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1,2,4-Triazinylmethylphenyl Glucoside as Novel *C*-Aryl Glucoside SGLT2 Inhibitors†

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Novel *C*-aryl glucoside SGLT2 inhibitors containing triazine motif were designed and synthesized for biological evaluation. Among the compounds assayed, triazine containing methoxy moiety **18** demonstrated the best *in vitro* inhibitory activity against *h*SGLT2 in this series to date (IC_{50} = 24.9 nM).

Key Words : SGLT, Dapagliflozin, Triazine, Glucoside, Diabetes

Introduction

Diabetes has become an increasing concern to the world's population. In 2007, approximately 246 million people were considered diabetic, with an additional 7 million people diagnosed with the disease every year. Type 2 diabetes is the most common disorder of glucose homeostasis, answering for almost 90-95% of all cases of diabetes.

Sodium-dependent glucose cotransporters (SGLTs) couple the transport of glucose against a concentration gradient with the simultaneous transport of Na^+ down a concentration gradient. It is believed that 90% of renal glucose reuptake is facilitated by SGLT2.[1]

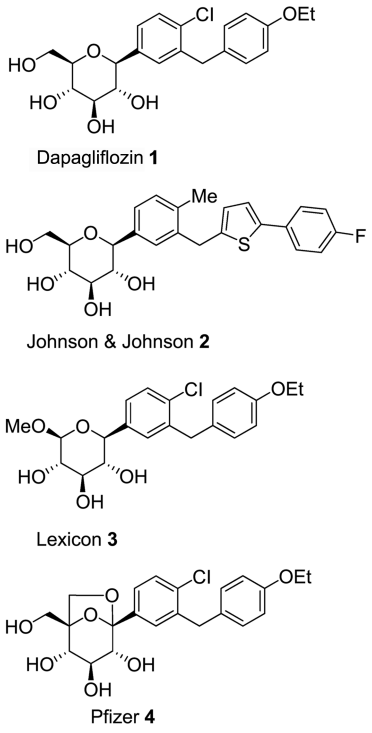


Figure 1. Structures of *C*-aryl glucoside SGLT2 inhibitors.

Bristol-Myers Squibb has identified dapagliflozin **1**, a potent, selective SGLT2 inhibitor for the treatment of type 2 diabetes (Figure 1). Currently, dapagliflozin is the most advanced SGLT2 inhibitor in clinical trials. On the other hand, Mitsubishi Tanabe, in collaboration with Johnson & Johnson, is developing canagliflozin **2**, another novel *C*-glucoside-derived SGLT2 inhibitor. In addition, several compounds originated from Boehringer Ingelheim, Lexicon, Astellas, and Pfizer are reported to be in various phase of clinical trials, respectively.

In the present study, *C*-glucosides bearing a heteroaromatic ring were exploited in order to develop novel SGLT2 targeting antidiabetic agents. We envisioned that replacement of the distal ring of dapagliflozin **1** with a heterocyclic ring might improve the physicochemical properties, thereby providing a high possibility for clinical development. Along this line, the structure of dapagliflozin was modified into proposed structure **5**, bearing a 1,2,4-triazine or fused 1,2,4-triazine ring as shown in Figure 2. Herein, we report the design, synthesis and biological evaluation of triazinylmethylphenyl glucoside congeners.

Experimental

NMR spectra were obtained on a Varian 400-MR (400 MHz ^1H, 100 MHz ^{13}C) spectrometer. NMR spectra were

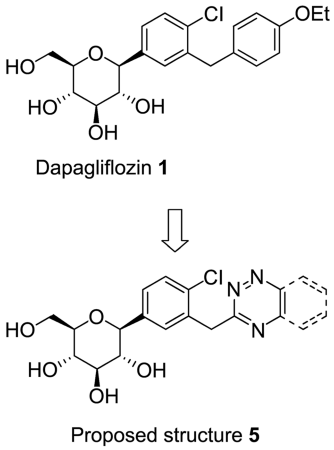


Figure 2. Exploration of distal ring.

†This paper is dedicated to Professor Eun Lee on the occasion of his honourable retirement.

recorded in ppm (δ) relative to tetramethylsilane (δ = 0.00) as an internal standard unless stated otherwise and are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, m = multiplet, and br = broad), coupling constant, and integration. Mass spectra were obtained with an Agilent 6110 quadruple LC-MSD (ESI+).

(2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-Chloro-3-((7-methylbenzo[e][1,2,4]triazin-3-yl)methyl)phenyl)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (11). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.46 (m, 2H), 7.42-7.31 (m, 3H), 7.05 (d, J = 8.8 Hz, 1H), 4.33 (s. 2H), 4.10 (d, J = 9.6 Hz, 1H), 3.88-3.84 (m, 1H), 3.68 (dd, J = 12.1, 9.9 Hz, 1H), 3.48-3.26 (m, 6H), 2.43 (s, 3H). MH + 455 (Na adduct).

(2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-Chloro-3-((5-phenyl-1,2,4-triazin-3-yl)methyl)phenyl)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (18). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.38 (m, 1H), 7.30-7.22 (m, 2H), 7.12 (d, J = 7.6 Hz, 1H), 4.41 (d, J = 2.4 Hz, 2H), 4.23 (s, 3H), 4.15 (d, J = 9.6 Hz, 1H), 3.85-3.79 (m, 1H), 3.61 (dd, J = 12.1, 9.9 Hz, 1H), 3.49-3.29 (m, 6H). MH + 420 (Na adduct).

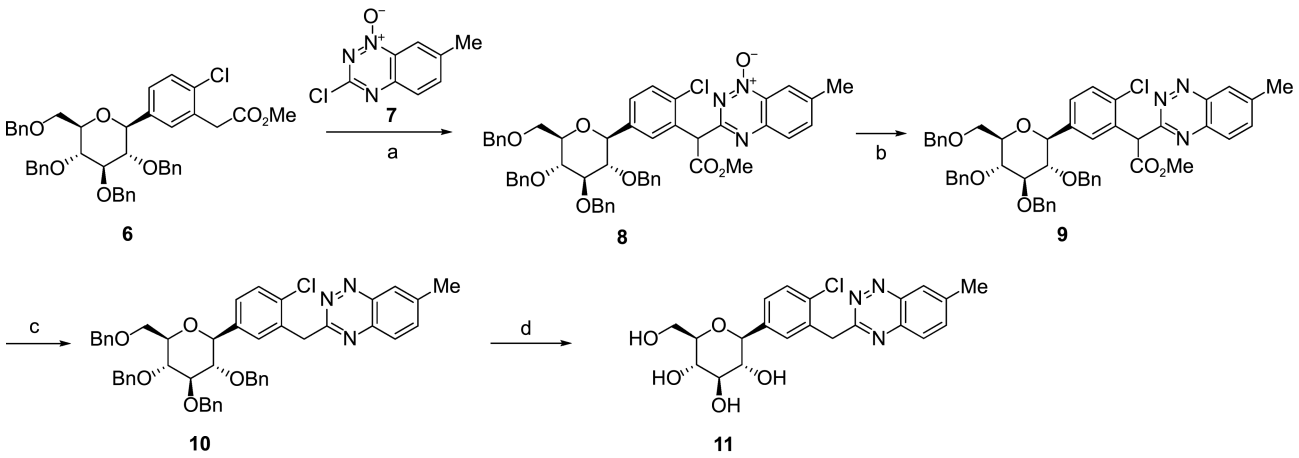
Cloning and Cell Line Construction for Human SGLT2. Human SGLT2 (*h*SGLT2) gene was amplified by PCR from cDNA-Human Adult Normal Tissue Kidney (Invitrogen, Carlsbad, CA). The hSGLT2 sequence was cloned into pcDNA3.1(+) for mammalian expression and were stably transfected into Chinese hamster ovary (CHO) cells. SGLT2-expressing clones were selected based on resistance to G418 antibiotic (Geneticin®, Invitrogen, Carlsbad, CA) and activity in the ^{14}C-α-methyl-D-glucopyranoside (^{14}C-AMG) uptake assay.

Inhibitory Effects on Human SGLT2 Activities. For sodium-dependent glucose transport assay, cells expressing *h*SGLT2 were seeded into a 96-well culture plate at a density of 5×10^4 cells/well in RPMI medium 1640 containing 10% fetal bovine serum. The cells were used 1 day after plating. They were incubated in pretreatment buffer (10 mM HEPES, 5 mM Tris, 140 mM choline chloride, 2 mM KCl, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4) at 37 °C for 10 min. They were then incubated in uptake buffer (10 mM HEPES, 5 mM Tris, 140 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM ^{14}C-nonlabeled AMG pH 7.4) containing ^{14}C-labeled (8 uM) and inhibitor or dimethyl sulfoxide (DMSO) vehicle at 37 °C for 2 h. Cells were washed twice with washing buffer (pretreatment buffer containing 10 mM AMG at room temperature) and then the radioactivity was measured using a liquid scintillation counter. IC_{50} was determined by nonlinear regression analysis using GraphPad PRISM.

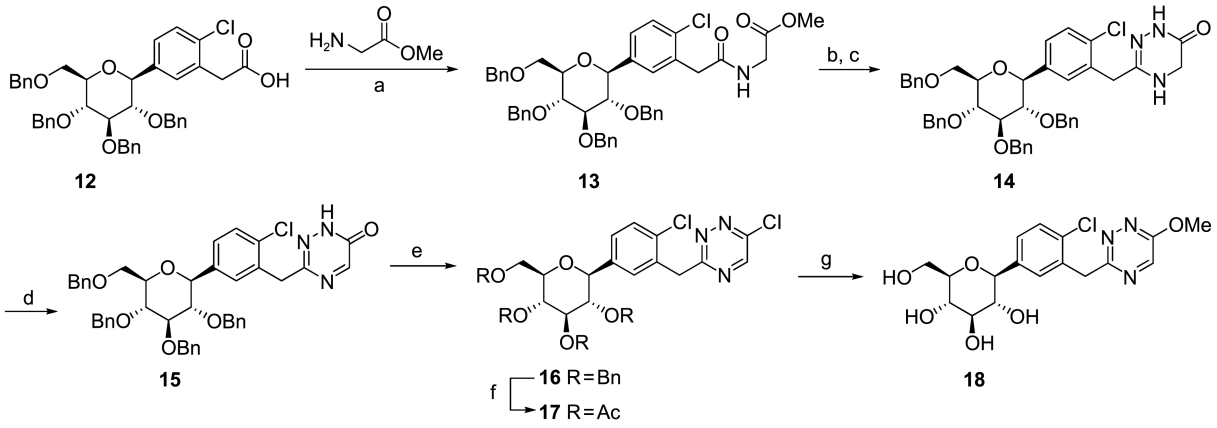
Results and Discussion

Preparation of the benzotriazine compound is described in Scheme 1. Thus, coupling of ester **6**[2] and 3-chloro-7-methylbenzo[*e*][1,2,4]triazine 1-oxide (**7**)[3,4] using NaH in the presence of DMF (*N,N*-dimethylformamide) produced the corresponding ester **8** in moderate yield (25%, 65% based on starting material recovered), which was reduced using Zn and NH_4Cl in an aqueous solution of dioxane to generate the ester **9** in 75% yield. Hydrolysis and concomitant decarboxylation using NaOH in a refluxed aqueous solution of methanol and THF (tetrahydrofuran) generated **10** in 73% yield. At last, total removal of benzyl groups using iodotrimethylsilane in the presence of CH_3CN at ambient temperature produced the target benzotriazine **11** in 52% yield over two steps.

We next extended our synthetic efforts for preparation of a novel triazine analog as shown in Scheme 2. Thus, previously reported carboxylic acid **12**[2] was coupled with methyl 2-aminoacetate hydrochloride in the presence of EDCI (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide), HOBt (1-hydroxybenzotriazole), and NMM (*N*-methylmorpholine) to provide the corresponding amide **13** in quantitative yield. Amide **13** smoothly underwent hydrazinolysis and subsequent cyclization to afford **14** in 77% yield over two steps. Oxidation of **14** with DDQ (2,3-dichloro-5,6-dicyano-*p*-benzoquinone) in CH_2Cl_2, and subsequent treatment of the resulting **15** with $POCl_3$ in toluene at 70 °C produced the key intermediate **16** in 35% yield over two steps. The remaining



Scheme 1. Reagents and conditions: (a) NaH (2 eq), DMF, rt, 25% yield 65% starting material recovered; (b) Zn (10 eq), NH_4Cl (10 eq), aq dioxane, rt, 75%; (c) NaOH (4 eq), THF/H_2O/MeOH (1/1/1), reflux, 73%; (d) TMSI (6 eq), CH_3CN, rt, 52%.



Scheme 2. Reagents and conditions: (a) EDCI, HOBT, NMM, CH_2Cl_2; (b) hydrazine, MeOH, 60 °C; (c) NH_4OAc, AcOH, reflux; 77% overall yields (2 steps); (d) DDQ, CH_2Cl_2; (e) $POCl_3$, toluene, 70 °C, 35% overall yield (2 steps); (f) TMSOTf (4 eq), Ac_2O (6 eq), CH_2Cl_2, 56%; (g) NaOMe, MeOH, 94%.

tasks involve introduction of methoxy group at C-6 position of triazine and deprotection of benzyl groups on glucose ring. Initial attempt to install methoxide and subsequent removal of benzyl groups using BCl_3 turned out to be troublesome. However, use of TMSOTf (trimethylsilyl trifluoromethanesulfonate) in combination with acetic anhydride uneventfully provided the peracetate **17**. Subsequently, introduction of methoxy group at C-6 position of triazine and removal of acetates on glucose proceeded smoothly ($NaOCH_3$, CH_3OH) to provide the target triazine **18** in one-pot reaction in a high yield (94%).

The cell-based SGLT2 AMG (Methyl-α-D-glucopyranoside) inhibition assay was performed to evaluate the inhibitory effects of all prepared compounds on *h*SGLT2 activities.[5,6] While the parent dapagliflozin **1** shows highly potent inhibitory activity against *h*SGLT2 (IC_{50} = 0.49 nM), neither of the analog structure results in better inhibitory activity against *h*SGLT2 (IC_{50} = 491 nM for **11** *vs* IC_{50} = 24.9 nM for **18** via in-house assay).[7] Based on this *in vitro* SGLT2 inhibitory activity data, it is apparent that the nitrogen atoms in the triazinyl moiety are not contributing positively to the inhibitory activity of triazinyl analogs of dapagliflozin. Especially, bicyclic benzotriazine reduces the inhibitory activity against *h*SGLT2 in two orders of magnitude, suggesting that flat and rigid bicyclic moiety is not tolerant at this region.

Conclusion

In summary, metabolically more stable *C*-glucosides bearing triazine ring as a potential antidiabetic agent was exploited. Among the compounds tested, triazine containing methoxy moiety **18** showed the better *in vitro* inhibitory activity against *h*SGLT2 in this series to date (IC_{50} = 24.9 nM). Replacement of the distal ring of dapagliflozin with a triazine ring appears to weaken in vitro inhibitory activity against *h*SGLT2 in an order of magnitude. However, the information obtained from this triazine series should help to design more effective SGLT2 inhibitors that are structurally related.

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7. Each compound **11** or **18** is one of the representatives in the series of triazine that we prepared. Although the other compounds were not mentioned due to company's policy, all of the prepared triazine derivatives showed IC_{50} values, ranging between 24.9~833 nM.