文章编号:1001-6880(2016)4-0481-05

植物内生菌 *Plantactinospora* sp. NEAU-gxj3 中一个新的酰胺类化合物

郭晓为[1,2],张 继[2],李建宋[2],张 辉[3],齐 欢[3],向文胜[3,4],王相晶[2],吴立军[1*],王继栋[3*]

[1]哈尔滨医科大学药学院,哈尔滨 150081;[2] 东北农业大学生命科学学院,哈尔滨 150030;

[3]浙江海正药业股份有限公司,台州 318000;[4] 中国农业科学院植物保护研究所,北京 100193

摘 要:对植物内生菌 *Plantactinospora* sp. NEAU-gxj3 的次级代谢产物进行了研究,分离得到 3 个化合物,经 1D 和 2D NMR、MS 波谱分析及与文献数据的比较鉴定三个化合物分别为一个新的酰胺类化合物,(2*E*,7*Z*)-9-hydroxy-4,6,8-trimethylnona-2,7-dienamide(**1**)和两个已知化合物,抗生素 U-62162(**2**)和 salternamide C(**3**)。对三个化合物的细胞毒活性实验结果表明化合物 **2** 对人白血病细胞株 K562、人肺腺癌 A549 细胞和人肝癌细胞株 HepG2 表现出较强的细胞毒活性,其 IC_{50} 分别为 6.8、32 μg/mL 和 19 μg/mL。

关键词:*Plantactinospora* sp.;新的酰胺类化合物;手霉素类抗生素;抗生素 U-62162;salternamide C

中图分类号:R284.1;R284.2;Q93 **文献标识码**:A DOI:10.16333/j.1001-6880.2016.4.002

A New Amide Metabolite from Endophytic *Plantactinospora* sp. NEAU-gxj3

GUO Xiao-wei[1,2], ZHANG Ji[2], LI Jian-song[2], ZHANG Hui[3], QI Huan[3], XIANG Wen-sheng[3,4], WANG Xiang-jing[2], WU Li-jun[1*], WANG Ji-dong[3*]

[1]*College of Pharmacy, Harbin Medical University, Harbin 150081, China*; [2]*School of Life Science, Northeast Agricultural University, Harbin 150030, China*; [3]*Zhejiang Hisun Pharmaceutical Co., Ltd., Taizhou 318000, China*; [4]*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China*

Abstract: A new amide metabolite, (2*E*,7*Z*)-9-hydroxy-4,6,8-trimethylnona-2,7-dienamide (**1**), together with two known manumycin-type compounds, antibiotic U-62162 (**2**) and salternamide C (**3**) were obtained from the fermentation broth of endophytic *Plantactinospora* sp. NEAU-gxj3. Their structures were determined on the basis of spectroscopic analysis, including 1D and 2D NMR as well as ESI-MS and comparison with literature data. Bioassay of cytotoxic activities results showed that **2** demonstrated potent cytotoxic activity against human leukemia cell line K562, human lung adenocarcinoma cell line A549 and human liver hepatocellular carcinoma cell line HepG2 with an IC_{50} value of 6.8, 32 μg/mL and 19 μg/mL, respectively.

Key words: *Plantactinospora* sp.; new amide metabolite; manumycin-type antibiotic; antibiotic U-62162; salternamide C

Introduction

Microorganisms possess a tremendous capacity to produce structurally diverse natural products with remarkable activities, rendering them as a prolific source of pharmaceutical leads and therapeutic agents[1,2]. However, in the last decade, the rate of new microbial compound identification is rapidly decreased, and the search for novel pharmaceutical compounds from microorganisms has become more difficult and expensive[2]. Therefore, many efforts have been moved towards the microorganisms from unusual and unexplored habitants, such as endothytic environment[3]. As part of our continuous screening for more active natural products from endophytic microorganisms, a new amide metabolite, (2*E*,7*Z*)-9-hydroxy-4,6,8-trimethylnona-2,7-dienamide (**1**), together with two known manumycin-type

Received: December 15, 2015 Accepted: January 12, 2016

Foundation item: This research was financially supported by grants from the National Outstanding Youth Foundation (31225024); National Natural Science Foundation of China (31471832, 31171913, 31500010, 31572070 and 31372006); National Key Technology R&D Program (2012BAD19B06); Chang Jiang Scholar Candidates Program for Provincial Universities in Heilongjiang (CSCP).

* Corresponding Author Tel: 86-451-86681103; E-mail: wulijun_111@hotmail.com; jdwang@hisunpharm.com

compounds, antibiotic U-62162 (**2**) and salternamide C (**3**) were obtained from the fermentation broth of endophytic *Plantactinospora* sp. NEAU-gxj3. In this study, we report the fermentation, isolation, structural elucidation and biological activity of **1**-**3**.

Materials and Methods

General

^{1}H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer (Bruker, Rheinstetten, Germany) with tetramethylsilane (TMS) as internal standard; ^{1}H and ^{13}C NMR assignments were supported by ^{1}H-^{1}H COSY, HSQC, NOESY and HMBC experiments; Electrospray ionization mass spectrometry (ESI-MS) and high resolution electrospray ionization mass spectroscopy (HR-ESI-MS) spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Waters Co, Milford, MA, USA); UV spectra were obtained from a Varian CARY 300 BIO spectrophotometer; IR spectra were measured with a Nicolet Magna FT-IR 750 spectrometer (υ_{max} in cm^{-1}); The analytic (Zorbax SB-C_{18}, 5 μm, 250 × 4.6 mm i.d) and semi-preparative (Zorbax SB-C_{18}, 5 μm, 250 × 9.4 mm i.d) RP-HPLC were conducted on an Agilent 1100 series; Commercial silica gel (Qingdao Haiyang Chemical Group, Co., 100-200 mesh) and Sephadex LH-20 gel were used for column chromatography; Spots were detected under UV or by heating after spraying with sulfuric acid-ethanol, 5∶95 (v/v).

Strain material

Strain *Micromonospora* sp. NEAU-gxj3 was isolated from soybean root [*Glycine max* (L.) Merr.] collected from Harbin, Heilongjiang Province, China. It was identified as the genus *Plantactinospora* by Professor Wen-Sheng Xiang at School of Life Science, Northeast Agricultural University, Harbin, China. The strain has been deposited in the German Collection Microorganisms and Cell Culture (DSMZ), with accession number of DSM 46832. The GenBank/EMBL/DDBJ database accession number of the 16S rRNA sequence of strain NEAU-gxj3 is KM359703.

Fermentation, extraction and isolation

The strain *Plantactinospora* sp. NEAU-gxj3 was grown and maintained on the oatmeal agar [International Streptomyces Project (ISP) medium No. 3] and incubated for 6-7 days at 28 ℃[4]. Then, a total of 10 mL of sterile water was added to the slant of the ISP3 medium. The spores were scraped off and transferred into a sterile tube containing glass beads, and the resulting spore suspension was then filtered through six layers of sterile cheesecloth and adjusted to 10^7-10^8 cfu/ mL. A volume of 2.0 mL of the spore suspension was inoculated into each of four 1-L Erlenmeyer flasks, which contain 250 mL of seed medium and then incubated at 28 ℃ for 48 h, shaken at 250 rpm. The seed medium consisted of glucose 4 g, maltodextrin 10 g, yeast extract 4 g, $CaCO_3$ 2 g in 1.0 L water and pH 7.2-7.4. Subsequently, 1 L of the culture was transferred into a 50-L fermentor containing 30 L of the producing medium consisting of sucrose 10%, yeast extract 0.5%, soybean powder 2.5%, peptone 0.4%, $CaCO_3$ 0.3%, $MgSO_4 \cdot 7H_2O$ 0.1%, $FeSO_4 \cdot 7H_2O$ 0.006%, K_2HPO_4 0.03%, $MnSO_4 \cdot H_2O$ 0.2%, $CoCl_2 \cdot 6H_2O$ 0.05%, and pH 7.0 before sterilization. The fermentation was conducted at 28 ℃ for 7 days stirred at 100 rpm with an aeration rate of 900 L of air per hour.

The final 30 L of the broth from a 50 L fermentor was filtered and the resulting cake was washed with water (3 L) and subsequently extracted with MeOH (3 L). The supernatant and the wash water were subjected to a Diaion HP-20 resin column eluting with 95% EtOH (5 L). The MeOH extract and the EtOH eluents were evaporated under reduced pressure to 1 L at 50 ℃ and the resulting concentrate was extracted three times using an equal volume of EtOAc. The combined EtOAc phase was concentrated under reduced pressure to yield 20 g of oily substances. The residual oily substance was chromatographed on silica gel and eluted with a $CHCl_3$-MeOH mixture (100∶0-60∶40, v/v). The fractions eluted with the $CHCl_3$-MeOH mixture (90∶10, v/v) were combined and evaporated to obtain fraction I. The fractions eluted with the $CHCl_3$-MeOH mixture (85∶15, v/v) were pooled and concentrated to give fraction II. The fraction I was subjected to Sephadex LH-20 gel column eluting with MeOH to give subfraction I, which was further isolated by semi-preparative HPLC eluting

with CH_3CN-H_2O (60∶40, v/v) with a flow rate of 1.5 mL/min at a room temperature to obtain compounds **2** (t_R 18.1 min, 25 mg) and **3** (t_R 20.2 min, 10 mg). The fraction II was subjected to Sephadex LH-20 gel column eluting with MeOH to give subfraction II, which was subsequently purified by semi-preparative HPLC using a solvent containing a CH_3CN-H_2O mixture (25∶75, v/v) with a flow rate of 1.5 mL/min at a room temperature to give compound **1** (t_R 20.5 min, 15 mg).

Cytotoxicity assay

Cytotoxic activities of **1**, **2** and **3** against human leukemia cell line K562, human lung adenocarcinoma cell line A549 and human liver hepatocellular carcinoma cell line HepG2 were evaluated using the CCK-8 colorimetric method[8]. Briefly, all kinds of cell lines were routinely cultured in DMEM containing 10% calf serum at 37 ℃ for 4 hours, in a humidified atmosphere of 5% CO_2 incubator. The adherent cells at their logarithmic growth stage were digested, and were inoculated onto 96-well culture plate at a density of 1.0×10^4 cells/well for the determination of proliferation. Test compounds were added to the medium after 24 hours and incubation was continued for 48 hours. Coloration substrate, cell counting kit-8 (CCK-8, Dojindo), was added to the medium followed by further incubation for 3 hours. Absorbance at 450 nm with a 600 nm reference was measured thereafter. Media and DMSO control wells, in which compound was absent, were included in all the experiments in order to eliminated the influence of DMSO. The inhibitory rate of cell proliferation was calculated by the following formula:

$$\text{Growth inhibition (\%)} = [OD_{control} - OD_{treated}]/OD_{control} \times 100$$

The cytotoxicity of compound on tumor cells was expressed as IC_{50} values (the drug concentration reducing by 50% the absorbance in treated cells, with respect to untreated cells) and was calculated by LOGIT method.

Results and Discussion

Structural elucidation

Compound **1** Amorphous solid, UV (EtOH) λ_{max} nm 202 (log 4.17); IR (KBr): v_{max} 3331, 2967, 1678, 1606, 1403, 1209, 1007, 863 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.80 (1H, d, J = 15.5 Hz, H-2), 6.74 (1H, dd, J = 15.5, 7.8 Hz, H-3), 2.30 (1H, m, H-4), 1.30 (2H, m, H-5), 2.46 (1H, m, H-6), 5.01 (1H, d, J = 9.7 Hz, H-7), 4.15 (1H, d, J = 11.7 Hz, H-9a), 4.02 (1H, d, J = 11.7 Hz, H-9b), 1.75 (3H, s, H-10), 0.85 (3H, d, J = 6.2 Hz, H-11), 1.00 (3H, d, J = 6.7 Hz, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.5 (s, C-1), 120.9 (d, C-2), 151.7 (d, C-3), 34.4 (d, C-4), 44.0 (t, C-5), 29.9 (d, C-6), 134.5 (d, C-7), 132.9 (s, C-8), 61.4 (t, C-9), 21.3 (q, C-10), 21.5 (q, C-11), 19.8 (q, C-12); HR-ESI-MS (m/z 212.1644 $[M+H]^+$, calculated for $C_{12}H_{22}NO_2$, 212.1645). The molecular formula of **1** was detennined to be $C_{12}H_{21}NO_2$ on the basis of HR-ESI-MS analysis, indicating 3 degrees of unsaturation. The IR spectrum revealed that **1** possessed a conjugated carbonyl group (1678 cm^{-1}) and a hydroxy group (3331 cm^{-1}). The ^1H NMR (400 MHz, $CDCl_3$) data showed three olefinic resonances in the deshielded region at δ_H 5.01 (1H, d, J = 9.7 Hz), 5.80 (1H, d, J = 15.5 Hz), 6.74 (1H, dd, J = 15.5, 7.8 Hz), one oxygenated methylene group at δ_H 4.15 (1H, d, J = 11.7 Hz), 4.02 (1H, d, J = 11.7 Hz), one aliphatic methylene at δ_H 1.30 (2H, m), two aliphatic methines at δ_H 2.30 (1H, m), 2.47 (1H, m), one olefinic methyl at δ_H 1.75 (3H, brs), two aliphatic methyls at δ_H 1.00 (3H, d, J = 6.7 Hz) and 0.85 (3H, d, J = 6.2 Hz). The ^{13}C NMR spectrum showed one carbonyl carbon at δ_C 168.5 (s), one olefinic quaternary carbon at δ_C 132.9 (s), three olefinic methines at δ_C 151.7 (d), 134.5 (d) and 120.9 (d), one oxygenated methylene at δ_C 61.4 (t), one aliphatic methylene at δ_C 44.0 (t), three methyls at δ_C 21.5 (q), 21.3 (q) and 19.8 (q) in addition to two aliphatic methines at δ_C 34.4 (d) and 29.9 (d). The correlations of ^1H-^1H COSY and HMBC in compound **1** were determined in this elucidation (Fig. 1). The ^1H-^1H COSY correlations of H-2/H-3/H-4/H_2-5/H-6/H-7 established the backbone core from C-2 to C-7. The doublet methyl groups C-11 and C-12 were connected to C-6 and C-4, respectively, by the ^1H-^1H COSY correlations of H_3-11/H-6 and H_3-12/H-

4. Furthermore, the HMBC correlations from H_2-9 and H_3-10 to the olefinic double-bond carbon C-7 and C-8 secured the connectivity from C-7 to C-8 bearing a methyl and an oxygenated methylene groups. Strong HMBC correlations from H-2 and H-3 to the carbonyl carbon C-1 at δ_C 168.5 in combined with the molecular formula indicated that an amide functional group was located on C-2 position. The geometry of the C-2 double bond was determined to be *E* by observation of the *trans* ^{1}H-^{1}H coupling constant ($J_{2,3}$ = 15.5 Hz) between H-2 and H-3. The geometry of the C-7-C-8 double bond was assigned as *Z* based on the downfield chemical shift of C-10 (δ_C 21.3). The stereogenic centers of C-4 and C-6 were assigned as those of (*E*)-4, 6, 8-trimethylnona-2, 7-dienamide, which was obtained from the salternamide C (**3**)[5]. Consequently, the structure of **1** was established as shown in Fig. 1. NMR data and detailed experimental data of **1** is available free of charge via the Internet at http://www.trcw.ac.cn.

Compound **2** Pale yellow oil, UV (EtOH) λ_{max} nm 233 (log 4.09), 280 (log 3.83); $[\alpha]_D^{25}$ +23.4 (*c* 0.17, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data see Table 1; HR-ESI-MS (*m/z* 442.2197 $[M+Na]^+$, calculated for $C_{23}H_{33}NO_6Na$, 442.2200). The molecular formula of **2** was deduced as $C_{23}H_{33}NO_6$ based on the HR-ESI-MS analysis, indicating 8 degrees of unsaturation. Analysis of the IR spectrum indicated the presence of hydroxyl and

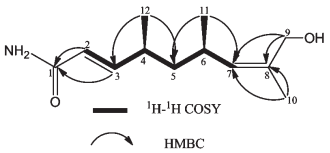

Fig. 1 Chemical structure, key ^{1}H-^{1}H COSY and HMBC correlations of 1

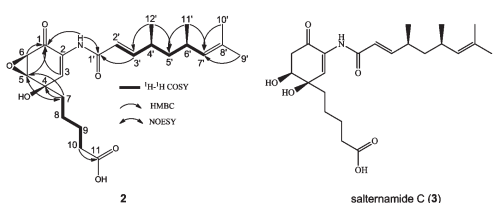

Fig. 2 Chemical structures of 2 and 3 and key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of 2

Table 1 NMR data for 2 (in $CDCl_3$) and 3 (in CD_3OD)

position	δ_H (*J* in Hz)		δ_C (ppm)
	2	**3**	**2**
1			188.8 (s)
2			128.3 (s)
3	7.42 (1H,s)	7.60 (1H,s)	128.5 (d)
4			70.9 (s)
5	3.63 (1H,br s)	3.99 (1H,dd,7.8,3.1)	58.0 (d)
6a	3.61 (1H,br s)	2.84 (1H,dd,16.9,7.8)	53.3 (d)
6b		2.74 (1H,dd,16.9,3.1)	
7	1.84 (2H,m)	1.88 (1H,m)	39.6 (t)
		1.74 (1H,m)	
8	1.42 (2H,m)	1.51 (2H,m)	22.5 (t)
9	1.67 (1H,m)	1.69 (1H,m)	24.8 (t)
	1.63 (1H,m)	1.63 (1H,m)	
10	2.37 (2H,m)	2.33 (2H,m)	33.5 (t)
11			177.8 (s)
1′			165.1 (s)
2′	5.81 (1H,d,15.3)	6.12 (1H,d,15.4)	121.0 (d)
3′	6.84 (1H,dd, 15.3,7.7)	6.78 (1H,dd, 15.4,7.7)	153.5 (d)
4′	2.31 (1H,m)	2.33 (1H,m)	34.3 (d)
5′a	1.31 (1H,m)	1.33 (1H,m)	43.9 (t)
5′b	1.25 (1H,m)	1.29 (1H,m)	
6′	2.39 (1H,m)	2.42 (1H,m)	30.1 (d)
7′	4.83 (1H,d,9.4)	4.86 (1H,d,9.3)	130.7 (d)
8′			130.4 (s)
9′	1.67 (3H,brs)	1.66 (3H,brs)	25.8 (q)
10′	1.60 (3H,brs)	1.60 (3H,brs)	18.0 (q)
11′	0.90 (3H,d,6.6)	0.92 (3H,d,6.5)	21.4 (q)
12′	1.01 (3H,d,6.6)	1.04 (3H,d,6.5)	19.1 (q)
NH	7.56 (1H,s)		

carbonyl functionalities with IR absorption at 3363 and 1675 cm^{-1}, respectively. The ^{1}H NMR (400 MHz, $CDCl_3$) data (Table 1) showed two downfield proton signals at δ_H 7.56 (1H,s), 7.42 (1H,s), one trans-double bond at δ_H 6.84 (1H,dd,*J* = 15.3,7.7 Hz), 5.82 (1H,d,*J* = 15.3 Hz), two olefinic methyls at δ_H 1.67 (3H,s) and 1.60 (3H,s), two aliphatic methyls at δ_H 1.01 (3H,d,*J* = 6.6 Hz) and 0.90 (3H,d,*J* = 6.6 Hz). The ^{13}C NMR spectra with DEPT experiments (Table 1) showed six quaternary carbons [including two olefinic carbons at δ_C 130.4 (s) and 128.3 (s), three carbonyl carbons at δ_C 188.8 (s), δ_C 177.8 (s) and 165.1 (s), one oxygenated

quaternary carbon at δ_C 70.9 (s)], four olefinic methines at δ_C 153.5 (d), 130.7 (d), 128.5 (d) and 121.0 (d), two oxygenated methines at δ_C 58.0 (d) and 53.3 (d), five aliphatic methylenes at δ_C 43.9 (t), 39.6 (t), 33.5 (t), 24.8 (t) and 22.5 (t), four methyls at δ_C 25.8 (q), 21.4 (q), 19.1 (q) and 18.0 (q) in addition to two aliphatic methines at δ_C 34.3 (d) and 30.1 (d). Full assignments of the ^1H and ^{13}C NMR spectra were consolidated through the use of ^1H-^1H COSY, HSQC and HMBC experiments. The ^1H-^1H COSY correlations of H_2-7/H_2-8/H_2-9/H_2-10 and the observed HMBC correlations (Fig. 2) from H_2-10 to C-11 revealed the presence of a pentanoic acid moiety. The trimethyl nonadienamide chain was established by the ^1H-^1H COSY correlations of H_3-11′/H-6′, H_3-12′/H-4′, H-2′/H-3′/H-4′/H-5′/H-6′/H-7′ and the HMBC crossing peaks from the NH signal (δ_H 7.56) and H-3′ to C-1′ (δ_C 165.1), from H_3-9′ and H_3-10′ to C-7′ and C-8′. By detailed comparison of the ^1H and ^{13}C NMR data (Table 1) of **2** with those of antibiotic U-62162[6,7] suggested that **2** was identical to antibiotic U-62162. The NOESY correlation (Fig. 2) of H-5 and H_2-7 further confirmed the structure of **2**.

Compound **3** Pale yellow oil, ^1H NMR (400 MHz, $CDCl_3$) data is shown in Table 1; ESI-MS *m/z* 444 [M + Na]$^+$. Its structure was elucidated as salternamide C (Fig. 2) by analysis of its spectroscopic data and comparison with literature values[5].

Cytotoxic activity

The results showed that **2** demonstrated strong cytotoxic activity against K562 with an IC_{50} value of 6.8 μg/mL, and moderate cytotoxic activity against A549 and HepG2 with IC_{50} values of 32 μg/mL and 19 μg/mL, respectively. However, **1** and **3** exhibited no significant cytotoxic activity against these three cell lines with IC_{50} values of > 100 μg/mL.

Conclusion

In conclusion, a new amide metabolite, (2*E*,7*Z*)-9-hydroxy-4,6,8-trimethylnona-2,7-dienamide (**1**), together with two known manumycin-type compounds antibiotic U-62162 (**2**) and salternamide C (**3**) were obtained from the fermentation broth of endophytic *Plantactinospora* sp. NEAU-gxj3. 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 literatures. Bioassay results showed that **2** demonstrated potent cytotoxic activity against K562, A549 and HepG2. However, **1** and **3** exhibited no significant cytotoxic activity against these three cell lines. Comparison of the cytotoxicity of compound **2** with that of compound **3** further confirmed that the 5,6-epoxide unit is an critical pharmacophore for the bioactivity of manumycins[9-12].

References

1 Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod*, 2012, 75: 311-335.

2 Subramani R, Aalbersberg W. Culturable rare actinomycetes: diversity, isolation and marine natural product discovery. *Appl Microbiol Biotechnol*, 2013, 97: 9291-9321.

3 Palaniyandi SA, Yang SH, Zhang L, *et al*. Effects of actinobacteria on plant disease suppression and growth promotion. *Appl Microbiol Biotechnol*, 2013, 97: 9621-9636.

4 Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol*, 1966, 16: 313-340.

5 Kim SH, Shin Y, Lee SH, *et al*. Salternamides A-D from a Halophilic *Streptomyces* sp. Actinobacterium. *J Nat Prod*, 2015, 78: 836-843.

6 Slechta L, Cialdella JI, Mizsak SA, *et al*. Isolation and characterization of a new antibiotic U-62162. *J Antibiot*. 1982, 35: 556-560.

7 Alcaraz L, Taylor RJK. The first synthesis of the *Streptomyces* derived antibiotic U-62162. *Chem Commun*, 1998, 29: 1157-1158.

8 Wang J, Zhang H, Yang X, *et al*. HS071, a new furan-type cytotoxic metabolite from *Streptomyces* sp. HS-HY-071. *J Antibiot*, 2008, 61: 623-626.

9 Rui Z, Sandy M, Jung B, *et al*. Tandem enzymatic oxygenations in biosynthesis of epoxyquinone pharmacophore of manumycin-type metabolites. *Chem Biol*, 2013, 20: 879-887.

10 Hu Y, Floss HG. New type II manumycins produced by *Streptomyces nodosus* ssp. *asukaensis* and their biosynthesis. *J Antibiot*, 2001, 54: 340-348.

11 Rui Z, Petrícková K, Skanta F, *et al*. Biochemical and genetic insights into asukamycin biosynthesis. *J Biol Chem*, 2010, 285: 24915-24924.

12 Li F, Maskey RP, Qin S, *et al*. Chinikomycins A and B: isolation, structure elucidation, and biological activity of novel antibiotics from a marine *Streptomyces* sp. Isolate M045. *J Nat Prod*, 2005, 68: 349-353.