The Journal of Antibiotics (2019) 72:241–245
https://doi.org/10.1038/s41429-019-0143-6

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

BRIEF COMMUNICATION

Two new glutarimide antibiotics from *Streptomyces* sp. HS-NF-780

Xue-Li Zhao[1] · Han Wang[1] · Zheng-Lian Xue[2] · Jian-Song Li[3] · Huan Qi[3] · Hui Zhang[3] · Tian Zhao[1] · Ji-Dong Wang[2,3] · Wen-Sheng Xiang[1]

Received: 27 October 2018 / Revised: 8 January 2019 / Accepted: 11 January 2019 / Published online: 29 January 2019

Abstract

Two new glutarimide antibiotics, 9-methylstreptimidone 2-α-D-glucopyranoside (**1**), and hydroxyiso-9-methylstreptimidone (**2**), along with a known compound, 9-methylstreptimidone (**3**), have been isolated from the broth of *Streptomyces* sp. HS-NF-780. 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. By modified Mosher's method and acid hydrolysis, the absolute configurations of compounds **1** and **2** were established. Compounds **1** and **2** exhibited moderate cytotoxic activity.

The antibiotics of the glutarimide group are structurally characterized by the presence of glutarimide ring bearing a side chain at the 1-position [1]. A number of distinct substances belonging to this class such as 9-methylstreptimidones [2], cycloheximide [3, 4], and migrastatin [5, 6] have been isolated from various species of *Streptomyces* [7]. Among them, 9-methylstreptimidone showed strong inhibitory activity against yeasts and filamentous fungi [1]. Besides, 9-methylstreptimidone inhibited NO production and iNOS expression in LPS-stimulated RAW264.7 cells, and induced apoptosis in Jurkat cells and adult T-cell leukemia cells, similar to other NF-κB inhibitors [8]. In the course of our screening program for novel microbe-derived bioactive secondary metabolites, two new glutarimide derivatives named 9-methylstreptimidone 2-α-D-glucopyranoside (**1**) and hydroxyiso-9-methylstreptimidone (**2**), along with a known compound, 9-methylstreptimidone (**3**) (Fig. 1), were isolated from the culture broth of *Streptomyces* sp. HS-NF-780. In this paper, we describe the fermentation, isolation, structure elucidation, and bioactivity of the two new compounds.

The producing strain HS-NF-780 was isolated from a soil sample collected from Linyi, Shandong province, China using the standard dilution plate method. The strain was identified as the genus *Streptomyces* because its 16S rRNA sequence (accession no: MH362834 in the GenBank) exhibited a high-sequence similarity of 100% with that of *Streptomyces* sp. NEAU-BGG209 (accession no: MG820043).

Strain HS-NF-780 was grown and maintained for 7 days at 28 °C on the YMS medium consisting of yeast extract 4.0 g, malt extract 10.0 g, glucose 4.0 g, $CoCl_2{\cdot}6H_2O$ 0.005 g and agar 20.0 g in 1.0 l tap water at pH 7.0. The stock culture was transferred into 1 l Erlenmeyer flasks containing 250 ml of the seed medium and incubated at 28 °C for 48 h on a rotary shaker at 250 r.p.m. The seed medium was composed of glucose 4.0 g, malt extract 10.0 g and yeast extract 4.0 g in 1.0 l tap water, pH 7.0. All of the media were sterilized at 121 °C for 20 min. The seed culture (5%) was transferred into 1 l Erlenmeyer flasks containing 25% volume of production medium. The production medium was composed of glucose 1%, soluble starch 4%, yeast extract 0.4%, malt extract 1%, $CaCO_3$ 0.2%, $FeSO_4{\cdot}7H_2O$ 0.1%, $ZnSO_4{\cdot}7H_2O$ 0.1%, and $MnCl_2{\cdot}4H_2O$ 0.1% at pH 7.2–7.4. The flasks were incubated at 28 °C for 7 days, shaken at 250 r.p.m.

The final 20 l fermentation broth was filtered to separate mycelial cake and supernatant. The mycelial cake was washed with water (3 l) and subsequently extracted with

Supplementary information The online version of this article (https://doi.org/10.1038/s41429-019-0143-6) contains supplementary material, which is available to authorized users.

✉ Ji-Dong Wang
jdwang@hisunpharm.com

✉ Wen-Sheng Xiang
xiangwensheng@neau.edu.cn

[1] Life Science and Biotechnology Research Center, School of Life Science, Northeast Agricultural University, Harbin, China

[2] College of Biochemical Engineering, Anhui Polytechnic University, Wuhu, China

[3] Zhejiang Key Laboratory of Antifungal Drugs, Zhejiang Hisun Pharmaceutical Co., Ltd, Taizhou, China

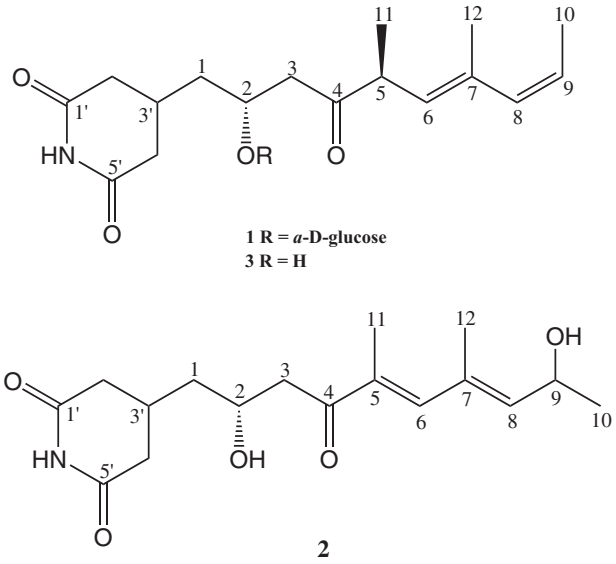

Fig. 1 Structures of compounds **1**, **2,** and **3**

MeOH (3 l) and the supernatant was subjected to a Diaion HP-20 resin (Mitsubushi Chemical, Tokyo, Japan) column eluting with 95% EtOH (5 l). The MeOH extract and the EtOH eluents were evaporated under reduced pressure at 55 °C to yield the crude extract. The crude extract was chromatographed on a silica gel (Qingdao Haiyang Chemical Group, Qingdao, China; 100-200 mesh) column and successively eluted with a stepwise gradient of $CHCl_3$/MeOH (100:0-50: 50, v/v) to give three fractions (Fr.1-Fr.3) based on the TLC profiles. TLC was performed on silica-gel plates (HSGF254, Yantai Chemical Industry Research Institute, Yantai, China) with solvent system of $CHCl_3$/MeOH (9:1, v/v) and the developed TLC plates were observed under a UV lamp at 254 nm or by heating after spraying with sulfuric acid-ethanol, 5:95 (v/v). The Fr.2 was subjected to another silica gel column eluted with $CHCl_3$/MeOH (95:5-60:40, v/v) to give six fractions (Fr.2-1-Fr.2-6). The Fr.2-4 was further purified by semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm, 250 × 9.4 mm inner diameter; 1.5 ml min^{-1}; 254 nm; Agilent, PaloAlto, CA, USA) eluting with CH_3CN/H_2O (20:80, v/v) to obtain compound **1** (t_R 24.68 min, 18.0 mg). The Fr.2-3 was separated by semi-preparative HPLC eluting with CH_3CN/H_2O (25:75, v/v) to afford compound **2** (t_R 20.13 min, 14.2 mg). The Fr.1 was subjected to a Sephadex LH-20 (GE Healthcare, Glies, UK) column eluted with $CHCl_3$/MeOH (1:1, v/v) and detected by TLC to give two subfractions (Fr.1-1-Fr.1-2). The Fr.1-2 was isolated by semi-preparative HPLC eluting with CH_3CN/H_2O (35:65, v/v) to give compound **3** (t_R 25.06 min, 23.5 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 (Bruker, Rheinstetten, Germany). The ESI-MS and HR-ESI-MS spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Waters Co, Milford, MA, USA).

Compound **1** was isolated as colorless oil with $[\alpha]_D^{25} + 125$ (*c* 0.03, EtOH) and UV (EtOH) λ_{max} nm (log ε): 233 (4.02). Its molecular formula was determined to be $C_{23}H_{35}NO_9$ by HRESIMS at *m/z* 492.2207 $[M + Na]^+$ (calcd as 492.2204 for $C_{23}H_{35}NO_9Na$) and NMR data (Table 1). In the IR spectrum of **1**, absorption at 3447, 3197, and 1689 cm^{-1} indicated the presence of hydroxyl, imide, and carbonyl groups, respectively. Analysis of ^1H NMR spectrum (Table 1) of **1** revealed the presence of three olefinic protons at δ_H 5.19 (1 H, br d, J = 9.8 Hz), 5.48 (1 H, m), 5.82 (1 H, br d, J = 11.6 Hz), one anomeric proton at δ_H 4.87 (1 H, d, J = 3.9 Hz), one oxygenated methylene at δ_H 3.64 (1 H, dd, J = 11.9, 5.3 Hz), 3.78 (1 H, dd, J = 11.9, 2.1 Hz), five oxygenated methine protons from δ_H 3.27 to δ_H 4.19, two olefinic methyls at δ_H 1.77 (3 H, dd, J = 7.2, 2.6 Hz), 1.86 (3 H, d, J = 1.2 Hz), one doublet aliphatic methyl at δ_H 1.14 (3 H, d, J = 6.8 Hz). The ^{13}C NMR and DEPT135 spectra (Table 1) of **1** showed 23 resonances attributable to one carbonyl carbon at δ_C 212.1, two amide carbonyl carbons at δ_C 175.4 and 175.4, three sp^2 methines at δ_C 125.8, 129.7, 134.1, one sp^2 quaternary carbon at δ_C 136.7, one anomeric carbon at δ_C 99.1, five oxygen-bearing aliphatic methines between 71.6 and 75.0 ppm, one oxygenated methylene at δ_C 62.5, two aliphatic methines at δ_C 28.2, 48.2, four aliphatic methylenes at δ_C 37.9, 39.2, 41.4, 44.8, and three methyl carbons at δ_C 15.0, 16.7, 17.5. Comparison of the ^1H and ^{13}C NMR data (Table 1) of **1** with those of **3** revealed significant similarities. The differences between **1** and **3** were that **1** showed six extra ^{13}C resonances. The six extra ^{13}C resonances were postulated to glucose moiety according to one doublet anomeric proton (δ_H 4.87), one oxygenated methylene (δ_H 3.64, 3.78) and four oxygenated methine protons. The relatively small $^3J_{HH}$ value (3.9 Hz) of the anomeric proton suggested an α linkage [9]. The linkage of the glucose to the aglycone was established by the HMBC correlation (Fig. 2) from H-1″ to C-2. The NOESY correlations (Fig. 2) between H-5 and H_3-12, H-6 and H-8 demonstrated the geometry of $\Delta^{6,7}$ was *E*. In the ^1H NMR spectrum of **1**, the coupling constant between H-8 and H-9 was 11.6 Hz, indicating the geometry of $\Delta^{8,9}$ was *Z*. Furthermore, the presence of glucose was evidenced by the acid hydrolysis. Compound **1** (2.5 mg) was dissolved in 2 ml of 2 M HCl and heated for 2 h at 80 °C, followed by neutralization with $NaHCO_3$. The reaction mixture was extracted with $CHCl_3$ to separate a sugar moiety-containing aqueous fraction from the aglycone-containing fraction. The aqueous fraction was identified by cochromatography with authentic glucose on TLC analysis using ethyl acetate/pyridine/glacial acetic acid/H_2O (8: 5: 1: 1.5, v/v) as a developing solvent. Spots were detected by heating after spraying with sulfuric acid-ethanol (5:95, v/v). The identical R_f values of the aqueous fraction

Table 1 The NMR spectroscopic data of compounds **1–3**

Position	1 (in CD_3OD)		2 (in CD_3OD)		3 (in $CDCl_3$)
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	1.50 m 1.73 m	41.4	1.51 m1.56 m	42.9	1.29 m 1.54 m
2	4.19 m	72.6	4.20 m	66.8	4.08 m
3	2.72 dd (17.6, 7.5)	44.8	2.81 dd (16.2, 4.6)	46.3	2.55 m
	2.96 dd (17.6, 4.6)		2.98 dd (16.2, 7.6)		2.64 m
4		212.1		203.2	
5	3.55 m	48.2		137.2	3.62 m
6	5.19 br d (9.8)	129.7	6.99 s	144.7	5.12 d (9.6)
7		136.7		133.5	
8	5.82 br d (11.6)	134.1	5.63 d (8.2)	140.4	5.74 d (11.7)
9	5.48 m	125.8	4.66 m	65.2	5.42 m
10	1.77 dd (7.2, 1.8)	15.0	1.28 d (6.3)	23.4	1.72 dd (7.2, 1.7)
11	1.14 d (6.8)	16.7	1.95 s	13.2	1.11 d (6.8)
12	1.86 d (1.2)	17.5	1.92 s	16.7	1.80 d (1.2)
1′′		175.4		175.6	
2′	2.31 m 2.60 m	39.2	2.36 m 2.75 m	38.0	2.24 m 2.70 m
3′	2.35 m	28.2	2.38 m	28.7	2.40 m
4′	2.31 m 2.77 m	37.9	2.38 m2.68 m	39.2	2.24 m 2.70 m
5′		175.4		175.5	
1″	4.87 d (3.9)	99.1			
2″	3.34 m	73.2			
3″	3.27 t (12.4)	71.6			
4″	3.57 m	74.5			
5″	3.52 m	75.0			
6″	3.78 dd (11.9, 2.1)	62.5			
	3.64 dd (11.9, 5.3)				

with that of authentic glucose indicated that the sugar moiety of **1** was glucose. The aglycone-containing fraction was subjected to reverse phase HPLC for analysis, with a Zorbax B-C18 column (Agilent 1100, 250 × 9.4 mm inner diameter, 5 µm), mobile phase of CH_3CN/H_2O (35:65, v/v), flow rate at 1.5 ml min^{-1}, and detection wavelength at 220 nm. Under these conditions, the aglycone-containing fraction gives peak at t_R (min) = 25.06. The peak of the known compound 9-methylstreptimidone (**3**) was detected at t_R (min) = 25.11. The retention time of the aglycone moiety of **1** was in good agreement with **3**, which suggested that the aglycone moiety of **1** was 9-methylstreptimidone. In order to determine the absolute configuration of the glucose moiety in **1**, the sugar residue was dissolved in pyridine (3 ml) containing L-cysteine methyl ester hydrochloride (1 mg) and heated at 60 °C for 1 h. A total of solution of *O*-torylisothiocyanate (25 µl) was added to the mixture, which was heated at 60 °C for a further 1 h. The mixture was analyzed by reversed-phase HPLC (Amethyst C18-H, 5 µm, 250 × 4.6 mm inner diameter; 0.8 ml min^{-1}; 250 nm) at 35 °C eluting with CH_3CN/H_2O (25:75, v/v). Under these conditions, standard sugar gave peak at t_R (min) = 21.658 for D-glucose. The peak of the sugar residue was detected at t_R (min) = 21.789, which identified as D-glucose by comparison with the retention time of the authentic sample [10–12].

Compound **2** was isolated as pale yellowish oil with optical rotation of $[\alpha]_D^{25} + 105$ (*c* 0.03, EtOH) and UV (EtOH) λ_{max} nm (log ε): 276 (4.10). HR-ESI-MS showed a molecular ion peak at *m/z* 346.1625 $[M + Na]^+$ (calcd for $C_{17}H_{25}NO_5Na$, 346.1625), indicating a molecular formula of $C_{17}H_{25}NO_5$. In the IR spectrum of **2**, absorption at 3419 and 1684 cm^{-1} indicated the presence of hydroxyl and carbonyl groups, respectively. Analysis of ^1H NMR spectrum (Table 1) of **2** revealed the presence of two olefinic protons at δ_H 5.63 (1 H, d, J = 8.2 Hz), 6.99 (1 H, s), two oxygenated methine protons at δ_H 4.20 (1 H, m), 4.66 (1 H, m), two olefinic methyl carbons at δ_H 1.92 (3 H, s), 1.95 (3 H, s), one doublet aliphatic methyl at δ_H 1.28 (3 H, d, J = 6.3 Hz). The ^{13}C NMR and DEPT135 spectra (Table 1) of **2** showed 17 resonances attributable to one carbonyl carbon at δ_C 203.2, two amide carbonyl carbons at δ_C 175.5 and 175.6, two sp^2 methines at δ_C 140.4, 144.7,

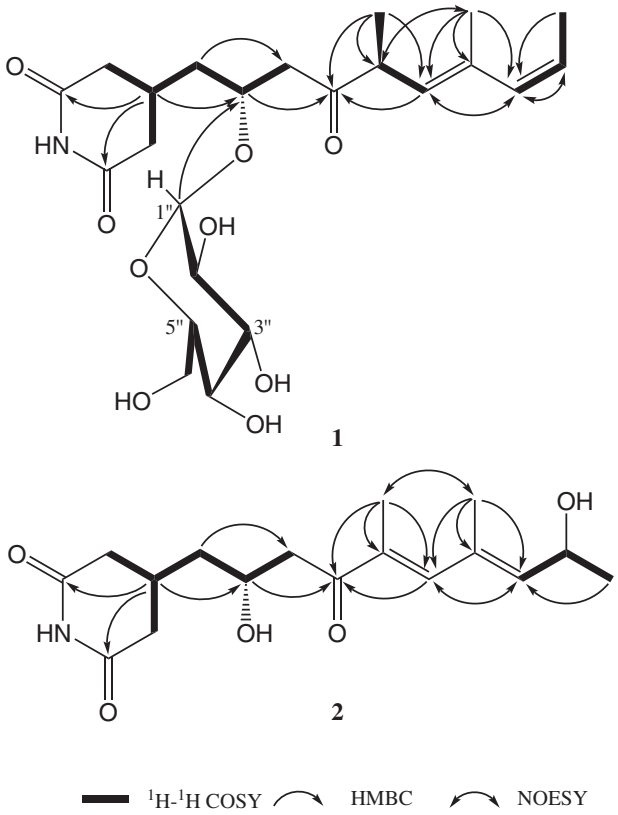

Fig. 2 Key ^{1}H-^{1}H COSY, HMBC, and NOESY correlations of compounds **1** and **2**

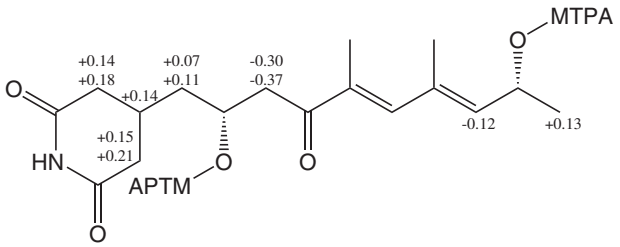

Fig. 3 $\Delta\delta$ values for the MTPA esters (**1a**, **1b**); $\Delta\delta$ (ppm) $= \delta_{S\,\mathbf{1a}} - \delta_{R\,\mathbf{1b}}$

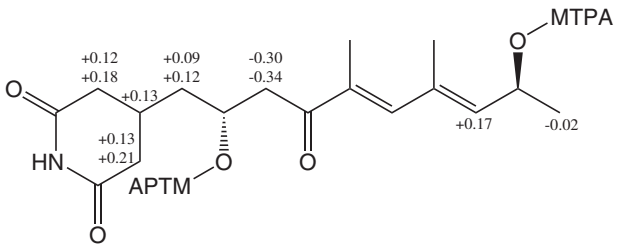

Fig. 4 $\Delta\delta$ values for the MTPA esters (**2a**, **2b**); $\Delta\delta$ (ppm) $= \delta_{S\,\mathbf{2a}} - \delta_{R\,\mathbf{2b}}$

two sp^2 quaternary carbons at δ_C 133.5, 137.2, one aliphatic methine at δ_C 28.7, two oxygenated methines at δ_C 65.2, 66.8, four methylenes at δ_C 38.0, 39.2, 42.9, 46.3, and three methyl resonances at δ_C 13.2, 16.7, 23.4. The complete assignment of all ^{1}H and ^{13}C NMR spectral data of **2** was subsequently accomplished by the ^{1}H-^{1}H COSY, HSQC and HMBC spectra. The correlations between H-3′/H_2-1/H-2/H_2-3, H-8/H-9/H_3-10 protons in the ^{1}H-^{1}H COSY spectrum (Fig. 2) indicated the presence of two structural units of C-3′–C-3 and C-8–C-10. The observed HMBC correlations (Fig. 2) from H-2, H-6 to C-4, from H_3-11 to C-4, C-5, C-6, from H_3-12 to C-6, C-7, C-8 established the linkage of C-3′–C-10. A glutarimide ring was defined by two amide carbonyl carbons at δ_C 175.5 and 175.6, which were coupled long range in an HMBC experiment to the protons (Table 1) of a four protons pair of methylene signals at δ_H 2.36 (1 H, m), 2.38 (1 H, m), 2.68 (1 H, m), 2.75 (1 H, m), which were in turn coupled in a ^{1}H-^{1}H COSY spectrum to a single methine proton signal at δ_H 2.38 (1 H, m) [13]. On the basis of the above spectroscopic data, a gross structure of **2** was established (Fig. 1). The NOESY correlation (Fig. 2) between H_3-11 and H_3-12 demonstrated the geometry of $\Delta^{5,6}$ was *E*. The geometry of $\Delta^{7,8}$ was also assigned as *E* by the NOESY correlation between H-6 and H-8. The absolute configuration of **2** was determined by the modified Mosher's method [14, 15]. To a solution of compound **2** (2.5 mg) in dry pyridine (200 µL) was added (−)-MTPA chloride (15 µl), and the solution was stirred at room temperature for 1 h. The reaction mixture was fractionated by semi-preparative HPLC eluting with CH_3CN/H_2O (80:20, v/v) to afford (*S*)-MTPA esters **1a** (t_R 19.82 min, 1.2 mg) and **2a** (t_R 20.39 min, 1.0 mg). In the same way, by using (+)-MTPA chloride, the compound **2** (2.5 mg) was converted into a mixture. The mixture was isolated by semi-preparative HPLC eluting with CH_3CN/H_2O (85:15, v/v) to obtain (*R*)-MTPA esters **1b** (t_R 26.18 min, 1.0 mg) and **2b** (t_R 27.43 min, 1.0 mg). Based on the MTPA determination rule, calculating $\triangle\delta$ (ppm) $= \delta_{S\,\mathbf{1a}} - \delta_{R\,\mathbf{1b}}$ (Fig. 3), the absolute configuration at C-2 was assigned as *R* and the absolute configuration at C-9 was assigned as *R*. However, calculating $\triangle\delta$ (ppm) $= \delta_{S\,\mathbf{2a}} - \delta_{R\,\mathbf{2b}}$ (Fig. 4), the absolute configuration at C-2 was assigned as *R* and the absolute configuration at C-9 was assigned as *S*. It showed that **2** was a C-9 epimeric mixture. Chiral HPLC analysis of **2** with a CElluose-C column and mobile phase of CO_2/MeOH further indicated that **2** was the epimeric mixture in a ratio of ~3:2.

Compound **3** was isolated as pale yellowish oil. Its structure was elucidated as 9-methylstreptimidone by analysis of its ^{1}H NMR and ESI-MS spectral data (Table 1) and comparison with literature values [16].

The cytotoxicity of **1** and **2** was assayed for growth-inhibition activity in vitro against three human tumor cell lines, human erythroleukemia cell line K562, human breast cancer cell line MCF-7, and human colon carcinoma cell line HCT-116 according to the CCK8 colorimetric method as reported in our previous papers [17, 18] using

Table 2 Cytotoxic activity of **1** and **2** against selected human tumor cell lines

Compounds	IC_{50} (μg ml^{-1})		
	K562	MCF-7	HCT-116
1	28.37	28.79	34.83
2	36.47	34.68	36.76
doxorubicin	1.17	0.79	0.45

doxorubicin as positive control. The results (Table 2) demonstrated that the two new compounds possessed moderate cytotoxic activity towards the three tumor cell lines.

Acknowledgements This work was supported in part by grants from the National Key Research and Development Program of China (No. 2017YFD0201606).

Compliance with ethical standards

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

References

1. Kim BS, Moon SS, Hwang BK. Isolation, antifungal activity, and structure elucidation of the glutarimide antibiotic, streptimidone, produced by *Micromonospora coerulea*. J Agri Food Chem. 1999;47:3372–80.
2. Allen MS, Becker AM, Rickards RW. The glutarimide antibiotic 9-methylstreptimidone: structure, biogenesis and biological activity. Aust J Chem. 1976;29:673–9.
3. Whiffen AJ, Bohonos N, Emerson RL. The production of an antifungal antibiotic by *Streptomyces griseus*. J Bacteriol. 1946;52:610–1.
4. Johnson F, Starkovsky NA, Gurowitz WD. Glutarimide antibiotics. VII. The synthesis of *dl*-neocycloheximide and the determination of the cyclohexanone ring stereochemistry of cycloheximide, its isomers, and inactone. J Am Chem Soc. 1965;87:3492–3500.
5. Nakae K, et al. Migrastatin, a novel 14-membered lactone from *Streptomyces* sp. MK929-43F1. J Antibiot. 2000;53:1228–30.
6. Shan D, et al. Synthetic analogues of migrastatin that inhibit mammary tumor metastasis in mice. Proc Natl Acad Sci USA. 2005;102:3772–6.
7. Ji X, et al. Synthesis and antiviral activities of synthetic glutarimide derivatives. Chem Pharm Bull. 2010;58:1436–41.
8. Ishikawa Y, Tachibana M, Matsui C, Obata R, Umezawa K, Nishiyama S. Synthesis and biological evaluation on novel analogs of 9-methylstreptimidone, an inhibitor of NF-κB. Bioorg Med Chem Lett. 2009;19:1726–8.
9. Patra S, et al. Structural characterization of an immunoenhancing heteropolysaccharide isolated from hot water extract of the fresh leaves of *Catharanthus rosea*. Carbohyd Polym. 2010;81:584–91.
10. Tanaka T, Nakashima T, Ueda T, Tomii K, Kouno I. Facile discrimination of aldose enantiomers by reversed-phase HPLC. Chem Pharm Bull. 2007;55:899–901.
11. Bang S, et al. New aromatic compounds from the fruiting body of *Sparassis crispa* (Wulf.) and their inhibitory activities on PCSK 9 mRNA expression. J Agri Food Chem. 2017;65:6152–7.
12. Zheng Q, Koike K, Han LK, Okuda H, Nikaido T. New biologically active triterpenoid saponins from *Scabiosa tschiliensis*. J Nat Prod. 2004;67:604–13.
13. Hochlowski JE, Whittern DN, Hill P, Mcalpine JB. Dorrigocins: novel antifungal antibiotics that change the morphology of *ras*-transformed NIH/3T3 cells to that of normal cells. II. isolation and elucidation of structure. J Antibiot. 1994;47:870–4.
14. Kubota NK, et al. Two new analogues of the cytotoxic substance BE-52211 from *Streptomyces* sp. J Nat Prod. 2004;67:85–7.
15. Ngo QT, et al. Lactones from the pericarps of *Litsea japonica* and their anti-inflammatory activities. Bioorg Med Chem Lett. 2018;28:2109–15.
16. Cheng C, Liu Q, Chen L, Jin W, Si S, Li D. Isolation, structure determination and biological activity of a new glutarimide antibiotic, S632A3. J Asian Nat Prod Res. 2006;8:55–60.
17. Wang J, Zhang H, Yang X, Zhou Y, Wang H, Bai H. HS071, a new furan-type cytotoxic metabolite from *Streptomyces* sp. HS-HY-071. J Antibiot. 2008;61:623–6.
18. Wang J, et al. Five new epothilone metabolites from *Sorangium cellulosum* strain So0157-2. J Antibiot. 2009;62:483–7.