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NOTE

JBIR-52, a new antimycin-like compound, from *Streptomyces* sp. ML55

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GRP78/Bip is a molecular chaperone in the endoplasmic reticulum (ER) induced by ER stress that promotes protein folding and has an important role as a survival factor in solid tumors by providing resistance to both chemotherapy and hypoglycemic stress.[1] Thus, specific downregulators of GRP78 expression can reasonably be expected to become promising drugs in cancer chemotherapy.[2] In the course of our screening program for downregulators of GRP78 expression, we have isolated versipelostatin A-F,[3–11] prunustatin A,[12,13] JBIR-04, -05[14] and JBIR-06.[15] Further screening resulted in the isolation of a new inhibitor designated as JBIR-52 (**1**, Figure 1) from culture of a JBIR-06 producer, *Streptomyces* sp. ML55.[15,16] In this paper, we report the isolation, structure elucidation and brief biological activity of a new member of antimycin, **1**.

Streptomyces sp. ML55 was cultured on a rotary shaker (220 r.p.m.) at 27 °C for 5 days in 500-ml Erlenmeyer flasks containing 100 ml of a production medium consisting of 2% glycerol (Nacalai Tesque, Kyoto, Japan), 1% molasses (Dai-Nippon Meiji Sugar, Tokyo, Japan), 0.5% casein (Kanto Chemical, Tokyo, Japan), 0.1% polypepton (Nihon Pharmaceutical, Tokyo, Japan), 0.4% $CaCO_3$ (Kozaki Pharmaceutical, Tokyo, Japan) (pH 7.2 before sterilization). The mycelium from the culture broth (2 l) was extracted with Me_2CO (400 ml). After concentration *in vacuo*, the residue was extracted twice with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The dried residue (1.99 g) was applied to normal-phase MPLC (Purif-Pack SI-60, size:60, Moritex, Tokyo, Japan) and developed with a *n*-hexane–EtOAc linear gradient system (0–100% EtOAc), and peak detection was carried out by UV absorption at 254 nm. The 60–75% EtOAc eluate (470 mg) was further chromatographed on normal-phase MPLC (Purif-Pack SI-60, size:20, Moritex) with *n*-hexane–EtOAc (80:20). A portion (44.5 mg) of the fraction (388 mg) including both **1** and JBIR-06 was finally purified by preparative reversed-phase HPLC using an L-column2 ODS (20 i.d.×150 mm, Chemical Evaluation Research Institute, Tokyo, Japan) with 60% CH_3CN–H_2O containing 0.1% formic acid (flow rate, 9.5 ml min^{-1}) to yield **1** (1.7 mg, retention time (Rt) 28.0 min) and JBIR-06 (2.3 mg, Rt 37.0 min).

Compound **1** was obtained as a white powder ($[\alpha]_D$ +40.0, *c* 0.07, 29 °C in MeOH, UV (MeOH) λ_{max} (ε) 225 (22 500), 336 nm (4050)). The IR spectrum of **1** revealed the characteristic absorptions of esters (ν_{max} 1750, 1280 cm^{-1}), amide (ν_{max} 1645 cm^{-1}), hydroxyl and/or amide NH (ν_{max} 3400 cm^{-1}) groups. The HR electron spray ionization MS spectrum of **1** gave the $(M+H)^+$ ion at *m/z* 549.2429 (calcd. for $C_{27}H_{37}N_2O_{10}$, 549.2448) consistent with a molecular formula of $C_{27}H_{36}N_2O_{10}$. Direct connectivity between protons and carbons was established by the heteronuclear single quantum coherence spectrum and the ^{13}C and ^1H NMR spectral data for **1** are shown in Table 1. The observed double-quantum-filtered (DQF)-COSY and constant time heteronuclear multiple bond correlation (HMBC)[17] spectra established four partial structures.

The sequence from an oxymethine proton 2-H (δ_H 5.46) to 11-H (δ_H 1.64), which in turn coupled to two methyl protons 12-H (δ_H 0.95) and 13-H (δ_H 0.94), through 10-H (δ_H 1.84, 1.72) in the DQF-COSY spectrum established a 3-methyl-1-oxybutyl moiety. A doublet methyl proton 27-H (δ_H 1.33) and a low-field shifted methine proton 9-H (δ_H 3.32), which were spin–spin coupled to each other, were each long-range coupled to an ester carbonyl carbon C-8 (δ_C 170.2) and a ketone carbonyl carbon C-1 (δ_C 202.1), 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 Figure 1b. Thus, a 2,6-dimethyl-3-oxo-4-oxyheptanoic acid moiety was elucidated as a partial structure of **1** (Figure 1b).

The proton sequence between the aromatic protons 22-H (δ_H 8.58), 23-H (δ_H 6.97) and 24-H (δ_H 7.36) indicated the presence of a 1,2, 3-trisubstituted benzene ring moiety. An amide proton 21-NH (δ_H 7.97) was coupled to an aldehyde proton 25-H (δ_H 8.52), which was

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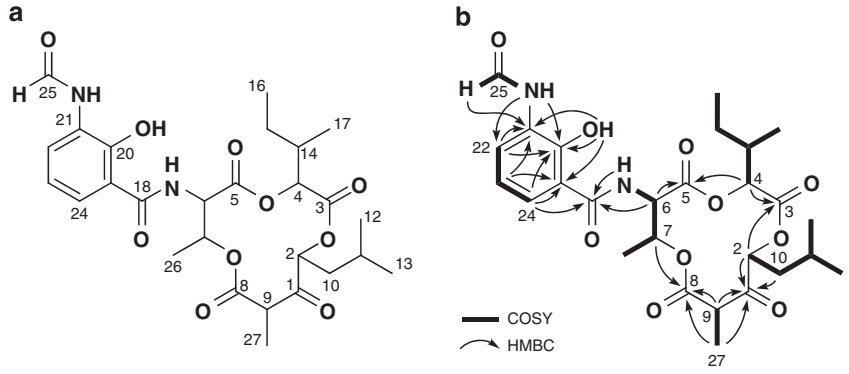

Figure 1 (**a**) Structure of JBIR-52 (**1**) (**b**) correlations in DQF-COSY (bold line) and constant time heteronuclear multiple bond correlation (arrow) spectra of **1**.

Table 1 ^{13}C and ^{1}H NMR data for **1**

	δ_C	δ_H
1	202.1	
2	79.1	5.46 (dd, 8.0, 4.5)
3	167.7	
4	77.6	5.30 (d, 6.5)
5	168.8	
6	55.4	5.29 (dd, 8.5, 3.5)
7	73.1	5.60 (qd, 6.5, 3.5)
8	170.2	
9	49.6	3.32 (q, 7.0)
10	42.1	1.84 (ddd, 14.5, 9.0, 4.5); 1.72 (ddd, 14.5, 8.0, 4.5)
11	24.5	1.64 (m)
12	22.8	0.95 (d, 6.5)
13	21.7	0.94 (d, 6.5)
14	37.0	2.05 (m)
15	24.6	1.57 (m); 1.31 (m)
16	10.9	0.94 (t, 7.0)
17	14.9	0.99 (d, 7.0)
18	170.2	
19	112.7	
20	150.6	
21	127.2	
22	125.0	8.58 (dd, 8.0, 1.0)
23	119.1	6.97 (t, 8.0)
24	120.5	7.36 (dd, 8.0, 1.0)
25	159.4	8.52 (d, 1.5)
26	15.9	1.38 (d, 6.5)
27	12.3	1.33 (d, 7.0)
6-NH		7.10 (br d, 8.5)
20-OH		12.58 (br s)
21-NH		7.97 (br s)

^{13}C (150 MHz) and ^{1}H (600 MHz) NMR spectra were taken on a Varian NMR System 600 NB CL in $CDCl_3$, and the solvent peak was used as an internal standard (δ_C 77.0, δ_H 7.26 p.p.m.).

considered to connect directly with this amide nitrogen atom from its ^{13}C chemical shift (δ_C 159.4). The aldehyde proton and the aromatic proton 23-H were long-range coupled to an aromatic carbon C-21 (δ_C 127.2). These results suggested that a formamide group is 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, indicating that this carbonyl carbon is substituted at C-19 (δ_C 112.7). The aromatic protons 22-H and 24-H in addition to a phenolic hydroxyl proton 20-OH (δ_H 12.58) were long-range coupled to an aromatic carbon C-20 (δ_C 150.6). 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 (Figure 1b) 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.[12–15]

The sequence from an amide proton 6-NH (δ_H 7.10) to a methyl proton 26-H (δ_H 1.38) through an α-methine proton 6-H (δ_H 5.29) and an oxymethine proton 7-H (δ_H 5.60) was observed in the DQF-COSY spectrum of **1**. In addition to these correlations, the long-range couplings from the methine proton 6-H to a carbonyl carbon C-5 (δ_C 168.8) 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 5.30) and an ester carbonyl carbon C-3 (δ_C 167.7) along with the sequence from 4-H to 16-H (δ_H 0.94) through 14-H (δ_H 2.05), which was additionally coupled to a methyl proton 17-H (δ_H 0.99), and 15-H (δ_H 1.57, 1.31), established a 3-methyl-2-oxypentanoic acid moiety (Figure 1b). The connectivity of these partial structures was elucidated by the long-range couplings between 2-H and C-3, 4-H and C-5 and 7-H and C-8. In this manner, the planar structure of **1** was determined as shown in Figure 1. JBIR-52 is structurally related to JBIR-06 in which the dimethyl residue is replaced by a methyl residue at the position of C-9.

To evaluate the inhibitory activity of **1** against GRP78 expression induced by 2-deoxyglucose as an ER stress, we used reporter gene assay system utilizing luciferase gene described previously.[5] The human fibrosarcoma HT1080 cells transformed with the luciferase reporter gene driven by the GRP78 promoter when treated with 10 mM of 2-deoxyglucose, produced four times more luciferase than did the untreated control. In this evaluation system, **1** reduced the expression of the reporter gene with an IC_{50} value of 137 nM, which is almost the same as that of JBIR-06 (IC_{50} value, 262 nM[14]). Contrary to the weak activity of 12-membered macrocyclic derivatives, 15-membered macrocyclic derivatives such as prunustatin A showed more potent activities (IC_{50} values, 1.9 nM[12]). It has been reported that GRP78 protects epithelial cells from ATP depletion.[18] These results suggested that the coordinate action of ER and mitochondrial respiration exerts protective action against both ER and mitochondrial stress. Hence, the activities of GRP78 suppression by these compounds were considered to depend on respiratory inhibition due to salicylic

acid moiety.[14] These results provide us the interesting information that the size of macrocyclic structure determines the inhibitory activity of GRP78 expression by salicylic acid function. Studies in the detailed biological activities are now underway.

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