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Exploration of SAR regarding glucose moiety in novel *C*-aryl glucoside inhibitors of SGLT2

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ABSTRACT

In order to investigate SAR regarding glucose moiety in novel *C*-aryl glucoside SGLT2 inhibitors containing a thiazole motif, a series of chemical modifications on glucose was conducted to explore potential utility as a suitable replacement of glucose per se. Among the compounds prepared, deshydroxy **29** (IC_{50} = 7.01 nM) demonstrated the best in vitro inhibitory activity against SGLT2 in this series to date. But, none of the compounds were better than the parent molecule **5** (IC_{50} = 1.75 nM).

Diabetes has become an increasing concern to the world's population. In 2010, approximately 285 million people around the world will have diabetes, corresponding to 6.4% of the world's adult population, with a prediction that the number of people with diabetes will have grown to 438 million by 2030.[1] Type 2 diabetes is the most common disorder of glucose homeostasis, accounting for approximately 90–95% of all cases of diabetes.[2]

Sodium-dependent glucose cotransporters (SGLTs) couple the transport of glucose against a concentration gradient with the simultaneous transport of Na^+ down a concentration gradient.[3] It is estimated that 90% of renal glucose reabsorption is facilitated by SGLT2.[4]

Bristol-Myers Squibb has identified dapagliflozin **1**, a potent, selective SGLT2 inhibitor for the treatment of type 2 diabetes.[5–7] At present, dapagliflozin **1** is the most advanced SGLT2 inhibitor in clinical trials.[8] On the other hand, Johnson & Johnson (**2**), Lexicon (**3**), and Pfizer (**4**) are reported to be in various phase of clinical trials (Fig. 1).[9]

In the previous study, C-glucosides bearing a heterocycle ring were exploited in order to develop novel SGLT2 targeting antidiabetic agents, since we envisioned that replacement of the distal or proximal ring of dapagliflozin **1** with a heterocyclic ring might improve the overall physicochemical properties of SGLT2 inhibitors.[10] Based on the structure of dapagliflozin, the distal ring was modified into thiazole. A series of lead optimization efforts led to the discovery of thiazole **5** bearing a furanyl moiety as shown in Figure 2.[11] In the present study, a variety of modifications, especially C-6 position on glucose (as shown in **6**) were conducted to establish SAR on carbohydrate pharmacophore based on structure of potent thiazole **5**.[12] Along this line, we report the synthesis and biological evaluation of thiazolylmethylphenyl glucoside congeners herein.

Preparation of the thiazole compound is described in Scheme 1. Thus, previously reported carboxylic acid **7**[10a,b] was coupled with 2-amino-1-(furan-2-yl)ethanone hydrochloride in the presence of EDCI (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide), HOBt (1-hydroxybenzotriazole), and NMM (4-methylmorpholine) to provide the corresponding amide **9** in 82% yield. Amide **9** smoothly underwent requisite cyclization by the action of Lawesson's reagent in refluxing THF (tetrahydrofuran) to result in thiazole **10**. Total removal of benzyl protections was accomplished with BCl_3 at 0 °C to produce a target compound **5** in 70% yield (Scheme 1).

As shown in Scheme 2, functionalization was initiated with alcohol **11**, which was prepared from **10** via a selective debenzylation.[13] Oxidation of the alcohol **11** to aldehyde **12** was achieved through Dess–Martin periodinane.[14] Aldehyde **12** turned out to be a versatile intermediate. This aldehyde was treated with DAST (diethylaminosulfur trifluoride)[15] to provide the corresponding difluoromethyl **13**. Aldehyde **12** was also treated with a Grignard reagent to provide the corresponding alcohols (**14**–**16**). Alternatively, aldehyde **12** was further oxidized to acid **17** by use of sodium chlorite and monobasic potassium phosphate in aqueous *t*-BuOH. Acid **17** was transformed into ester **18** by trimethylsilyldiazomethane. Ester **18** was then treated with excess Grignard reagent to tertiary alcohol **19**. Ester **18** also smoothly underwent

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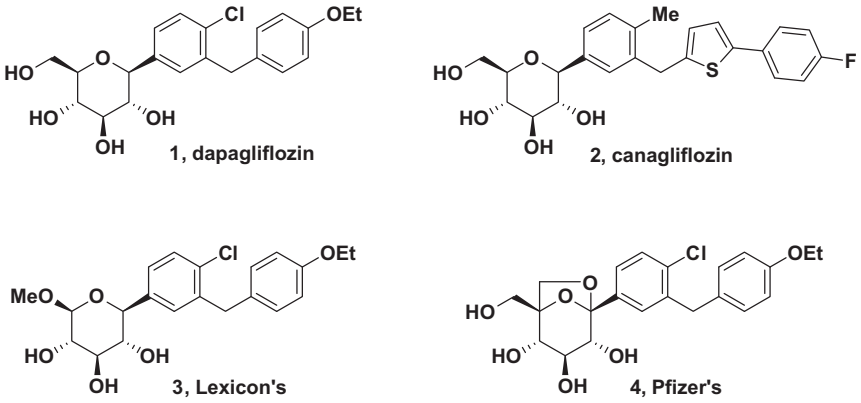


Figure 1. Structures of *C*-aryl glucoside SGLT2 inhibitors.

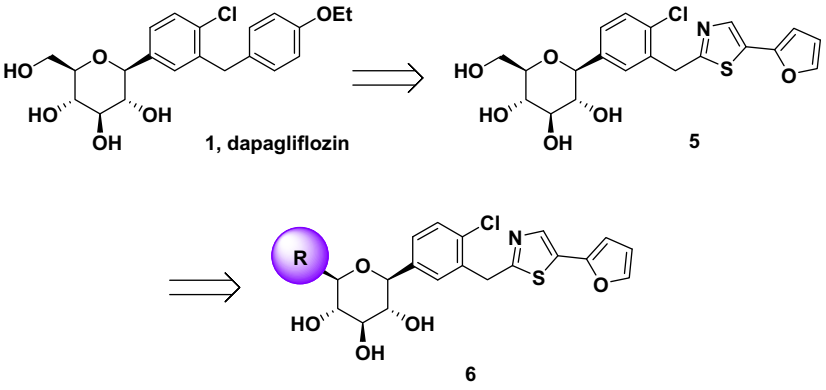
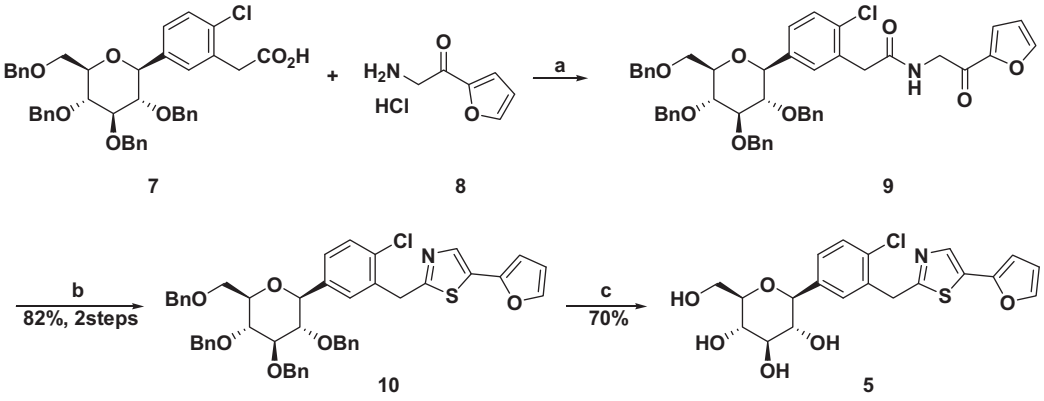


Figure 2. Exploration of C-6 position of glucose moiety in novel C-glucoside bearing thiazole **5**.

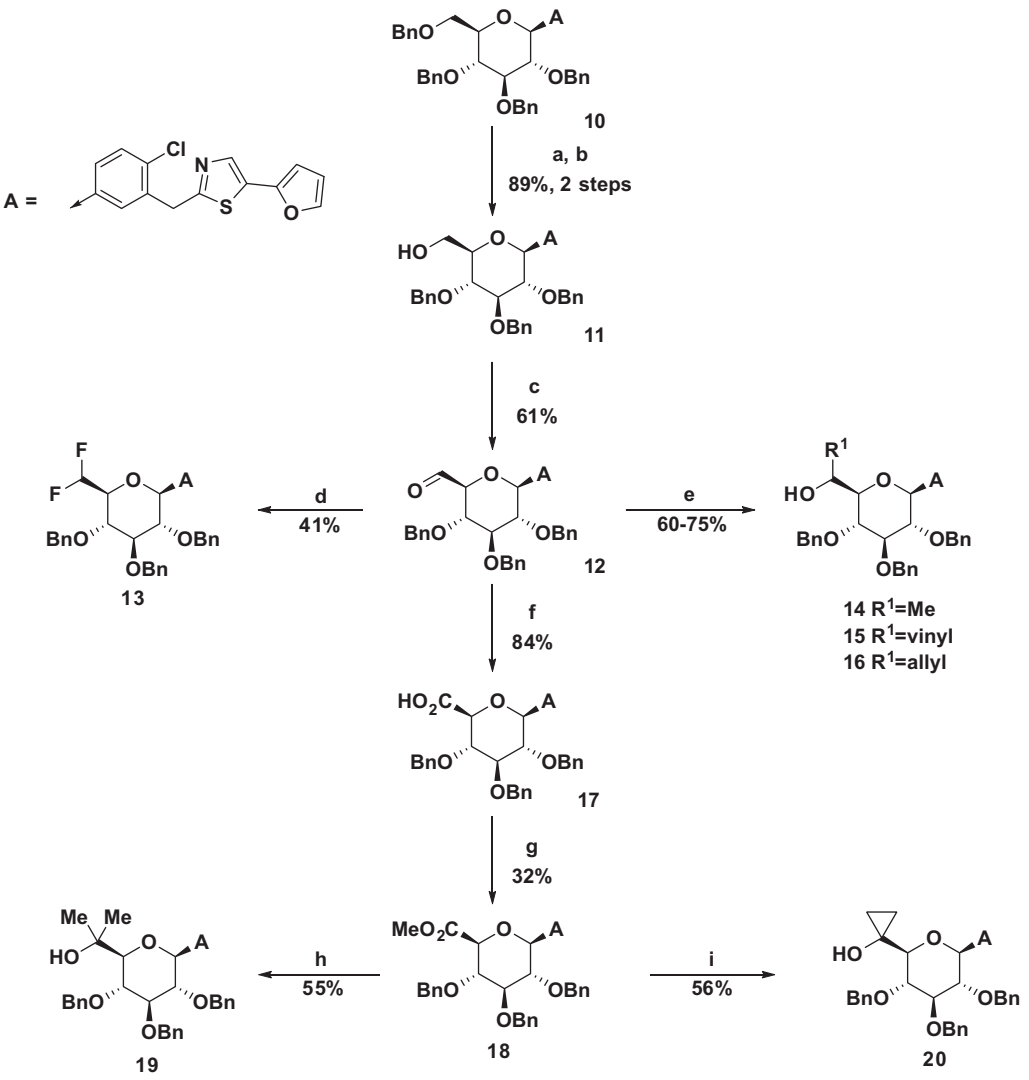


Scheme 1. Reagents and conditions: (a) EDCI, HOBt, NMM, DMF, rt; (b) Lawesson's reagent, THF, 85 °C; (c) BCl_3(1 M in CH_2Cl_2), CH_2Cl_2, 0 °C.

Kulinkovich cyclopropanation to generate the corresponding cyclopropanol **20**.[16] Finally, deprotection of benzyl groups was accomplished with BCl_3 at 0 °C to produce the corresponding target compounds as previously described.

Alternatively, free alcohol form **5** was utilized to provide various glucose analogs as shown in Scheme 3. Thus, **5** was selectively tosylated with tosyl chloride in the presence of 2,6-lutidine to produce tosylate **21**. Diverse nucleophiles were used to generate the glucose analogs as shown in Scheme 3. For example, compounds containing 1,2,4-triazole (**26**) or tetrazole (**27**, **28**) were obtained by treating tosylate **21** with 1,2,4-triazole or tetrazole in the presence of a suitable base such as cesium carbonate or triethylamine, respectively. Also iodide **24**, which was prepared by treatment of tosylate **21** with NaI, was reduced to **29** by action of hydrogen in the presence of catalytic Raney-Nickel.[17]

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.[18,19] Exploration of the SAR began by removing C-6 hydroxyl group at the glucose of 4-chloro-3-((5-(furan-2-yl)thiazol-2-yl)methyl)phenyl **5**. Table 1 shows the structure–activity relationship upon alteration of the C-6 substituent at the glucose employing only the β-anomer. For instance, deshydroxy **29** showed fourfold decrease of inhibitory activity (**29**, IC_{50} = 7.01 nM), compared with parent **5** (IC_{50} =



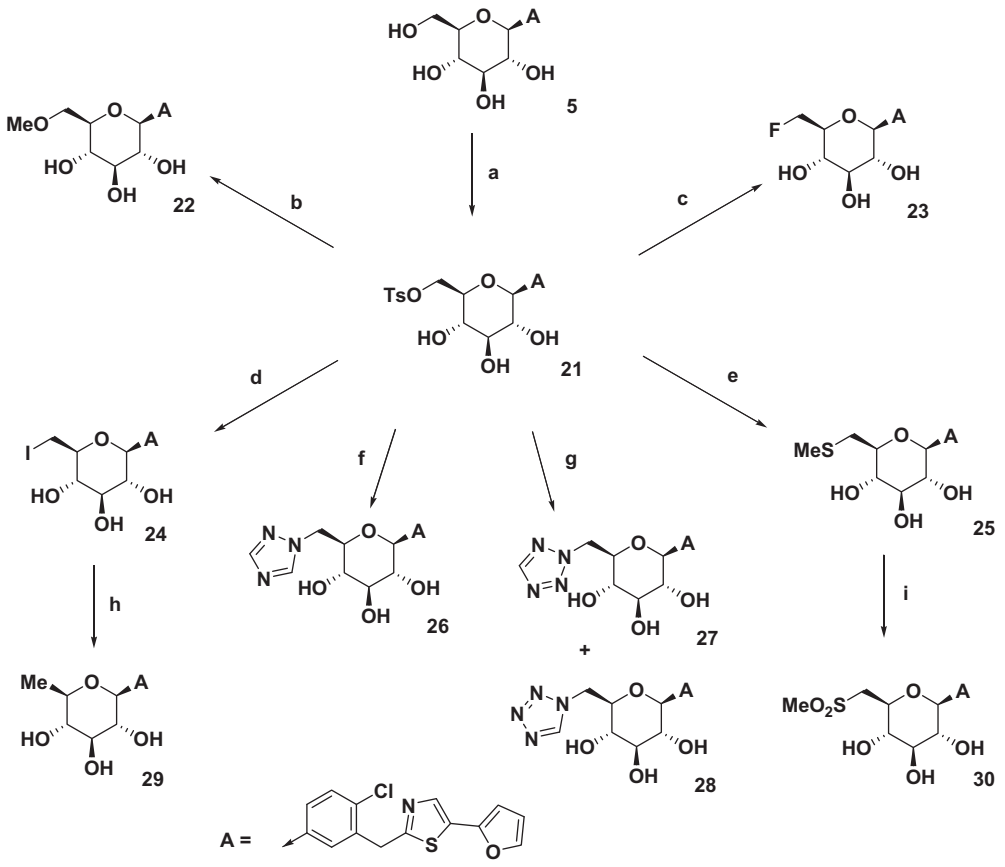
Scheme 2. Reagents and conditions: (a) TMSOTf, Ac_2O, CH_2Cl_2, −55 °C, 1 h; (b) NaOMe, MeOH, rt, 2 h; (c) Dess–Martin Periodinane, CH_2Cl_2, rt, 2 h; (d) DAST, CH_2Cl_2, −78 °C to rt, 15 h; (e) R^1MgBr, THF, 0 °C, 3 h; (f) KH_2PO_4, $NaClO_2$, 2-methyl-2-butene, aq *t*-BuOH, rt, 15 h; (g) $TMSCH_2N_2$, CH_2Cl_2, 0 °C to rt, 15 h; (h) MeMgCl, THF, 0 °C, 3 h; (i) EtMgCl, $Ti(Oi\text{-}Pr)_4$, THF, 0 °C, 3 h.

1.75 nM). Addition of an extra methyl group at C-6 glucose deteriorated activity by 8- to 14-fold, dependent on the stereochemistry at C-6 on glucose (**32a**, IC_{50} = 13.5 nM; **32b**, IC_{50} = 23.8 nM). This effect was also observed as the vinyl group was introduced at the position as exemplified in **33a** and **33b** (**33a**, IC_{50} = 54.9 nM; **33b**, IC_{50} = 54.8 nM). This adverse steric effect became even more evident as *gem*-dimethyl group was introduced at the position as exemplified in **36** (**36**, IC_{50} = 89.5 nM). Cyclopropyl group, which is rigid and compact, demonstrates twofold increase of inhibitory activity against *h*SGLT2 (**37**, IC_{50} = 48.9 nM), but it is still threefold less active than monosubstituted compound **32a** (**32a**, IC_{50} = 13.5 nM), likely suggesting that increased steric crowdedness is not favorable in the region. Carbonyl groups on the glucose were also explored. Ester **35** showed 30-fold decrease in inhibitory activity against *h*SGLT2 (**35**: IC_{50} = 59.1 nM), likewise ketone **38** showed even 60-fold decrease in activity, implying that sp^2 character at this particular position is not that favorable. Fluorides appear to be tolerated to some degree and demonstrate remote possibility for replacement of the hydroxyl group (IC_{50} = 10.6 nM for monofluoride **23** and IC_{50} = 9.55 nM for difluoride **31**).

Next, a series of simple alkylated compounds was explored (**22**, **39**, **40**, Table 1). This series exhibited decrease in inhibitory activities against *h*SGLT2 compared with the parent compound **5**. For instance, methyl ether **22** (IC_{50} = 23.5 nM) showed 13-fold decrease in activity than the parent **5** (IC_{50} = 1.75 nM). Ethyl ether **39** even further reduced inhibitory activity against *h*SGLT2 (**39**, IC_{50} = 68.7 nM). Thioethers appear to be slightly more active than the ether counterparts as exemplified in **25** and **41** (**25**, IC_{50} = 18.6 nM; **41**, IC_{50} = 31.9 nM). However, oxidation of methyl thioether **25** to sulfone **30** demonstrated significant loss of activity such as two orders of magnitude (**30**, IC_{50} = 1450 nM), implying that sulfonyl functionality in the region is not tolerated. Additionally, substitution with triazole (**26**), tetrazole (**27**, **28**) was performed. These analogs showed moderate inhibitory activity against *h*SGLT2, but none improved potency relative to the simple glucose **5**. Among the azole compounds, tetrazoles proved to be more potent than triazole as shown in Table 1, implying that an extra nitrogen in tetrazole ring might be involved in shaping molecule more compact, thereby improving inhibitory activity.

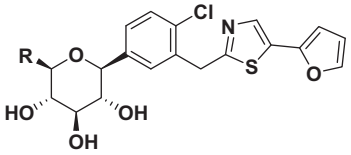
Overall, modifications of the C-6 on glucose appear to weaken in vitro inhibitory activity against *h*SGLT2 perhaps for unfavorable steric environments at the glucose ring position.

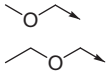
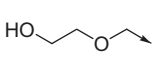
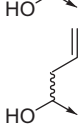
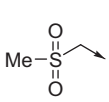
In summary, a variety of modifications at the C-6 position on glucose was conducted in the present study to establish SAR on



Scheme 3. Reagents and conditions: (a) TsCl, 2,6-lutidine, rt, 15 h, 78%; (b) NaOMe, MeOH, rt, 15 h, 36%; (c) KF, 1,2-ethanediol, 100 °C, 20 h, 11%; (d) NaI, 2-butanone, 80 °C, 2 h, 48%; (e) NaSMe, DMF, rt, 4 h, 45%; (f) 1,2,4-triazole, Cs_2CO_3, DMF, rt, 3 d, 14%; (g) tetrazole, Et_3N, DMF, 80 °C, 3 days, 24%; (h) H_2, cat. Raney-Nickel, Et_2NH, MeOH, rt, 1 d, 31%; (i) *m*CPBA, CH_2Cl_2, 0 °C, 1 h, 66%.

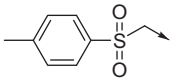
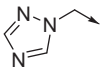
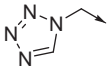
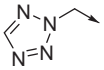
Table 1
In vitro inhibitory activity against *h*SGLT2



Compound	R	hSGLT2 IC_{50} (nm)	Compound	R	hSGLT2 IC_{50} (nm)
1 Dapagliflozin	–	0.49 ± 0.04	**23**		10.6
29		7.01	**31**		9.55
5		1.75	**22**		23.5
32a		13.5	**39**		68.7
32b		23.8	**40**		30.9
33a		54.9	**25**		18.6
33b		54.8	**41**		31.9
34		20.8	**30**		1450

(*continued on next page*)

Table 1 (*continued*)

Compound	R	hSGLT2 IC_{50} (nm)	Compound	R	hSGLT2 IC_{50} (nm)
36		89.5	**21**		156
37		48.9	**26**		471
35		59.1	**28**		68.9
38		110	**27**		106

carbohydrate pharmacophore based on structure of potent thiazole **5**. Among the compounds tested, deshydroxy **29** or difluoride **31** appear to be tolerated at this position to a degree, but none improved potency against *h*SGLT2.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.115.

References and notes

1. International Diabetes Federation *Diabetes Atlas*, 4th ed.; International Diabetes Federation: Montreal, Canada, 2009.
2. Dwarakanathan, A. *J. Insur. Med.* **2006**, *38*, 20.
3. Mackenzie, B.; Loo, D. D. *J. Biol. Chem.* **1996**, *20*, 32678.
4. Moe, O. W.; Berry, C. A.; Rector, F. C. In *The Kidney*; Brenner, B. M., Rector, F. C., Eds., 5th ed.; WB Saunders Co.: Philadelphia, 2000; p 375.
5. Ellsworth, B. A.; Meng, W.; Patel, M.; Girotra, R. N.; Wu, G.; Sher, P.; Hagan, D.; Obermeier, M.; Humphreys, W. G.; Robertson, J. G.; Wang, A.; Han, S.; Waldron, T.; Morgan, N. N.; Whaley, J. M.; Washburn, W. N. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4770.
6. Meng, W.; Ellsworth, B. A.; Nirschl, A. A.; McCann, P. J.; Patel, M.; Girotra, R. N.; Wu, G.; Sher, P. M.; Morrison, E. P.; Biller, A. A.; Zahler, R.; Deshpande, P. P.; Pullockaran, A.; Hagan, D. L.; Morgan, N.; Taylor, J. R.; Obermeier, M. T.; Humphreys, W. G.; Khanna, A.; Discenza, L.; Robertson, J. G.; Wang, A.; Han, S.; Wetterau, J. R.; Janovitz, E. B.; Flint, O. P.; Whaley, J. M.; Washburn, W. N. *J. Med. Chem.* **2008**, *51*, 1145.
7. Washburn, W. N. *J. Med. Chem.* **2009**, *52*, 1785.
8. According to a report from Business Wire on June 26, **2010**, dapagliflozin as add on therapy to insulin demonstrated improved glycemic control in patients with type 2 diabetes inadequately controlled with insulin.
9. (a) Nomura, S.; Sakamaki, S.; Hongu, M.; Kawanshi, E.; Koga, Y.; Sakamoto, T.; Yamamoto, Y.; Ueta, K.; Kimata, H.; Nakayama, K.; Tsuda-Tsukimoto, M. *J. Med. Chem.* **2010**, *53*, 6355; (b) Lexicon Pharmaceuticals, www.clinicaltrials.gov, 2009, NCT00962065.; (c) Mascitti, V. Presented at the 240th American Chemical Society National Meeting and Exposition, Boston, MA, August 2010; CARB 90.
10. (a) Lee, J.; Lee, S.-H.; Seo, H. J.; Son, E.-J.; Lee, S. H.; Jung, M. E.; Lee, M.; Han, H.-K.; Kim, J.; Kang, J.; Lee, J. *Bioorg. Med. Chem.* **2010**, *18*, 2178; (b) Kim, M. J.; Lee, J.; Kang, S. Y.; Lee, S.-H.; Son, E.-J.; Jung, M. E.; Lee, S. H.; Song, K.-S.; Lee, M.; Han, H.-K.; Kim, J.; Lee, J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3420; (c) Kang, S. Y.; Song, K.-S.; Lee, J.; Lee, S.-H.; Lee, J. *Bioorg. Med. Chem.* **2010**, *18*, 6069; (d) Lee, J.; Kim, J. Y.; Choi, J.; Lee, S.-H.; Kim, J.; Lee, J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7046.
11. Song, K.-S.; Lee, S. H.; Kim, M.; Seo, H.; Lee, J.; Lee, S.-H.; Jung, M. E.; Son, E. J.; Lee, M.; Kim, J.; Lee, J. *ACS Med. Chem. Lett.* doi:10.1021/ml100256c.
12. Modifications at other positions on glucose including C-2, C-3, C-4, and C-5 appear to devastate in vitro inhibitory activity against *h*SGLT2. See: (a) Washbrun, W. N. *Exp. Opin. Ther. Patents* **2009**, *19*, 1485; (b) Honda, T.; Oguchi, M.; Yoshida, M.; Okuyama, R.; Ogata, T. Abe, M.; Ueda, K.; Ohsumi, J.; Izumi, M. Patent EP2048105; 2009.
13. Kobertz, W. R.; Bertozzi, C. R.; Bednarski, M. D. *J. Org. Chem.* **1996**, *61*, 1894.
14. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.
15. Walba, D. M.; Razavi, H. A.; Clark, N. A. *J. Am. Chem. Soc.* **1988**, *110*, 8686.
16. (a) Wolan, A.; Six, Y. *Tetrahedron* **2010**, *66*, 3097; (b) Lee, J.; Kang, C. H.; Kim, H.; Cha, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 291.
17. Hanessian, S.; Simard, B. D.; Simard, D. *Tetrahedron* **2009**, *65*, 6656.
18. For 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 *h*SGLT2 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.
19. 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 μM) 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.[20,21]
20. Han, S.; Hagan, D. L.; Taylor, J. R.; Xin, L.; Meng, W.; Biller, S. A.; Wetterau, J. R.; Washburn, W. N.; Whaley, J. M. *Diabetes* **2008**, *57*, 1723.
21. Katsuno, K.; Fujimori, Y.; Takemura, Y.; Hiratochi, M.; Itoh, F.; Komatsu, Y.; Fujikura, H.; Isaji, M. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 323.