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Exploration of *O*-spiroketal *C*-arylglucosides as novel and selective renal sodium-dependent glucose co-transporter 2 (SGLT2) inhibitors

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ABSTRACT

A series of novel *O*-spiroketal *C*-arylglucosides have been prepared and evaluated in cell-based functional assays for activity against human sodium-dependent glucose co-transporters 1 and 2 (SGLT1 and 2). The core spiro[isobenzofuran-1,2′-pyran] structure proved to be an effective scaffold for diversification and a number of compounds with single digit nanomolar potency and high selectivity have been synthesized. Compound **5a** promoted glucosuria when administered in vivo in rats and produced a significant blood glucose reduction effect.

Type II diabetes mellitus (DM2) is an acquired metabolic disease in which the glycemic load resulting from excess nutrient consumption and dysregulated gluconeogenesis overwhelms the ability of pancreatic islet beta cells to produce sufficient insulin to maintain euglycemia. DM2 often appears in the setting of comorbidities associated with hyperglycemia and a sedentary lifestyle, including the constellation with obesity, dyslipidemia and hypertension known as metabolic syndrome. The renal sodium glucose linked transporter 2, SGLT2, has emerged as an attractive target for the management of DM2 in the context of metabolic syndrome.[1] As its name suggests, SGLT2 acts by cotransport of sodium ion and glucose to recover glucose from urine, thereby conserving a valuable metabolic resource under nutrient-limited conditions.[2] Blockade of this activity should release glucose into urine, thereby reducing circulating glucose levels. Individuals affected by the genetic syndrome Familial Renal Glucosuria (FRG) have mutations in SGLT2, sometimes resulting in a predicted complete loss of transporter activity, but have no obvious morbidities.[3] In extreme cases of FRG a syndrome of 'salt wasting' is observed, with hypotension and a slight elevation in aldosterone consistent with a homeostatic mechanism that has been engaged to retain sodium.[4] Selectivity for the inhibition of SGLT2 compared to the closely related SGLT1 is thought to be highly attractive because deficiency in SGLT1 leads to the human genetic disease Glucose Galactose Malabsorption syndrome, characterized by a severe diarrhea and failure to thrive.[5]

Following the initial disclosure by Tanabe Seiyaku of SGLT2-selective inhibitors patterned on the naturally occurring inhibitor phlorizin,[6,7] a large number of candidate inhibitors have been synthesized.[8] Members of of the O-glycoside class of SGLT2 inhibitor, including T-1095A,[6,7] sergliflozin-A (**1**),[9] remogliflozin[10] or their pro-drugs have been evaluated for the treatment of DM2 but have been found to require frequent dosing because of metabolic instability. Dapagliflozin (**2**), a *C*-aryl glucoside, has been identified as a potent and selective SGLT2 inhibitor with a favorable pharmacodynamic and pharmacokinetic profile in preclinical studies.[11]

The program described here was directed at the creation of metabolically robust compounds with high selectivity towards SGLT2. Modeling studies and analysis of the published X-ray structure of different classes of potential SGLT2 inhibitors,[12] led to the realization that the aryl ring (B ring) of the aglycone moiety was almost perpendicular to carbohydrate moiety (A ring) in the lowest energy conformation. A series of novel compounds (Fig. 1) that have a different core structure than reported inhibitors was

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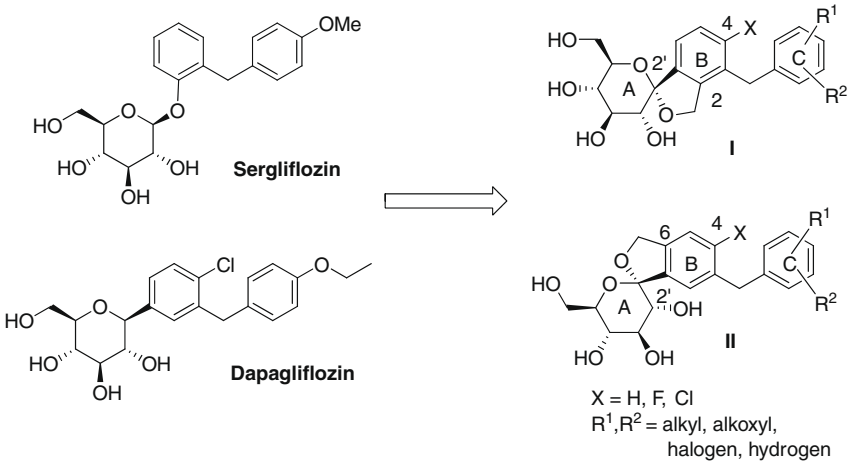
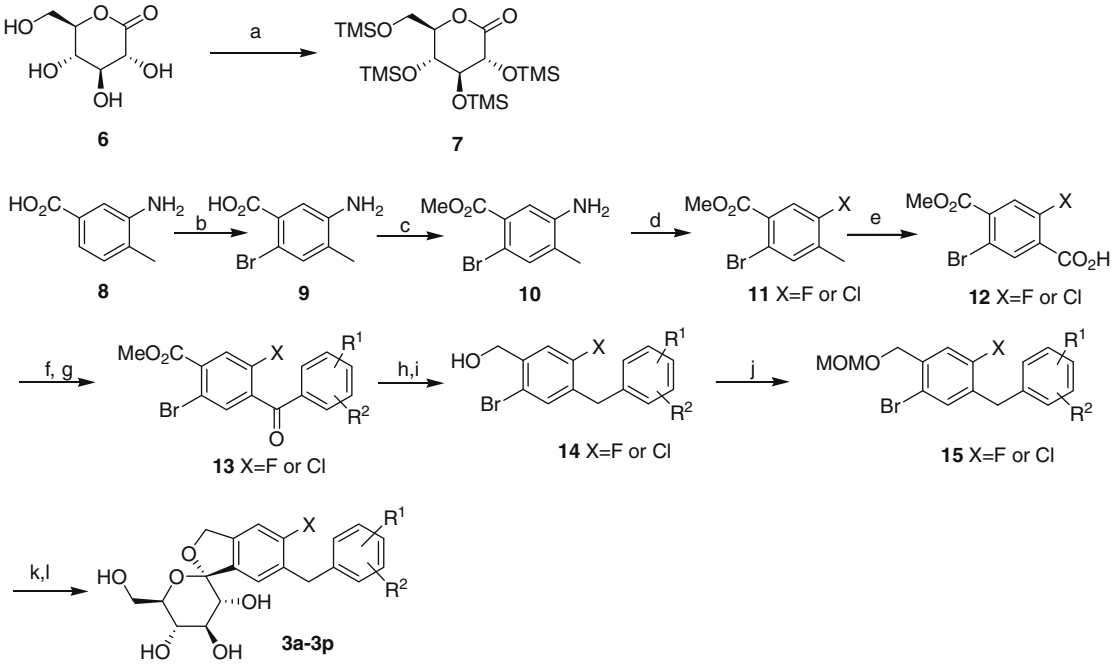


Figure 1. Origin and design of *O*-spiroketal *C*-arylglucosides SGLT2 inhibitors.

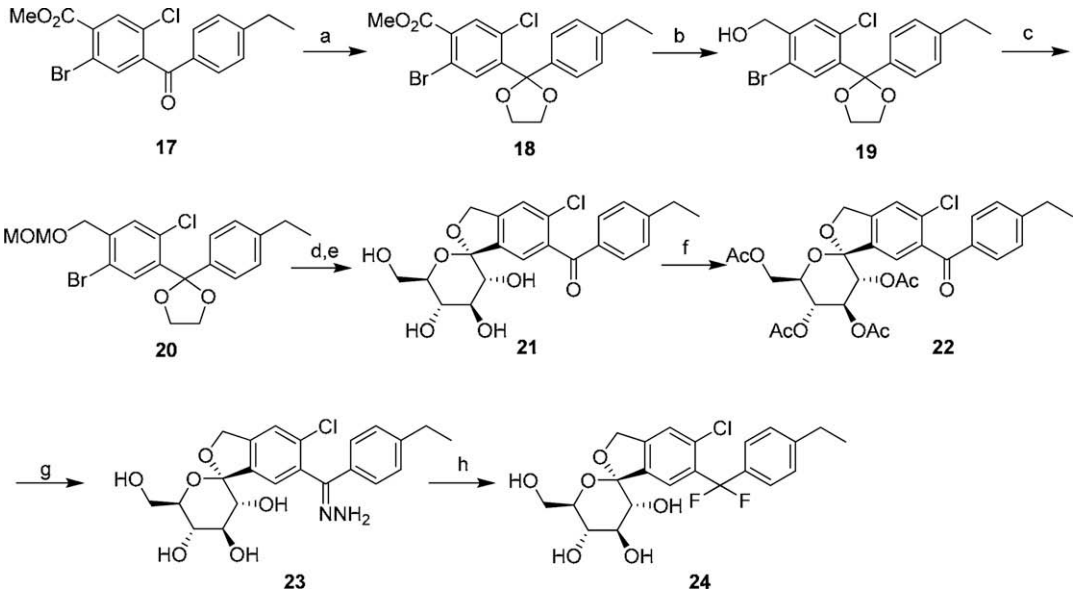
accordingly synthesized.[13] The central modifications were the formation of a *O*-spiro linkage between the glucose ring and the aglycone proximal phenyl ring, wherein the spiro structure expected to combine both the character of *C*-glucosides and *O*-glucosides, and the retention of a chlorine at the 4-position on the B ring, a substitution that had been found to be critical for high in vivo potency in preliminary studies. Retention of the 4-chloro substituent and the attainment of higher potency distinguishes the program discussed below from that disclosed by Kobayashi et al.[14] albeit at the price of a significant increase in synthetic difficulty attributable to the electron deficiency of the resulting B ring.

As part of our ongoing SAR studies,[15] *O*-spiroketal *C*-arylglucosides with varied substituents on the aglycone aryl rings were systematically prepared to search for possible favorable ligand–protein interactions. Through modulation of the electron density and the substitution pattern of the distal ring, inferences could be drawn as to which substituent would result in a favorable orientation with respect to the target.

The synthesis of **3a–3p** is shown in Scheme 1. Persilylated gluconolactone **7** was prepared in 93% yield by a slow addition of trimethylsilyl chloride to commercially available gluconolactone **6** in *N*-methylmorpholine and tetrahydrofuran.[16,17] Benzoates **11** were constructed through Sandmeyer reaction or Balz–Schiemann reaction conditions from aniline **10,**[18] which was prepared by bromination of commercially available 3-amino-4-methylbenzoic acid **8** followed by esterification of acid **9.** Oxidation of **11** with potassium permanganate in *tert*-butanol provided the key electron-deficient tetrasubstituted benzene **12**. Friedel–Crafts acylation of substituted benzenes with the benzoyl chloride prepared from acid **12** by treatment with oxalyl chloride generated the benzophenone **13**. Selective reduction of the ketone with triethylsilane and catalytic trifluoromethanesulfonic acid in trifluoroacetic acid provided



Scheme 1. Construction of the aglycones and synthesis of **3a**–**3p**. Reagents and conditions: (a) TMSCl, NMM, THF, 35 °C (93%); (b) NBS, DMF, 5 °C (87%); (c) $SOCl_2$, MeOH, reflux (99%); (d) $NaNO_2$, concd HCl, CuCl, 1,4-dioxane, H_2O, 0 °C (93%); or HBF_4, isoamyl nitrite, anhydrous EtOH, −10 °C, then xylene, reflux (50%); (e) KMnO4, *t*-BuOH, 18-crown-6, H_2O, reflux (73%); (f) $(COCl)_2$, DMF, DCM; (g) $AlCl_3$, R^1 and R^2 substituted phenyl, DCM; (h) Et_3SiH, CF_3SO_3H, TFA; (i) $NaBH_4$, MeOH, THF; (j) MOMCl, DIPEA, DCM, 0 °C–rt; (k) *n*-BuLi, THF, toluene, −78 °C, lactone **7**; (l) CH_3SO_3H, MeOH, −78 °C to rt (40–63%, two steps).



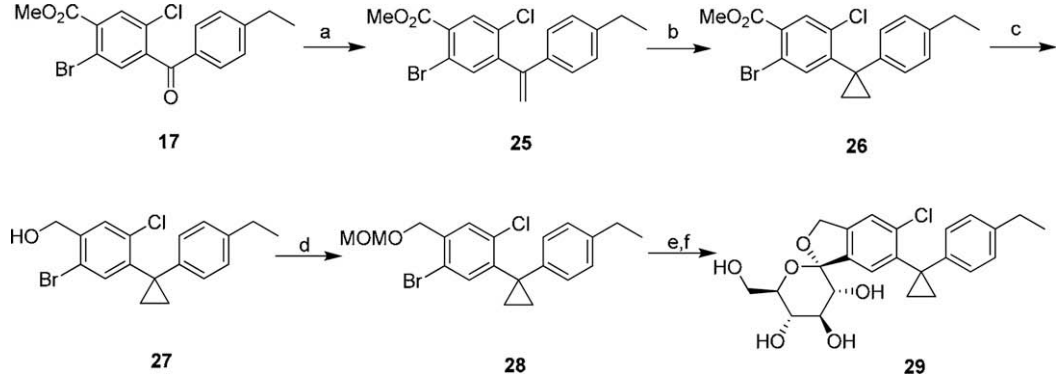
Scheme 2. Reagents and conditions: (a) 1,2-bis(trimethylsilyloxy)ethane, ethylene glycol, $TsOH{\cdot}H_2O$, toluene, reflux (34%); (b) $NaBH_4$, MeOH, THF, 65 °C; (c) MOMCl, DIPEA, DCM, 0 °C–rt (86%); (d) *n*-BuLi, THF, toluene, −78 °C, lactone **7**; (e) CH_3SO_3H, MeOH, −78 °C to rt (68%, two steps); (f) Ac_2O, pyridine, DMAP, DCM, rt; (g) $NH_2NH_2{\cdot}H_2O$, TsOH, EtOH, reflux; (h) DBH, HF/pyridine, DCM, −78 °C (36%, two steps).

the corresponding diarymethane which was further reduced with sodium borohydride to give the benzyl alcohol **14**. Protection of the primary alcohol with chloromethyl methyl ether gave the aglycone **15**. Lithium–halogen exchange of **15** followed by its addition to 2,3,4,6-tetra-*O*-trimethylsilyl-β-D-gluconolactone **7** gave a mixture of lactols which were converted in situ to the desired *O*-spiroketal *C*-arylglucosides **3a–p** by treatment with methanesulfonic acid in methanol.[19,20]

The suitability of substituents on the one atom bridge between rings B and C was also evaluated. Scheme 2 details the route to the difluoromethylene bridge, which was attained by conversion of ketone **17** to the ketal **18**, followed by reduction with sodium borohydride to give alcohol **19** and subsequent protection with chloromethyl methyl ether to yield the intermediate 1,3-dioxolane **20**. In a one-pot reaction, compound **21** was prepared from **20** using a synthetic approach similar to that outlined for the formation of compound **3a–p** in Scheme 1. An initial attempt to synthesize the target diaryldifluoromethane **24** by direct fluorination of tetra acetyl glucoside **22** with DAST failed, but preparation of the hydrazone **23** followed by fluorination with HF/pyridine complex with catalytic DBH afforded the desired compound.[20,21]

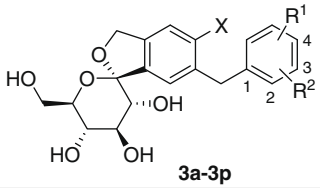
The synthesis of the cyclopropyl glucoside **29** is described in Scheme 3. Standard Wittig reaction conditions applied to ketone **17** gave the alkene **25**, which was subjected to modified Simmons–Smith reaction conditions to give the cyclopropyl compound **26.**[22] Reduction and protection of **26** provided the aglycone **28**. Condensation and cyclization as described above gave analogue **29** in moderate yield.[20]

The compounds were tested using cell-based SGLT functional assays.[23] The role of substituent groups on the 2-, 3- and 4-positions of the distal aryl ring was examined via compounds that encompassed alkoxyl, alkyl and halogen groups (Table 1). At the 4-position, methoxyl-substituted compound **3c** showed an IC_{50} value of 2.5 nM for human SGLT2 (*h*SGLT2) and only 80-fold selectivity vs. human SGLT1 (*h*SGLT1), whereas a 2.5-fold improvement in selectivity was found for ethoxyl-substituted compound **3b**. Replacing the ethyl substituent with isopropyl or *tert*-butyl gave compounds **3d–f**, with reduced binding affinity but increased affinity for SGLT2 over SGLT1. In particular, the high selectivity of **3g** can be expected to favorably impact the predicted gastrointestinal side effect profile.[24] All *mono*-, *para*-substituted R^1 analogues **3a–h**, whether the 4-position on the proximal ring was chlorine

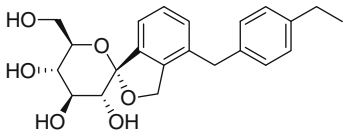


Scheme 3. Reagents and conditions: (a) $(C_6H_5)_3PCH_3I$, *t*-BuOK, toluene, rt (60%); (b) Et_2Zn, TFA, CH_2I_2, DCM, −15 °C (58%); (c) $NaBH_4$, MeOH, THF, 65 °C (98%); (d) MOMCl, DIPEA, DCM, 0 °C–rt; (e) *n*-BuLi, THF, toluene, −78 °C, lactone **7**; (f) CH_3SO_3H, MeOH, −78 °C to rt (34%, two steps).

Table 1
O-Spiroketal *C*-arylglucosides (**3a–p**): exploration of the aglycone proximal and distal ring substituents (X, R^1 and R^2)[23]



3a-3p



4[15]

Compound	X	R^1	R^2	*h*SGLT2 IC_{50} (nM)	*h*SGLT1 IC_{50} (μM)	Selectivity (*h*SGLT2/*h*SGLT1)
2	NA	NA	NA	6.7	0.89	132
3a	H	4-OEt	H	71	10–100	>140
3b	Cl	4-OEt	H	6.5	1.3	200
3c	Cl	4-OMe	H	2.5	0.2	80
3d	Cl	4-Et	H	6.6	0.6	91
3e	Cl	4-*i*Pr	H	7.1	2.5	352
3f	Cl	4-*t*Bu	H	13	9.7	742
3g	Cl	4-OCF_3	H	0.3	3.1	10,333
3h	F	4-Et	H	8.6	5.3	616
3i	Cl	4-OEt	3-Cl	68	10.5	154
3j	Cl	4-OEt	2-Cl	>1000	10–100	ND
3k	Cl	4-OEt	2-Me	>1000	1–10	ND
3l	Cl	4-OMe	2-Me	>1000	10–100	ND
3m	Cl	4-F	2-Me	~100	1–10	ND
3n	Cl	4-F	2-OEt	1000	10–100	ND
3o	Cl	3-Me	2-F	0.8	1.5	1875
3p	Cl	3-Me	2-OMe	~10	1–10	ND
4	NA	NA	NA	>>1000	ND	ND

or fluorine, had equivalent or increased potency and selectivity for SGLT2 inhibition relative to dapagliflozin **2,** possibly because of the greater conformational constraints imposed by the spiro scaffold.

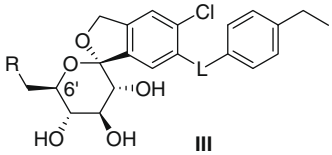
Di-substitution at the 2- and 4-positions of the distal aryl ring, (Table 1, analogue **3j–n)** resulted in products having significantly lower SGLT2 inhibitory activities, perhaps due to the increased steric bulk at the 2-position. Analogs **3o–p** exhibited a binding affinity to SGLT2 that was unexpected from the SAR profile of reported *C*-glucosides or *O*-glucosides in which the *meta* position of the distal aryl ring was substituted with a methyl group.[25] Compound **3o**, in which the *meta* position is methyl and *ortho* position is fluorine, had ~8-fold higher potency and more than 14-fold higher selectivity for inhibition of SGLT2 compared with dapagliflozin **2**.

Modification of the spacer moiety between the proximal and distal rings of **3d** afforded the high potency compounds **21**, **24** and **29** (Table 2). Interestingly, compound **29** exhibited a fivefold higher potency and a 50-fold more selectivity for SGLT2 inhibition compared to **3d**, perhaps due to a more favorable orientation of the distal ring induced by the conformational effects of the cyclopropyl group.

The SAR of replacements for the glucose primary hydroxyl group was investigated as outlined in Scheme 4, leading to **5a–f** (Table 2). Direct and selective fluorination of **3d** with DAST in dichloromethane provided compound **5a**. Compounds **5b–e** could be obtained by S_N2 substitution of tosylate **16** which was formed from **3d** by reaction with *p*-toluenesulfonyl chloride and 2,6-lutidine. Reduction of azide **5e** with triphenylphosphine generated the amine **5f** in moderate yield.[20]

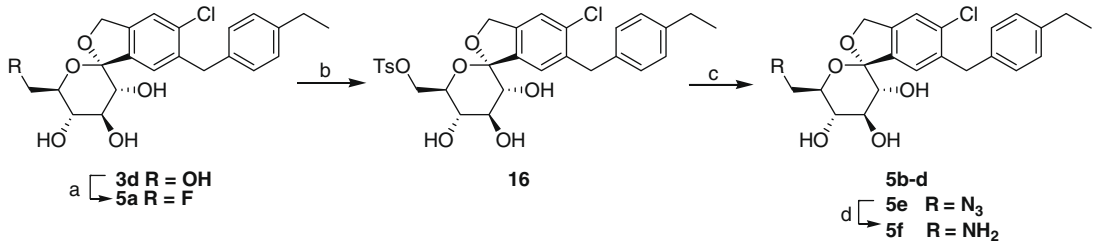
Replacement of the 6′ hydroxyl group with fluorine gave a similar in vitro profile to compound **3d**. The primary amine compound **5f** had greatly reduced SGLT2 inhibitory activity. Replacement of the hydroxyl by a methoxyl group, compound **5b** appeared to yield comparable SGLT2 potency and more selectivity, but the more bulky trifluoroethoxyl group of compound **5c** reduced potency at

Table 2
O-Spiroketal *C*-arylglucosides (III) SAR exploration of the aglycone spacing element (L) and 6′-position substituent (R)[23]



III

Compound	R	L	*h*SGLT2 IC_{50} (nM)	*h*SGLT1 IC_{50} (μM)	Selectivity (*h*SGLT2/*h*SGLT1)
5a	F	CH_2	3.8	0.7	184
5b	CH_3O	CH_2	5.8	2.7	466
5c	CF_3CH_2O	CH_2	70	14	200
5d	CH_3COO	CH_2	43	3.5	82
5e	N_3	CH_2	10–100	1–10	ND
5f	NH_2	CH_2	100–1000	>100	ND
21	OH	CO	22	43	1955
24	OH	CF_2	7	1.6	229
29	OH		1.3	6.0	4615



Scheme 4. Reagents and conditions: (a) DAST, DCM, −70 °C (61%); (b) *p*-toluenesulfonyl chloride, 2,6-lutidine, rt (66%); (c) CH_3ONa; or CF_3CH_2ONa; or NaOAc; or NaN_3, DMF (78%); (d) PPh_3, THF/H_2O(4:1), rt (60%).

least 10-fold less potent compared to the parent compound **3d**. Acetylation led to compound **5d** with both reduced potency and selectivity.

Oral administration of a single dose of 25 mg/kg of **3d** to CD1 mice produced severe diarrhea after 6 h. Oral administration of a single dose of 1.0 mg/kg of **5a** to normal Sprague-Dawley rats induced loss of 1220 mg of glucose per 200 g body weight over 24 h, which appeared to be a ~120-fold elevation in glucose disposal relative to vehicle controls. In a separate experiment, a 44% AUC reduction in blood glucose level versus controls was observed in 2 h after a single 1 mg/kg oral dose of **5a** was administrated to normal C57BL/6 J mice with starting blood glucose levels of 68–86 mg/dL. The above correlation of SGLT2 inhibition, glucosuria and blood glucose-lowering effects of compound **5a** suggest the series may be merit further exploration.

In summary, we have developed a novel series of SGLT2 inhibitors, in which the core scaffold is spiro[isobenzofuran-1,2′-pyran]. An SAR exploration shows good inhibitory activity and high selectivity for SGLT2 that we attribute, in part, to a greater conformational constraint imposed by the spiro system. Further investigation of the in vitro and in vivo SAR of *O*-spiroketal *C*-aryglucosides will be reported in due course.

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23. A plasmid bearing the human full-length SGLT1 coding sequence in the pDream 2.1 mammalian expression vector was purchased from GenScript Corporation. A full-length human SGLT2 cDNA (GenScript Corporation) was cloned into the pEAK15 mammalian expression vector. Human SGLT1 expression plasmid DNA was transiently transfected into COS-7 cells (American Type Culture Collection) using Lipofectamine 2000 (Invitrogen Corporation). Transfected cells were evaluated for SGLT1 activity by methyl-α-D-[U-^{14}C]glucopyranoside (AMG) uptake assay and cryopreserved until use. Plasmid containing human SGLT2 was linearized and stably transfected into HEK293.ETN cells. SGLT2-expressing clones were selected based on resistance to puromycin (Invitrogen Corporation) and activity in AMG uptake assay. Cells expressing SGLT1 or SGLT2 were seeded on 96-well ScintiPlates (PerkinElmer, Inc.) in DMEM containing 10% FBS (1×10^5 cells per well in 100 μL medium) incubated at 37 °C under 5% CO_2 for 48 h prior to the assay. Cells were washed twice with 150 μL of either sodium buffer (137 mM NaCl, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM tris(hydroxymeth-yl) aminomethane/*N*-2-hydroxyethylpiperazine-*N*′-ethane sulfonic acid [Tris/Hepes], pH 7.2) or sodium-free buffer (137 mM *N*-methyl-glucamine, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM Tris/Hepes, pH 7.2). Test compound in 50 μL each of sodium or sodium-free buffer containing 40 μCi/mL methyl-α-D-[U-^{14}C]glucopyranoside (Amersham Biosciences/GE Healthcare) was added per well of a 96-well plate and incubated at 37 °C with shaking for either 2 h (SGLT1 assay) or 1.5 h (SGLT2 assay). Cells were washed twice with 150 μL of wash buffer (137 mM *N*-methylglucamine, 10 mM Tris/Hepes, pH 7.2) and methyl-α-D-[U-^{14}C]glucopyranoside uptake was quantitated using a TopCount scintillation counter (PerkinElmer, Inc.). Inhibitors were assayed at eight concentrations in triplicates. Sodium-dependent glucopyranoside uptake was calculated by subtracting the values obtained with sodium-free buffer from those obtained using sodium buffer. In general, ratios of sodium-dependent to sodium-independent AMG uptake in SGLT1 and SGLT2 expressing cells were 10–15 and 15–20, respectively. Results of AMG uptake were analyzed using GraphPad Prism (Intuitive Software for Science). IC_{50} calculations were performed using nonlinear regression with variable slope. As a reference standard, a derivative of dapagliflozin was routinely included in the assays. In 26 independent evaluations, the reference compound inhibited SGLT2 activity by 69.7 ± 9.6% and SGLT1 by 72.7 ± 6.7% at 10 nM and 10 μM, respectively.
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