 2a and Supplementary Figure S7) correlations from H₂-2 (δ_H 2.99 and 3.15) to C-1 (δ_C 173.8) and C-4 (δ_C 141.5), from H-3 (δ_H 5.51) to C-1, from H-5 (δ_H 4.81) to C-4 and C-7 (δ_C 135.6), from H₃-31 (δ_H 1.66) to C-3 (δ_C 118.0), C-4 and C-5 (δ_C 72.2), from H-8 (δ_H 6.69) to C-7 and C-32 (δ_C 12.5), from H₃-32 (δ_H 1.88) to C-6 (δ_C 140.5), C-7 and C-8 (δ_C 148.0), from H-10 (δ_H 7.37) to C-12 (δ_C 200.6) and C-33 (δ_C 11.8), from H-13 (δ_H 5.40) to C-12, from H₃-33 (δ_H 1.88) to C-10 (δ_C 146.3), C-11 (δ_C 131.4) and C-12, from H-14 (δ_H 5.34) to C-12 established the connectivity between C-1 to C-30, which was the presence of 5,13,21,23,27-pentahydroxyl-4,7,11,22,24-pentamethyl-12-oxotriaconta-3,6,8,10,14,16,18,28-octaenoic acid. Finally, the HMBC correlation from H-21 (δ_H 5.16) to C-1 and remaining unsaturation indicated the esterification between C-1 and C-21.

The geometries of double bonds and relative configurations of **1** were established from coupling constants and the ROESY correlations (Fig. 2b and Supplementary Figure S8). The coupling constants were 15.0 Hz between H-8 (δ_H 6.69) and H-9, 14.5 Hz between H-14 and H-15 (δ_H 6.63), 14.9 Hz between H-16 (δ_H 6.07) and H-17 (δ_H 6.28), 15.2 Hz between H-18 (δ_H 6.11) and H-19, respectively. The ROESY correlations were observed between H₃-31 and H-5, between H-5 and H₃-32, between H₃-32 and H-9, between H-9 and H₃-33, between H-6 (δ_H 5.66) and H-8, between H-8 and H-10, between H-10 and H-13, between H-13 and H-15, between H-15 and H-17, between H-14 and H-16, between H-16 and H-18, resulting in the geometry of double bonds, were all *E*-configuration in the 22-membered macrolide. However, the relative configurations of C-5 and

Table 1 ^1H and ^{13}C NMR spectral data of hamuralides A (**1**) and B (**2**) in CD_3OD

No.	Hamuramicin A (1)				Hamuramicin B (2)			
	δ_{C} mult.		δ_{H} mult. (J in Hz)		δ_{C} mult.		δ_{H} mult. (J in Hz)	
1	173.8	C			173.8	C		
2	34.3	CH ₂	2.99	dd (16.6, 7.4)	34.2	CH ₂	2.99	dd (16.7, 7.0)
			3.15	dd (16.6, 7.4)			3.16	dd (16.7, 7.0)
3	118.0	CH	5.51	m	118.0	CH	5.51	dd (7.0, 7.0)
4	141.5	C			141.5	C		
5	72.2	CH	4.81	d (9.2)	72.2	CH	4.81	d (9.5)
6	140.5	CH	5.66	d (9.2)	140.5	CH	5.66	d (9.5)
7	135.6	C			135.6	C		
8	148.0	CH	6.69	d (15.0)	148.0	CH	6.69	d (15.0)
9	124.0	CH	6.58	dd (15.0, 10.8)	124.0	CH	6.58	dd (15.0, 10.7)
10	146.3	CH	7.37	d (10.8)	146.3	CH	7.37	d (10.7)
11	131.4	C			131.4	C		
12	200.6	C			200.6	C		
13	76.7	CH	5.40	d (9.5)	76.7	CH	5.41	d (10.0)
14	132.1	CH	5.34	dd (9.5, 14.5)	132.1	CH	5.34	dd (10.0, 14.5)
15	137.8	CH	6.63	dd (10.5, 14.5)	137.8	CH	6.63	dd (14.5, 10.6)
16	130.6	CH	6.07	dd (14.9, 10.5)	130.6	CH	6.07	dd (10.6, 15.3)
17	135.9	CH	6.28	dd (14.9, 10.6)	135.9	CH	6.29	dd (15.3, 10.7)
18	133.4	CH	6.11	dd (10.6, 15.2)	133.4	CH	6.11	dd (10.7, 15.0)
19	133.6	CH	5.61	ddd (15.2, 10.4, 4.8)	133.6	CH	5.61	ddd (15.0, 10.1, 4.3)
20	37.0	CH ₂	2.33	ddd (10.4, 10.4, 15.4)	37.1	CH ₂	2.34	ddd (10.1, 10.3, 15.0)
			2.62	br.d (15.4)			2.62	br.d (15.0)
21	77.5	CH	5.16	ddd (10.4, 6.8, 2.4)	77.5	CH	5.16	br.dd (8.4, 15.3)
22	40.4	CH	1.82	ddq (6.8, 3.4, 6.8)	40.4	CH	1.83	m
23	76.3	CH	3.28	m	76.4	CH	3.28	m
24	37.5	CH	1.57	m	37.5	CH	1.56 ^c	m
25	29.0	CH ₂	1.18	m	29.4	CH ₂	1.15	m
			1.7 ^a	m			1.76	m
26	35.8	CH ₂	1.32	m	35.6	CH ₂	1.34	m
			1.7 ^a	m			1.56 ^c	m
27	68.2	CH	4.41	m	72.4	CH	3.53	m
28	135.1	CH	5.37	ddd (10.4, 10.4, 1.7)	40.5	CH ₂	1.40	m
29	126.1	CH	5.53	ddd (1.2, 6.9, 10.4)	19.9	CH ₂	1.47	m
30	13.4	CH ₃	1.68	dd (1.7, 6.9)	14.5	CH ₃	0.93	t (6.8)
31	14.5	CH ₃	1.66	s	15.5	CH ₃	1.69	s
32	12.5	CH ₃	1.88 ^b	s	12.5	CH ₃	1.88 ^d	s
33	11.8	CH ₃	1.88 ^b	s	11.8	CH ₃	1.88 ^d	s
34	9.4	CH ₃	0.89	d (6.8)	9.4	CH ₃	0.90	d (6.8)
35	16.3	CH ₃	0.82	d (6.8)	16.4	CH ₃	0.83	d (6.8)

NMR spectra were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C

mult. multiplicity

^{a-d} Overlapped

C-13 were not determined, although the coupling constants were 9.2 Hz between H-5 and H-6, 9.5 Hz between H-13 and H-14, respectively. The stereochemistry of the two hydroxyl groups at C-5 and C-13 was able to be both

orientation, owing to the flexible 22-membered ring. A previous report of pulvomycin showed that the relative stereochemistry at C-5 and C-13 using nOe [17], plus the absolute configuration of pulvomycin, was confirmed by

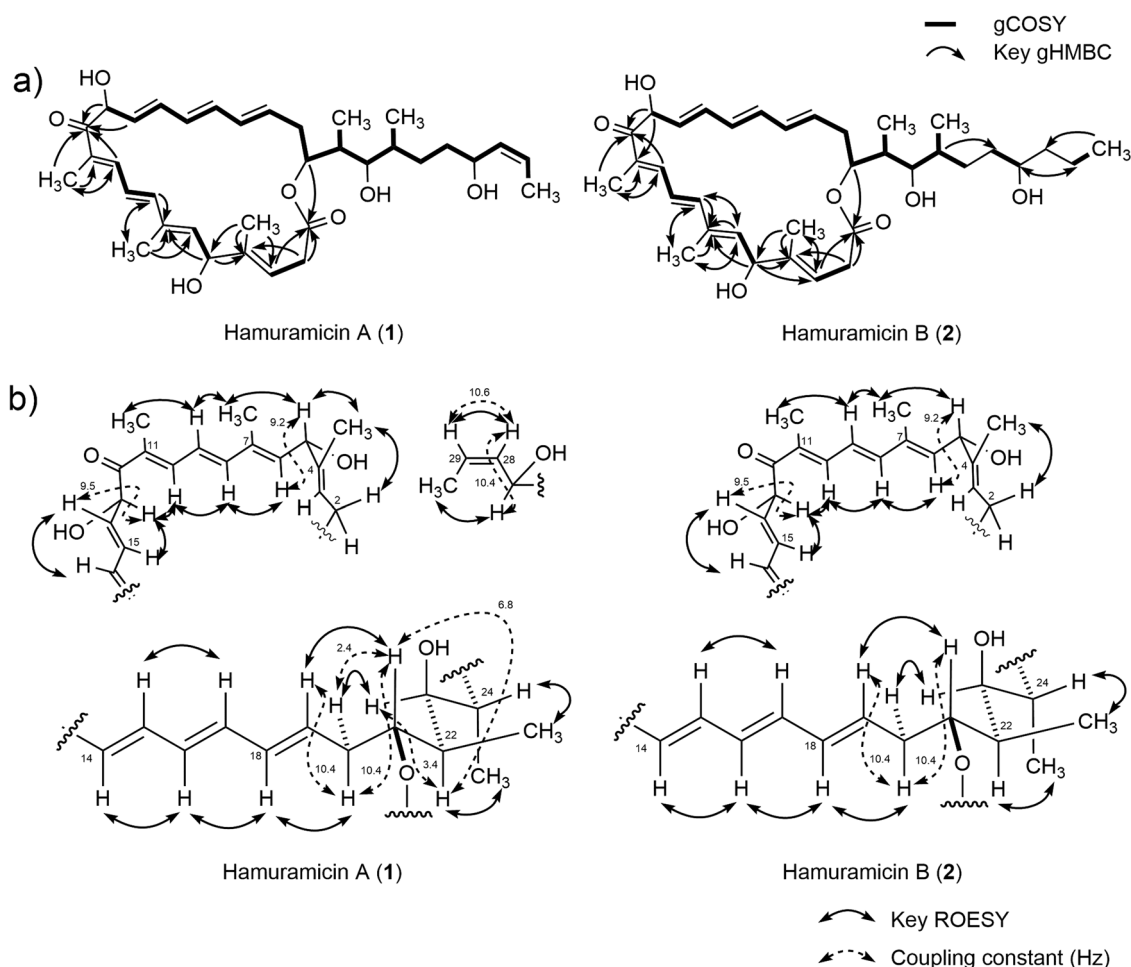


Fig. 2 Structure elucidation of hamuramicins A (1) and B (2). **a** gCOSY and key gHMBC correlations. **b** Key ROESY correlations and coupling constants

crystallographic analysis with an elongation factor (EF-) Tu (PDB entry 2C78) [18]. Likewise, the ROESY correlations were observed between H-18 and H₂-20 (δ_{H} 2.33), between H₂-20 (δ_{H} 2.62) and H-23 (δ_{H} 3.28), between H-19 (δ_{H} 5.61) and H-21, between H-22 (δ_{H} 1.82) and H₃-35 (δ_{H} 0.82), between H-24 (δ_{H} 1.57) and H₃-34 (δ_{H} 0.89), between H-27 (δ_{H} 4.41) and H₃-30 (δ_{H} 1.68), between H-28 (δ_{H} 5.37) and H-29 (δ_{H} 5.53). The small coupling constants between H-20_{eq} and H-21 (2.4 Hz), and between H-22 and H-23 (3.4 Hz) would support their syn-relationship, and the large coupling constants between H-19 and H-20_{ax} (10.4 Hz), between H-20_{ax} and H-21 (10.4 Hz) and between H-21 and H-22 (6.8 Hz) would support their anti-relationship. In addition, a coupling constant with 10.6 Hz between H-28 and H-29 suggested 28Z. We tried to elucidate the absolute configuration of C-27 using modified Mosher's method but, unfortunately, the sample was decomposed. Thus, the geometries of double bonds and relative configurations of 1

was elucidated to be 3E, 6E, 8E, 10E, 14E, 16E, 18E, 21R*, 22S*, 23R*, 24R* and 28Z (Fig. 2b).

Structure elucidation of hamuramicin B (2)

Compound 2 contained a lot of impurities. The NMR analysis revealed approximately a 2:1 mixture. The minor compound, which is very similar to structure of 2, could not be determined. Significant differences of ¹H and ¹³C NMR data were observed at the midpoint of the 27-position (Supplementary Figure S9). We concluded that the minor compound may be a diastereomer of 2.

Compound 2 was obtained as a pale yellow powder ($[\alpha]_{\text{D}}^{23} -166$; *c* 0.1, MeOH) and readily soluble in CHCl₃, CH₂Cl₂, acetonitrile, acetone, MeOH and DMSO. It showed UV absorption (Supplementary Figure S10) maxima at 330 nm (ϵ 16010), 275 nm (ϵ 47720) and 265 nm (ϵ 43870) in MeOH. The IR absorption (Supplementary Figure S11) at 1716 and 3432 cm⁻¹ indicated the presence of ketones and

Table 2 Biological activity of hamuralides A (1) and B (2), The MIC values against eight bacteria

	MIC value ($\mu\text{g/mL}$)	
	Hamuramicin A (1)	Hamuramicin B (2)
<i>Bacillus subtilis</i> ATCC6633	>256	>256
<i>Kocuria rhizophia</i> ATCC9341	4	4
<i>Staphylococcus aureus</i> ATCC6538P	>256	>256
<i>Escherichia coli</i> NIHJ	>256	>256
<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> KB88	4	2
<i>Klebsiella pneumoniae</i> ATCC10031	>256	>256
<i>Proteus vulgaris</i> NBRC3167	>256	>256
<i>Pseudomonas aeruginosa</i> NBRC12582	>256	>256

hydroxyl groups, respectively. The molecular formula of **2** was elucidated by HR-ESI-MS to be $\text{C}_{35}\text{H}_{52}\text{O}_7$ (found m/z 583.3620 $[\text{M} - \text{H}]^-$, calcd. m/z 583.3635), requiring 10 degrees of unsaturation. The physico-chemical properties and NMR spectral data were similar to those of **1** (Table 1, Supplementary Figures S12, S13 and S14). The structure of **2** indicated that it was a reductant of **1**, according to the absence of a double bond at the H-28 position in NMR spectra coupled with decreased degrees of unsaturation. The planar structure of **2** was further elucidated to be a reductant of **1** at the 28 position using COSY and HMBC (Fig. 2a and Supplementary Figures S15 and S16), and the geometries of double bonds and relative configurations of **2** was the same as **1** by data of ROESY (Supplementary Figure S17) correlation and coupling constants (Fig. 2b). We tried to elucidate the absolute configuration of the H-27 using modified Mosher's method but the sample was decomposed.

Biological activity

Compounds **1** and **2** were evaluated for antimicrobial activity and cytotoxicity (Tables 2 and 3). Compounds **1** and **2** showed growth inhibition activity against *Kocuria rhizophia* ATCC9341 and *Xanthomonas oryzae* pv. *oryzae* KB88 but they showed no antimicrobial activities against six other bacteria at a concentration of 256 $\mu\text{g/mL}$ using the agar dilution method [19]. However, both compounds

Table 3 The IC_{50} values of cytotoxicity against five adherent cell lines

	Cytotoxicity (μM)	
	Hamuramicin A (1)	Hamuramicin B (2)
HeLa S3	1.1	1.3
A549	2.3	1.4
PANC-1	5.0	4.5
HT29	5.0	5.9
H1299	8.8	17.8

broadly showed cytotoxicity against five adherent cell lines. The similar pulvomycin was evaluated for antimicrobial activity by Landini et al [20]. The report showed that MIC values of pulvomycin were 2 $\mu\text{g/mL}$ and 8 $\mu\text{g/mL}$ against *Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* L165, respectively. However, **1** and **2** exhibited no antimicrobial activity against *B. subtilis* ATCC6633 and *S. aureus* ATCC6538P. It is suggested that the side chain is important for the expression of broad antibacterial activity.

This is the first report that new secondary metabolites from the cultured broth of the genus *Allostreptomyces* have been isolated. It reinforces the belief that there is significant possibility of discovering a significant number of useful compounds from culture broths of endophytic non-*Streptomyces* organisms.

Materials and methods

Fermentation

A loop of strain K12-0794 cells grown on an agar slant was inoculated into 10 mL of the seed medium consisting of 2.4% starch, 0.1% glucose, 0.3% peptone, 0.3% meat extract, 0.5% yeast extract, 0.4% CaCO_3 and incubated until sufficient growth at 280 rpm at 27 °C. The cultured broth was stored at -28 °C as a frozen stock. Two hundred microliters of the frozen stock were inoculated into 10 mL of fresh seed medium with 5 pieces of glass beads (ϕ 6.3–7.8 mm) in test tubes for 3 days at 280 r.p.m. at 27 °C. Two milliliters of the seed culture were inoculated into 100 mL of the seed medium in 500-mL Erlenmeyer flasks and cultured for 4 days at 210 rpm at 27 °C. The seed (1.2 L) culture was inoculated into 20 L of fermentation medium consisting of 2.0% soluble starch, 1.0% defatted wheat germ, 0.3% meat extract, 0.4% CaCO_3 and 0.08% Disform® as a defoaming agent in 30-L jar fermenter and incubated for 4 days (agitation, 200 rpm; temperature, 27 °C; aeration, 0.5 v/v/m).

General experiments

NMR spectra were measured by a Varian XL-400 spectrometer (Agilent Technologies Japan Ltd., CA, USA), with ^1H NMR at 400 MHz and ^{13}C NMR at 100 MHz in CD_3OD . The chemical shifts are expressed in ppm and referred to CHD_2OD (3.31 p.p.m.) in the ^1H NMR spectra and to CD_3OD (49.0 p.p.m.) in the ^{13}C NMR spectra. ESI-MS spectra were measured with a JMS AX-505 HA mass spectrometer (JEOL Ltd., Tokyo, Japan). IR spectra (KBr) were taken on a FT-210 Fourier transform infrared spectrometer (Horiba Ltd., Kyoto, Japan). UV spectra were measured with a Hitachi U-2801 spectrophotometer (Hitachi Ltd., Tokyo, Japan). Optical rotation was measured with a JASCO P-2200 polarimeter (JASCO Co., Tokyo, Japan).

Antimicrobial activities

The agar dilution method was carried out according to the method recommended by CLSI¹⁹. Antimicrobial activities of **1** and **2** against eight microorganisms, *B. subtilis* ATCC6633, *K. rhizophila* ATCC9341, *S. aureus* ATCC6538P, *Escherichia coli* NIHJ, *X. oryzae* pv. *oryzae* KB88, *Klebsiella pneumoniae* ATCC10031, *Proteus vulgaris* NBRC3167 and *Pseudomonas aeruginosa* NBRC12582, were evaluated. The cultured broths of test microorganisms were diluted to log 6.0 CFU/mL and were spotted with Mueller Hinton II agar containing **1** or **2** (final concentration: 0.25–256 $\mu\text{g}/\text{mL}$). All microorganisms, except *X. oryzae*, were incubated at 37 °C for 24 h. *X. oryzae* was incubated at 27 °C for 48 h.

Cytotoxicity

Cytotoxic evaluation of **1** and **2** used five adherent human cell lines, HeLa S3 (human cervical cancer cell line), HT29 (human colorectal adenocarcinoma cell line), A549 (human adenocarcinoma cell line derived from lung cancer), H1299 (human non-small cell lung carcinoma cell line) and PANC-1 (human cell line derived from pancreatic cancer). All cell lines were incubated in DMEM medium (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 10% FBS and 1% penicillin streptomycin at 37 °C under 5% CO_2 . The cultivated cell lines (5×10^4 cells per well) were seeded in 96-well plates. After overnight culture, MeOH solution of **1** or **2** was added into each well. After 2 days of incubation at 37 °C, WST-8 solution was added to each well and kept for 4 h at 37 °C. The absorbance of each well was measured using a Corona Grating Microplate Reader SH-9000 (Corona Electric Co. Ltd., Ibaraki, Japan) at 460 nm.

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Compliance with ethical standards

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

References

1. Tiwari K, Gupta RK. Rare actinomycetes: a potential storehouse for novel antibiotics. *Crit Rev Biotechnol.* 2012;32:108–32.
2. Ōmura S, et al. A new alkaloid AM-2282 OF *Streptomyces* origin. Taxonomy, fermentation, isolation and preliminary characterization. *J Antibiot.* 1977;30:275–82.
3. Ōmura S, et al. Clostomicins, new antibiotics produced by *Micromonospora echinospora* subsp. *Armeniaca* subsp. nov. I. Production, isolation, and physico-chemical and biological properties. *J Antibiot.* 1986;39:1407–1412.
4. Ōmura S, Otaguro K, Nishikiori T, Ōiwa R, Iwai Y. Setamycin, a new antibiotic. *J Antibiot.* 1981;34:1253–6.
5. Nakashima T, et al. Mangromicins A and B: structure and anti-trypanosomal activity of two new cyclopentadecane compounds from *Lechevalieria aerocolonigenes* K10-0216. *J Antibiot.* 2014;67:253–60.
6. Nakashima T, Kamiya Y, Iwatsuki M, Takahashi Y, Ōmura S. Mangromicins, six new anti-oxidative agents isolated from a culture broth of the actinomycete, *Lechevalieria aerocolonigenes* K10-0216. *J Antibiot.* 2014;67:533–9.
7. Nakashima T, et al. Mangromicin C, a new analog of mangromicin. *J Antibiot.* 2015;68:220–2.
8. Kimura T, et al. Anti-trypanosomal compound, sagamilactam, a new polyene macrocyclic lactam from *Actinomadura* sp. K13-0306. *J Antibiot.* 2016;69:818–24.
9. Nakashima T, Takahashi Y, Ōmura S. Search for new compounds from Kitasato microbial library by physicochemical screening. *Biochem Pharmacol.* 2016;134:42–55.
10. Matsumoto A, Takahashi Y. Endophytic actinomycetes: promising source of novel bioactive compounds. *J Antibiot.* 2017;70:514–9.
11. Takahashi Y. Continuing fascination of exploration in natural substances from microorganisms. *Biosci Biotech Biochem.* 2017;81:6–12.
12. Qin S, et al. Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. *Appl Microbiol Biotechnol.* 2011;89:457–73.
13. Inahashi Y, et al. Spoxazomicins A-C, novel antitrypanosomal alkaloids produced by an endophytic actinomycete. *Streptosporangium oxazolinicum* K07-0460T. *J Antibiot.* 2011;64:303–7.
14. Nakashima T, et al. Trehangelins A, B and C, novel photo-oxidative hemolysis inhibitors produced by an endophytic actinomycete, *Polymorphospora rubra* K07-0510. *J Antibiot.* 2013;66:311–7.
15. Inahashi Y, et al. Actinoallolides A-E, new anti-trypanosomal macrolides, produced by an endophytic actinomycete. *Actinoallomurus fulvus* MK10-036. *Org Lett.* 2015;17:864–7.
16. Huang MJ, et al. *Allostreptomyces psamosilenae* gen. nov., sp. nov., an endophytic actinobacterium isolated from the roots of *Psamosiline tunicoides* and emended description of the family

- Streptomyces [Waksman and Henrici (1943)AL] emend. Rainey et al. 1977, emend. Kim et al. 2003, emend Zhi et al. 2009. *Int J Syst Evol Microbiol.* 2017;67:288–93.
17. Smith JR, et al. Structure revision of the antibiotic pulvomycin. *J Am Chem Soc.* 1985;107:2849–57.
 18. Parmeggiani A, et al. Structure basis of the action of pulvomycin and GE2270 A on elongation factor Tu. *Biochemistry.* 2006;45:6846–57.
 19. Clinical and Laboratory Standards Institute, 2005. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Proposed Guideline M45-P*, CLSI, Wayne, USA
 20. Landini P, Bandera M, Soffientini A, Goldstein BP. Sensitivity of elongation factor Tu (EF-Tu) from different bacterial species to the antibiotics efrotomycin, pulvomycin and MDL 62879. *J Gen Microbiol.* 1933;139:769–74.