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Biosynthesis of Novel Glucosides Geldanamycin Analogs by Enzymatic Synthesis S

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Two new glucosides (**1** and **2**) of geldanamycin (GA) analogs were obtained from in vitro glycosylation by UDP-glycosyltransferase (YjiC). Based on spectroscopic (HR-ESI-MS, 1D, and 2D-NMR) analyses, the glucosides were elucidated as 4,5-dihydro-7-*O*-descarbamoyl-7-hydroxyl GA-7-*O*-β-D-glucoside (**1**) and ACDL3172-18-*O*-β-D-glucoside (**2**). Furthermore, the water solubility of compounds **1** and **2** was about 215.2 and 90.7 times higher respectively, than that of the substrates. Among compounds **1**–**4**, only **3** showed weak antiproliferative activity against four human tumor cell lines: MDA-MB-231, SMMC7721, HepG2, and SW480 (IC_{50}: 13.6, 15.1, 31.8, and 22.7 μM, respectively).

Keywords: Geldanamycin analogs, glycosyltransferase, water solubility, cytotoxicity

Geldanamycin (GA), a 19-member macrocyclic lactam, was first isolated in 1970 from *Streptomyces hygroscopicus* var. *geldanus* var. *nova* [2]. GA was identified as the first natural product inhibitor of heat shock protein (Hsp) 90 that exhibits potent anticancer activities in various cancer cell lines and xenograft models [13, 15, 17]. However, the use of GA as a chemotherapeutic agent has not proceeded owing to its severe hepatotoxicity, metabolic instability, and poor water solubility [5, 16]. Good water solubility often leads to good drug potency and advantageous pharmacokinetic profiles. Therefore, water-soluble GA derivatives with improved pharmacological profiles and pharmacokinetic properties are needed [1, 18].

Glycosylation has been an effective tool for improving the water solubility, biological potency, and pharmacokinetic properties of many natural products [4, 14]. Recently, we reported several novel GA analogs using biosynthetic approaches and semisynthesis [6-10]. Here, we report the enzymatic synthesis of two novel GA glucosides using the UDP-glycosyltransferase (UGT) YjiC, a UGT from *Bacillus licheniformis* DSM-13, which has been reported to successfully glycosylate geldanamycin analogs, flavonoids, resveratrol, and polyketide macrolides [3, 11, 12, 18].

Two decarbamoyl-GA analogs, 4,5-dihydro-7-*O*-descarbamoyl-7-hydroxyl GA (**3**) and ACDL3172 (**4**), which were isolated from the carbamoyl transferase gene *(gel8)*-inactivated mutant of *S. hygroscopicus* JCM4427, were used as substrates of the glycosylation reaction (Fig. 1) [6, 9]. The in vitro

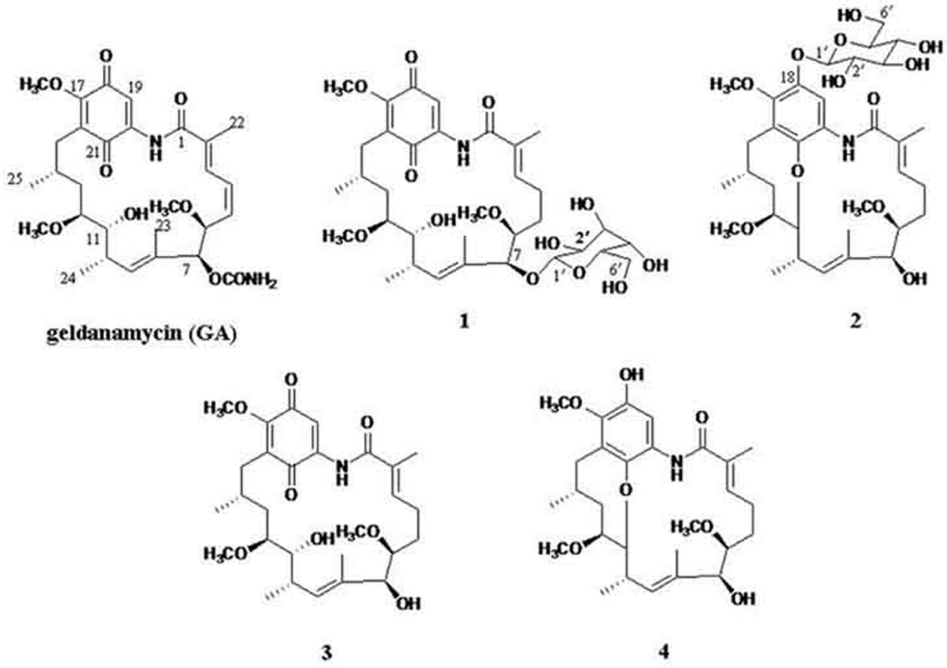

Fig. 1. Structures of geldanamycin and compounds **1** - **4**.

glycosylation reactions were analyzed by HPLC and LC-MS, which showed the formation of new glycosylated products peaks (Figs. S1 and S2). In particular the LC-MS analysis confirmed that the new products (**1** and **2**) were mono-glucosides of the substrates (**3** and **4**), respectively.

The YjiC UGT enzyme expression vector (pET302-YjiC) was obtained from Prof. Jae Kyung Sohng of Sun Moon University. The details of the method for expression and purification of YjiC are described in a previous report [18]. The in vitro glycosylation reactions were performed in 40 ml of reaction buffer (100 mM Tris-HCl, pH 8.8) containing 1 mM $MgCl_2 \cdot 6H_2O$, 8 ml of MeOH, 50 μg/ml of purified UGT enzyme (YjiC), 3 mM UDP-Glc, and 3 mM substrate (**3** or **4**), as previously reported. After a 16 h incubation at 28°C, the reaction was quenched by adding an equal volume of EtOAc and mixed by vortexing. Aliquots were centrifuged at 3,000 rpm for 20 min, and the EtOAc extract was used for analysis. Then, the EtOAc extract was further purified by reversed-phase HPLC (Waters 2535Q semi-prep HPLC) using a SunFire C_{18} column (250 × 10 mm) with 40% acetonitrile (CH_3CN:H_2O, 4.5 ml/min) to yield compounds **1** (5.4 mg) and **2** (4.1 mg), respectively. To determine the water solubility, excess substrates (**3** and **4**) and their glycosylated products (**1** and **2**) were mixed with 25 μl of distilled water in an Eppendorf tube at room temperature and sonicated to maximize the solubility of each compound. After sonication for 30 min and centrifugation at 12,000 rpm for 10 min, insoluble material was removed. The aqueous solution was diluted and analyzed by HPLC. The HPLC peaks were integrated and the sample solution concentration was calculated as previously described [18, 19]. The human breast cancer cell line MDA-MB-231, human hepatocellular carcinoma cell lines SMMC7721 and HepG2, and human colon adenocarcinoma cell line SW480 were seeded in 96-well plates at a density of 5,000 cells/well for 1 day. The cells were incubated with the compounds at various concentrations for 72 h. Geldanamycin was used as a positive control. The cell viability was evaluated by a standard MTT assay [18].

Compound **1** was obtained as a white amorphous solid. Its HR-ESI-MS exhibited a pseudo molecular ion peak at *m/z* 680.3128 $[M-H]^-$, consistent with the molecular formula, $C_{34}H_{51}NO_{13}$. The NMR data of **1** are comparable to those of GA, and indicate the absence of the carbamoyl group and C-4,5 double bond of GA. In the ^{13}C-NMR spectrum of **1**, the presence of signals at δ_c 184.4 and 183.8 correspond to the benzoquinone ring at the C-18 and C-21 positions. An analysis of the 1D and 2D NMR data suggested that **1** was related to **3**, except for a glucose moiety (Table 1) [6]. The anomeric proton signal at δ_H 4.14 (1H, d, J = 7.9 Hz, H-1′) is consistent with the β-configuration of glucose in **1**, based on the coupling constant (7.9 Hz). The position of the

Table 1. NMR data for compounds **1** and **2** in DMSO-d_6.

	Compound **1**		Compound **2**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	—	164.9	—	164.5
2	—	132.7	—	123.6
3	6.11, s	139.1	6.65, dd (9.8, 4.3)	139.0
4	2.28, m; 2.19, m	24.1	2.42, m; 2.20, m	24
5	1.62, m	29.5	1.68, overlap; 1.41, m	30.5
6	3.27, m	82.1	2.94, m	80.2
7	3.79, d (6.6)	88.6	3.75, m	80.7
8	—	132.9	—	134.7
9	5.31, d (9.2)	133.3	5.38, d (9.3)	133.3
10	2.54, overlap	32.9	2.59, m	33.4
11	3.41, overlap	73.9	3.56, d (4.9)	72.9
12	3.07, m	80.2	3.30, m	83.2
13	1.38, m; 1.27, m	34.2	1.68, m	30.6
14	1.44, m	28.2	1.70, m	35.6
15	2.42, m; 2.32, m	31.3	3.02, d (12.9); 2.62, m	32.4
16	—	128.4	—	118.4
17	—	156.9	—	146.3
18	—	184.4	—	149.1
19	6.81, s	110.7	7.34, s	104.3
20	—	141.1	—	136.9
21	—	183.8	—	144.1
22	1.78, s	12.9	2.07, s	12.9
23	1.51, s	12.7	1.44, s	11.4
24	0.83, d (6.8)	13.9	0.93, d (6.7)	15.1
25	0.89, overlap	21.9	0.75, d (6.7)	21.3
6-OCH_3	3.39, s	58.9	3.41, s	59.3
12-OCH_3	3.17, s	56.3	3.32, s	56.8
17-OCH_3	3.97, s	61.6	3.80, s	61.6
NH	9.03, brs	—	**	—
1′	4.14, d (7.9)	103.4	4.87, d (7.4)	101.6
2′	2.98, m	74.6	3.38, overlap	77.34
3′	3.08, overlap	77.5	3.31, m	73.9
4′	3.10, overlap	70.4	3.17, t (5.0)	70.4
5′	3.12, overlap	77.1	3.38, overlap	77.5
6′	3.57, m; 3.43, dt (11.2, 5.4)	61.5	3.75, m; 3.47, dt (11.8, 6.0)	61.32

^{1}H and ^{13}C NMR spectral data (δ) were obtained at 700 and 175 MHz, respectively. **Not observed.

glucose moiety attached to aglycone was confirmed by an HMBC experiment. The HMBC correlation between the anomeric proton H-1′ (δ_H 4.14) and C-7 (δ_C 88.6) indicated that a β-glucose unit was attached to the C-7 position (Fig. S3). Meanwhile, the positions of the functional groups were determined with HMBC correlations (6-OCH_3/C-6, 12-OCH_3/C-12, 17-OCH_3/C-17, H-22/C-1, C-2, C-3, H-23/C-8, C-9, and H-24/C-9, C-10, C-11) (Fig. S3). Therefore, the structure of **1** was identified as 4,5-dihydro-7-*O*-descarbamoyl-7-hydroxyl GA-7-*O*-β-D-glucoside (Fig. 1).

Table 2. Water solubility of the substrates and their glucosides (**1** and **2**).

Compounds	Solubility in water (mg/ml)	MW	Solubility in water (mM)	Relative solubility
1	192.2	681.7	282.0	215.2
3	0.68	519.6	1.31	1.0
2	70.66	665.7	106.1	90.7
4	0.59	503.6	1.17	1.0

Table 3. Antiproliferative activity (IC_{50}, μM) of the compounds[a].

Compounds	MDA-MB-231	SMMC7721	HepG2	SW480
3	13.6	15.1	31.8	22.7
Geldanamycin[b]	0.6	0.1	1.7	0.5

[a]Compounds **1**, **2**, and **4** were inactive (IC_{50} > 100 μM).

[b]Geldanamycin was used as a positive control.

Compound **2** was obtained as a dark brown amorphous solid. Its HR-ESI-MS profile exhibited a pseudo-molecular ion peak at *m/z* 666.3497 $[M+H]^+$, consistent with the molecular formula, $C_{34}H_{51}NO_{12}$. The ^1H- and ^{13}C-NMR spectra of **2** were very similar to those of the tricyclic GA analog, ACDL3172 (**4**), except for the presence of a glucose moiety (Table 1) [9]. The anomeric proton signal at δ_H 4.87 (1H, d, J = 7.4 Hz, H-1′) of **2** was assigned as the β-configuration. Meanwhile, the HMBC correlation between the anomeric proton H-1′ (δ_H 4.87) and C-18 (δ_C 149.1) indicated that a glucose moiety was attached to C-18 of aglycone (Fig. S3). Furthermore, a comprehensive analysis of the 2D-NMR data of **2** permitted the complete assignments of its protons and carbons, and the structure of **2** was identified as ACDL3172-18-*O*-β-D-glucoside (Fig. 1).

Two new glucosides (**1** and **2**) of GA analogs were obtained from in vitro glycosylation using a UDP-glycosyltransferase (YjiC). The solubility of the novel glucosides **1** and **2** in water was determined by comparing with those of the substrates. The water solubility of substrates **3** and **4** was found to be 1.31 mM and 1.17 mM, respectively (Table 2). In contrast, the solubility of the glycosylated products (**1** and **2**) in water was 282.0 mM and 106.1 mM, respectively, about 215.2 and 90.7 times higher than that of the substrates. Our data suggest that the attachment of a glucose moiety to these substrates by enzymatic synthesis greatly enhanced their water solubility.

Compounds **1–4** were evaluated for antiproliferative activities against MDA-MB-231, SMMC7721, HepG2, and SW480 cell lines using the MTT assay. Only compound **3** exhibited weak antiproliferative effects, with IC_{50} values ranging from 13.6 to 31.8 μM (Table 3). The in vitro antiproliferative activity of glucoside compound **1** was reduced relative to that of the substrate, which is consistent with previous studies [18]. In some cases, such cytotoxic compounds can be inactivated by glycosylation and reactivated for cytotoxic activity by glycosidase [1].

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