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ARTICLE

Dolyemycins A and B, two novel cyclopeptides isolated from *Streptomyces griseus* subsp. *griseus* HYS31

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Abstract

Two novel cyclopeptides with special skeleton, namely, dolyemycins A (**1**) and B (**2**) were isolated from *Streptomyces griseus* subsp. *griseus* HYS31 by bio-guided isolation. Their structures were elucidated by detailed analysis of spectroscopic data. These two compounds were cyclopeptides containing eleven amino acids including five unusual amino acids (hydroxyglycine, 3-hydroxyleucine, 3-phenylserine, *β*-hydroxy-*O*-methyltyrosine, 2,3-diaminobutyric acid) in both of them and an extra nonprotein amino acids (3-methylaspartic acid) in Dolyemycin B only. Dolyemycins A and B performed antiproliferative activity against human lung cancer A549 cells with IC_{50} values of 1.0 and 1.2 μM, respectively.

Introduction

Nearly two thirds of all known antibiotics are produced by actinomycetes [1]. They exhibit antibacterial [2], anti-tumor [3], insecticide [4] and many other activities. Streptomyces are the main producers of secondary metabolites of actinomycetes, and these compounds have a variety of different structures [1, 5]. Peptide antibiotics can be mostly produced by *Bacillus* sp. [6] and *Streptomyces* sp. [7]. They exhibit antimicrobial activity, anti-tuberculosis, and surface activity, which endows them with a wide range of applications in the fields of pharmaceuticals and agriculture [6, 8–10]. These peptide antibiotics are widely concerned by researchers, such as surfactin, fengycin, iturin, and daptomycin [11–13]. Among them, daptomycin is the first FDA-approved cyclic lipopeptide antibiotic, and may be one of the most important antibiotics in the past 50 years [14–16].

Actinomycete HYS31 was isolated from soil and preserved by our laboratory. The methanol extract (MeOH) of HYS31 biomass exhibited antiproliferative activity against human lung cancer cells and human leukemia cells in preliminary study. Two cyclopeptides, namely dolyemycins A and B, with novel skeleton were isolated from actinomycete HYS31 by bio-guided isolation. Their structures were determined by detailed analysis of spectroscopic data. The half maximal inhibitory concentration (IC_{50}) of compounds **1** and **2** against human lung cancer A549 cells was 1.0 and 1.2 μM, respectively. We describe herein the details of the structural characterization of these two compounds and their biological activities.

Results

Identification of the producing strain

Streptomyces sp. HYS31 was isolated from soil and preserved by our laboratory. The strain was identified as *Streptomyces griseus* subsp. *griseus* according to its morphological characteristics, biochemical characteristics, and partial sequence of its 16S rDNA.

Fermentation

The strain HYS31 was first cultured on Coates medium from agar slants for 3 days, then the healthy colonies were generated and inoculated into six 250-ml shake flasks,

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which contained 50 ml of seed medium per flask. After cultured under 180 r.p.m. at 28 °C for 25 h, the seed medium was inoculated into 5 l fermenter that contained 3.0 l of fermentation medium with 10% inoculum concentration. After 6 days, the fermentation broth was centrifuged at 8000 r.p.m. for 5 min to obtain the biomass. Repeat steps above for five times to collect biomass.

Extraction and isolation

The biomass was extracted with 80% MeOH three times, each for 12 h. The 80% MeOH extract was centrifuged at 8000 r.p.m. for 5 min to obtain the supernatant, and then evaporated in vacuum to remove MeOH. The remaining aqueous solution was extracted with equal volume EtOAc three times, each for 24 h. The EtOAc layer was combined and concentrated in vacuum. The EtOAc extract (7.40 g) was obtained and subjected to silica gel column chromatography (CC) eluted with $CHCl_3$/MeOH system (99:1, 95:5, 90:10, 88:12, 80:20, 70:30, 60:40, 40:60, and 0:100, v/v). The human lung cancer cell line A549 was used as biological activity guide to determine active fractions by the Cell Counting Kit-8. The active fraction of $CHCl_3$/MeOH 9:1 (3.90 g) was then applied to Sephadex LH-20 CC eluted with $CHCl_3$/MeOH system (1:1, v/v) to obtain nine active fractions. The active fraction A3 was analyzed and prepared by HPLC (Waters 1525/2487, cosmoil C18, 5 μm, 20 × 250 mm, 7 ml min^{-1}, UV detection 235 nm, $t_{R1} = 27$ min, $t_{R2} =$ 39 min) eluted with 50% CH_3CN to yield compound **1** (13.9 mg) and compound **2** (10.8 mg).

Structural elucidation of compounds 1 and 2

Compound **1** was obtained as white amorphous powder. It gave an $[M + Na]^+$ peak in the high-resolution electrospray-ionization mass spectrometry (HR-ESI-MS) at *m/z* 1491.6542 (calcd for $C_{73}H_{92}N_{14}O_{19}Na$, 1491.6561), indicating the presence of 35° of unsaturation. The IR spectrum of **1** displayed absorption bands for the amino group (3300 cm^{-1}), phenyl group and amide carbonyl group (1657, 1531 cm^{-1}). The 1D NMR spectra (in CD_3OD) showed twenty-one quaternary C-atoms including thirteen carbonyl groups (δ_C 171–178), one connected with oxy-aromatic carbon (δ_C 161.0), and other seven quaternary C-atoms; thirty-seven methines including fifteen saturated *O*-methines or *N*-methines at δ_H 3.8–5.5, twenty atoms at δ_H 5.7–8.0, δ_C 114–141 belonged to aromatic rings or olefinic groups, and two aliphatic atoms (δ_H 1.73, 1.86); seven methylenes; eight methyl groups (including one methoxyl at δ_H 3.66, s, 3 H; δ_C 55.7); ten amide NH (δ_H 7.37, 7.48, 7.76, 7.89, 7.94, 8.02, 8.13, 8.48, 8.97, 9.59). These spectroscopic data suggested that **1** was a peptide derivative containing at least ten amino-acid residues and an unsaturated side chain. Amino acid residues (designated as A to K, Fig. 2) were determined by 1D and 2D NMR data.

The structure of **1** was confirmed by 2D NMR data analysis. The proton and corresponding carbon resonances in the 2D NMR spectra of **1** were assigned by the gradient heteronuclear single-quantum coherence (gHSQC) experiment.

The ^1H-^1H correlation spectroscopy (COSY) of **1** showed coupling correlations of NH-2 (δ_H 7.48)/H-2 (δ_H 4.46)/H-3 (δ_H 3.81)/H-4 (δ_H 1.73)/H_3-5 (δ_H 0.84)/H_3-6 (δ_H 1.04) together with the HMBC correlations of H-3 (δ_H 3.81) to C-1 (δ_C 171.1) and their shifts revealed the presence of a 2-amino-3, 3-dimethylbutanoic acyl group [17] (the residue of 3-hydroxyleucine, assigned as residue A). Similarly, the structure of amino acid residue B was determined as leucine based on the ^1H-^1H COSY correlations of (NH-8 (δ_H 8.47)/H-8 (δ_H 4.34)/H_2-9 (δ_H 1.61, 1.83)/H-10 (δ_H 1.86)/H_3-11 (δ_H 0.93), and H_3-12 (δ_H 0.99)) and key HMBC correlations of H_2-9 (δ_H 1.61, 1.83) to C-7 (δ_C 177.7). Residues A and B were connected by a peptide bond which was supported by the HMBC correlations of NH-2 (δ_H 7.48) to C-7 (δ_C 177.7) (C = O).

A hydroxyglycine (residue C) was found to link with residue B by a peptide bond, which was supported by the ^1H-^1H COSY correlation of NH-14 (δ_H 8.97) / H-14 (δ_H 5.44) and the HMBC correlation of NH-14 (δ_H 8.97) and NH-8 (δ_H 8.47) to C-13 (δ_C 173.5), respectively.

The presence of an indole ring was determined according to the linkages of H-19 (δ_H 7.58)/H-20 (δ_H 6.92)/H-21 (δ_H 7.06)/H-22 (δ_H 7.30) in ^1H-^1H COSY and correlations from H-20 (δ_H 6.92) to C-18a (δ_C 128.6), H-21 (δ_H 7.06) to C-22 (δ_C 112.4), H-19 (δ_H 7.58) to C-18 (δ_C 110.8), H-23 (δ_H 7.19) to C-18 (δ_C 110.8) and C-22a (δ_C 138.1) in HMBC spectrum. Furthermore, the ^1H-^1H COSY correlations confirmed linkages of NH-16 (δ_H 8.13)/H-16 (δ_H 4.74)/H_2-17 (δ_H 3.17, 3.35). The HMBC correlation from H-16 (δ_H 4.74) to C-18 (δ_C 110.8) indicated the indole ring was linked to H_2-17, which belonged to a substitutional alanine (due to the HMBC correlation of H_2-17 (δ_H 3.17, 3.35) to C-15 (δ_C 175.0). Therefore, the structure of amino acid residue D was determined as tryptophan.

A di-substituted benzene ring bearing a methoxyl group was confirmed based on the linkages of H-28/32 (δ_H 6.68, 2H)/H-29/31 (δ_H 6.57, 2H) in ^1H-^1H COSY and correlations from H-29/31 (δ_H 6.57, 2H) to C-27 (δ_C 133.0), H-28/32 (δ_H 6.68, 2H) to C-30 (δ_C 161.0) and H_3-33 (δ_H 3.66, 3H) to C-30 (δ_C 161.0). The ^1H-^1H COSY correlations confirmed linkages of NH-25 (δ_H 7.37)/H-25 (δ_H 4.78)/H-26 (δ_H 4.49), and the shift of δ_H 4.49 of H-26 suggested that C-26 was an *O*-methines. In addition, the correlation from H-25 (δ_H 4.78) to C-27 (δ_C 133.0) in HMBC indicated the di-substituted benzene ring was linked to C-26, and the correlation from H-26 (δ_H 4.49) to C-24 (δ_C 171.6) revealed the amino acid

Table 1 Spectra data for compounds **1** and **2**

Position	**1**		Position	**2**	
	δ_C, type	δ_H (J in Hz)		δ_C	δ_H (J in Hz)
3-Hydroxyleucine (A)			3-Hydroxyleucine (A)		
1	171.1, C		1	171.2	
2	56.7, CH_2	4.46, d (6.9)	2	56.8	4.45, d (7.3)
3	76.8, CH	3.81, d (9.1)	3	76.8	3.81, dd (9.3, 1.3)
4	32.5, CH	1.73, m	4	32.5	1.72, m
5	19.0, CH3	0.84, d (6.7)	5	19.0	0.84, d (6.7)
6	19.7, CH_3	1.04, d (6.6)	6	19.7	1.04, d (6.6)
NH		7.48, m	NH		7.47, brs
Leu (B)			Leu (B)		
7	177.7, C		7	177.7	
8	56.7, CH_2	4.34 overlap	8	56.8	4.35 overlap
9	41.2, CH_2	1.61 m, 1.83 m	9	41.2	1.60 m, 1.83 m
10	26.1, CH	1.86, m	10	26.1	1.87, m
11	21.8, CH_3	0.93, d (6.4)	11	21.8	0.92, d (6.4)
12	23.3, CH_3	0.99, d (6.4)	12	23.3	0.99, d (6.4)
NH		8.47, d (4.4)	NH		8.49, d (4.9)
Hydroxyglycine (C)			Hydroxyglycine (C)		
13	173.5, C		13	173.5	
14	74.6, CH	5.44, d (6.9)	14	74.6	5.42, d (6.3)
NH		8.97, d (6.2)	NH		9.01, d (6.7)
Trp (D)			Trp (D)		
15	175.0, C		15	175.0	
16	55.6, CH	4.74, dd (9.1, 4.4)	16	55.7	4.73, dd (9.4, 4.3)
17	29.4, CH_2	3.17, dd (14.7, 9.2), 3.35, s	17	29.3	3.17, dd (14.7, 9.4), 3.36, m
18	110.8, C		18	110.8	
18a	128.6, C		18a	128.6	
19	119.7, CH	7.58, d (7.9)	19	119.7	7.59, d (7.9)
20	120.0, CH	6.92, t (7.5)	20	120.0	6.92, t (7.5)
21	122.4, CH	7.06, t (7.5)	21	122.5	7.07, t (7.5)
22	112.4, CH	7.30, overlap	22	112.4	7.29, d (5.2)
22a	138.1, C		22a	138.1	
23	125.2, CH	7.19, s	23	125.2	7.19, s
NH		8.13, d (8.0)	NH		8.17, d (8.2)
β-Hydroxy-*O*-methyltyrosine (E)			β-Hydroxy-*O*-methyltyrosine (E)		
24	171.6, C		24	171.6	
25	59.2, CH	4.78, d (7.0)	25	59.2	4.79, m
26	74.9, CH	4.49, d (6.9)	26	74.9	4.49, d (6.8)
27	133.0, C		27	132.9	
28	129.1, CH	6.68, d (8.2)	28	129.1	6.64, d (5.6)
29	114.5, CH	6.57, d (8.5)	29	114.5	6.55, d (6.8)
30	161.0, C		30	161.0	
31	114.5, CH	6.57, d (8.5)	31	114.5	6.55, d (6.8)
32	129.1, CH	6.68, d (8.2)	32	129.1	6.64, d (5.6)
33	55.6, CH_3	3.66, s	33	55.6	3.65, s

Table 1
(continued)

Position	**1**		Position	**2**	
	δ_C, type	δ_H (J in Hz)		δ_C	δ_H (J in Hz)
NH		7.37, d (10.0)	NH		7.40, s
Pro (F)			Pro (F)		
34	174.0, C		34	174.1	
35	61.8, CH	4.34, overlap	35	61.8	4.35, overlap
36	30.2, CH_2	1.96, dd (13.1, 6.4), 1.65, m	36	30.2	1.97, dd (19.4, 9.6), 1.68, brs
37	24.8, CH_2	1.77, m, 1.41, m	37	24.9	1.76, m, 1.42, m
38	48.2, CH_2	3.72, m	38	48.2	3.72, dd (17.1, 8.6)
3-Phenylserine (G)			3-Phenylserine (G)		
39	171.8, C		39	171.8	
40	58.3, CH	4.51, d (9.0)	40	58.5	4.52, d (8.8)
41	74.0, CH	4.58, (d, 10.0)	41	73.7	4.56, (d, 10.1)
42	143.3, C		42	143.3	
43	127.4, CH	7.42, d (7.4)	43	127.3	7.38, overlap
44	129.2, CH	7.34, t (7.6)	44	129.3	7.38, overlap
45	128.4, CH	7.26, d (7.5)	45	128.5	7.22, m
46	129.2, CH	7.34, t (7.6)	46	129.3	7.38, overlap
47	127.4, CH	7.42, d (7.4)	47	127.3	7.38, overlap
NH		7.76, d (8.4)	NH		7.89, d (8.5)
Gly (H)			Gly (H)		
48	171.6, C		48	171.8	
49	43.5, CH_2	3.46, d (17.5), 4.09, overlap	49	43.9	3.46, d (17.0), 4.09, overlap
NH		7.90, overlap	NH		7.67, brs
Asp (I)			3-Methylaspartic acid (I*)		
50	172.9, C		50	177.7	
51	50.9, CH	5.05, s	51	54.8	5.18, m
52	38.1, CH_2	2.93, dd (16.0, 10.3), 3.36, t (4.0)	52	42.3	3.27, dd (14.8, 7.3)
53	173.7, C		53	172.8	
NH		8.02, d (9.4)	54	14.5	1.24, d (7.1)
			NH		7.97, d (10.3)
Ala (J)			Ala (J)		
54	175.2, C		55	175.6	
55	53.1, CH	4.09, overlap	56	53.1	4.09, overlap
56	16.6, CH_3	1.45, overlap	57	17.0	1.48, d (7.4)
NH		7.95, overlap	NH		7.90, m
2,3-Diaminobutyr-ic acid (K)			2,3-Diaminobutyri-c acid (K)		
57	176.7, C		58	176.4	
58	62.2, CH	5.12, s	59	62.2	5.09, s
59	70.6, CH	5.49, q (6.7)	60	70.6	5.48, q (6.6)
60	17.6, CH_3	1.36, d (6.8)	61	17.7	1.36, d (6.8)
NH		9.59, s	NH		9.56, s
Pyridyl acryloyl chain			Pyridyl acryloyl chain		
61	172.4, C		62	172.2	

Table 1
(continued)

Position	**1**		Position	**2**	
	δ_C, type	δ_H (J in Hz)		δ_C	δ_H (J in Hz)
62	122.4, CH	7.24, d (16.0)	63	122.6	7.25, d (15.9)
63	140.4, CH	7.90, overlap	64	140.0	7.82, d (15.8)
64	134.4, C		65	134.3	
65	127.7, CH	7.95, overlap	66	127.7	7.94, m
66	130.3, CH	7.30, overlap	67	130.4	7.31, d (6.1)
67	131.0, CH	7.14, dd (5.2, 3.7)	68	131.0	7.15, m
68	139.5, C		69	139.3	
69	129.1, CH	6.46, d (11.3)	70	129.0	6.36, d (11.3)
70	130.1, CH	5.74, dq (14.0, 7.0)	71	130.3	5.83, dq (13.1, 6.5)
71	14.5, CH_3	1.45, overlap	72	14.5	1.51, d (6.8)

^{1}H NMR data (δ_H) were measured in MeOH-d4 at 600 MHz. Proton coupling constants (J) in Hz are given in parentheses. ^{13}C NMR data (δ_C) were measured in MeOH-d4 at 125 MHz

residue E was a β-hydroxy-O-methyltyrosine. Residues C, D, and E were sequentially connected on the base of the key correlations from H-14 (δ_H 5.44) to C-15 (δ_C 175.0) and H-16 (δ_H 4.74) to C-24 (δ_C 171.6).

A dipeptide structural moiety composed by a proline (residue F) and 3-phenylserine (residue G) could be determined by the ^{1}H-^{1}H COSY (Table 1) together with the key HMBC correlations of H-35 (δ_H 4.34)/C-34 (δ_C 174.0), H-44/46 (δ_H 7.34, 2H)/C-42 (δ_C 143.3) and H-43/47 (δ_H 7.42, 2H)/C-41 (δ_C 74.0), as well as by their chemical shifts. The saturated O-methines of C-41 was determined by comparing its chemical shift with that of 3-hydroxylphenylalanine. Due to the 3-hydroxylphenylalanine was also one of the seven residues for the skeleton of vancomycin [18, 19], therefore, the NMR data for residue F of compound **1** could determine by comparing its NMR data. This dipeptide group was supported to connect with residue E on the base of the HMBC correlation from H-26 (δ_H 4.49) to C-34 (δ_C 174.0).

A tripeptide structural moiety with the constituents of glycine (residue H), aspartic acid (residue I), and alanine (residue J) was based on their chemical shifts and the ^{1}H-^{1}H COSY (Table 1) and key HMBC correlations. Especially, based on the HMBC correlations of NH-49 (δ_H 7.90)/C-50 (δ_C 172.9) and H-51 (δ_H 5.05)/C-54 (δ_C 175.2) showed the solid evidence for the linkage of residue H-I-J one by one. This tripeptide was conformed to linked to the 3-phenylserine (residue G) by the HMBC correlation from H-40 (δ_H 4.51) to C-48 (δ_C 171.6).

All analysis above allowed us to establish the connection between 10 fragments as A-B-C-D-E-F-G-H-I-J.

An unusual 2, 3-diaminobutyric acid (DABA) residue (K) was verified in compound **1** based on the ^{1}H-^{1}H COSY correlations of NH-58 (δ_H 9.59)/H-58 (δ_H 5.12)/H-59 (δ_H 5.49)/H_3-60 (δ_H 1.36), and their chemical shifts were very similar to the DABA moiety in sansanmycins [20] and pacidamycins [21]. It was interesting to find that the residues A and J were all connected with DABA by peptide bonds based on the HMBC correlations of H-5+9 (δ_H 5.49) to C-1 (δ_C 171.1) and NH-55(δ_H 7.95) to C-57 (δ_C 176.7). Therefore, the main skeleton of **1** was illustrated as a cyclopeptide including five nonprotein amino acids which should be assembled by nonribosomal peptide synthetase (NRPSs).

Apart from the above signals to the cyclopeptide moiety, the analysis of remainder ^{1}H NMR and ^{13}C NMR were most likely to existi three spin systems including a substitutional α, β-unsaturated amide, a 2, 3-disubstituted pyridinyl, and a *cis*-propenyl. Furthermore, based on the HMBC correlations from H-70 (δ_H 5.74) to C-68 (δ_C 139.5), H-69 (δ_H 6.46) to C-64 (δ_C 134.4), H-62 (δ_H 7.24) to C-64 (δ_C 134.4), and H-63 (δ_H 7.90) to C-61 (δ_C 172.4), a pyridyl acryloyl side chain composed by those three coupling fragments were determined as (E)-3-(3-((Z)-prop-1-en-1-yl) pyridin-2-yl)acrylic acyl. The pyridyl acryloyl side chain was connected with fragment K according to the correlation between NH-58 (δ_H 9.59) and C-62 (δ_C 122.4) in HMBC spectrum. Finally, the planar structure of compound **1** was determined as Fig. 1.

The relative configuration of **1** was established by means of rotating frame Overhauser effect spectroscopy. The correlations of H-2/H-3, H-24/H-25 and H-40/H-41 revealed that the hydroxyl and amino groups in residues A, E, and G were bearing *cis* orientation. Furthermore, the correlations of H-2/H-59/NH-58 and H_3-60/H-58 supported the relative configuration of diamino groups of DABA oriented in opposite sides. Its absolute configuration was determined by X-ray diffraction analysis (Figs. 2 and 3).

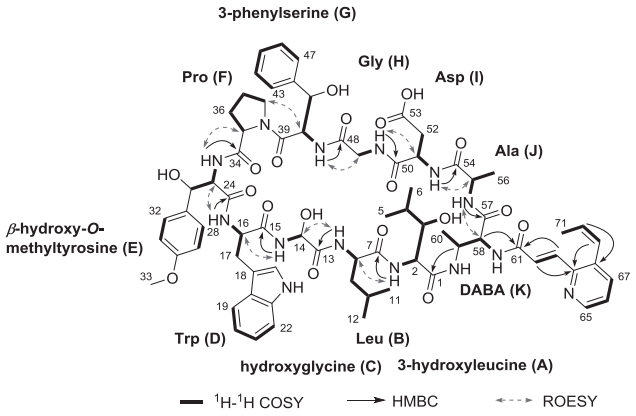

Fig. 1 Planar structure of dolyemycin A (**1**)

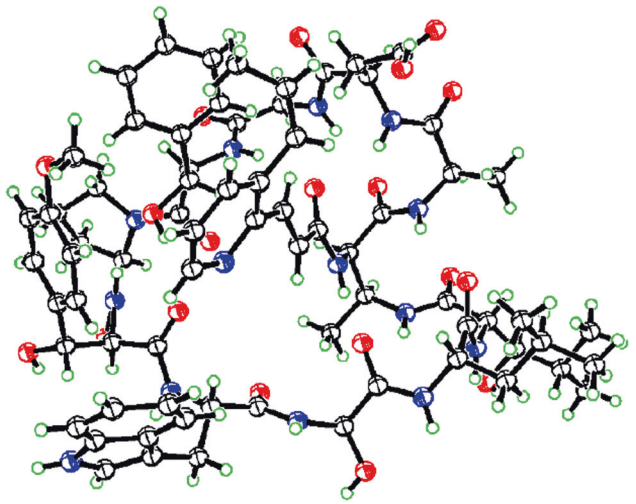

Fig. 2 X-ray crystallographic structure of dolyemycin A (**1**) (ORTEP drawing)

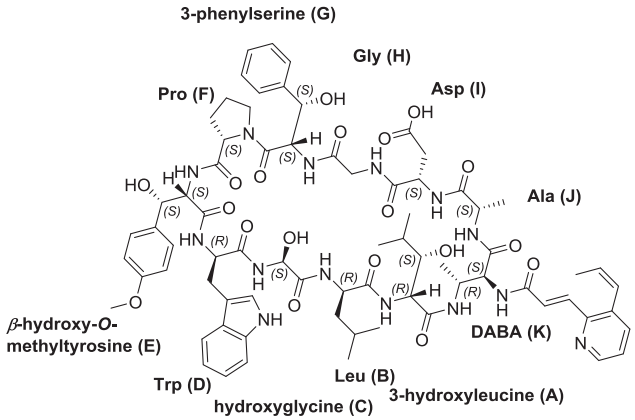

Fig. 3 Absolute configuration of dolyemycin A (**1**)

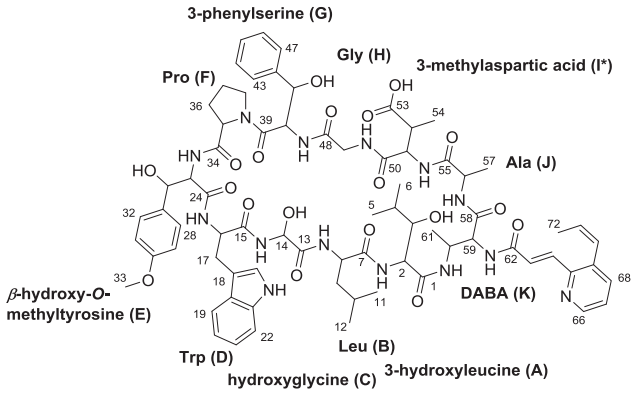

Fig. 4 Planar structure of dolyemycin B (**2**)

Compound **2** was obtained as white amorphous powder. Its molecular formula of $C_{74}H_{94}N_{14}O_{19}$ with 35° of unsaturation was established on base of the quasi-molecular ion at *m/z* 1483.6848 $[M+H]^+$ (calcd for $C_{74}H_{95}N_{14}O_{19}$, 1483.6898) peak in HR-ESI-MS. Compound **2** showed similar physicochemical property and spectroscopy features with that of **1** (see Table 1). Comparing the ^{13}C and DEPT spectra between compounds **1** and **2** revealed that compound **2** bearing most of the resonance signals of **1** except for the lack of a methylene signal at δ_C 38.1 corresponding to the aspartic acid (residue I) of **1**. Furthermore, by detail analysis of the NMR data of **2**, the signals of an additional quaternary carbon and one methyl atoms were found in the ^{13}C NMR spectrum, which suggested that the aspartic acid in **1** should be replaced by a 3-methylaspartic acid in **2** (Fig. 4). This suggestion was supported by the ^1H-^1H COSY correlations of H_3-54/H-52/H-51, as well as the HMBC correlations from H_3-54 (δ_H 1.24) to C-51 and C-53.

For compound **2** was obtained from the same strain, and the planar structure of **2** was very similar to that of **1** which suggested that the absolute configuration of **2** should keep the same to that of **1**. H-51 and H-52 showed *cis*-configuration based on the ROSEY correlation of H-51/H-52. However, its absolute configuration could not be determined due to the microscale of **2**.

From the structures of compounds **1** and **2**, they both belong to cyclopeptides with a ring structure constituted by eleven amino acids. By further analysis, we can also find that there were about 45% and 54% nonprotein amino acids in the structure of compound **1** and **2**, respectively. These characteristics in their structures made them very special in microbial peptide antibiotics.

X-ray crystallographic analysis of 1

The absolute configuration of **1** was determined using data collected on a Bruker APEX-II CCD diffractometer. The crystal was kept at 173.0 K during data collection. Using Olex2, the structure was solved with the ShelXT structure solution program using Intrinsic Phasing and refined with the ShelXL refinement package using Least Squares minimization. Crystallographic data for **1** has been deposited at the Cambridge Crystallographic Data Centre (**1**: CCDC 1560877). Copies of the data can be obtained free of charge

Table 2 The IC_{50} of **1** and **2** against human cancer cells

Compound	IC_{50} (μM)	
	A549	HL60
1	1.0	1.2
2	1.2	7.3

by application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 44-(0)1223−336033 or e-mail: deposit@ccdc.cam.ac.uk).

Crystal data of 1

Colorless crystal, $C_{146}H_{202}N_{28}O_{55}$ ($M = 3233.38$ g/mol): orthorhombic, space group $P2_12_12_1$ (no. 19), $a = 12.8498$ (4) Å, $b = 21.1505(7)$ Å, $c = 30.5807(9)$ Å, $V = 8311.2(4)$ Å^3, $Z = 2$, $T = 173.0$ K, μ (Cu Kα) = 0.838 mm^{-1}, $D_{calc} = 1.292$ g cm^{-3}, 68210 reflections measured ($5.08° \le 2\Theta \le 133.6°$), 14697 unique ($R_{int} = 0.1082$, $R_{sigma} = 0.0643$), which were used in all calculations. The final R_1 was 0.0688 ($I > 2\sigma$ (I)) and wR_2 was 0.2072 (all data).

Dolyemycin A (1)

White amorphous powder; $[\alpha]_D^{25} = +0.02$ (c 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 241 (3.1) nm; IR (KBr) cm^{-1}: 3333, 2960, 2933, 1657, 1531, 1455, 1249, 1204, 1138; HR-ESI-MS (positive-ionization mode) *m/z*: 1491.6542 $[M + Na]^+$, (calcd for $C_{73}H_{92}N_{14}O_{19}Na$, 1491.6561); ^{1}H and ^{13}C NMR: see Table 1.

Dolyemycin B (2)

White amorphous powder; $[\alpha]_D^{25} = +0.02$ (c 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 241 (3.1) nm; HR-ESI-MS (positive-ionization mode) *m/z*: 1483.6848 $[M + H]^+$, (calcd for $C_{74}H_{95}N_{14}O_{19}$, 1483.6898); ^{1}H and ^{13}C NMR: see Table 1.

Biological properties

The antiproliferative activities shown in Table 2 indicated that both **1** and **2** exhibited broad inhibitory activities against human cancer cells such as human lung cancer A549 cells and human leukemia HL60 cells. The most potent activity of **1** and **2** was observed against A549 human lung cancer cells with IC_{50} of 1.0 μM and 1.2 μM, respectively.

Discussion

Two cyclopeptides with novel skeleton were isolated from HYS31 by bio-guided isolation. The key structural features of them were characterized by spectroscopic analysis. They exhibited antiproliferative activities to human cancer cells such as human lung cancer cells and human leukemia cells. The most potent activity of **1** and **2** was observed against A549 human lung cancer cells with IC_{50} of 1.0 and 1.2 μM, respectively. The strain HYS31 was identified as *Streptomyces griseus* subsp. *griseus* according to its morphological characteristics, biochemical characteristics and partial sequence of its 16S rDNA.

Compounds **1** and **2** were two cyclopeptides with special structures isolated from secondary metabolites of an actinomycete. As novel cyclopeptides, their potential as biosurfactant and potential of other medical applications on antibacterial, antifungal and anti-tuberculosis are worthy of further study. Our findings also suggest that the strain *Streptomyces griseus* subsp. *griseus* HYS31 may have special metabolic pathway, which enable the produce of cyclopeptides with so many unusual amino acids. In this regard, further study on its fermentation products may lead us to find more novel compounds with stronger biological activity.

Methods

General

Fractions were monitored with TLC (HSGF 254, Yantai, People's Republic of China), and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in 95% ethanol. Column chromatography was performed on Sephadex LH-20 (Pharmacia) and silica gel (200–300 mesh, Yantai, People's Republic of China). HPLC purification was conducted on Waters 1525/2487 liquid chromatography. UV spectra were performed on a Beijing Purkinje–TU–1810 spectrophotometer. IR spectra were performed on a Magna–IR 550 spectrometer. NMR experiments were performed on Bruker AVANCE-600 and AVANCE-500 instruments. HR-ESI-MS spectrum was acquired using a Q-Tof micro LCTTM mass spectrometer.

Antiproliferative activity

The antiproliferative activities of the compounds were evaluated against the HL-60 and A549 cells by the Cell Counting Kit-8 (CCK-8). Briefly, cells were seeded into 96-well plates and grown for 24 h. Cells were then treated with increasing concentrations of compounds and grown for further 72 h. At the end of exposure time, 10 μl CCK8 (Dojindo, Kumamoto, Japan) was added to each well and the plates were kept in the incubator for 4 h, then measured at 450 nm using multiwell spectrophotometer (SpectraMax, Molecular Devices, USA). The inhibition rate was

calculated as (1-A_{450} treated/A_{450} control) × 100%. The cytotoxicity of compounds was expressed as an IC_{50}, determined by the Logit method. Doxorubicin was used as positive control.

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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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