J. Antibiot. 61(4): 241–244, 2008

NOTE

THE JOURNAL OF ANTIBIOTICS

A Novel Antimycin-like Compound, JBIR-06, from *Streptomyces* sp. ML55

Jun-ya Ueda, Aya Nagai, Miho Izumikawa, Shuhei Chijiwa, Motoki Takagi, Kazuo Shin-ya

Received: February 15, 2008 / Accepted: March 19, 2008

Abstract A novel compound of antimycin family, JBIR-06 (**1**), was isolated from *Streptomyces* sp. ML55. The structure of **1** was established as a twelve-membered macrocyclic skeleton with a 3-(formylamino)-2-hydroxybenzamide based on the spectroscopic data. Compound **1** inhibited the expression of GRP78 induced by 2-deoxyglucose at the IC_{50} value of 250 nM.

Keywords antimycin, JBIR-06, molecular chaperone, GRP78, *Streptomyces* sp.

GRP78, which is well-known as a molecular chaperone in the endoplasmic reticulum (ER), also plays an important role as a survival factor in solid tumors, due to its acquisition of a resistant mechanism against both chemotherapy and hypoglycemic stress [1]. Thus, specific down-regulators of GRP78 transcription can reasonably be expected to become promising drugs in cancer chemotherapy [2]. In the course of our screening program for down-regulators of GRP78/Bip molecular chaperone expression, we isolated versipelostatin [3], prunustatin A [4, 5], JBIR-04, -05 [6] and SW-163A [6]. Further screening resulted in the isolation of a novel inhibitor of GRP78 expression, designated as JBIR-06 (**1**, Fig. 1), a new member of antimycin [7] family from mycelium of *Streptomyces* sp. ML55 [8]. In this paper, we report the isolation, structure elucidation, and brief biological activities of **1**.

The *Streptomyces* sp. ML55 was cultured at 27°C for 5 days in 500-ml Erlenmeyer flasks containing a medium consisting of 2.0% glycerin (Nacalai Tesque), 1.0% molasses (Dai-Nippon Meiji Sugar), 0.5% casein (KANTO CHEMICAL), 0.1% polypepton (NIHON PHARMACEUTICAL), 0.4% $CaCO_3$ (KOZAKAI PHARMACEUTICAL) (pH 7.2 before sterilization). The mycelium from the culture broth (2.0 liters) was extracted with Me_2CO (400 ml). After concentrated *in vacuo*, the residue was extracted with EtOAc for two times. The organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. The dried residue was applied to normal-phase MPLC (Purif-Pack SI-60, Moritex) and developed with a

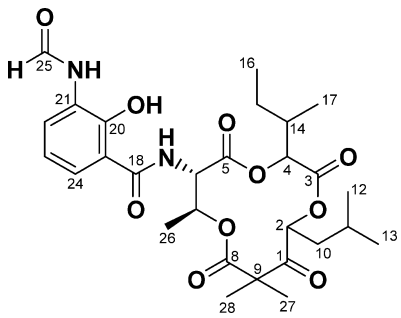

Fig. 1 Structure of JBIR-06 (**1**).

K. Shin-ya (Corresponding author)**:** Biological Information Research Center (BIRC), National Institute of Advanced Industrial Science and Technology (AIST), 2-42 Aomi, Koto-ku, Tokyo 135-0064, Japan, E-mail: k-shinya@aist.go.jp

M. Takagi (Corresponding author)**, J. Ueda, A. Nagai, M. Izumikawa, S. Chijiwa:** Japan Biological Information Research Center (JBIRC), Japan Biological Informatics Consortium (JBIC), 2-42 Aomi, Koto-ku, Tokyo 135-0064, Japan,
E-mail: motoki-takagi@aist.go.jp

Table 1 Physico-chemical properties of JBIR-06 (**1**)

Appearance	Light brown amorphous solid
Melting point[a]	90.0～97.5°C
$[\alpha]_D^{22}$ (MeOH)[b]	−30.0° (*c* 0.04)
Molecular formula	$C_{28}H_{38}N_2O_{10}$
HR-ESI-MS (*m/z*)[c]	found: 563.2609 $[M+H]^+$
	calcd: 563.2605 for $C_{28}H_{39}N_2O_{10}$
UV λ_{max} (MeOH) nm (ε)[d]	225 (22700), 334 (3760)
IR ν_{max} ($CHCl_3$) cm^{-1}[f]	1738, 1644, 1527, 1281, 1211

[a] Melting point was determined with a Yanagimoto micro melting point apparatus. [b] Optical rotation was operated on a HORIBA SEPA-300 polarimeter. [c] HR-ESI-MS data were recorded on a Waters LCT-Premier XE. [d] UV spectrum was measured on a HITACHI U-3200 spectrophotometer. [f] IR spectrum was obtained using a HORIBA FT-720 spectrophotometer.

hexane - EtOAc linear gradient system (0～100% EtOAc), and the peak detection was carried out by UV absorption at 254 nm. The 60～75% EtOAc eluate was subjected to preparative reversed-phase HPLC (mobile phase: 65% MeCN - H_2O; column: Senshu Pak PEGASIL ODS 20 i.d.×250 mm; detector: Waters 2996 photodiode array detector and Waters 3100 mass detector) to yield **1** (1.5 mg; Rt, 42.1 minutes).

The physico-chemical properties of **1** are summarized in Table 1. Compound **1** was obtained as light brown amorphous solid (MP 90.0～97.5°C) and its molecular formula was determined to be $C_{28}H_{38}N_2O_{10}$ by HR-ESI-MS. The IR spectrum showed absorbance for esters and amides (1738, 1644, 1211 cm^{-1}). The direct connectivity between protons and carbons was established by the HSQC spectrum and the ^{13}C- and ^{1}H-NMR spectral data for **1** are shown in Table 2. The DQF-COSY and HMBC spectra established four partial structures (Fig. 2).

The sequence from an oxymethine proton 2-H (δ_H 5.61) to 11-H (δ_H 1.62), which in turn coupled to two methyl protons 12-H (δ_H 0.95) and 13-H (δ_H 0.941), through 10-H (δ_H 1.84, 1.72) in the COSY spectrum established a 3-methylbutyl moiety. Two singlet methyl protons 27-H (δ_H 1.36) and 28-H (δ_H 1.17) were each long-range coupled to an ester carbonyl carbon C-8 (δ_C 171.9), a quaternary carbon C-9 (δ_C 53.5) and a ketone carbonyl carbon C-1 (δ_C 204.8), which in turn long-range coupled to 2-H and 10-H. These HMBC correlations indicated the successive connectivity of C-2, C-1, C-9 and C-8 as shown in Fig. 2. Thus, a 2,2,6-trimethyl-3-oxo-4-oxyheptanoate moiety was elucidated as a partial structure of **1** as shown in Fig. 2.

The proton sequence between the aromatic protons 22-H (δ_H 8.58), 23-H (δ_H 6.97) and 24-H (δ_H 7.33) indicated the presence of a 1,2,3-trisubstituted benzene ring moiety.

Table 2 ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) data for **1**

	δ_H	δ_C
1		204.8
2	5.61 (dd, 7.6, 4.7)	77.8
3		168.9
4	5.268 (d, 6.2)	77.4
5		167.8
6	5.270 (dd, 8.5, 3.2)	55.3
7	5.68 (qd, 6.5, 3.2)	72.6
8		171.9
9		53.5
10	1.84 (ddd, 14.5, 8.7, 4.7)	43.0
	1.72 (ddd, 14.4, 7.6, 5.6)	
11	1.62 (m)	24.59
12	0.95 (d, 6.5)	21.9
13	0.941 (d, 6.5)	22.7
14	2.04 (m)	37.1
15	1.57 (m)	24.56
	1.31 (m)	
16	1.00 (d, 6.8)	11.1
17	0.939 (t, 7.3)	15.1
18		170.2
19		112.7
20		150.5
21		127.4
22	8.58 (dd, 7.9, 1.0)	124.9
23	6.97 (t, 8.1)	119.1
24	7.33 (dd, 8.1, 1.0)	120.2
25	8.52 (br s)	159.0
26	1.37 (d, 6.5)	15.9
27	1.36 (s)	23.2
28	1.17 (s)	20.6
6-NH	7.06 (br d, 8.5)	
20-OH	12.58 (br s)	
21-NH	7.92 (br s)	

NMR spectra were recorded on a Varian NMR System 600 NB CL. The Sample was dissolved in $CDCl_3$, and the solvent peak was used as an internal standard (δ_H 7.24 and δ_c 77.0).

An amide proton 21-NH (δ_H 7.92) was coupled to an aldehyde proton 25-H (δ_H 8.52), which was considered to connect directly with this amide nitrogen atom from its ^{13}C chemical shift (δ_C 159.0). The aldehyde proton and the aromatic proton 23-H were long-range coupled to an aromatic quaternary carbon C-21 (δ_C 127.4). These results suggested that the formamide group was substituted at the position of C-21. The aromatic proton 24-H was long-range coupled to a carbonyl carbon C-18 (δ_C 170.2) at the *peri* position, which indicated that this carbonyl carbon was substituted at C-19 (δ_C 112.7). The aromatic protons 22-H

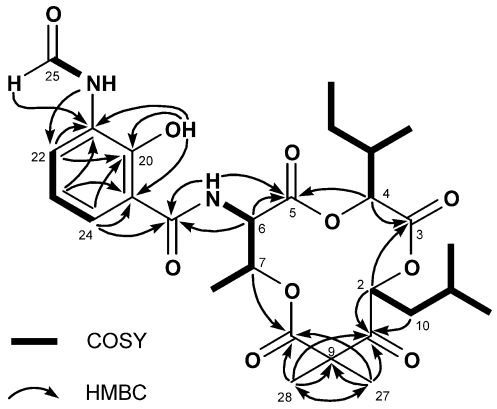

Fig. 2 Key correlations in 2D NMR (^{1}H-^{1}H COSY and HMBC).

and 24-H in addition to a phenolic hydroxy proton 20-OH (δ_H 12.58) were long-range coupled to an aromatic carbon C-20 (δ_C 150.5). By taking into consideration these ^{13}C chemical shifts of aromatic carbons, an oxygen atom should be substituted at the C-20 position. Other ^{1}H-^{13}C long-range couplings as shown in the Fig. 2 and the UV spectrum of **1** also suggested the existence of the 3-(formylamino)-2-hydroxybenzoyl moiety, which is the same chromophore as those of the antimycin related compounds [4～7].

The sequence from an amide proton 6-NH (δ_H 7.06) to a methyl proton 26-H (δ_H 1.37) through an α-methine proton 6-H (δ_H 5.270, δ_C 55.3) and an oxymethine proton 7-H (δ_H 5.68) was observed in the DQF-COSY spectrum of **1**. In addition to these correlations, the long-range couplings from the methine proton 6-H and the amide proton 6-NH to a carbonyl carbon C-5 (δ_C 167.8) and the amide carbonyl carbon C-18 were observed. These results established a threonine residue and its connectivity with the chromophore moiety.

The remaining substructure was also determined by the interpretation of ^{1}H-^{1}H spin couplings and ^{1}H-^{13}C long-range couplings as follows. The long-range coupling between an oxymethine proton 4-H (δ_H 5.268) and an ester carbonyl carbon C-3 (δ_C 168.9), along with the sequence from 4-H to 16-H (δ_H 1.00) through 14-H (δ_H 2.04), which was additionally coupled to a methyl proton 17-H (δ_H 0.939), and 15-H (δ_H 1.57, 1.31) observed in the DQF-COSY spectrum of **1**, established a 3-methyl-2-oxypentanoate moiety as shown in Fig. 2. The connectivity of these partial structures were elucidated by the long-range couplings between 2-H and C-3, 4-H and C-5, 7-H and C-8, respectively. In this manner, the planar structure of **1** was determined as shown in Fig. 1. Although nine-, fifteen-, and eighteen-membered macrocyclic analogs, such as antimycin A, neoantimycin and respirantin, respectively, were frequently isolated [4～7, 9～11], **1** is the first example which consists of a twelve-membered macrocyclic skeleton attached with a 3-(formylamino)-2-hydroxybenzoyl in amide linkage.

The absolute configuration of the threonine moiety of **1** was determined as follows. Compound **1** (0.12 mg) was hydrolyzed with 3 N NaOH (0.2 ml) for 10 minutes at 95°C. Then, 6 N HCl was added to this reaction mixture to adjust the pH to 2～3 followed by the extraction with EtOAc. The aqueous layer was further subjected to acid hydrolysis (6 N HCl, 0.5 ml) for 12 hours at 120°C to obtain the threonine residue. After acid hydrolysis, the reaction solution was adjusted to neutral and evaporated *in vacuo*. To determine the absolute configuration of the threonine residue, Marfey's method was adopted [12]. The residue was dissolved in 0.1 M $NaHCO_3$ aq (0.1 ml) and successively 10 mM N^{α}-(5-fluoro-2,4-dinitrophenyl)-L-alaninamide (FDAA) in Me_2CO (0.1 ml) was added. The mixture was kept at 70°C for 10 minutes with frequent shaking. After workup with the addition of 0.2 N HCl, the filtered reaction mixture was subjected to Waters UPLC® system (Acquity UPLC® BEH C_{18} 1.7 μm, 2.1×50 mm, 10% MeCN - H_2O with 0.1% formic acid). The standard D- and L-threonine and D,L-*allo*-threonine were reacted with FDAA in the same manner as described above. The threonine residue obtained from the lysate was determined to be L-threonine (Rt: 10.7 minutes; L-Thr, 10.5 minutes; D-Thr, 29.1 minutes; L-*allo*-Thr, 11.3 minutes; D-*allo*-Thr, 17.2 minutes).

The human fibrosarcoma cell line HT1080 cells which were transformed with the luciferase reporter gene under the regulation of the GRP78 promoter, produced luciferase by four fold greater quantity than did the control when treated with 10 mM of 2-deoxyglucose [13]. In this evaluation system, **1** reduced the expression of the reporter gene with the IC_{50} value of 262 nM. In the same manner, the structurally related compounds prunustatin A and SW-163A exhibited inhibitory activities in this assay with IC_{50} values of 1.9 nM and 9.1 nM, respectively. Since antirespiratory agents such as antimycin, alloaureothin [14], rotenone, oligomycin and verrucosidin [15, 16] show the inhibitory activities against GRP78 expression, we briefly evaluated reducing activities of **1**, prunustatin A and SW-163A in DPPH radical scavenging assay system [17]. In this assay, **1**, prunustatin A and SW-163A reduced the DPPH radical by 56.2, 54.5 and 54.5%, respectively, at the concentration of 20 μM. Since antirespiratory activities of these compounds are almost the same and the inhibitory activities against GRP78 expression of **1** was 130

times less than that of prunustatin A, the size of ring system is considered to play a significant role to exhibit the inhibitory effects against GRP78 expression induced by the treatment of 2-deoxyglucose. These results suggested that antirespiratory effect is not the active center of these compounds. Although the action mechanism for the inhibitory effects against GRP78 expression of versipelostatin was investigated detailed [13], its target is still unknown. Clarifying the target of these compounds is expected to discover the novel and undiscovered pathway of GRP78 expression.

Acknowledgement This work was supported by the grant from the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

References

1. Katschinski DM, Jacobson EL, Wiedemann GJ, Robins HI. Modulation of VP-16 cytotoxicity by carboplatin and 41.8°C hyperthermia. J Cancer Res Clin Oncol 127: 425–435 (2001)
2. Imaizumi K, Miyoshi K, Katayama T, Yoneda T, Taniguchi M, Kubo T, Tohyama M. The unfolded protein response and Alzheimer's disease. Biochem Biophys Acta 1536: 85–96 (2001)
3. Park HR, Furihata K, Hayakawa Y, Shin-ya K. Versipelostatin, a novel GRP/Bip molecular chaperone down-regulator of microbial origin. Tetrahedron Lett 43: 6941–6945 (2002)
4. Umeda Y, Chijiwa S, Furihata K, Furihata K, Sakuda S, Nagasawa H, Watanabe H, Shin-ya K. Prunustatin A, a novel GRP78 molecular chaperone down-regulator isolated form *Streptomyces violaceoniger*. J Antibiot 58: 206–209 (2005)
5. Umeda Y, Furihata K, Sakuda S, Nagasawa H, Ishigami K, Watanabe H, Izumikawa M, Takagi M, Doi T, Nakao Y, Shin-ya K. Absolute structure of prunustatin A, a novel GRP78 molecular chaperone down-regulator. Org Lett 9: 4239–4242 (2007)
6. Izumikawa M, Ueda J, Chijiwa S, Takagi M, Shin-ya K. Novel GRP78 molecular chaperone expression down-regulators JBIR-04 and -05 isolated from *Streptomyces violaceoniger*. J Antibiot 60: 640–644 (2007)
7. Watanabe K, Tanaka T, Fukuhara K, Miyairi N, Yonehara H, Umezawa H. Blastmycin, a new antibiotic from *Streptomyces* sp. J Antibiot 10: 39–45 (1957)
8. Ueda J, Togashi T, Matsukura S, Nagai A, Nakashima T, Komaki H, Anzai K, Harayama S, Doi T, Takahashi T, Natsume T, Kisu Y, Goshima N, Nomura N, Takagi M, Shin-ya K. A novel nuclear export inhibitor JBIR-02, a new piericidin discovered from *Streptomyces* sp. ML55. J Antibiot 60: 459–462 (2007)
9. Urushibata I, Isogai A, Matsumoto S, Suzuki A. Respirantin, a novel insecticidal cyclodepsipeptide from *Streptomyces*. J Antibiot 46: 701–703 (1993)
10. Caglioti L, Misiti D, Mondelli R, Selva A, Arcamone F, Cassinelli G. The structure of neomycin. Tetrahedron 25: 2193–2221 (1969)
11. Takahashi K, Tsuda E, Kurosawa K. SW-163A and B, novel immunosuppressants produced by *Streptomyces* sp. J Antibiot 54: 867–873 (2001)
12. Adamson JG, Hoang T, Crivici A, Lajoie GA. Use of Marfey's reagent to quantitate racemization upon anchoring of amino acids to solid supports for peptide synthesis. Anal Biochem 202: 210–214 (1992)
13. Park H-R, Tomida A, Sato S, Tsukumo Y, Yun J, Yamori T, Hayakawa Y, Tsuruo T, Shin-ya K. Blockade of survival response to glucose deprivation for selective killing of tumor cells. J Natl Can Inst 96: 1300–1330 (2004)
14. Ueda J, Hashimoto J, Nagai A, Nakashima T, Komaki H, Anzai K, Harayama S, Doi T, Takahashi T, Nagasawa K, Natsume T, Takagi M, Shin-ya K. New aureothin derivative, alloaureothin, from *Streptomyces* sp. MM23. J Antibiot 60: 321–324 (2007)
15. Choo S-J, Park H-R, Ryoo I-J, Kim J-P, Yun B-S, Kim C-J, Shin-ya K, Yoo I-D. Deoxyverrucosidin, a novel GRP78/BiP down-regulator, produced by *Penicillium* sp. J Antibiot 58: 210–213 (2005)
16. Park H-R, Ryoo I-J, Choo S-J, Hwang J-H, Kim J-Y, Cha M-R, Shin-ya K, Yoo I-D. Glucose-deprived HT-29 human colon carcinoma cells are sensitive to verrucosidin as a GRP78 down-regulator. Toxicology 229: 253–261 (2007)
17. Yoshida T, Mori K, Hatano T, Okumura T, Uehara I, Komagoe K, Fujita Y, Okuda T. Studies on inhibition mechanism of autoxidation by tannins and flavonoids. V. Radical-scavenging effects of tannins and related polyphenols on 1,1-diphenyl-2-picrylhydrazyl radical. Chem Pharm Bull 37: 1919–1921 (1989)