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Cholesterol-derived novel anti-apoptotic agents on the structural basis of ginsenoside Rk1

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ABSTRACT

Design and synthesis of cholesterol-derived anti-apoptotic agents were described. The synthesized cholesterol analogs designed on the structural basis of ginsenoside Rk1 inhibited the undesirable apoptosis of human endothelial cells, which are induced by a vascular injury. In particular, analogue **1** possessing 4,6-di-*O*-acetyl-2,3-dideoxyhex-2-enopyran linked to hydroxyl group of cholesterol exhibited the most effective anti-apoptotic activities at both 5 and 10 μg/ml.

Homeostasis in tissues of multi organism can be achieved by proper balance between cell proliferation and apoptosis.[1] Many pathological phenomenons such as cancer,[2] autoimmunity,[3] sepsis,[4] and neuro degenerative diseases[5] were observed at the collapse of this equilibrium. Adequate maintenance of endothelial cells constructing the inner cellular lining of blood vessels are involved in vascular homeostasis for health.[6] It has been known that vascular endothelial cell (VEC) apoptosis can induce an alteration in the integrity of vessels and the function of endothelium. This programmed cell death may contribute to pathogenesis of a variety of human diseases, such as atherosclerosis, allograft vasculopathy, and congestive heart failure (CHF).[7] Therefore, apoptosis inhibition has been considered as a new approach for treatment of vascular disorder. In addition, it has recently been revealed that ginsenoside Rg3 isolated from the root of *genus* Panax (ginseng), which exerts many pharmacological activities such as anti-tumor, anti-inflammatory, neuroprotection against the cerebral ischemia-induced injury,[8] has anti-apoptotic activity.[9] Moreover, our efforts for development of the potent anti-apoptotic agents have driven discovery of ginsenoside Rk1, which exhibit potent anti-apoptotic activity in human umbilical vein endothelial cell (HUVEC) lines.[10] However, instability of Rk1 under acidic conditions and the extract process-dependent composition of ginsenosides limited studies on biological functions and underlying mechanisms of their biological activities.[11] Up to date, most researches on natural ginsenosides has focused on their biological activities. Few studies on structure–activity relationship (SAR) with the synthetic ginsenoside analogues was reported.[12] Thus, we were interested in the anti-apoptosis associated SAR of the ginsenoside Rk1 analogues.[13] On the basis of our preliminary studies that the glycosyl moiety at C3 of Rk1 is crucial for the VEC apoptosis inhibiting effect, we

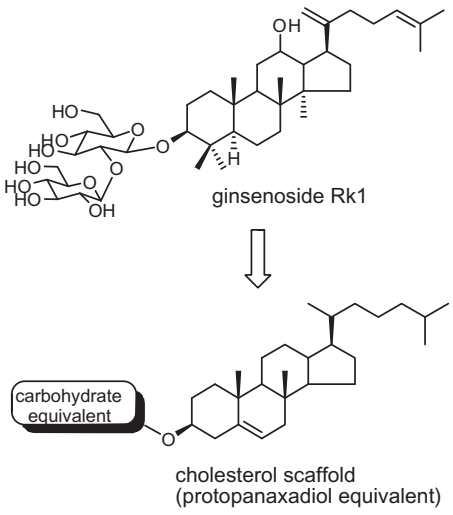

Figure 1. Cholesterol analogues based on ginsenoside Rk1.

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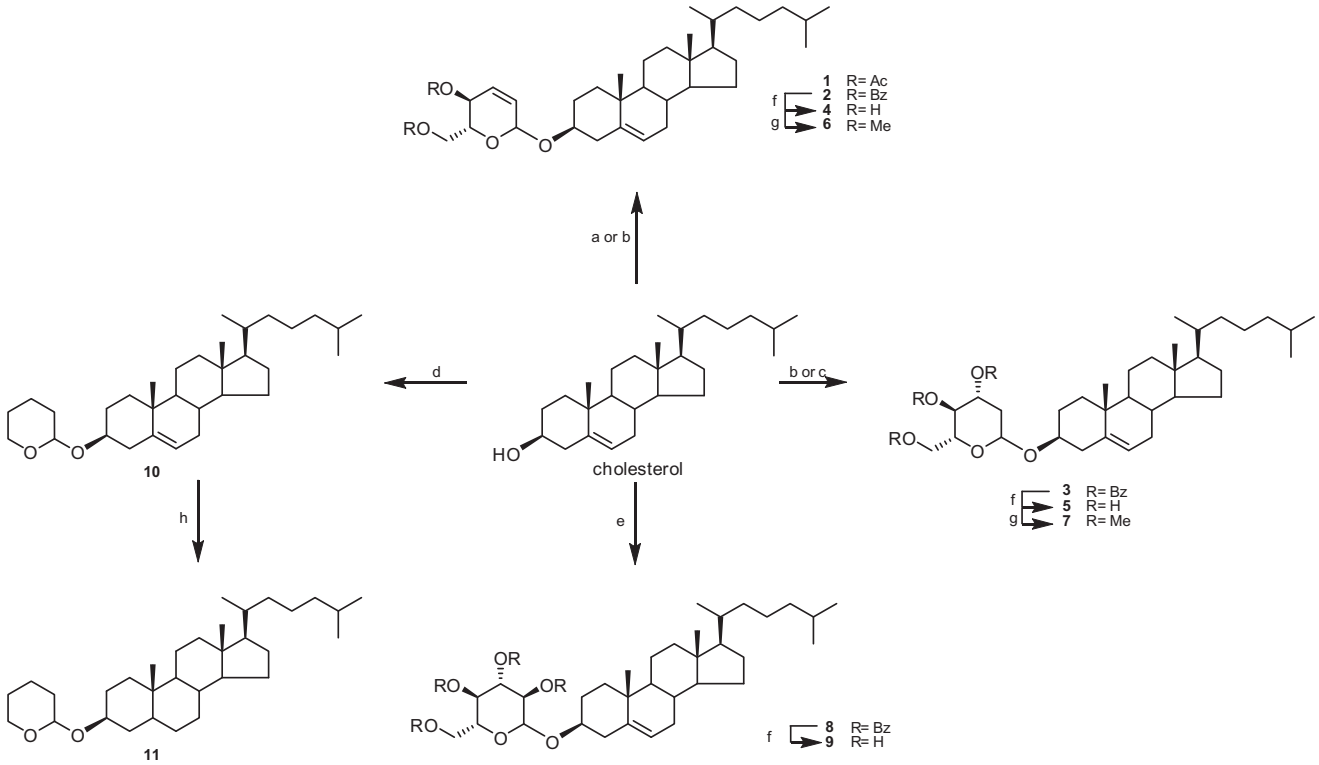
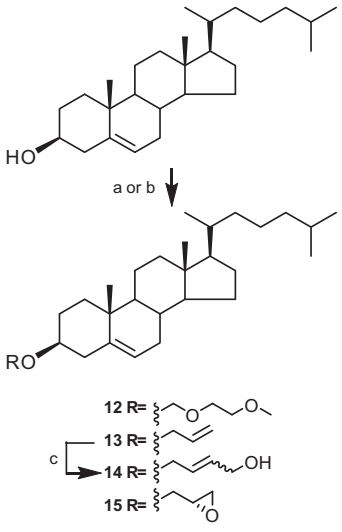


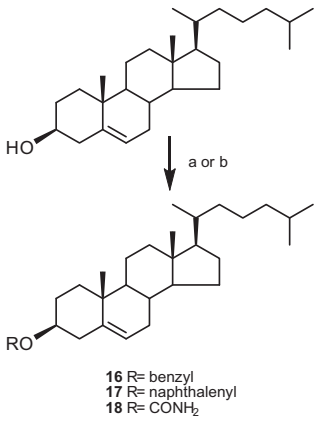
Scheme 1. Reagents and conditions: (a) 3,4,6- *tri*-*O*-acetyl-D-glucan, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 43%; (b) 3,4,6-*tri*-*O*-benzoyl-D-glucan, TsOH, CH_2Cl_2, 26%; (c) 3,4,6-*tri*-*O*-methoxy-D-glucan, CAN, CH_3CN; (d) DHP, PPTS, CH_2Cl_2, 68%; (e) 2,3,4,6-tetrabenzoyl-D-gluconosyl bromide, AgOTf, CH_2Cl_2, −20 °C, 45%; (f) NaOMe, MeOH/CH_2Cl_2, 60%; (g) MeI, NaH, THF, 0 °C, 32%; (h) H_2, Pd/C, EtOAc, 98%.

attempted to develop novel and structurally simplified VEC apoptosis inhibitors by introducing the carbohydrate equivalents. In addition, we selected cholesterol scaffold to substitute for the protopanaxadiol backbone of Rk1 in terms of structural similarity and ready accessibility. Taking together these, we have conducted design and synthesis of a series of cholesterol analogues. We also investigated their inhibitory effects on the serum deprivation-induced apoptosis of human endothelial cells (HECs). We herein report our successful results.

As an equivalent of the glycosyl unit, cyclic ether (hydropyran), acyclic ether (alkyl ether) and aryl moieties were introduced to C3 alcohol of the cholesterol scaffold (Fig. 1). Our synthesis commenced with an introduction of dihydro- or tetrahydropyran moieties to C3 alcohol of cholesterol. Dihydropyran analogue **1** was prepared by reaction of 3,4,6-*tri*-*O*-acetyl-D-glucal with cholesterol in the presence of boron trifluoride. Treatment of *tri*-*O*-benzoyl glucal with cholesterol in acidic media yielded a mixture of **2** and **3**, which could be separated by column chromatography. Removal of benzoyl group of **2** and **3** by using NaOMe afforded **4** and **5**, which could be further transformed into **6** and **7** by methylation (NaH, iodomethane), respectively. Analogue **9** was prepared by reaction of cholesterol with 2,3,4,6-tetrabenzoyl-D-glucopyranosyl bromide in the presence of silver triflate,[14] followed by



Scheme 2. Reagents and conditions: (a) $^{i}Pr_2NEt$, CH_2Cl_2, MEMCl, 48% for **12**; (b) NaH, THF/DMF (1:1), allyl bromide, reflux, 36% for **9**; 2*S*-(+)-glycidol tosylate, 45% for **15**; (c) Grubbs' 2nd catalyst, 1,4-butene diol, CH_2Cl_2, reflux, 30%.



Scheme 3. Reagents and conditions: (a) NaH, TBAI, aryl bromide, THF, reflux, 30%; (b) $Cl_3CCONCO$, $CHCl_3$, 31%.

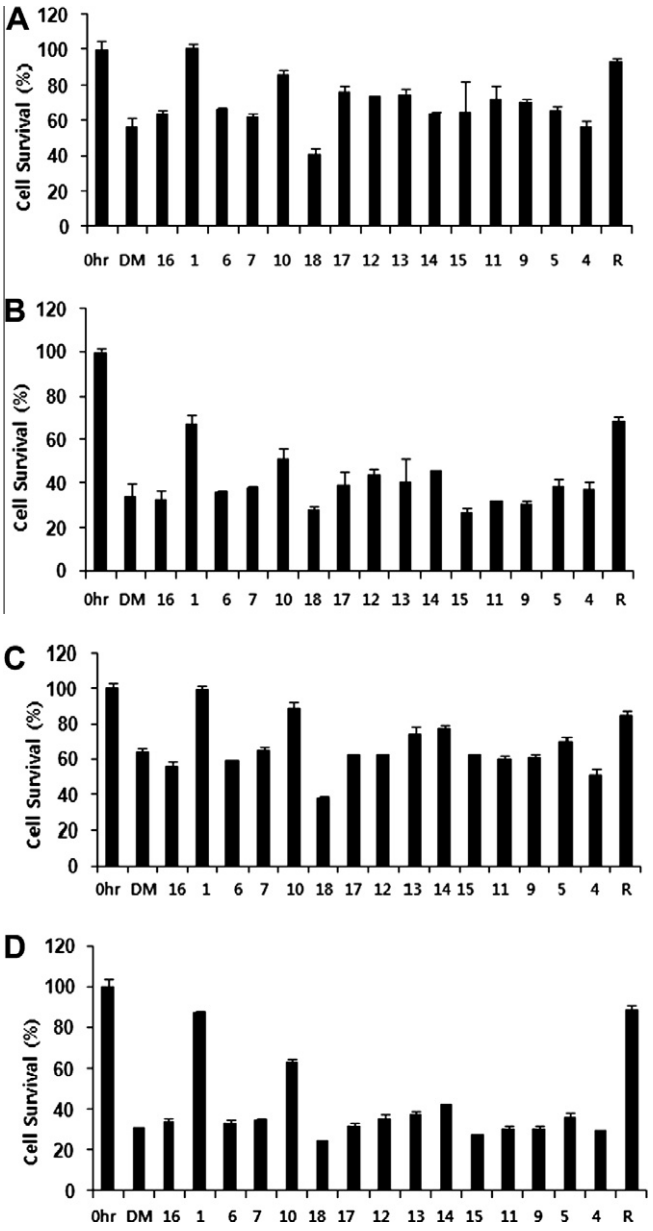


Figure 2. Effects of the cholesterol analogs on apoptosis of HUVECs. (A) 5 μg/ml for 24 h treatment, (B) 5 μg/ml, 48 h, (C) 10 μg/ml 24 h, (D) 10 μg/ml, 48 h treatment. 0 h, the viability of the cells cultured in the medium without any derivatives. DM, the viability of the cells cultured in the medium containing DMSO used as a vehicle control. R, the viability treated with Rk1.

debenzoylation. Reaction of cholesterol with 3,4-dihydro-*2H*-pyran in the presence of pyridinium *p*-toluene sulfonate in CH_2Cl_2 afforded **10**, which was readily converted to **11** by Pd/C catalyzed hydrogenation (Scheme 1).

Next, we synthesized a series of cholesterol analogues linked with the acyclic, or alkyl ether moieties. Analogue **12** was synthesized by reaction with MEMCl in the presence of Hunig's base. Allylation of cholesterol by treatment with sodium hydride and allyl bromide in refluxing THF/DMF provided allyl ether **13**. Cross metathesis of **13** with 1,4-butene diol by employing Grubbs' 2nd generation catalyst afforded **14**. Reaction of cholesterol with sodium hydride and then with (*2S*)-glycidol tosylate afforded **15** (Scheme 2).

To confirm the spatial influence of glycosyl residue, aryl moiety as a carbohydrate equivalent was also introduced to the cholesterol scaffold. Analogues **16** and **17** were prepared by O-benzylation and O-naphthylation of cholesterol under reaction conditions of sodium hydride and TBAI in refluxing THF. Treatment of cholesterol with trichloroacetyl isocyanate gave carbamate **18** (Scheme 3).

Inhibitory effect of the cholesterol analogues were investigated on the growth factor deprivation-induced HUVEC cell death using the MTT colorimetric method at concentration of 5 μg/ml and 10 μg/ml for 24 h and 48 h, respectively. The result of MTT assay is summarized in Figure 2. It is noticeable that the acetyl dihydropyran derivative **1** was more potent than ginsenoside Rk1 known as one of the potent anti-apoptotic agent. In contrast, analogues **5** and **6** possessing methoxy or hydroxy dihydropyran exhibited less potent activity compared to Rk1. This result revealed that the acyl group of the carbohydrate equivalents is important to manifest the inhibitory effect of the cholesterol analogues. The tetrahydropyran analogue **10** also exhibited comparable activity to that of Rk1 after 24 h treatment. However it was slightly less potent than Rk1 after 48 h treatment. Analogues **4**, **7**, and **9** possessing the substituted pyran moiety did not show any significant inhibitory activity. Analogues, **12**, **13**, **14**, and **17** possessing the acyclic carbohydrate equivalents exhibited moderate inhibitory activities at the concentration of 5 μg/ml. It is also noteworthy that the tetrahydropyran analogue **10** was more potent than the dihydrocholesteryl tetrahydropyran analog **11**.

For further investigation of the cell protective effect of analogues **1** and **10**, their anti-apoptotic effect on cell viability in human retina endothelial cells (HRECs) was examined. In addition, their dose-dependent activities were confirmed by the cell survival counting assay, which was performed at 0.1 μg/ml to 10 μg/ml, respectively. The concentration-dependent cell viabilities by both analogues **1** and **10** are well revealed in Figure 3.

The anti-apoptotic effects of analogues **1** and **10** were further confirmed by their inhibitory effects on nuclear DNA condensation and fragmentation in VECs, which were analyzed by

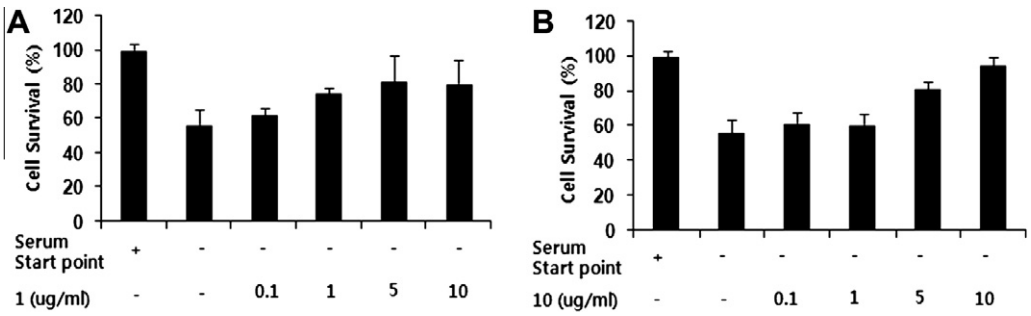


Figure 3. Inhibitory effects by analogs **1,** and **10** on apoptosis of HRECs. (A) Treatment with analog **1** at sequential concentration from 0.1 to 10 μg/ml for 48 h. (B) Treatment with analog **10** at sequential concentration from 0.1 to 10 μg/ml for 48 h.

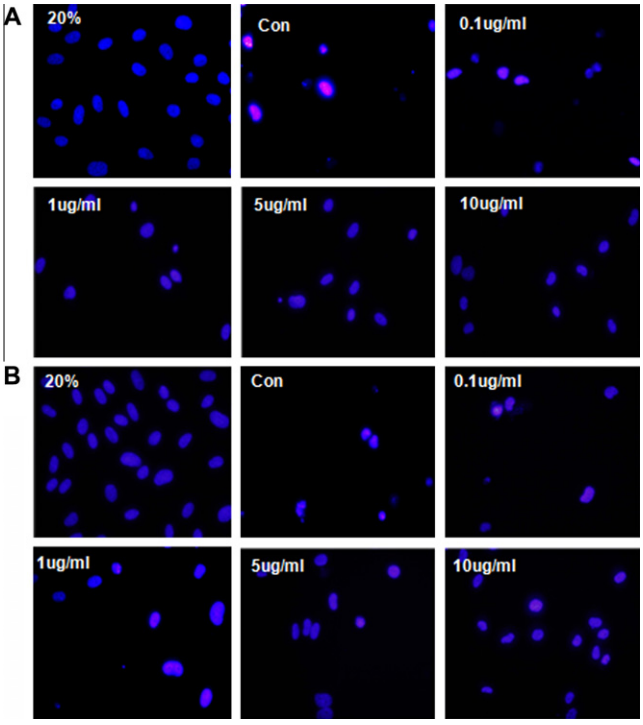


Figure 4. Changes in nuclear morphology accessed by DAPI staining (A) Cells treated with **1** of various concentrations for 48 h. (B) Cells treated with **10** of various concentrations for 48 h.

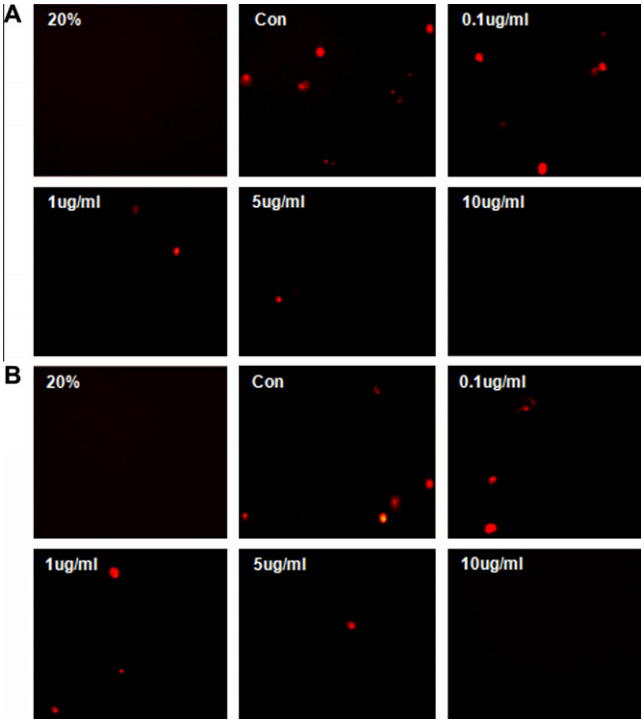


Figure 5. TUNEL assays at various concentrations of **1** and **10**. (A) Cells treated with **1** of various concentrations for 48 h. (B) Cells treated with **10** of various concentrations for 48 h.

4′-6-diamino-2-phenylindole (DAPI) staining and TUNEL assay. In consistent with the MTT assay results, the nuclear staining of HECs with DAPI revealed increased cell survivals by **1** or **10** after 48 h of serum deprivation. TUNEL assay specifically detecting DNA fragmentation also revealed that analogues **1** and **10** suppressed the apoptotic cell death induced by serum deprivation in a dose-dependent manner (Figs. 4 and 5).

In conclusion, we identified a series of potent anti-apoptotic agents derived from easily accessible cholesterol. In particular, analogues **1** and **10** possessing the cholesterol scaffold and the cyclic carbohydrate equivalents exhibited excellent cell survival activities, which are equipotent to that of ginsenoside Rk1. In addition, these analogues exhibited dose-dependent cell viability on HREC line. The excellent human endothelial cell protective activities of our novel Rk1-based anti-apoptotic agents envisage its valuable application for treatment of vascular disorders. Currently, systematic studies on their therapeutic applications including in vivo test of the potent cholesterol analogs as well as their mechanistic aspects are in good progress.

Acknowledgement

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