



BRIEF COMMUNICATION

Two new lankacidin-related metabolites from *Streptomyces* sp. HS-NF-1178

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Received: 10 August 2017 / Revised: 27 October 2017 / Accepted: 17 November 2017 / Published online: 18 January 2018
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Abstract

Two new lankacidin-related metabolites, 2,18-seco-lankacidinol A (**1**), 2,18-seco-lankacidinol B (**2**) and a known compound, lankacidinol (**3**), were isolated from the fermentation broth of *Streptomyces* sp. HS-NF-1178. Their structures were determined on the basis of spectroscopic analysis, including 1D and 2D NMR techniques as well as ESI-MS and comparison with data from the literature. These two new compounds, especially compound **1**, exhibited potent antitumor activity.

Lankacidin-group antibiotics, produced by organism *Streptomyces rochei*, are a class of unique 17-membered macrocyclic antibiotics different from traditional even-membered macrolides^{1, 2}. These antibiotics and their derivatives showed antimicrobial activity against various Gram-positive bacteria, *Neisseria gonorrhoeae*, *Vibrio cholerae* and *Xanthomonas oryzae*³. More importantly, they exhibited strong effect on staphylococcal infection in mice by oral or intraperitoneal administration⁴. In addition, they also displayed considerable in vivo antitumor activity against certain cell line models such as L1210 leukemia, melanoma B16 and 6C3 HED/OG lymphosarcoma⁵. In the course of hunting for new microbe-derived bioactive secondary metabolites, two new lankacidin-related metabolites, designated as 2,18-seco-lankacidinols A and B (**1-2**) and a known compound, lankacidinol (**3**) (Fig. 1), were isolated from the fermentation broth of *Streptomyces* sp. HS-NF-1178. In this paper, the details of fermentation, isolation,

structure characterization and bioactivity of these two new compounds are described.

Strain *Streptomyces* sp. HS-NF-1178 was isolated from a soil sample collected from a pine forest in Tianmu Mountain of Hangzhou, Zhejiang province, China. The strain was identified as the genus *Streptomyces* because its 16S rRNA sequence (accession no: KY884722 in the GenBank) exhibited a high-sequence similarity of 99.93% with that of *Streptomyces* sp. NRRL-16374 (T) (accession no: DQ026660).

This strain was incubated for 6–8 days at 28 °C on YMS medium containing malt extract (Becton, Dickinson and Company, Franklin Lake, NJ, USA) 10.0 g, yeast extract (Oxoid, Basingstoke, UK) 2.0 g, KNO₃ 1.0 g and agar (Becton, Dickinson and company, Franklin Lake, NJ, USA) 20.0 g in 1.0 l tap water at pH 7.2–7.4. The strain of stock culture was transferred into 1 l Erlenmeyer flasks containing 25% volume of the seed medium and incubated at 28 °C for 48 h, shaken at 250 r.p.m. The seed medium consisted of glucose (Sinopharm Chemical Reagent, Shanghai, China) 4.0 g, malt extract 10.0 g, yeast extract 4.0 g and CaCO₃ 2.0 g in 1.0 l tap water, pH 7.2–7.4. All of the media were sterilized at 121 °C for 30 min. Then, 1 l of the culture was transferred into a 50 l fermentor containing 30 l of producing medium consisting of glucose 10 g, soluble amyllum (Haiyan Liuhe Starch Chemical Co, Ltd., Haiyan, China) 40 g, yeast extract 4 g, malt extract 10.0 g, CaCO₃ 2 g, MgSO₄·7H₂O 1 g, NaCl 1 g, KH₂PO₄ 2 g, pH 7.2–7.4. The fermentation was carried out at 28 °C for 6 days and stirred at 100 r.p.m. with an aeration rate of 700 l of air per hour.

The fermentation broth (30 l) was centrifuged to separate mycelial cake and supernatant. The mycelial cake was

Electronic supplementary material The online version of this article (<https://doi.org/10.1038/s41429-017-0004-0>) contains supplementary material, which is available to authorized users.

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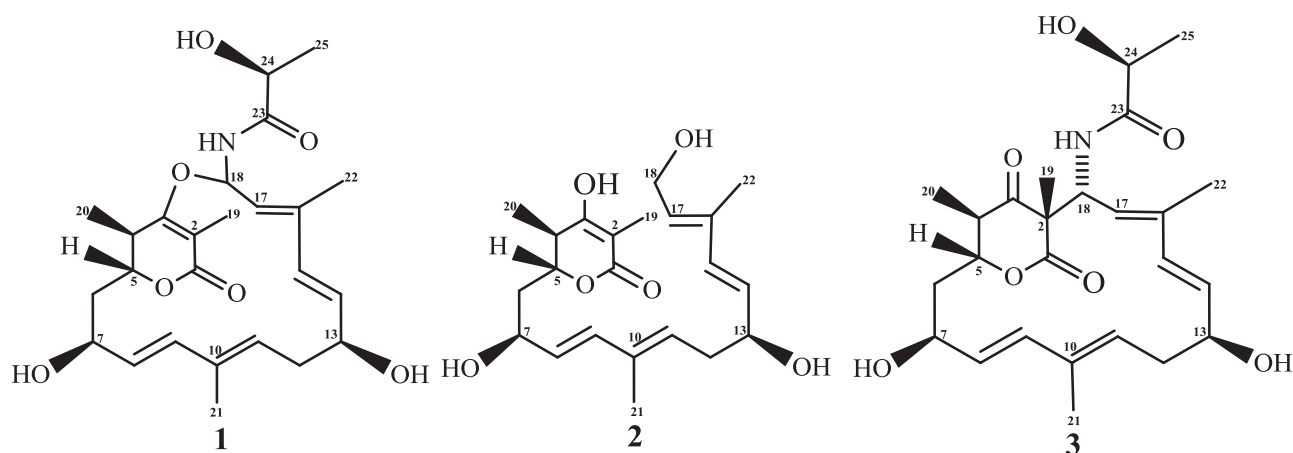


Fig. 1 Chemical structures of 2,18-seco-lankacidinol A (**1**), 2,18-seco-lankacidinol B (**2**) and lankacidinol (**3**)

Table 1 ^1H NMR data of compounds **1**, **2** and **3**

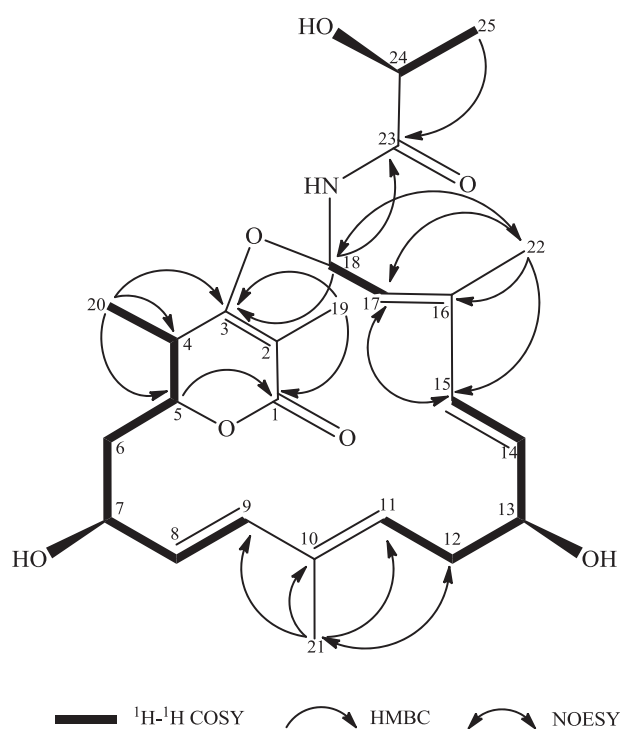
Position	δ_{H} (J in Hz)		
	1 (in CD_3OD)	2 (in CD_3OD)	3 (in DMSO-d_6)
1			
2			
3			
4	2.49 m	2.38 m	2.41 m
5	4.17 m	4.37 m	4.68 m
6	2.09 m	2.05 m	2.12 m
	1.80 m	1.70 m	1.96 m
7	4.30 dd (13.2, 6.8)	4.30 dd (14.0, 7.0)	4.17 m
8	5.58 m	5.57 m	5.51 dd (15.4, 9.4)
9	6.31 d (15.4)	6.28 d (16.0)	6.10 d (15.4)
10			
11	5.58 m	5.57 m	5.28 m
12	2.42 m	2.41 m	2.23 m
13	4.22 dd (12.4, 6.1)	4.18 dd (13.0, 6.4)	3.89 m
14	5.86 dd (15.5, 6.3)	5.69 dd (15.7, 6.6)	5.37 dd (15.8, 8.1)
15	6.29 d (15.5)	6.24 d 15.7	5.56 d (15.8)
16			
17	5.47 d (8.2)	5.61 m	4.73 d (10.9)
18	6.05 d (8.2)	4.21 d (6.7)	5.28 m
19	1.73 s	1.70 s	1.27 s
20	1.26 d (7.0)	1.12 d (7.1)	1.13 d (6.6)
21	1.78 br s	1.77 br s	1.42 br s
22	1.89 br s	1.77 br s	1.71 br s
23			
24	4.39 q (6.4)		3.96 m
25	1.36 d (6.7)		1.24 d (6.8)
7-OH			4.83 d (4.4)
13-OH			5.02 d (4.1)
24-OH			5.82 d (4.7)
NH			7.77 d (10.2)

extracted with MeOH (51) and the supernatant was subjected to a Diaion HP-20 resin (Mitsubishi Chemical Co., Ltd., Tokyo, Japan) column eluting with 95% EtOH (51). The MeOH extract and the EtOH eluents were evaporated under reduced pressure at 50 °C to yield a mixture (36.6 g). The crude extract was chromatographed on a silica gel (Qingdao Haiyang Chemical Group, Qingdao, Shandong, China; 100–200 mesh) column and successively eluted with a stepwise gradient of $\text{CHCl}_3/\text{MeOH}$ (100:0, 95:5, 90:10, 85:15, 80:20 and 70:30, v/v) to give four fractions (Fr.1–Fr.4) based on the TLC profiles. TLC was performed on silica-gel plates (HSGF254, Yantai Chemical Industry Research Institute, Yantai, China) with solvent system of $\text{CHCl}_3/\text{MeOH}$ (9:1, v/v). The Fr.2 eluted with $\text{CHCl}_3/\text{MeOH}$ (85:15, v/v) was subjected to a Sephadex LH-20 (GE Healthcare, Glies, UK) column eluted with $\text{CHCl}_3/\text{MeOH}$ (1:1, v/v) and detected by TLC to obtain three subfractions (Fr.2-1–Fr.2-3). Fr.2-2 was further isolated by preparative HPLC (Shimadzu LC-8A, Shimadzu-C18, 5 μm , 250 \times 20 mm inner diameter; 20 ml min^{-1} ; 220 nm/254 nm; Shimadzu, Kyoto, Japan) eluting with a stepwise gradient $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (15–33%, v/v, 25 min) to give four subfractions (Fr.2-2-1 to Fr.2-2-4) based on the retention time. Then Fr.2-2-3 (t_{R} 13.6 min) was purified by semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm , 250 \times 9.4 mm inner diameter; 1.5 ml min^{-1} ; 220 nm; Agilent, Palo Alto, CA, USA) eluting with $\text{CH}_3\text{CN}/\text{CH}_3\text{OH}/\text{H}_2\text{O}$ (20:20:60, v/v) to obtain compound **1** (t_{R} 18.3 min, 22 mg). Fr.2-2-4 (t_{R} 17.9 min) was separated by semi-preparative HPLC to yield compound **2** (t_{R} 30.3 min, 12.3 mg). Fr.2-2-2 (t_{R} 11.2 min) was isolated by semi-preparative HPLC to give compound **3** (t_{R} 10.4 min, 25.3 mg). ^1H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer (Rheinstetten, Germany). The ESI-MS and ESI-HRMS spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Milford, MA, USA).

Table 2 ^{13}C NMR data of compounds **1**, **2** and **3**

Position	δ_{C} (ppm)		
	1 (in CD_3OD)	2 (in CD_3OD)	3 (in $\text{DMSO}-d_6$)
1	170.8 (s)	172.4 (s)	171.0 (s)
2	97.6 (s)	97.4 (s)	56.9 (s)
3	172.2 (s)	175.4 (s)	211.7 (s)
4	38.3 (d)	37.9 (d)	46.3 (d)
5	79.4 (d)	76.4 (d)	75.4 (d)
6	42.0 (t)	39.7 (t)	37.8 (t)
7	70.8 (d)	70.6 (d)	68.5 (d)
8	129.8 (d)	129.7 (d)	130.9 (d)
9	137.3 (d)	137.4 (d)	136.5 (d)
10	136.1 (s)	136.0 (s)	135.6 (s)
11	129.4 (d)	129.8 (d)	128.1 (d)
12	37.4 (t)	37.5 (t)	37.8 (t)
13	73.0 (d)	73.3 (d)	73.5 (d)
14	135.0 (d)	132.3 (d)	132.5 (d)
15	134.3 (d)	135.5 (d)	133.3 (d)
16	140.1 (s)	135.9 (s)	137.4 (s)
17	129.3 (d)	131.7 (d)	125.7 (d)
18	83.6 (d)	59.4 (t)	50.5 (d)
19	8.6 (q)	8.7 (q)	20.5 (q)
20	16.7 (q)	11.0 (q)	9.7 (q)
21	12.8 (q)	12.9 (q)	12.7 (q)
22	12.9 (q)	12.6 (q)	12.9 (q)
23	177.5 (s)		174.3 (s)
24	74.4 (d)		67.8 (d)
25	17.4 (q)		21.6 (q)

Compound **1** was obtained as white powder with $[\alpha]_{\text{D}}^{25} +9$ (c 0.2, EtOH) and UV (EtOH) λ_{max} nm ($\log \epsilon$): 229 nm (3.97). It exhibited a molecular formula of $\text{C}_{25}\text{H}_{35}\text{NO}_7$ as deduced from the ESI-HRMS at m/z 462.2477 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{25}\text{H}_{36}\text{NO}_7$ 462.2486) in combination with the NMR data (Tables 1 and 2). The IR spectrum of **1** displayed absorption bands for hydroxyl (at 3395 cm^{-1}) and carbonyl (at 1712 cm^{-1}) groups. Analysis of ^1H NMR spectrum (Table 1) of **1** revealed the presence of two aliphatic methyl doublets (δ_{H} 1.26, 1.36), three olefinic methyls (δ_{H} 1.73, 1.78, 1.89), four oxygenated methine protons (δ_{H} 4.17, 4.22, 4.30, 4.39) in addition to seven downfield proton signals. The ^{13}C NMR and DEPT135 spectra (Table 2) of **1** showed 25 resonances attributable to three downfield carbons (δ_{C} 170.8, 172.2, 177.5), six sp^2 methines, two sp^2 quaternary carbons, five oxygen bearing methines, two methylenes, one methine, five methyl carbons in addition to a carbon signal at δ_{C} 97.6. The complete assignment of all ^1H and ^{13}C NMR spectral data of **1** was subsequently accomplished by the ^1H - ^1H COSY, HMQC and HMBC

**Fig. 2** Key ^1H - ^1H COSY, HMBC and NOESY correlations of 2,18-seco-lankacidinol A (**1**)

spectra. The ^1H - ^1H COSY correlations (Fig. 2) of $\text{H}_3\text{-}20/\text{H-}4/\text{H-}5/\text{H-}6/\text{H-}7/\text{H-}8/\text{H-}9$, $\text{H-}11/\text{H-}12/\text{H-}13/\text{H-}14/\text{H-}15$, $\text{H-}17/\text{H-}18$, $\text{H-}24/\text{H-}25$ indicated the four structural fragments (shown by thick lines) of C-20-C-9, C-11-C-15, C-17-C-18, C-24-C-25. The observed HMBC correlations (Fig. 2) from $\text{H}_3\text{-}21$ to C-9, C-10, C-11, from $\text{H}_3\text{-}22$ to C-15, C-16, C-17 established the linkage of C-20-C-18. The HMBC correlations of $\text{H}_3\text{-}19$ with C-1, C-2, C-3, H-5 with C-1 and $\text{H}_3\text{-}20$ with C-3 established the C-1-C-5 six-membered lactone ring moiety as shown in Fig. 2. The linkage of C-3 and C-18 through an oxygen atom was supported by the HMBC correlation from H-18 to C-3 and the downfield carbon resonance of C-3 (δ_{C} 172.2). The connection of C-18 and C-23 through a NH group was evident from the correlation of H-18 to C-23 in the HMBC spectrum and NMR data of C-18 (δ_{H} 6.05; δ_{C} 83.6). Taken the molecular formula of $\text{C}_{25}\text{H}_{35}\text{NO}_7$ into account, two hydroxyl groups were situated at C-7 and C-13, respectively. On the basis of the above spectroscopic analysis, a gross structure of **1** was elucidated as shown in Fig. 1. Thus, compound **1** was named 2, 18-seco-lankacidinol A with a different skeleton from lankacidinol A (**3**). The downfield shifting of C-2 and C-18 (δ_{C} 97.6 and 83.6, respectively, in **1**; δ_{C} 56.9 and 50.5 in **3**) as well as the upfield shifting of C-3 and C-19 (δ_{C} 172.2 and 8.6, respectively, in **1**; δ_{C} 211.7 and 20.5 in **3**) further confirmed the structural assignment of **1**. The olefin conformations of **1** were determined based on

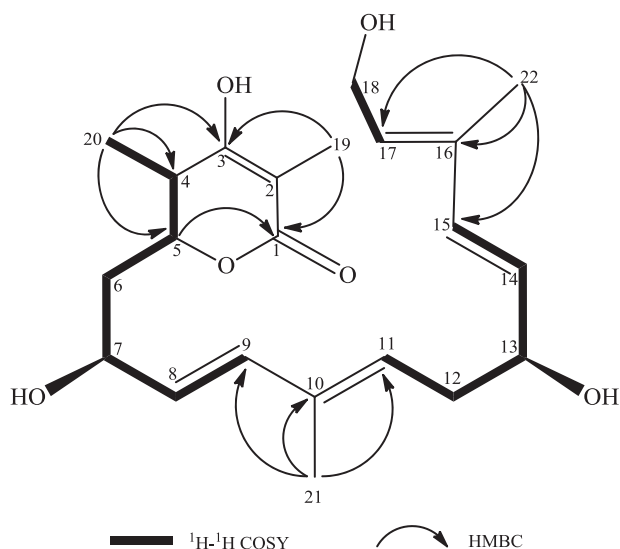


Fig. 3 Key ^1H - ^1H COSY and HMBC correlations of 2,18-seco-lankacidinol B (**2**)

the J values and NOESY experiment. The coupling constants of H-9 (δ_{H} 6.31, d, $J = 15.4$ Hz) and H-15 (δ_{H} 6.29, d, $J = 15.5$ Hz) unambiguously revealed double bond geometry at C-8 and C-14 to be both *trans*. In the NOESY spectrum, the crossing signals (Fig. 2) of H-17 to H-15, H₃-22 to H-18, H₃-21 to H₂-12 indicated the double bonds at C-10 and C-16 were both *trans*. The other chiral centers of **1** were assigned as described for lankacidinol.

Compound **2** was isolated as colorless oil with UV (EtOH) λ_{max} nm (log ϵ): 238 nm (4.34) and $[\alpha]_{\text{D}}^{25} + 1$ (c 0.1, EtOH). Its molecular formula was determined to be C₂₂H₃₂O₆ on the basis of the ESI-HRMS at m/z 391.2118 [M-H]⁻ (calcd for C₂₂H₃₁O₆ 391.2126). The IR spectrum of **2** showed absorption bands for hydroxyl (at 3418 cm⁻¹) and carbonyl (at 1711 cm⁻¹) groups. The ^1H NMR spectrum (Table 1) of **2** displayed a aliphatic methyl doublet (δ_{H} 1.12), three olefinic methyls (δ_{H} 1.70, 1.77, 1.77), three oxygenated methine protons (δ_{H} 4.18, 4.30, 4.37), one oxygenated methylene protons (δ_{H} 4.21) in addition to six downfield olefinic proton signals. The ^{13}C NMR and DEPT135 spectra (Table 2) of **2** showed 22 resonances attributable to two downfield carbons, six sp^2 methines, three sp^2 quaternary carbons, four methines (three oxygenated), three methylenes (one oxygenated) and four methyl carbons. Comparison of the ^1H and ^{13}C NMR data of **2** with those of **1** suggested that **2** had the same C-1 to C-22 structural unit as **1**. Obviously, one of the difference between **2** and **1** was that the 2-hydroxy-propionamido moiety in **1** was absent in **2** and one downfield methine of C-18 (δ_{C} 83.6) in **1** was replaced by one oxygenated methylene (δ_{C} 59.4) in **2**. Considered the molecular formula C₂₂H₃₂O₆ of **2**, the connection between C-3 and C-18 in **1**

Table 3 Antimicrobial activities of compounds **1**, **2** and **3**

Compound	Diameter of inhibition zones (mm)			
	1	2	3	Gentamicin
<i>Micrococcus luteus</i>	15	0	17	3
<i>Bacillus subtilis</i>	0	3	0	15
<i>Staphalococcus aureus</i>	0	2	13	6

was cleaved in **2** and two hydroxyl groups were attached at C-3 and C-18, respectively. Consequently, the planar structure of **2** was established and compound **2** was named 2, 18-seco-lankacidinol B. The correlations of H₃-20/H-4/H-5/H-6/H-7/H-8/H-9, H-11/H-12/H-13/H-14/H-15, H-17/H-18 in the ^1H - ^1H COSY spectrum (Fig. 3) and the observed HMBC correlated signals from H₃-19 to C-1, C-2, C-3, from H₃-20 to C-3, from H₃-21 to C-9, C-10, C-11 and from H₃-22 to C-15, C-16, C-17 further confirmed the above structure assignment of **2**. The coupling constants of H-9 (δ_{H} 6.28, d, $J = 16.0$ Hz) and H-15 (δ_{H} 6.24, d, $J = 15.7$ Hz) showed the double bond geometries at C-8 and C-14 to be *trans*. The highfield ^{13}C NMR chemical shifts of C-21 (δ_{C} 12.9), C-22 (δ_{C} 12.6), were very similar to those reported for lankacidinol A, lankacidinol C and 2,18-seco-lankacidinol A (**1**), indicated the double bonds at C-10 and C-16 both being *trans*. The other relative stereochemistry of **2** was assigned by analogy with **1**.

Compound **3** was obtained as white powder. Its structure was elucidated as lankacidinol by the analysis of its spectroscopic data (Tables 1 and 2) and comparison with literature values⁶.

The antimicrobial activities of compounds **1**, **2** and **3** were measured by disk diffusion method using gentamicin as a positive control⁷. Both compounds **1** and **3** were found to be active against *Micrococcus luteus* with broadness of the clear ring of 15 mm and 17 mm at 100 μg per 7 mm paper disks. Compound **3** showed activity against *Staphylococcus aureus* with broadness of the clear ring of 13 mm. Compound **2** exhibited weak inhibitory activity against *Bacillus subtilis* and *Staphylococcus aureus* with broadness of the clear ring of 3 and 2 mm (Table 3), respectively.

The cytotoxicity of compounds **1**, **2** and **3** were assayed for growth-inhibition activity in vitro against two human tumor cell lines, human lung tumor cells A549 and human prostate cancer cells PC-3 according to CCK8 colorimetric method as reported in our previous papers^{8, 9} using doxorubicin as positive control. The results (Table 4) showed that the two new compounds exhibited potent antitumor activities against two cancer cell lines (PC-3, A549).

Table 4 Cytotoxic activity of compounds **1**, **2** and **3** against selected human tumor cell lines

Compound	IC ₅₀ (μg/ml)			
	1	2	3	Doxorubicin
A549	39.9	78.7	>100	0.234
PC-3	37.9	11.1	>100	0.279

From a biosynthetic view, 2,18-seco-lankacidinol A (**1**) seems to be formed by a different cyclization route from lankacidin. It was reported that a nucleophilic attack of an enolate anion at C-3 on an imide at C-18 occurred through the C-2 and C-3 double bond in an oxidized metabolite of LC-KA05, which resulted in a lankacidin carbon skeleton¹⁰. In the case of 2,18-seco-lankacidinol A (**1**), a direct nucleophilic attack of an enolate anion at C-3 on an imide at C-18 formed a different skeleton with an ether linkage. This result has opened a way to create lankacidin-group antibiotics with ether linkage.

Acknowledgements This research was financially supported by grants from the National Natural Science Foundation of China (No. 31471832)

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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