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C-Aryl glycoside inhibitors of SGLT2: Exploration of sugar modifications including C-5 spirocyclization

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ABSTRACT

Modifications to the sugar portion of *C*-aryl glycoside sodium glucose transporter 2 (SGLT2) inhibitors were explored, including systematic deletion and modification of each of the glycoside hydroxyl groups. Based on results showing activity to be quite tolerant of structural change at the C-5 position, a series of novel C-5 spiro analogues was prepared. Some of these analogues exhibit low nanomolar potency versus SGLT2 and promote urinary glucose excretion (UGE) in rats. However, due to sub-optimal pharmacokinetic parameters (in particular half-life), predicted human doses did not meet criteria for further advancement.

Sodium glucose co-transporter 2 (SGLT2) is a sugar transport protein located on the luminal side of proximal tubules in the kidney. It is the major contributor to re-uptake of filtered glucose from pro-urine. By promoting urinary glucose excretion (UGE), inhibitors of SGLT2 show promise for the treatment of diabetes by attenuation of postprandial plasma glucose levels and by promoting weight loss via loss of calories.[1,2] With few exceptions,[3] inhibitors of SGLT2 owe their discovery to phlorizin (**1**), a naturally-occurring *O*-aryl glycoside that has long been known to cause glucosuria in animals and humans (Fig. 1).[4] Phlorizin is a fairly potent inhibitor of SGLT2 (IC_{50} = 36 nM),[5] with some selectivity against SGLT1, a related sugar transporter (IC_{50} = 330 nM). Unlike SGLT2, SGLT1 is not only expressed in the kidney, but is also found in the intestine and other tissues. Inhibition of SGLT1 may lead to undesirable gastrointestinal effects.[6] Early drug discovery efforts on SGLT2 focused on *O*-aryl glycosides related to phlorizin with the aim of increasing potency for SGLT2 and minimizing activity versus SGLT1.[1] Because of their susceptibility to the action of glycosidases, few *O*-aryl glycosides have survived beyond early stages of development due to poor oral bioavailability. The advent of C-aryl glycoside SGLT2 inhibitors, such as dapagliflozin (**2**)[5,7] solved the problem of glycosidase susceptibility by excising the glycoside oxygen linker (Fig. 1). Dapagliflozin is currently in Phase 3 clinical trials for the treatment of diabetes, leading a field of other *C*-aryl glycoside SGLT2 inhibitors in development.[1]

Continuing research to discover structurally distinct SGLT2 inhibitors has been devoted largely to modification of the diarylmethylene side chain of dapagliflozin.[1,8] In contrast, changes to the synthetically more challenging glycoside portion of the molecule have been less well explored[9–13] and developing structure–activity relationships (SAR) with respect to glycoside modification has been difficult owing to the limited amount of data typically provided in

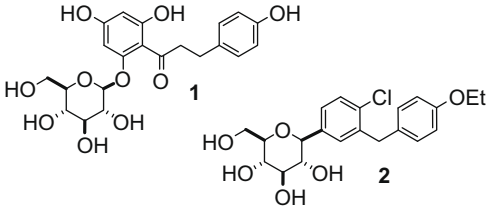

Figure 1.

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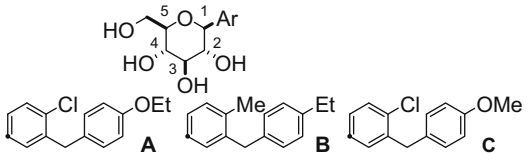
patent applications. Here, we report the SAR associated with systematic deletion and modification of each of the glycoside hydroxyl groups and the use of this information to design novel spirocyclic analogues in the *C*-aryl glycoside series.

Structures of final target compounds and associated SGLT2 and SGLT1 inhibition data are shown in Tables 1 and 2.[14] We explored the SAR using 4-methyl-3-(4-ethylbenzyl) and 4-chloro-3-(4-methoxybenzyl), side chains B and C, respectively (Table 1). Based on the very similar potencies of compounds **2**, **3**, and **4** against SGLT2 (IC_{50}s ~1–2 nM), we assumed that these aryl side chains were more or less interchangeable for the purpose of generating SAR versus SGLT2.

Reference compounds **2**,[5] **3**,[15] and **4**,[15] as well as analogue **5** were prepared by addition of the appropriately substituted 3-benzylphenyllithium to 2,3,4,6-tetra-*O*-trimethylsilyl-β-D-glucolactone.[16] Modifications at C-2 and C-4, providing compounds **6**, **7**, **10**,[9] **11**,[9] and **12**, were carried out as shown in Scheme 1, using a protecting group sequence described for **4** in the patent literature.[9] Analogues **8** and **9**, with changes at C-3, were synthesized as shown in Scheme 2. Compounds **13** and **14** were prepared as previously described.[10] Spiro analogue **16** was synthesized as shown in Scheme 3, the key step involving an intramolecular olefin metathesis reaction. Spirocyclopropyl compounds **17**–**18** were prepared via cyclopropanation of the corresponding 6-methylene intermediate (Scheme 4). Compound **15** and spirocycles **19**–**24** were obtained as recently described by Mascitti.[17]

The only C-1-modified compound prepared was **5**, substituting the benzylic hydrogen atom for OMe. The activity of this compound versus SGLT2 was significantly lower (~700-fold) than **3**. Changes at positions C-2, C-3, and C-4 (Table 1 and entries 6–12), including OH deletion (**6**, **8**, and **10**), conversion of OH to OMe (**7** and **12**) and inversion of stereochemistry (**9** and **11**) gave similar results, though the magnitude to which activity decreased due to C-4 modification was less (~20–180-fold). In distinct contrast to the changes explored at positions C-1–C-4, SGLT2 activity appeared relatively tolerant of modifications to the C-5 hydroxymethyl group as demonstrated by C-5 methyl analogue **13** (IC_{50} = 2.4 nM) and C-5 fluoromethyl analogue **14** (IC_{50} = 5.1 nM). Attachment of a second hydroxymethyl group at C-5 (**15**) produced a modest loss of activity (SGLT2 IC_{50} = 59 nM). Based on these results, we focused subsequent efforts on further structural exploration at C-5, including novel spiro analogues (Table 2). Whereas the result for spirotetrahydrofuran **16** was somewhat disappointing (SGLT2 IC_{50} = 53 nM), spirocyclopropyl compounds **17** and **18** displayed reasonably potent activity against the target (IC_{50} = 3.0 nM and 7.9 nM, respectively), warranting further profiling including counter screening against SGLT1, in vivo rat pharmacokinetics (PK)[18] and measurement of 24 h UGE following oral administration in rat. Compared to **2**, neither analogue was particularly selective for SGLT2 over SGLT1 (**17**: ~63-fold;

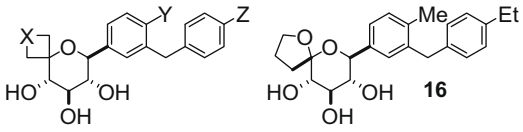
Table 1[14]



Compd	Sugar modification	Ar	SGLT2 IC_{50} (nM)	SGLT1 IC_{50} (nM)
2	None	A	1.4 ± 0.3 (5)	1200 ± 120 (4)
3	None	B	1.5[a] (2)	
4	None	C	1.0 ± 0.4 (11)	240 ± 40 (4)
5	C-1 H to OMe	B	1000 (1)	
6	C-2 OH to H	B	>10,000 (1)	
7	C-2 OH to OMe	C	140 (1)	
8	C-3 OH to H	B	2000[b] (2)	
9	C-3 epi-OH	B	3700 (1)	
10	C-4 OH to H	C	21 (1)	
11	C-4 epi-OH	B	139 ± 23 (3)	
12	C-4 OH to OMe	C	180 (1)	
13	C-5 CH_2OH to Me	C	2.4 ± 0.5 (5)	73[c] (2)
14	C-5 CH_2OH to CH_2F	C	5.1 (1)	
15	C-5 H to CH_2OH	C	59 (1)	6800[d] (2)

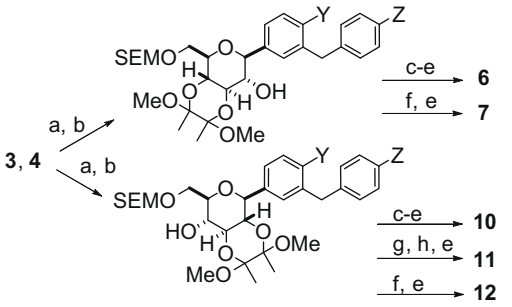
[a] 2.0 nM and 1.2 nM.
[b] 1650 nM and 2500 nM.
[c] 55 nM and 95 nM.
[d] 5600 nM and 8400 nM.

Table 2[14]

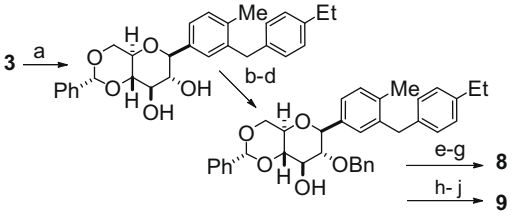


Compd	X	Y	Z	SGLT2 IC_{50} (nM)	SGLT1 IC_{50} (nM)
16		Me	Et	53 (1)	1100 (1)
17	Bond	Me	Et	3.0 ± 0.5 (5)	190 ± 40 (5)
18	Bond	Me	OMe	7.9 ± 3.5 (7)	370 ± 180 (6)
19	NH	Me	Et	5100 (1)	>10,000 (1)
20	SO_2	Me	Et	14 (1)	>10,000 (1)
21	O	Me	Et	3.4 ± 0.8 (3)	1500 (2)
22	O	Me	OMe	6.6 ± 2.5 (8)	1540 ± 180 (7)
23	O	Cl	OMe	23 ± 22 (5)	>9600 (5)
24	O	Cl	OEt	32 ± 79 (5)	5600 ± 930 (4)
25	Bond	Me	(C=O)Me		

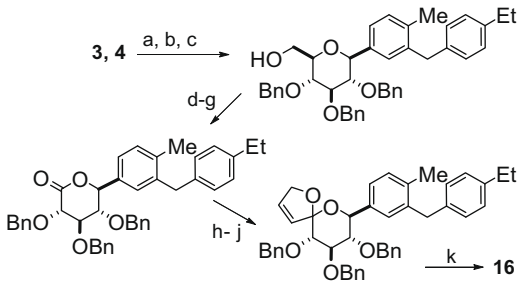
[a] 1100 nM and 1900 nM.



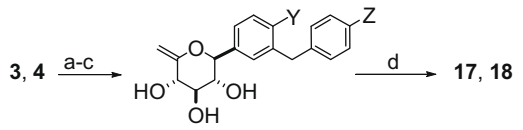
Scheme 1. Reagents and conditions: (a) 2,3-butanedione/$HC(OMe)_3$/$BF_3{\cdot}OEt_2$/60 °C/3 h; (b) SEMCl/iPr_2NEt/CH_2Cl_2/rt/overnight; (c) 1,1-thiocarbonyl-diimidazole/toluene/reflux/18 h; (d) AIBN/$(TMS)_3SiH$/120 °C/overnight; (e) TFA/H_2O/rt/1 h; (f) MeI/NaH/THF/rt/overnight; (g) Dess–Martin reagent/CH_2Cl_2/rt/overnight; (h) Na–Selectride/THF, −78 °C to rt/3 h.



Scheme 2. Reagents and conditions: (a) $PhCH(OMe)_2$/$HBF_4{\cdot}OEt_2$/DMF/rt/overnight; (b) Bu_2SnO/toluene/reflux/4 h, then PMBBr/$Bu_4N^+I^-$/reflux/overnight; (c) BnBr/$Bu_4N^+I^-$/DMF/0 °C to rt/overnight; (d) DDQ/CH_2Cl_2/rt/3 h; (e) NaH/imidazole/THF/reflux/3 h, then CS_2/reflux/0.75 h, then MeI/reflux/0.5 h; (f) Bu_3SnH/AIBN/toluene/reflux/1.25 h; (g) H_2 (3 atm)/$Pd(OH)_2$/HCl/EtOH/THF/rt/3 days; (h) Dess–Martin reagent/CH_2Cl_2/rt/overnight; (i) $NaBH_4$/MeOH/THF/0–10 °C/0.33 h; (j) as for Step g except 1 day reaction time.



Scheme 3. Reagents and conditions: (a) $PhCH(OMe)_2/HBF_4{\cdot}OEt_2$/DMF/rt/overnight; (b) BnBr/$Bu_4N^+I^-$/DMF/0 °C to rt/overnight; (c) $LiAlH_4/Et_2O/CH_2Cl_2$/0 °C then $AlCl_3$/reflux/3 h; (d) TsCl/Et_3N/DMAP/CH_2Cl_2/rt/24 h; (e) NaI/2-butanone/80 °C/overnight; (f) $CsCO_3$/DMF/80 °C/25 h; (g) $O_3/CH_2Cl_2/-78$ °C then Me_2S; (h) $CH_2CHMgBr$/THF/−78 °C/2 h; (i) CH_2CHCH_2OH/4 Å sieves/montmorillonite K-10 clay/0.5 h; (j) $(PCy_3)_2(PhCH)RuCl_2/CHCl_3$/rt/0.75 h; (k) Pd/10% HCO_2H in MeOH/rt/overnight.



Scheme 4. Reagents and conditions: (a) TsCl (1.5 equiv)/py/0 °C/overnight; (b) NaI (1.5 equiv)/2-butanone/80 °C/overnight; (c) NaOMe (9 equiv)/MeOH/0–45 °C; (d) CH_2I_2 (9 equiv)/Et_2Zn (1 M in hexane; 6 equiv)/CH_2Cl_2/−10 °C to rt/overnight.

18 ~47-fold; **2**: ~860-fold). In PK studies (Table 3), **17** and **18** showed moderate to very high plasma clearance in rats, a species that predicts the human clearance of **2** in humans quite well.[18] Unexpectedly, despite poor oral bioavailability, **17** (administered orally at 10 mg/kg) promoted 24 h UGE similar to the maximal UGE achieved with **2** in rats (10 mg/kg). We attribute the pharmacodynamic activity of **17** in rats to formation of a long-lived active metabolite, possibly ketone **25** (Table 2), which was unambiguously identified as a major metabolite in rats, as well as in rat, dog and human microsome preparations.

Concomitant with characterization of **17** and **18**, synthesis of additional C-5 spiro analogues continued. This effort provided azetidine **19** (SGLT2 IC_{50} = 5100 nM), dioxothietane **20** (SGLT2 IC_{50} = 14 nM), and oxetanes **21**–**24**. Based on the similar potencies of compounds **2**, **3**, and **4**, the range of activity within the oxetane series (SGLT2 IC_{50} ~3–30 nM) was surprising as was the comparatively weak activity of **24**, bearing the same aryl side chain as that in **2**. Compound **22**, having no potential for metabolism to a methyl ketone and being quite potent and selective for SGLT2 (IC_{50} = 6.6 nM), was selected for additional profiling.

In rat PK studies, **22** displayed high bioavailability (100%), and improved clearance (22.3 mL/min/kg) relative to cyclopropyl analogues **17** and **18**. However, in rat acute UGE studies, this compound gave disappointing results (Fig. 2). Despite increasing exposure with dose, **22** was unable to elicit 24 h UGE equal to **2**, even when dosed as high as 60 mg/kg. We attribute this result to a half-life that is short (1.2 h) compared to that of **2** (4.4 h).[19] With this shorter half-life, maintaining high percent inhibition of the target over the dosing interval is much more difficult. Indeed, our modeling of the pharmacokinetic–pharmacodynamic (PK/PD) relationship for SGLT2 indicates that the dose required to achieve the maximal rate of UGE increases exponentially in relation to decreasing half-life, in line with the in vivo results for **22**. From in vitro studies using human liver microsomes and hepatocytes (turnover rates and metabolite identification), it appears that **22** undergoes glucuronidation on the sugar moiety at an increased rate relative to **2**, leading to a comparatively short in vivo half-life.

Table 3
Rat pharmacokinetic parameters for key compounds

Compd	Dose (mg/kg; iv)	Cl (mL/min/kg)	Vdss (L/kg)	$t_{1/2}$ (h)	F^a (%)
2[19]	2	10.0	3.8	4.4	100
17	1	112	5.7	0.59	
18	1	48.9	2.6	0.61	
22	2	22.3	2.3	1.2	100

[a] 5 mg/kg po.

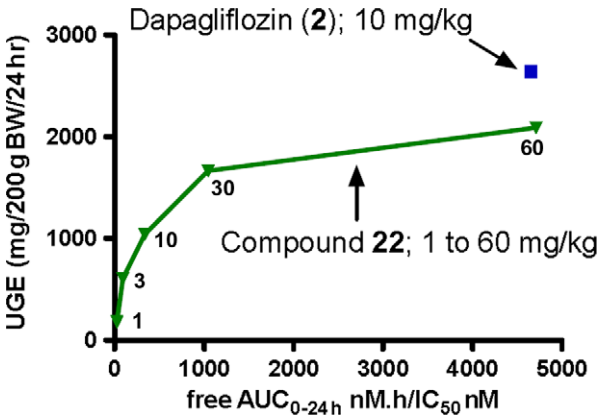


Figure 2. Rat 24 h UGE versus exposure, correcting for SGLT2 potency (BW = body weight). Male Sprague–Dawley rats (n = 6/group) were randomized to receive one of five doses of compound **22** (1, 3, 10, 30, 60 mg/kg) or dapagliflozin (**2**; 10 mg/kg). Following compound administration, urine was collected over 24 h for measurement of glucose excretion. Simultaneously, drug exposure was assessed in satellite animals. Compounds **22** and **2** achieved a maximal UGE of 2000–2500 mg/200 g BW, respectively.

In conclusion, based on these studies, we conclude that structural changes at the C-5 position in the *C*-aryl glycoside SGLT2 inhibitor series can be well tolerated.[20] The C-5 position is the only position whereby OH deletion does not lead to appreciable loss of SGLT2 inhibition. This information allowed the design of a series of novel C-5 spiro analogues, some of which exhibit low nanomolar potency versus SGLT2 and promote urinary glucose excretion (UGE) in rats. However, due to sub-optimal pharmacokinetics (half-life), predicted human doses did not meet criteria for further advancement.

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14. IC_{50} values were obtained by measuring inhibition of sodium-dependent methyl-α-D-[U-^{14}C]glucopyranoside (AMG) uptake in CHO cells stably expressing human SGLT1 or 2; 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.
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18. Rat PK studies were carried out in Sprague–Dawley rats. Allometric scaling of rat clearance for **2** provided an estimate of human clearance in good agreement with the value derived from published PK data for **2**.
19. Although we determined a half-life in rats for **2** similar to that reported in Ref. 3 (4.6 h), our measurements of Cl and Vdss in differ from those reported (4.8 mL/min/kg and 1.7 L/h, respectively).
20. Additional support for this conclusion is provided by the C-5 modifications reported in Ref. 13.