Brief Report

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Synthesis of solasodine glycoside derivatives and evaluation of their cytotoxic effects on human cancer cells

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ABSTRACT: Solasodine glycosides, such as solamargine, have been proved to be very important anti-cancer agents. In order to discover more potent cytotoxic agents and explore the preliminary structure activity relationship, a new series of solasodine glycosides 2-9 were synthesized *via* a transglycosylation strategy, and their cytotoxic activity against a panel of human cancer cell lines (MCF-7, KB, K562, and PC3 cells) were evaluated by MTT assays. The results indicated that compounds 2, 8, and 9 with the substitute moiety of rhamnose, 2-hydroxyethoxymethyl, and 1,3-dihydroxypropan-2-yloxy-methyl, respectively, exhibited quite strong anticancer activity. The underlying mechanism tests demonstrated that these compounds could induce apoptosis detected by DAPI staining, and Annexin V and propidium iodide binding. Cell cycle analysis indicated that the cancer cells were predominantly arrested at the G2/M phase when exposure to these compounds was examined by flow cytometry. These compounds may serve as lead candidates in the development of novel chemotherapeutics for cancer treatment.

Keywords: Saponins, solasodine, cytotoxicity, apoptosis

1. Introduction

Natural derived products which belong to the traditional medicinal system are affluent resources for new drug development. Steroidal glycosides are a series of natural products and display a wide spectrum of structural diversity with versatile biological activities, such as anti-inflammatory, antibacterial, antiparasitic, antifungal, and anticancer activities (*1-5*). Both the sugar residue and aglycone in the structure play very important roles in their biological performance (*6-8*).

Solasodine, a solanum type of steroid alkaloid with a C27 cholestane skeleton, was first discovered from the fruit of the devil's apple in free and conjugated forms (*9*). Solasodine conjugates displayed uneventful anticancer activity, but the mixture of the solasodine glycosides (BEC: 33% solasonine, 33% solamargine, and 34% of their corresponding di- and monoglycosides) discovered from the Devil's Apple plant (*9-15*) have been clinically applied as an antineoplastic agent.

Solasodine glycosides exhibited obvious anticancer activities when compared with its aglycone (*9-14*). Meanwhile, the cytotoxic activities of solasodine glycosides were generally dependent on the presence of sugars, in particular rhamnose (*9-21*), and were mediated by the rhamnose-binding receptors that occur on the membrane of cancer cells (*21*).

In order to discover more potent cytotoxic agents for cancer therapy with the simplified structures which were facilitated for industrial preparation, six monosaccharides including rhamnose as well as two open-loop saccharide analogues were introduced to solasodine and compounds **2-9** were then prepared accordingly (Scheme 1).

2. Materials and Methods

2.1. *Chemicals*

Solasodine **1** (Scheme 1) was synthesized using five straightforward sequential reactions as reported previously (*22*). Six glycosyl bromide **10-15** (Scheme 1) was then employed to assemble a small library of solasodine glycosides using classic Koenigs-Knorr glycosylation. In addition, two open-loop saccharide analogues were transferred to the solasodine using 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (DIEA) as a catalyst.

As shown in Scheme 1, glycosyl donors **10-14** were prepared from the corresponding benzoylated monosaccharide using a solution of 45% HBr/HOAc in CH_2Cl_2. Compound **15** was easily prepared from

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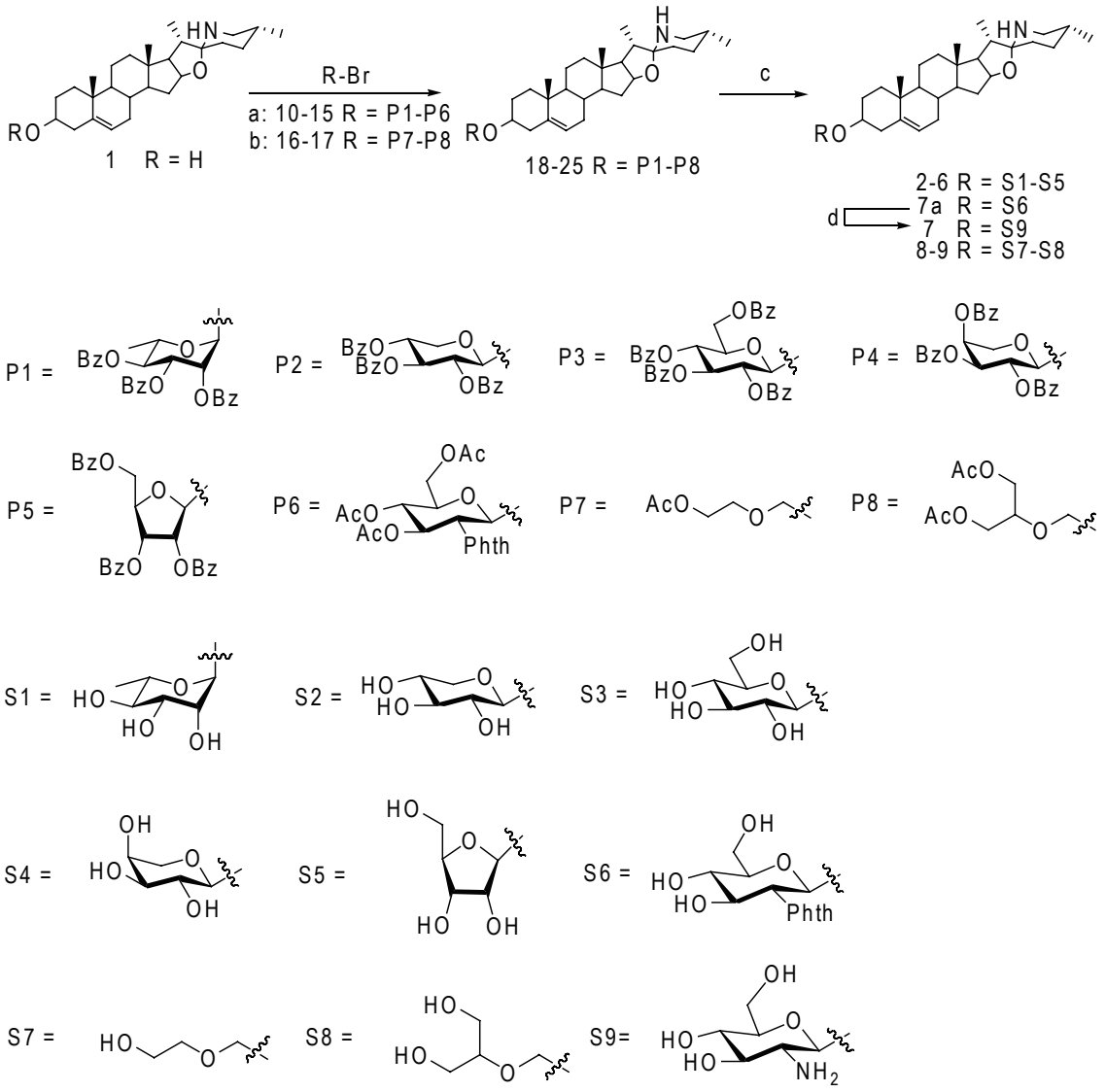


Scheme 1. Reagents and conditions. (a) AgOTf, 4 Å molecular sieve, −20°C, solasodine, 12 h; **(b)** DIEA, CH_2Cl_2, room temperature, 24 h; **(c)** CH_3OH/CH_3ONa, 4 h, 98%; **(d)** CH_3NH_2, 4 h, 97%.

commercial glucosamine hydrochloride (*23*). Compound **16** was synthesized from 1,3-dioxolane using AcBr, and **17** was prepared from 1,3-dioxolan-4-yl-methanol (*24*). And then each donor of **10-15** was condensed with solasodine **1**, respectively, with catalysis from silver trifluoromethanesulfonate (AgOTf) to provide protected **18-23**. Under the promotion of DIEA, coupling of open-loop donor **16** or **17** with solasodine **1** afforded the intermediates **24** and **25**, respectively. At last, MeOH/MeONa or CH_3NH_2 was used to hydrolyze the tosyl, acetyl and phthalic anhydride to provide target compounds **2-9**. The β-bond linkage between glycosyl and aglycone in compounds **2-7** was comfirmed. Specific information on synthesis of targeted compounds **2-9** is indicated in the Appendix.

2.2. *Cell lines and cell culture*

Human squamous cell carcinoma (KB), breast adenocarcinoma (MCF-7), myelogenous leukemia (K562), prostate cancer (PC3), human umbilical vein endothelial (ECV304), and human hepatocyte cells (HL7702) were maintained in RPMI 1640 medium (HyClone, Thermo Fisher Scientific Inc., Waltham, MA, USA). The medium was supplemented with 10% fetal bovine serum (FBS) (Gibco, Invitrogen, Carlsbad, CA, USA), 100 μg/mL penicillin and 100 μg/mL streptomycin. Cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C.

2.3. *MTT assay*

Cells were seeded into 96-well culture plates at a density of 4-5 × 10^3 cells per well and cultured for 24 h. Thereafter the cells were treated with various concentrations of tested compounds and incubated for 24-48 h. Cell viability was assessed by MTT assay. The cells were incubated with 5 mg/mL MTT solution at 37°C for 4 h, and the resulting crystals were dissolved

in dimethyl sulfoxide (DMSO). The optical density (OD) was measured using a plate microreader (Bio-Rad 680, Bio-Rad Co., Hercules, USA). Cell viability was calculated as follows:

$$\text{Cell viability (\%)} = 100 \times (OD_{sample} - OD_{blank})/(OD_{control} - OD_{blank})$$

2.4. *Cell morphology examination*

To assess the effect of compounds **2**, **8**, and **9** on apoptosis, MCF-7 cells were seeded in 24-well plates. After treatment with compounds **2**, **8** and **9** for 24 h cells were washed twice with PBS, and were observed under the microscope. Images were processed with Adobe Photoshop 7.0.

2.5. *4',6-Diamino-2-phenylindole (DAPI) staining*

MCF-7 cells were seeded onto 12-mm round, glass cover slips in 24-well plates. After 24 h treatment with compounds **2**, **8**, and **9**, cells were stained with 4 μg/mL DAPI for 10 min at room temperature. The cover slips containing cells were then mounted on microscope slides using mounting medium and analyzed with fluorescence microscopy. Fluorescence images were processed using AutoQuant X 2.1 software from Cybernatics, Inc. (Bethesda, MD, USA).

2.6. *Apoptosis analysis*

Apoptosis was evaluated with Annexin V/propidium iodide (PI) staining using an FITC Annexin V Apoptosis Detection Kit purchased from BD Biosciences (San Jose, CA, USA). MCF-7 cells were seeded at a density of 1×10^5/mL into 6-well plates. After 24 h incubation with compounds **2**, **8**, and **9**, the cells were washed twice with ice-cold phosphate buffered saline (PBS). Each cell sample was transferred to individual tubes and centrifuged at 200 × g for 5 min. The supernatant was removed, and the cells were resuspended in 400 μL of annexin V-FITC binding buffer and incubated at room temperature in the dark for 15 min with 5 μL annexin V-FITC and PI 10 μL (50 μg/mL) was added for another 5 min. Apoptosis was measured using flow cytometry (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and WinMDI 2.9 analysis software.

2.7. *Cell-cycle analysis*

Cell cycle was analyzed using flow cytometry of PI-stained cells. MCF-7 cells were cultured in the presence of different concentrations of compounds **2**, **8**, and **9**. After designated time intervals, cells were fixed in 70% ethanol overnight at 4°C and washed once with PBS. Then, the cells were incubated with 1 U/mL of RNase A (DNase free) for 30 min at 37°C and 10 μg/mL of PI for 1 h at room temperature in the dark. Cell cycle distribution was examined by flow cytometry, and data was analyzed using the Modfit program (Becton, Dickinson and Company).

2.8. *Statistical analysis*

All experiments were performed at least three times. Statistical analysis was performed with an analysis of variance (ANOVA) followed by the Turkey's *t*-test. $p < 0.05$ were considered statistically significant.

3. Results and Discussion

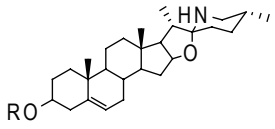
3.1. *Cytotoxic effects of synthesized compounds on human cancer cells*

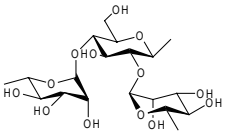
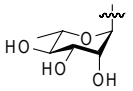
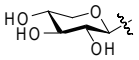
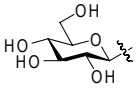
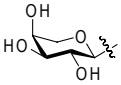
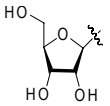
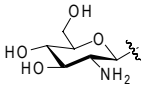
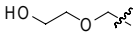
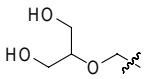
The anticancer effect of synthesized saponins **2-9** as well as solasodine **1** against human KB, K562, MCF-7, PC3, EVC304, and HL7702 cells were preliminarily evaluated by MTT assay (*25*). As shown in Table 1, compounds **2**, **3**, **8**, and **9** were found to possess relatively potent cytotoxic activity against PC3 cells, with IC_{50} values ranging from 7.2 to 18.4 μM. In K562 cells, compounds **2** and **9** with IC_{50} values of 18.8 and 17.0 μM showed obvious superiority to other synthesized compounds in suppressing cell proliferation. Similarly, compounds **2**, **8**, and **9** exhibited excellent proliferation inhibitory activity against MCF-7 cells, with IC_{50} values of 12.9 and 17 μM, respectively. On the other hand, compounds **2**, **8**, and **9** showed weak cytotoxicity in human normal cell lines ECV304 and HL7702 (Table 1). These results indicated that the saponins with rhamnose (*e.g.* compound **2**) or open-loop saccharide analogues (*e.g.* compounds **8** and **9**) exhibited more potent anticancer activity than the other derivatives synthesized in the current study.

3.2. *Compounds **2, 8, and 9** induced cell morphologic alteration in MCF-7 cells*

To examine the effect of compounds **2**, **8**, and **9** on cell morphology during cell death, MCF-7 cells were treated with various concentrations (0, 15, 30 μM) of these compounds for 24 h, respectively. As shown in Figure 1A, the morphological character of cells changed greatly. Cell shrinkage and membrane integrity loss appeared, which are typical features of apoptosis (*26,27*). Apoptotic cells were also defined on the basis of characteristic nuclear morphology including condensation of chromatin producing crescent shapes around the periphery of the nucleus and apoptotic bodies (*28,29*). As shown in Figure 1B, the non-treated cells had rounded and intact nuclei with diffuse DAPI staining, while the cells treated with compounds **2**, **8**, and **9** had nuclei that were smaller and brighter

Table 1. The cytotoxicity of compounds 1-9 on human cell lines



Compound	R	IC_{50} (μM)					
		KB	K562	MCF-7	PC3	ECV304	HL7702
Solamargine (*Ref. 30*)		7.8	8.0	8.2	5.9	ND	ND
1	H-	ND	ND	ND	13.6	ND	ND
2		29.1	18.8	14.2	18.4	34.0	36.0
3		ND	51.0	53.0	16.5	ND	ND
4		ND	43.0	77.0	27.9	ND	ND
5		ND	44.0	64.0	23.1	ND	ND
6		ND	ND	ND	66.9	ND	ND
7		ND	37.0	98.0	21.9	ND	ND
8		28.9	112.0	12.9	17.4	> 40	> 40
9		29.4	17.0	26.0	7.2	> 40	> 40

ND: not detected.

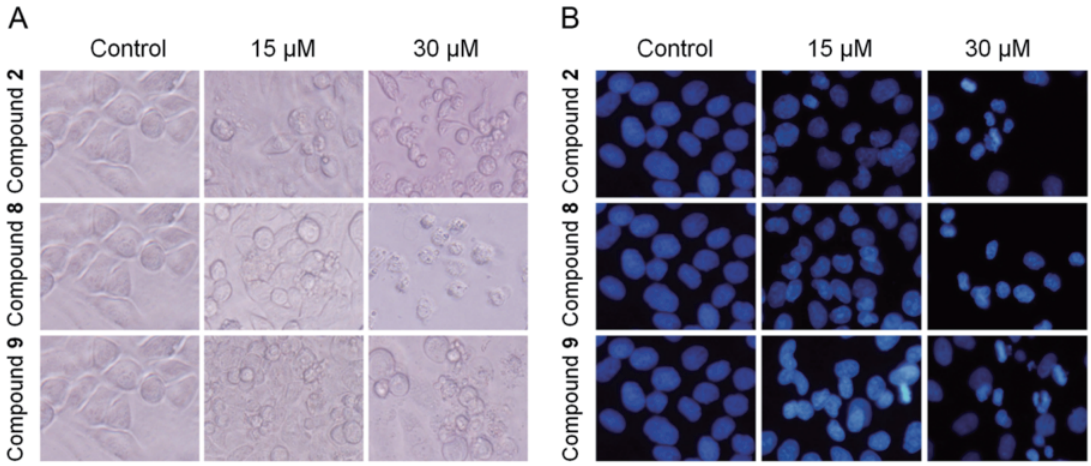


Figure 1. Morphological changes of MCF-7 cells after treatment with compounds 2, 8, and 9, respectively, for 24 h. Cell morphology was examined directly under light microscopy **(A)** or detected *via* fluorescence microscopy after the cells were stained with DAPI **(B)**.

stained with condensed chromatin forming crescent-shaped profiles around the periphery of the nucleus or separate globular structures (apoptotic bodies). The result implied that compounds **2**, **8**, and **9** induced cell apoptosis in MCF-7 cells.

3.3. *Quantification of apoptotic cells solicited by compounds **2**, **8**, and **9** in MCF-7 cells*

To further quantify the extent of compounds **2**, **8**, **9**-induced apoptosis, MCF-7 cells double stained with annexin V and PI were analyzed by flow cytometry. The analysis results revealed that the proportion of cells stained with annexin V increased with compounds **2**, **8**, and **9** treatment (Figure 2). The percentages of apoptotic cells increased from 0.88%, 0.07%, and 0.07% to 8.58%, 9.16%, and 10.56% when cells were exposed to 15 μM compounds **2**, **8**, and **9**, respectively, for 24 h. The proportion of apoptotic cells went up to 30.36%, 36.71%, and 34.18% when cells were treated with 30 μM of the above agents.

3.4. *Compounds **2**, **8**, and **9** induced cell cycle arrest at G_2/M phase in MCF-7 cells*

To determine whether compounds **2**, **8**, and **9** exerted cell growth inhibition activity *via* the induction of cell cycle arrest in addition to apoptosis, we next focused on the effect of compounds **2**, **8**, and **9** on the cell cycle of MCF-7 cells. We observed that compounds **2**, **8**, and **9** treatment led to cells accumulating in the G_2/M phase compared with non-treated cells (Figure 3). The ratio of cells in the G_2/M phase following the treatment with 15 μM compounds **2**, **8**, and **9** for 24 h increased from 6.60%, 5.79%, and 4.35% to 11.18%, 11.41%, and 7.71%, respectively. The values rose to 14.33%, 17.26%, and 17.84%, respectively, when cells were incubated with 30 μM of the above compounds for 24 h.

In summary, solasodine **1** together with its eight glycoside derivatives **2-9** were synthesized *via* a Koenigs-Knorr strategy. The preliminary structure-activity relationship (SAR) analysis indicated that

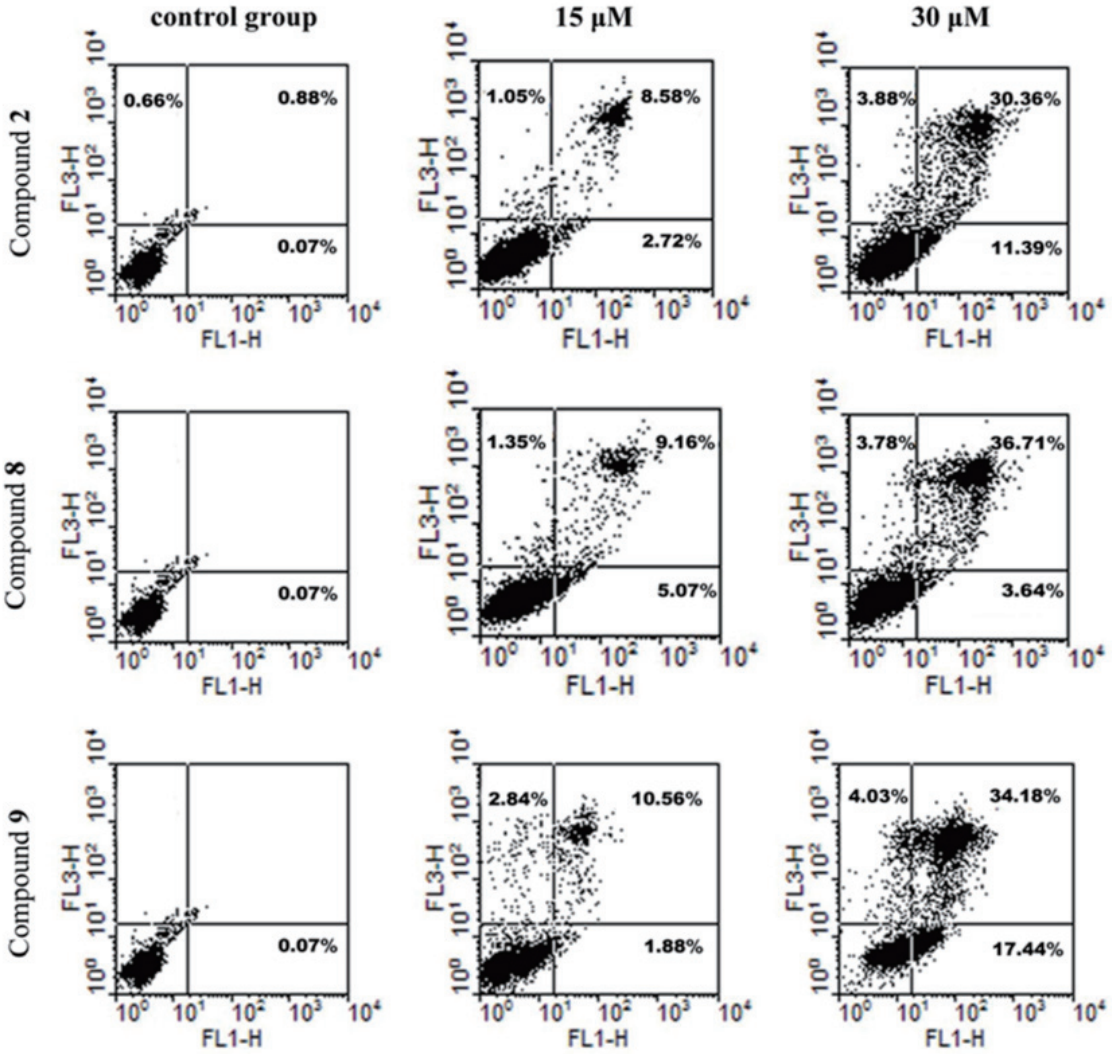


Figure 2. Flow cytometry analysis of cell apoptosis. MCF-7 cells were treated with compounds **2**, **8**, and **9**, respectively, for 24 h and then stained with Annexin-V/PI.

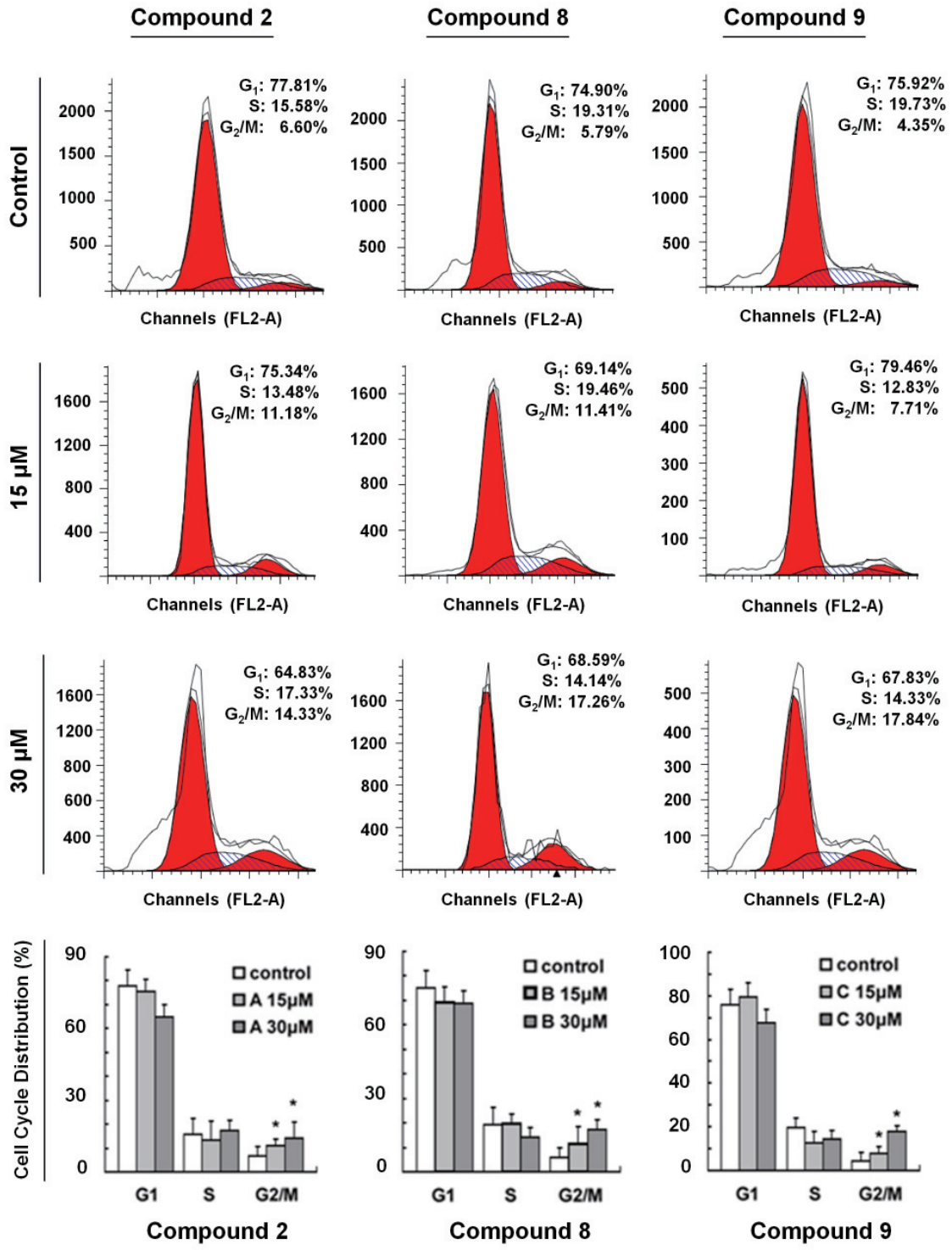


Figure 3. DNA content analysis. MCF-7 cells were treated with compounds **2**, **8**, and **9**, respectively, for 24 h and then stained with PI. The fluorescence of PI-stained cells was analyzed using flow cytometry. Cell cycle distribution was analyzed based on the DNA content. * $p < 0.05$ *vs*. control.

the rhamnose substituent was essential and the chain substituents in compounds **8** and **9** were also very important for its anticancer activity. The other sugar substituted derivatives exhibited less anticancer activity. These results were consistent with the previous report (*21*). Furthermore, it was demonstrated that the chain and rhamnose substituents exhibited similar cytotoxic potency. Our underlying work suggested that compounds **2**, **8**, and **9** have the ability to kill cancer cells *via* a form of apoptosis-like cell death. Moreover, compounds **2**, **8**, and **9**-treated cells were arrested predominantly at the G_2/M phase. This study demonstrated that compounds **2**, **8**, and **9** may merit further investigation as potential therapeutic leads for the development of novel anticancer drugs. Much more work concerning the biochemical mechanism is ongoing.

Acknowledgements

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Appendix

Flash column chromatography was performed on silica gel (200-300 mesh, Qindao Ocean Chemical Co., Qingdao, Shandong, China). Analytical thin-layer chromatography (TLC) was carried out on precoated

Silica Gel 60 F_{254} plates (E. Merck, Darmstadt, Germany) with detection by fluorescence and/or by charring with 30% (v/v) H_2SO_4 in EtOH. Dichloromethane was distilled from CaH_2. The chemicals and materials were purchased from Alfa-Aesar (Ward Hill, MA, USA) and were used as received. NMR spectra were recorded on a Bruker Avance DRX 600 spectrometer (Bruker BioSpin, Rheinstetten, Germany) at 600 (^{1}H) and 150 (^{13}C) MHz, respectively. Elucidations of chemical structures were based on ^{1}H, ^{13}C, ^{1}H-^{1}H COSY, HMBC, and HMQC NMR experiments. Signals are reported as follows: s (singlet), d (doublet), t (triplet), q (quintet), m (multiplet), and coupling constants are reported in Hz (hertz). Melting points were determined in an X-6 melting-point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter (Waltham, MA, USA). Mass spectral data (HR-ESI-MS and HR-FAB-MS) were obtained on LTQ-Orbitrap XL (Thermo Fisher Scientific Inc.) and JEOL JMS-DX-303HF (JEOL, Tokyo, Japan) in the positive ion mode, respectively.

*Synthesis of compound **1** (Ref. 28)*

Yield 18%, white solid; ^{1}H-NMR (600 MHz, $CDCl_3$): 5.37 (br. s, H-C(6)); 4.31 (m, Ha-C(16)); 3.54 (m, Ha-C(3)); 2.68 (m, CH_2-(26)); 1.19 (s, Me-(19)); 1.10 (s, Me-(21)); 0.97 (s, Me-(27)); 0.84 (s, Me-(18)). ESI-MS: 414.3 ($[M + H]^+$).

*Synthesis of compounds **10-14***

HBr/HOAc (1.0 mmol) was added to a solution of each of the protected sugars (0.5 mmol) in 4 mL CH_2Cl_2 at 0°C. The reaction mixture was stirred at 0°C for 12 h, the progress of the reaction was monitored by TLC. The solvent was removed under reduced pressure to afford a residue. Purification of the residue on a silica gel column with a 6:1 solution of petroleum ether-EtOAc gave **10-14** (yield 40-50%, oil).

*Synthesis of compound D-glucopyranosyl bromide **(15)***

Triethylamine (1.7 mL, 11.6 mmol) was added to the suspension of D-galactosamine hydrochloride (1 g, 4.65 mmol) in DMF (30 mL). After 20 min stirring at room temperature, phtalic anhydride (688 mg, 4.65 mmol) was added to this solution, and the mixture was stirred for 2 h at 50°C under N_2 atmosphere. The reaction mixture was allowed to stand until its temperature dropped to room temperature, excess amounts of triethylamine (5 mL) and acetic anhydride (5 mL) were added. After a 2-day stirring at room temperature under a N_2 atmosphere, the reaction mixture was concentrated in vacuo, mixed with water (150 mL), extracted three times with petroleum ether-EtOAc 1:1, and dried over Na_2SO_4. The organic layer was concentrated and separated by silicagel column chromatography (petroleum ether-EtOAc 1:1) to give the protected D-galactosamine (1.82 g, 82%). The resulting protected D-galactosamine (1.395 g, 2.92 mmol) was mixed with acetic anhydride (0.69 mL, 7.31 mmol), and treated with HBr-AcOH (30%, 16 mL, excess) with ice-cooling. After stirring for an hour at room temperature under a N_2 atmosphere, the mixture was concentrated in vacuo. The reaction mixture was mixed with saturated $NaHCO_3$, extracted three times with CH_2Cl_2, dried over Na_2SO_4, and concentrated to give **15** (1.41 g, 97%, Crude).

*Synthesis of compounds **16** and **17***

AcBr (5.05 mmol) was added to a solution of each of the acetyl protected rings (5 mmol) in 6 mL anhydrous CH_2Cl_2 at room temperature. The mixture was stirred at the same temperature for 12 h. The solvent was removed under reduced pressure to afford **16** and **17** (yield 70-80%, oil).

*Synthesis of compounds **18-23***

AgOTf (33 mg, 0.13 mmol) was added to a stirred mixture of brominated sugar (1.01 mmol) and solasodine (0.414 g, 1.0 mmol) in anhydrous CH_2Cl_2 (8 mL) with a 4 Å molecular sieve at −20°C under a N_2 atmosphere. The reaction mixture was stirred under this condition for 90 min. The solution was filtered and the solvent was evaporated under reduced pressure. The residue was subjected to silica column chromatography with a 40:1 solution of CH_2Cl_2-MeOH to afford **18-23** as white solids (yield 20-70%).

*Synthesis of compounds **24** and **25***

Ethyldiisopropylamine (DIEA) (3 mL) was added to a solution of solasodine (0.414 g, 1 mmol) in 5 mL CH_2Cl_2 at room temperature. After stirring for 15 min, compounds **16** and **17** (1.01 mmol) were added to the mixture and stirred for 12 h. The mixture was concentrated and purified by silica column chromatography with a 40:1 solution of CH_2Cl_2-MeOH to afford **24** and **25** (yield 70-80%).

*Synthesis of compounds **2-6**, **7a**, **8**, and **9***

Compounds **18-25** were dissolved in a solution of MeOH-CH_2Cl_2 (2:1, 10 mL). To the solution was added 2 g/100 mL MeONa/MeOH until a pH 9-10 was attained. The mixture was stirred at room temperature for 4 h, neutralized with Amberlite IR-120 (H^+), filtered and concentrated. The residue was added to a silica gel column with a 45:1 solution of CH_2Cl_2-MeOH to afford compounds **2-6**, **7a**, **8**, and **9** (yield 95-100%).

Solasodine 3-O-L-rhamnoside (**2**). ^{1}H-NMR (DMSO, 600 MHz) δ: 0.76 (3H, s, Me-18), 0.89 (3H, s, Me-27), 0.97 (3H, s, Me-21), 1.10 (3H, s, Me-19),

2.64 (2H, m, H-26), 3.54 (1H, s, H-3), 4.54 (1H, s, H-16), 4.71 (1H, d, J = 19.2 Hz, H-1′), 5.33 (1H, s, H-6); ^{13}C-NMR (DMSO, 600 MHz) δ: 140.7; 121.6; 98.6; 75.8; 71.4; 71.1; 69.0; 56.1; 49.8; 41.9; 40.5; 39.4; 38.5; 36.9; 32.2; 32.0; 31.2; 29.7; 20.7; 19.5; 18.6; 18.4. ESI-MS: 559.8 ([M + H]$^+$).

Solasodine 3-O-D-xylosidase (**3**). ^{1}H-NMR (DMSO, 600 MHz) δ: 0.76 (3H, s, Me-18), 0.89 (3H, s, Me-27), 0.97 (3H, s, Me-21), 1.10 (3H, s, Me-19), 2.67 (2H, m, H-26), 3.63 (1H, s, H-3), 4.54 (1H, s, H-16), 4.94 (1H, d, J = 17.4 Hz, H-1′), 5.33 (1H, s, H-6); ^{13}C-NMR (DMSO, 600 MHz) δ: 140.93; 121.4; 105.2; 102.2; 97.3; 83.2; 77.7; 73.7; 70.0; 61.6; 56.4; 49.8; 46.2; 41.5; 40.5; 38.7; 37.2; 36.8; 32.2; 31.9; 31.4; 29.8; 28.0; 20.7; 19.5; 18.7; 16.2; 15.0. ESI-MS: 545.7 ([M + H]$^+$).

Solasodine 3-O-D-galactosidase (**4**). ^{1}H-NMR (DMSO, 600 MHz) δ: 0.74 (3H, s, Me-18), 0.86 (3H, d, J = 7.2 Hz, Me-27), 0.97 (3H, s, Me-21), 1.05 (3H, t, J = 7.2 Hz, Me-19), 2.37 (2H, m, H-26), 3.61 (1H, s, H-3), 4.17 (1H, s, H-16), 4.18 (1H, d, J = 17.4 Hz, H-1′), 5.32 (1H, m, H-6); ^{13}C-NMR (DMSO, 600 MHz) δ: 141.0; 121.6; 101.8; 78.3; 77.2; 75.5; 74.0; 71.0; 68.6; 62.8; 60.9; 56.5; 47.6; 41.3; 38.8; 36.8; 29.8; 19.9; 19.6; 16.6; 15.8. ESI-MS: 575.7 ([M + H]$^+$).

Solasodine 3-O-L-arabinoside (**5**). ^{1}H-NMR (CD_3OD, 600 MHz) δ: 0.83 (3H, s, Me-18), 0.87 (3H, d, J = 6.0 Hz, Me-27), 0.99 (3H, d, J = 7.2 Hz, Me-21), 1.04 (3H, s, Me-19), 2.64 (2H, m, H-26), 3.58 (1H, m, H-3), 3.70 (2H, m, H-2′), 3.96 (2H, m, H-1′), 4.32 (1H, s, H-16), 5.37 (1H, s, H-6); ^{13}C-NMR (CD_3OD, 600 MHz) δ: 140.0; 122.0; 100.7; 98.5; 78.5; 72.7; 71.6; 67.6; 65.2; 62.7; 56.5; 50.1; 47.5; 41.3; 40.5; 39.8; 38.7; 37.2; 36.9; 34.0; 32.1; 31.4; 30.1; 29.6; 20.9; 19.4; 16.4; 15.2. ESI-MS: 545.7 ([M + H]$^+$).

Solasodine 3-O-D-riboside (**6**). ^{1}H-NMR (DMSO, 600 MHz) δ: 0.75 (3H, s, Me-18), 0.88 (3H, d, J = 6.6 Hz, Me-27), 0.96 (3H, s, Me-21), 1.07 (3H, d, J = 6.0 Hz, Me-19), 2.69 (2H, s, H-26), 3.67 (1H, s, H-3), 4.53 (1H, m, H-16), 4.85 (1H, t, J = 6.0 Hz, H-1′), 5.33 (1H, d, J = 4.8 Hz, H-6); ^{13}C-NMR (DMSO, 600 MHz) δ: 140.4; 121.7; 98.7; 97.9; 76.3; 70.9; 68.5; 63.7; 61.4; 55.8; 54.9; 49.3; 48.5; 45.2; 41.0; 40.3; 38.7; 38.2; 36.8; 31.7; 31.4; 31.0; 30.1; 20.3; 19.1; 18.3; 17.3; 15.8. ESI-MS: 545.7 ([M + H]$^+$).

Solasodine 3-O-(2′-hydroxyethoxy)methyl ether (**8**). ^{1}H-NMR (CD_3OD, 600 MHz) δ: 0.84 (3H, s, Me-18), 0.87 (3H, d, J = 6.0 Hz, Me-27), 0.98 (3H, d, J = 4.8 Hz, Me-21), 1.05 (3H, s, Me-19), 2.66 (2H, m, H-26), 3.50 (1H, m, H-3), 3.75 (4H, m, H-2',3'), 4.32 (1H, s, H-16), 4.81 (2H, m, H-1′), 5.38 (1H, s, H-6); ^{13}C-NMR (CD_3OD, 600 MHz) δ: 140.5; 121.7; 98.3; 94.2; 71.0; 62.3; 56.5; 50.1; 41.2; 40.5; 40.0; 39.4; 37.1; 36.9; 32.2; 31.4; 28.8; 20.8; 19.4; 16.5; 15.4. ESI-MS: 487.7 ([M + H]$^+$).

Solasodine 3-O-(1′,3′-dihydroxypropan-2′-yloxy)methyl ether (**9**). ^{1}H-NMR (DMSO, 600 MHz) δ: 0.75 (3H, s, Me-18), 0.85 (3H, m, Me-27), 0.97 (3H, s, Me-21), 1.05 (3H, t, J = 7.2 Hz, Me-19), 3.09 (1H, m, H-2′), 3.24 (2H, m, H-26), 3.61 (1H, m, H-3), 4.38 (1H, t, J = 4.8 Hz, H-16), 5.32 (1H, d, J = 4.8 Hz, H-6); ^{13}C-NMR (DMSO, 600 MHz) δ: 140.9; 120.6; 95.8; 94.5; 79.4; 76.7; 61.6; 60.7; 59.1; 58.7; 56.4; 55.5; 54.9; 41.4; 40.9; 37.2; 36.7; 32.2; 31.4; 29.2; 20.7; 19.5; 16.4; 15.2. ESI-MS: 487.7 ([M + H]$^+$).

Synthesis of compound 7

$CH_3NH_2 \cdot H_2O$ (1 mL) was added to a solution of compound **7a** (1 mmol) in 3 mL anhydrous MeOH at room temperature. The mixture was stirred at this temperature for 4 h. The mixture was concentrated and purified by silica column chromatography with a 40:1 solution of CH_2Cl_2-MeOH to afford **7** (yield: 90%).

Solasodine 3-O-glucosaminidase (**7**). ^{1}H-NMR (DMSO, 600 MHz) δ: 0.75 (3H, s, Me-18), 0.78 (3H, d, J = 6.6 Hz, Me-27), 0.88 (3H, d, J = 6.6 Hz, Me-21), 0.98 (3H, s, Me-19), 2.67 (2H, m, H-26), 3.66 (1H, d, J = 10.8 Hz, H-3), 4.56 (1H, s, H-16), 5.21 (1H, s, H-1′), 5.34 (1H, d, J = 4.2 Hz, H-6); ^{13}C-NMR (DMSO, 600 MHz) δ: 140.7; 121.7; 98.0; 77.7; 73.9; 70.5; 65.5; 62.7; 61.2; 59.0; 56.8; 50.0; 41.4; 38.3; 37.2; 36.8; 34.2; 32.2; 31.5; 29.5; 20.8; 19.9; 19.6; 16.6; 15.7. ESI-MS: 574.8 ([M + H]$^+$).