A Novel Versipelostatin Analogue, Versipelostatin F Isolated from *Streptomyces versipellis* 4083-SVS6

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Abstract A novel versipelostatin (**1**) analogue, versipelostatin F (**2**) was isolated from *Streptomyces versipellis* 4083-SVS6. The structure of **2** was determined by the analyses of the spectroscopic data. Compound **2** inhibited the expression of GRP78 induced by 2-deoxyglucose with an IC_{50} value of $0.3\ \mu\text{M}$, which is 10-times more potent compared with that of **1**.

Keywords *Streptomyces versipellis*, versipelostatin, molecular chaperone, GRP78

GRP78 is a molecular chaperone in the endoplasmic reticulum (ER) induced by ER stress that promotes protein folding and plays an important role as a survival factor in solid tumors by providing resistance to both chemotherapy and hypoglycemic stress [1]. Therefore, specific down-regulators of GRP78 transcription can reasonably be expected to become promising drugs in cancer chemotherapy. In the course of our screening program for down-regulators of GRP78 molecular chaperone expression, we have isolated versipelostatin (**1**) [2–4], new members of the neoantimycin family, prunustatin A [5, 6], JBIR-04, -05 [7] and -06 [8]. Compound **1** is the first compound which has been found to down-regulate GRP78 expression induced by ER stress. Although the biological activities of **1**, namely its inhibitory effects against GRP78 expression, were investigated in detail [9], its target is still

unknown. Further investigation for congeners of **1** resulted in the isolation of a novel derivative, versipelostatin F (**2**), which showed stronger inhibitory activity against GRP78 expression than that of **1**. In this paper, we report herein the isolation, structure elucidation and its brief biological activity of **2**.

The versipelostatin-producing strain, *S. versipellis* 4083-SVS6 was cultured at 27°C for 5 days in 50-liter jar fermentors containing 30 liters of a medium consisting of 2.5% starch, 1.5% soy bean meal, 0.2% dry yeast and 0.4% CaCO_3 (pH 6.2 before sterilization). The fermentation broth (60 liters) was centrifuged and the supernatant was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The dried residue was subjected to normal-phase MPLC (Purif-Pack SI-60, Moritex) by stepwise elution with a CHCl_3 -MeOH (5.0%, 10%, 20% MeOH, successively) solvent system. The 10% MeOH eluate (210 mg) was separated by reversed-phase MPLC (Purif-Pack ODS-100, Moritex) with a H_2O -MeOH linear gradient system and further purified by preparative reversed-phase HPLC using a XBridge Prep C18 column (5.0 μm OBD, 19 i.d. $\times$ 150 mm, Waters) with 60% aqueous MeOH containing 0.4% diethylamine (flow rate, 10 ml/minute) to yield versipelostatin F (**2**, 31 mg; Rt, 23.2 minutes). The structure of **2** (Fig. 1) was mainly elucidated by NMR spectral analysis as follows.

The physico-chemical properties of **2** are summarized in Table 1. Compound **2** was obtained as a colorless amorphous solid and its molecular formula was determined

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Fig. 1 Structures of versipelostatin (**1**) and versipelostatin F (**2**).

to be $C_{61}H_{94}O_{18}$ by HR-ESI-MS. The IR spectra revealed the characteristic absorption of a carbonyl ($\nu_{\max}$ 1703 cm^{-1}) and hydroxyl ($\nu_{\max}$ 3433 cm^{-1}) groups. The ^{13}C - and ^1H -NMR data of **2** are shown in Tables 2 and 3. Comparison of these ^{13}C - and ^1H -NMR data of **2** with those of **1** together with 2D NMR spectral analysis such as DQF-COSY and HMBSC spectra, in which key correlations were summarized in Fig. 2, revealed that the aglycon of **2** consisted of the same skeleton as that of **1** as shown in Fig. 2. The relative stereochemistry of a cyclohexene and an octalone moieties in **2** were confirmed to be the same as those of **1** from NOE analyses. Since the ^{13}C - and ^1H -NMR signals for the aglycon part of **2** are superimposable with those of **1**, the absolute stereochemistry of the aglycon in **2** was concluded to be the same as that of **1**.

The three anomeric signals C-1' (δ_{C} 100.0, δ_{H} 5.32), C-1'' (δ_{C} 103.7, δ_{H} 5.44) and C-1''' (δ_{C} 99.3, δ_{H} 5.68) suggested the presence of three sugar moieties. All the proton signals for those sugars were assigned by DQF-COSY spectrum with 1D-TOCSY technique, while each corresponding carbon signal was assigned by HSQC experiment (Table 3). The sequence from an anomeric proton 1'-H to a terminal methyl proton 6'-H (δ_{H} 1.41, δ_{C} 18.55) through methylene protons 2'-H (δ_{H} 2.48, 2.00; δ_{C} 39.9), oxymethine protons 3'-H (δ_{H} 4.65, δ_{C} 67.7), 4'-H (δ_{H} 3.63, δ_{C} 82.6) and 5'-H (δ_{H} 4.38, δ_{C} 68.74) was established from the analyses of DQF-COSY. A long-range ^1H - ^{13}C coupling from 1'-H to C-5' established a 2,6-dideoxyhexopyranosyl moiety. Large spin coupling constants derived from di-axial orientations among these protons ($J_{1',2'_{\text{ax}}} = 9.4$ Hz, $J_{4',5'_{\text{ax}}} = 9.6$ Hz) determined this sugar moiety as a β -digitoxopyranoside. In the same manner, another β -digitoxopyranoside unit was also confirmed. Remaining sugar unit was elucidated as follows. The sequence from the anomeric proton 1''-H to a methyl proton 6''-H (δ_{H} 1.62, δ_{C} 18.53) through oxymethine protons 2''-H (δ_{H} 4.57, δ_{C} 68.1), 3''-H (δ_{H} 3.81, δ_{C} 82.2),

Table 1 Physico-chemical properties of **2**

Appearance	Colorless amorphous solid
Melting point ^a	177.0~185.0°C
Optical rotation ^b	$[\alpha]_{\text{D}}^{24} -54.8^\circ$ (c 1.0, MeOH)
HR-ESI-MS ^c (m/z)	
found	1113.6354 ($\text{M}-\text{H}^-$)
calcd	1113.6362 ($\text{C}_{61}\text{H}_{93}\text{O}_{18}$)
IR ^d (KBr) $\nu_{\max}$ cm^{-1}	3433, 1703, 1628

^a Melting point was determined with a Yanagimoto micro melting point apparatus and are uncorrected. ^b Optical rotation was measured on a HORIBA SEPA-300 polarimeter. ^c HR-ESI-MS measurement was carried out on a Waters LCT Premier XE mass spectrometer. ^d IR spectra was obtained on a HORIBA FT-720 Fourier transform infrared spectrometer.

4''-H (δ_{H} 4.36, δ_{C} 77.9) and 5''-H (δ_{H} 4.42, δ_{C} 68.75) was established from the analyses of DQF-COSY. The long-range ^1H - ^{13}C couplings from 1''-H to C-5'' and a methoxyl proton 3''-O-Me (δ_{H} 3.29) to C-3'' established a 3-O-methyl-6-deoxyhexopyranosyl moiety. Spin coupling constants among di-axial oriented protons ($J_{3'',4''} \approx 9.6$ Hz, $J_{4'',5''} \approx 9.3$ Hz) and the typical ^{13}C - ^1H coupling constant for α orientation at C-1'' ($J_{\text{C-H}} = 167.8$ Hz) revealed the existence of an α -acofriopyranoside (3-O-methyl- α -rhamnopyranoside) moiety. The ^1H - ^{13}C long-range correlations from three anomeric protons H-1', H-1'', and H-1''' to C-19, C-4', and C-4'', respectively, concluded a trisaccharide, O - β -digitoxopyranosyl-(1 $\rightarrow$ 4)- O - α -acofriopyranosyl-(1 $\rightarrow$ 4)- β -digitoxopyranoside, attached to C-19 via an oxygen atom as shown in Fig. 3. Thus, the structure of **2** was determined as shown in Fig. 1.

To evaluate the inhibitory activity of **2** against GRP78 expression induced by 2-deoxyglucose (2-DG) as an ER stress, we used a reporter gene assay system utilizing luciferase gene described previously [9]. The human fibrosarcoma HT1080 cells transformed with the luciferase reporter gene driven by the GRP78 promoter when treated with 10 mM of 2-DG, produced four times more luciferase than did the untreated control. In this evaluation system, **2** suppressed 2-DG-induced GRP78 expression in a dose-dependent manner with an IC_{50} value of 0.3 μM and had no effect on the activity under normal growth condition. Contrary to the potent inhibitory activity of **2**, **1** showed the less potent inhibitory activity with an IC_{50} value of 4.0 μM . Thus, **2** had 10-times more potent activity than that of **1**. These results suggested that the α -acofriopyranosyl residue in the sugar moieties plays an important role for the inhibitory activity. Compound **1** inhibited the growth of several cancer cell lines under hypoglycemic condition and demonstrated the *in vivo* antitumor activity on a xenograft

Table 2 ^{13}C - and ^1H -NMR data for aglycon moiety of **1** and **2**

Position	1		2	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}
1	173.2		174.5	
2	102.4		99.9	
3	201.2		200.6	
4	58.5		58.7	
5	40.2	2.88 (t, 10.8)	41.1	2.78 (m)
6	50.6	2.73 (m)	50.4	2.70 (m)
7	211.2		212.3	
8	52.0	3.30 (dd, 14.2, 5.9); 2.93 (m)	51.2	3.32 (m); 2.85 (dd, 14.9, 9.6)
9	72.3	4.11 (td, 9.8, 6.1)	71.8	4.20 (m)
10	49.1	2.69 (br t, 11.7)	48.8	2.59 (m)
11	122.2 ^b	6.39 (br s)	122.3	6.38 (br s)
12	134.8 ^b		135.9 ^b	
13	57.1	4.21 (m)	56.3	4.53 (br s)
14	140.6		140.1	
15	136.9	5.96 (d, 9.5)	136.9	6.09 (d, 9.3)
16	39.0	2.73 (m)	38.6	2.75 (m)
17	33.5	1.74 (m); 0.90 (br t, 12.6)	33.1	1.82 (m); 1.05 (m)
18	34.8	2.09 (m)	34.8	2.16 (m)
19	89.8	3.10 (br d, 4.2)	90.2	3.29 (m)
20	31.1	1.43 (m)	31.0	1.73 (m)
21	29.7	1.72 (m); 1.28 (m)	29.9	1.78 (m); 1.29 (m)
22	19.6	1.47 (m)	18.9	1.43 (m)
23	32.9	1.80 (m)	32.9	1.56 (m); 1.54 (m)
24	41.2		40.3	
25	128.3	5.52 (br s)	127.8	5.59 (br s)
26	134.7 ^b		134.4	
27	32.9	2.40 (m)	33.8	2.34 (m)
28	37.5	2.57 (m); 1.97 (m)	37.5	2.68 (m); 1.81 (m)
29	86.4		85.9	
30	199.8		200.0	
31	24.8	2.94 (m); 2.49 (pentet, 7.5)	24.8	2.81 (m); 2.53 (m)
32	12.8	1.35 (t, 7.8)	12.5	1.27 (m)
33	16.5	1.16 (d, 6.6)	17.8	1.22 (m)
34	23.5	1.73 (br s)	23.3	1.75 (m)
35	22.8	2.19 (m); 2.14 (m)	22.7	2.18 (m)
36	15.4	1.07 (t, 7.5)	15.1	1.12 (t, 6.8)
37	64.4	4.01 (dd, 10.5, 3.3); 3.77 (dd, 10.5, 7.3)	64.5	4.01 (dd, 10.3, 2.7); 3.79 (m)
38	17.2	1.11 (d, 6.6)	17.4	1.28 (d, 7.3)
39	19.5	0.66 (br d, 6.1)	19.0	0.75 (br s)
40	22.5	1.26 (s)	23.4	1.22 (m)
41	21.9	1.69 (br s)	22.3	1.71 (s)
42	20.1	1.21 (d, 7.3)	20.2	1.18 (d, 7.3)

^{13}C - (125 MHz) and ^1H - (500 MHz) NMR spectra were taken on a Varian NMR System 500 NB CL in pyridine- d_5 , and the solvent peak was used as an internal standard (δ_{C} 123.5, δ_{H} 7.19). ^b These carbon signals were assigned by HSQC and/or HMBC experiments.

Fig. 2 Key correlations in DQF-COSY (bold line) and HMBC (arrow) spectra of aglycon of **2**.

Table 3 ^{13}C - and ^1H -NMR data for sugar moiety of **2**

Position	δ_{C}	δ_{H}
Digitoxose		
1'	100.0	5.32 (br d, 9.4)
2'	39.9	2.48 (br d, 9.4); 2.00 (br t, 9.4)
3'	67.7	4.65 (br s)
4'	82.6	3.63 (dd, 9.6, 1.6)
5'	68.74	4.38 (dq, 9.6, 6.4)
6'	18.55	1.41 (d, 6.4)
Acofriose (3-O-methylrhamnose)		
1''	103.7	5.44 (s)
2''	68.1	4.57 (br s)
3''	82.2	3.81 (dd, 9.8, 2.7)
4''	77.9	4.36 (br t, 9.4)
5''	68.75	4.42 (dq, 9.1, 6.1)
6''	18.53	1.62 (d, 6.1)
3''-O-Me	56.8	3.29 (s)
Digitoxose		
1'''	99.3	5.68 (br d, 9.1)
2'''	39.8	2.48 (br d, 9.5); 1.95 (br t, 9.5)
3'''	68.67	4.44 (br s)
4'''	74.2	3.58 (dd, 9.5, 2.9)
5'''	70.0	4.09 (dq, 9.5, 6.4)
6'''	18.9	1.43 (d, 6.4)

^{13}C - (125 MHz) and ^1H - (500 MHz) NMR spectra were taken on a Varian NMR System 500 NB CL in pyridine- d_6 , and the solvent peak was used as an internal standard (δ_{C} 123.5, δ_{H} 7.19).

model [9]. Therefore, it is expected that **2** would exhibit stronger inhibitory activity *in vitro* and *in vivo*.

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Fig. 3 Key correlations in DQF-COSY (bold line) and HMBC (arrow) spectra of trisaccharide moiety of **2**.

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