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Pyrimidinylmethylphenyl glucoside as novel *C*-aryl glucoside SGLT2 inhibitors

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ABSTRACT

Novel *C*-aryl glucoside SGLT2 inhibitors containing pyrimidine motif were designed and synthesized for biological evaluation. Among the compounds assayed, pyrimidine containing methylthio moiety **11g** demonstrated the best in vitro inhibitory activity against SGLT2 in this series to date (IC_{50} = 10.7 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 by 2030 the number of people with diabetes will have grown to 438 million.[1] Type 2 diabetes is the most common disorder of glucose homeostasis, accounting for nearly 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, a potent, selective SGLT2 inhibitor for the treatment of type 2 diabetes.[5–7] At present, dapagliflozin is the most advanced SGLT2 inhibitor in clinical trials.[8] On the other hand, Mitsubishi Tanabe, in collaboration with Johnson and Johnson, is developing canagliflozin, another novel C-glucoside-derived SGLT2 inhibitor.[9] In addition, Boehringer Ingelheim, Lexicon, Astellas, and Pfizer are reported to be in various phases of clinical trials (Fig. 1).[10]

In the present study, C-glucosides bearing a heteroaromatic ring were exploited in order to develop novel SGLT2 targeting antidiabetic agents with better biological activities and pharmacological properties than dapagliflozin.[11] Along this line, the structure of dapagliflozin was modified into **A** or **B**, bearing a pyrimidine ring as shown in Figure 2. Herein, we report the design, synthesis and biological evaluation of pyrimidinylmethylphenyl glucoside congeners.

Preparation of the pyrimidine compound is described in Scheme 1. Thus, a mixture of alcohols **1**[11a,b] was converted to the required beta-isomer **2** using three steps involving benzoylation, separation through recrystallization from isopropyl alcohol, and subsequent hydrolysis with lithium hydroxide in aqueous mixture of methanol–THF in about 60% yield. The alcohol **2** thus obtained was converted to bromide **3** using PBr_3 in the presence of a catalytic amount of pyridine in quantitative yield. Suzuki–Miyaura coupling[12] of the key intermediate bromide **3** with boronic acid such as 2-(methylthio)pyrimidin-5-ylboronic acid (**4**)[13] generated pyrimidinylmethylphenyl compound **5** in quantitative yield. Subsequent deprotection of the four benzyl groups using BBr_3 in methylene chloride produced the target compound **6k** in approximately 40% yield.

We next extended a method previously adopted by us[11b] for preparation of novel pyrimidinyl analogs as shown in Scheme 2. Thus, coupling of ester **7**[11a,b] with 5-bromo-2-chloropyrimidine (**8**) using NaH in the presence of DMF produced the corresponding ester, which was hydrolyzed and decarboxylated using LiOH in an aqueous solution of THF and methanol to generate the key intermediate **9** in 66% yield over two steps. Methylthio group was then introduced smoothly under microwave conditions in approximately 50% yields to produce the compound of structure **10**. At last, deprotection of benzyl groups was accomplished in two steps. For example, **10** was treated with TMSOTf and acetic anhydride to provide the corresponding tetraacetate, which was subsequently hydrolyzed to yield the target compound **11** in 58% yields over two steps.

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.[14,15] Exploration

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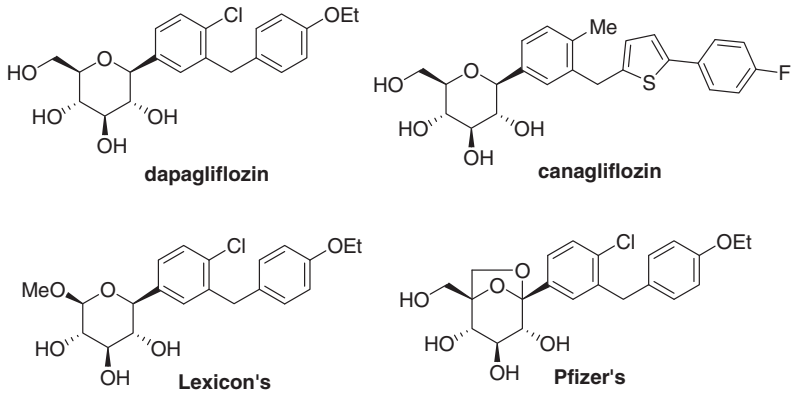


Figure 1. Structures of *C*-aryl glucoside SGLT2 inhibitors.

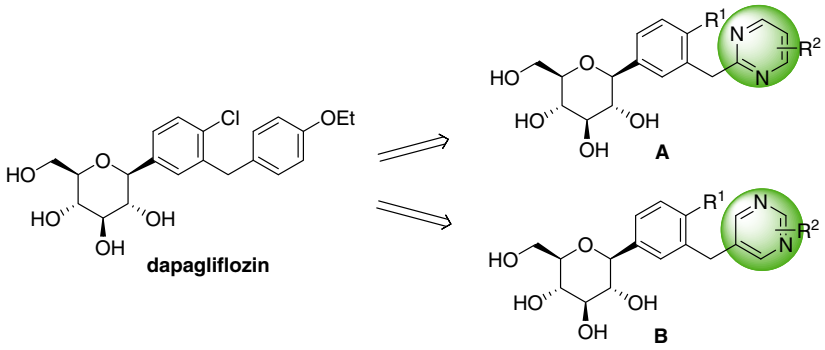
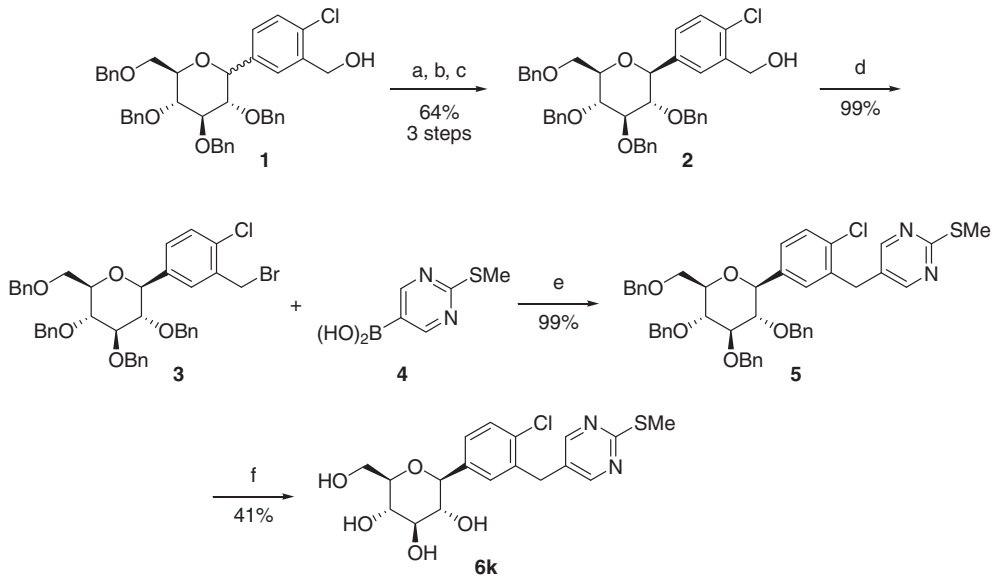
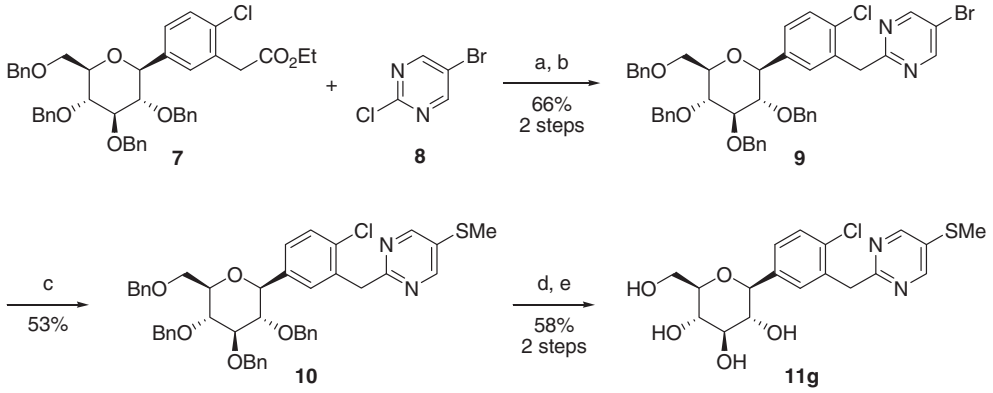


Figure 2. Exploration of C-glucoside bearing pyrimidine at the distal ring.



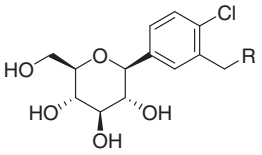
Scheme 1. Reagents and conditions: (a) benzoyl chloride, pyridine, rt; (b) IPA (selective crystallization); (c) LiOH·H_2O, THF, MeOH, H_2O, rt; (d) PBr_3, cat. pyridine, ether, rt; (e) $Pd(PPh_3)_4$, Cs_2CO_3, toluene, EtOH, 120 °C; (f) BBr_3, CH_2Cl_2, 0 °C.

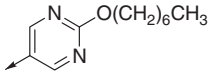
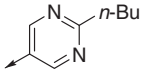
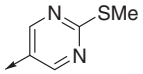
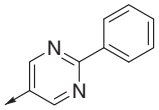
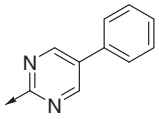
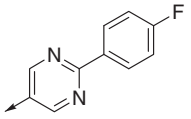
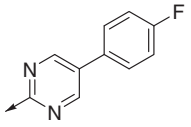
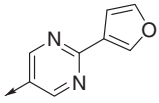
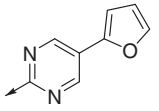
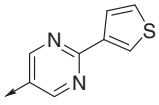
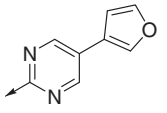
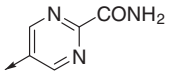
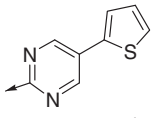
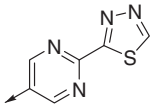
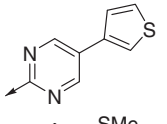
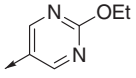
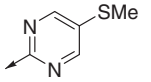
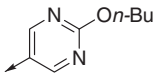
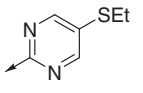
of the SAR began by replacing the phenyl moiety at the distal ring position of dapagliflozin with pyrimidine moiety.[19] Table 1 shows the structure–activity relationship upon alteration of the substituent at the distal pyrimidine ring employing only the β-anomer. Initially, *n*-butyl on the pyrimidine ring showed relatively poor activity (**6a**, IC_{50} = 8870 nM). However, replacement for this moiety with phenyl group **6b** improved the inhibitory activity against *h*SGLT2 (IC_{50} = 124 nM). Fluorophenyl on the pyrimidine ring displayed comparable inhibitory activity against *h*SGLT2 (IC_{50} = 119 nM). Meanwhile, substitution of phenyl with thiophene exhibited slightly better activity against *h*SGLT2 (**6e**: IC_{50} = 113 nM) than phenyl (**6b**, IC_{50} = 124 nM). On the other hand, furan **6d** and thiadiazole **6g** demonstrated twofold lower in vitro inhibitory activity (IC_{50} = 246 nM for **6d**, IC_{50} = 223 nM for **6g**) than



Scheme 2. Reagents and conditions: (a) NaH, DMF, 0 °C to rt; (b) LiOH·H_2O, THF, MeOH, H_2O, rt; (c) NaSMe, NMP, MW (170 °C); (d) TMSOTf, Ac_2O, −30 °C to rt; (e) NaOMe, MeOH, rt.

Table 1
In vitro inhibitory activity against *h*SGLT2



Compound	R	*h*SGL2 IC_{50} (nM)[a]	Compound	R	*h*SGL2 IC_{50} (nM)[a]
Dapagliflozin	—	0.49 ± 0.04[b]	**6j**		130
6a		8870	**6k**		76.0
6b		124	**11a**		73.6
6c		119	**11b**		107
6d		246	**11c**		36.8
6e		113	**11d**		34.5
6f		189	**11e**		17.1
6g		223	**11f**		64.4
6h		46.9	**11g**		10.7
6i		447	**11h**		39.4

[a] These data were obtained by single determinations.
[b] The IC_{50} value was obtained by in-house multiple determinations.

phenyl, likely suggesting that increased polarity is not that favorable in the region. Alkoxy groups on the pyrimidine were also explored. As a matter of fact, ethoxy group showed favorable inhibitory activity against *h*SGLT2 (**6h**: IC_{50} = 46.9 nM), which is particularly reminiscent of dapagliflozin. As the number of carbons increase, the inhibitory activity against *h*SGLT2 becomes fluctuated. For example, the tenfold drop in activity is observed in varying the length of the alkoxy chain from ethyl (**6h**: IC_{50} = 46.9 nM) to *n*-butyl (**6i**: IC_{50} = 447 nM), whereas the threefold increase in activity is observed from *n*-butyl (**6i**: IC_{50} = 447 nM) to *n*-heptyl (**6j**: IC_{50} = 130 nM). But none improved inhibitory activity relative to the simple ethoxy analog **6h**. Methylthio group for this region also appears to be tolerant (IC_{50} = 76 nM for **6k**), primarily implying that small lipophilic groups are favored at this location.

Next, isomeric pyrimidine series of compounds such as 4-chloro-3-(5-(methylthio)pyrimidin-2-yl)methylphenyl was explored (**11a**–**11h**, Table 1). This series exhibited increase in inhibitory activities against *h*SGLT2 compared with the previous pyrimidine series such as 4-chloro-3-(2-(methylthio)pyrimidin-5-yl)methylphenyl. For instance, phenylpyrimidine **11a** (IC_{50} = 73.6 nM) showed twofold increase in activity than the counterpart **6b** (IC_{50} = 124 nM). As more clear demonstration, two pairs of examples are presented, including furans **6d** (IC_{50} = 246 nM), **11d** (IC_{50} = 34.5 nM) as well as methylthiopyrimidines **6k** (IC_{50} = 76 nM), **11g** (IC_{50} = 10.7 nM), demonstrating sevenfold increase of activity, respectively. Among the substituents tested up to date, the best result was obtained when thiomethyl is installed at C-5 at pyrimidine as shown in **11g** (IC_{50} = 10.7 nM).[18] However, increasing the number of carbons from methylthio **11g** to ethylthio **11h** exhibited threefold drop in inhibitory activity against *h*SGLT2 (**11g**: IC_{50} = 10.7 nM; **11h**: IC_{50} = 39.4 nM).

Thus, replacement of the distal ring of dapagliflozin with a pyrimidine ring appears to weaken in vitro inhibitory activity against *h*SGLT2 perhaps for unfavorable electronic environments at the distal ring position. However, the information obtained from this pyrimidine series should help to design more effective SGLT2 inhibitors that are structurally related.

In summary, metabolically more stable C-glucosides bearing pyrimidine ring as a potential antidiabetic agent was exploited. Among the compounds tested, pyrimidine containing methylthio moiety **11g** showed the best in vitro inhibitory activity against *h*SGLT2 in this series to date (IC_{50} = 10.7 nM).

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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: Philadelphia, 2000; pp 375–415.
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. 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**. doi:10.1021/jm100332n.
10. (a) Boehringer Ingelheim Pharmaceuticals, www.clinicaltrials.gov, 2010, NCT01164501. (b) Lexicon Pharmaceuticals, www.clinicaltrials.gov, 2009, NCT00962065. (c) Astellas Pharma Inc., www.clinicaltrials.gov, 2010, NCT01135433. (d) Mascitti, V. Presented at the 240th American Chemical Society National Meeting and Exposition, Boston, MA, August 22–26, 2010; Medi-151.
11. (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.
12. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
13. To the solution of 5-bromo-2-chloropyrimidine (1.00 g, 5.17 mmol) in THF (20 mL) was added sodium methanethiolate (544 mg, 7.75 mmol). The reaction mixture was stirred at 60 °C overnight and evaporated in vacuo. The residue was purified by flash column chromatography (Biotage SP1™) to provide 5-bromo-2-(methylthio)pyrimidine (1.01 g, 95%) as a white solid (MH+ 205). To the solution of 5-bromo-2-methylthiopyrimidine (1.01 g, 4.92 mmol) in THF (20 mL) was added trimethylborate (0.84 mL, 7.39 mmol) at −78 °C. To the reaction mixture was added *n*-BuLi (2.5 M in hexane, 3.0 mL, 7.5 mmol) at −78 °C for 1 h and warmed-up to −20 °C. The mixture was quenched by 1 N aqueous HCl solution at −20 °C and evaporated in vacuo. The residue was poured into water (50 mL) and extracted with EtOAc (50 mL × 2). The organic layer was dried over $MgSO_4$ and evaporated in vacuo to provide 2-(methylthio)pyrimidin-5-ylboronic acid (538 mg, 64%) as a yellow solid: ^{1}H NMR (400 MHz, CD_3OD) δ 8.76 (s, 2H), 2.57 (s, 3H).
14. 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.
15. 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.[16,17]
16. 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.
17. Katsuno, K.; Fujimori, Y.; Takemura, Y.; Hiratochi, M.; Itoh, F.; Komatsu, Y.; Fujikura, H.; Isaji, M. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 323.
18. *(2S,3R,4R,5S,6R)-2-(4-Chloro-3-((5-(methylthio)pyrimidin-2-yl)methyl)phenyl)-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol* (**11g**): white solid; mp 107 °C (MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (s, 2H), 7.35 (d, J = 2.0 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.21 (dd, J = 8.4, 2.0 Hz, 1H), 4.98 (br, 1H), 4.95 (d, J = 5.2 Hz, 1H), 4.83 (d, J = 5.6 Hz, 1H), 4.45 (t, J = 5.6 Hz, 1H), 4.32 (d, J = 15.6 Hz, 1H), 4.26 (d, J = 15.6 Hz, 1H), 3.97 (d, J = 9.2 Hz, 1H), 3.68–3.64 (m, 1H), 3.40 (quint, J = 6.0 Hz, 1H), 3.25–3.17 (m, 2H), 3.15–3.06 (m, 2H), 2.50 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.61, 154.64, 139.34, 135.10, 132.38, 131.34, 131.00, 128.30, 127.66, 81.15, 80.58, 78.29, 74.55, 70.27, 61.31, 48.51, 42.13, 14.47; MS (ESI) m/z 413 $(M+H)^+$, 435 $(M+Na)^+$.
19. In this Letter, SGLT2 inhibition data were obtained by single determinations. Whenever we use dapagliflozin as our reference in our in-house assay, the SGLT2 inhibition for dapagliflozin has showed a certain number in the close range (IC_{50} = 0.49 ± 0.04 nM) in each different assay. Thus, we trust that all SAR discussions in the manuscript are scientifically valid.