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Aglycone exploration of *C*-arylglucoside inhibitors of renal sodium-dependent glucose transporter SGLT2

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ABSTRACT

Inhibition of sodium-dependent glucose transporter 2 (SGLT2), the transporter that is responsible for renal re-uptake of glucose, leads to glucosuria in animals. SGLT-mediated glucosuria provides a mechanism to shed excess plasma glucose to ameliorate diabetes-related hyperglycemia and associated complications. The current study demonstrates that the proper relationship of a 4′-substituted benzyl group to a β-*1C*-phenylglucoside is important for potent and selective SGLT2 inhibition. The lead *C*-arylglucoside (**7a**) demonstrates superior metabolic stability to its *O*-arylglucoside counterpart (**4**) and it promotes glucosuria when administered in vivo.

Diabetes is a disease characterized by episodic bouts of hyperglycemia for which alternative complementary treatments are needed.[1] Attenuation of renal glucose recovery by inhibition of sodium-dependent glucose transporters (SGLTs) has been suggested as a means to ameliorate hyperglycemia.[2,3] Under normal conditions, excess renal capacity exists to ensure complete glucose recovery from the glomerular filtrate; however, inhibition of SGLT diminishes this capability, resulting in glucosuria. Although the more ubiquitously expressed SGLT1 plays a contributing role, current evidence suggests that the primary effector for renal glucose recovery is SGLT2, which is expressed on the luminal surface of the renal proximal tubules.[4] Selective SGLT2 inhibitors are desired since inhibition of SGLT1, which is predominantly expressed in the gut to absorb glucose and galactose, should produce gastrointestinal disturbances.[5,6] The risk of hypoglycemia due to selective SGLT2 inhibition is expected to be minimal, since the normal counter-regulatory mechanisms would be maintained.

The *O*-arylglucoside natural product phlorizin **1** is a non-selective potent SGLT inhibitor (Fig. 1).[7] Phlorizin protects diabetic animal models against hyperglycemia and associated glucose toxicity; however, poor oral bioavailability due to β-glucosidase-mediated metabolism in the gut necessitates subcutaneous administration.[8] Structural modification of phlorizin by researchers at Tanabe Seiyaku led to the selective *O*-arylglucoside SGLT2 inhibitor T-1095A (**2**).[9] When administered po in rodent models of diabetes as the 6-*O*-methylcarbonate prodrug T-1095, the resulting glucosuria induced by T-1095A, following liberation by liver esterases, markedly ameliorated glycemic levels.[3] Subsequently, we[10] and researchers at Kissei Pharmaceutical Co, Ltd[11] have reported that *O*-arylglucosides of *o*-benzylphenols are potent selective SGLT2 inhibitors. However, the susceptibility of **4**, our lead compound of this series, to β-glucosidase metabolism resulted in species-dependent exposure and glucosuric efficacy.

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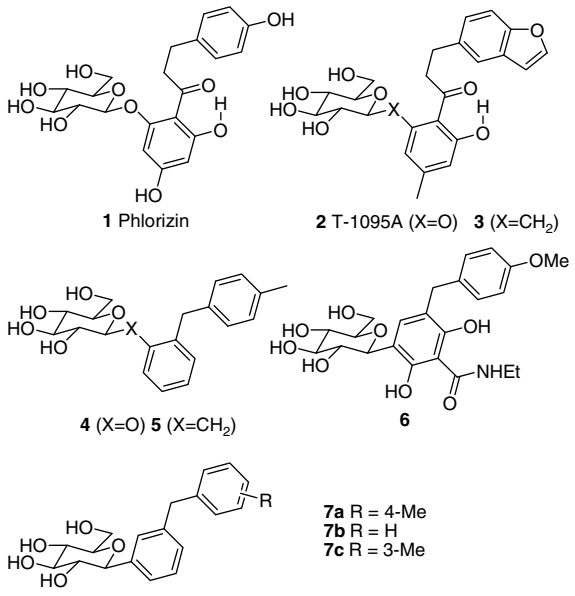


Figure 1. Structures of SGLT inhibitors.

In an attempt to increase the metabolic stability of the glucosyl linkage of *O*-arylglucoside **4**, we synthesized *C*-benzylglucoside **5**.[12] This compound displayed a significant loss in SGLT2 activity (75-fold) as compared to compound **4**. Link et al. reported that a similar modification of **2** to generate the carbon analog **3** produced a >20-fold loss in potency.[13] Together, these findings imply that the isosteric replacement of the oxygen glucosidyl link with a methylene greatly attenuated previously favorable ligand–protein interactions. Possibly, the greater conformational freedom of **3** and **5** contributed to the reduction in SGLT2 affinity due to removal of the conformational constraints imposed by the *exo*-anomeric effect.[14,15]

Fortuitously, an alternative lead for *C*-glucoside-derived SGLT2 inhibitors surfaced upon characterization of **6**, a minor *C*-arylglucoside side-product that was generated during our SGLT2 program.[10] Of particular interest was the *meta* presentation of the glucosyl and benzyl appendages of **6** rather than the typical *ortho* presentation of *O*-glucoside-derived inhibitors. The promising activity of **6** (EC_{50} SGLT2 = 1300 nM) and selectivity (>6-fold vs SGLT1), despite the presence of polar substituents that had been found to be unfavorable in the *O*-glucoside SAR, prompted the synthesis of **7a**, the counterpart of **4**. The in vitro profile of **7a** was extremely encouraging: SGLT2 EC_{50} = 22 nM; >600-fold selectivity vs. SGLT1. The importance of a *para* substituent on the distal ring became readily apparent upon comparison of **7a** to the parent **7b** or *meta* isomer **7c** thereby underscoring the role of the substituent to properly orient the distal ring to achieve high affinity. A similar bias for *para* substitution of the distal ring had been observed for *O*-arylglucoside analogs of both **4** and dihydrochalcones reported by Hongu et al.[16]

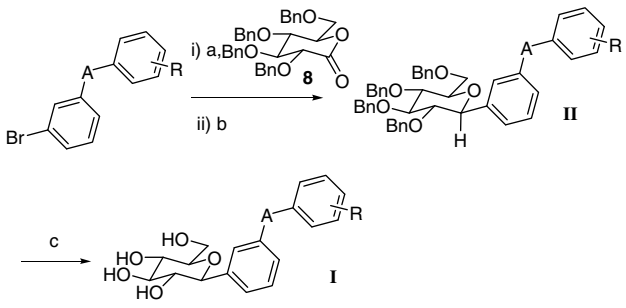
These findings prompted a systematic study of *meta*-*C*-arylglucosides with varying linkers to evaluate proper placement of the distal aryl ring. The assumption was that high-affinity SGLT2 inhibitors require not only the distal aryl ring to bear a lipophilic substituent but also the distal ring that assumes an orientation such that the lipophilic substituent can occupy a favorable binding pocket. Therefore, to ensure that proper conclusions were drawn regarding the consequences of introduction of a zero, one, two or three methylene spacer, three derivatives of each were prepared in which the distal ring was either unsubstituted or bore a *m*-methyl or *p*-methyl group.

The β-*C*-arylglucosides depicted in Scheme 1 were synthesized via the method of Czernecki and Ville[17] Bihovsky et al.[18] and Jaramillo and Knapp,[19] with the limitation that the aglycone functionality be resistant to the strongly basic and strongly acidic conditions employed in this route. Bromodiarylmethanes, prepared by methods reported in the literature,[20] were lithiated and then added to 2,3,4,6-tetra-*O*-benzylgluconolactone[21] (**8**). Reduction of the resultant lactol generated predominantly β-linked glucosides[22] (formula **II**) that were deprotected by hydrogenolysis to give compounds of formula **I** in which A is a methylene.

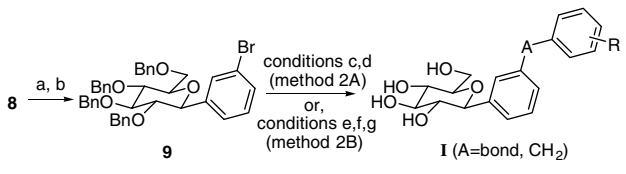
Alternatively, as outlined in Scheme 2, the series of aryl glucosides **I** in which A is a methylene could be obtained by conversion of 2,3,4,6-tetra-*O*-benzyl-1-(3-bromo)phenyl-1-deoxyglucose (**9**) to the corresponding aryl-trimethylstannane, followed by palladium-catalyzed coupling to benzylhalides.[23] The corresponding biphenylglucosides **I** in which A is a bond were obtained as depicted in Scheme 2 by coupling of the versatile intermediate **9** with arylboronic acids. Intermediate **9** was prepared by addition of 3-lithiobromobenzene to gluconolactone **8** followed by reduction of the lactol.

Other aglycones were synthesized as outlined in Scheme 3; in some cases, an unsaturated linker was carried through the synthesis whereupon catalytic hydrogenation concomitantly deprotected the glucosyl benzyl ethers and reduced the double bond. Lewis acidic conditions were used to deprotect benzyl ethers in compounds with aglycone functionality that were incompatible with hydrogenolytic deprotection.[24,25]

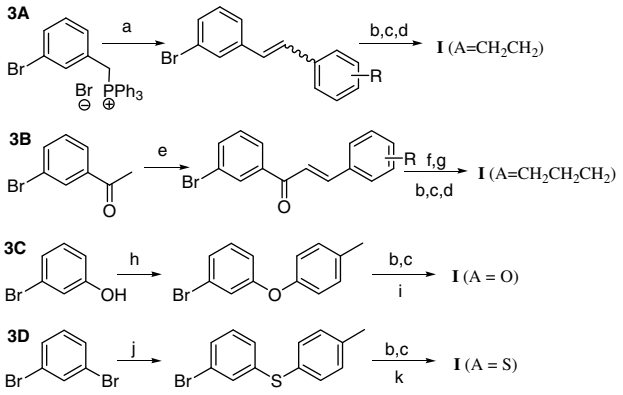
The accompanying table (Table 1) summarizes the structure–activity consequences upon alteration of the spacer moiety between the *C*-glucoside proximal and distal rings. Variation of the spacer from one (**7**) to zero (**10**), two (**11**) or three methylenes (**12**) reduced affinity ~3-fold for the unsubstituted (R = H) and *m*-methyl-substituted examples. In contrast, changing the methylene spacer of **7a** from one to zero (**10c**), two (**11c**), or to three methylenes (**12c**) reduced the binding affinity of the *p*-methyl-substituted analogs 13-, 19-, and 29-fold, respectively. The unique



Scheme 1. General route employed in the synthesis of *C*-arylglucosides. Reagents and conditions: (a) BuLi, THF, −78 °C, then **8** (41–64%). (b) BF_3OEt_2, Et_3SiH, CH_3CN, −30 °C (69–83%). (c) H_2, $Pd(OH)_2$ (33–95%).

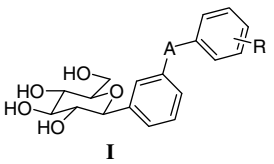


Scheme 2. Synthesis of *C*-glycosyl bromo-aglycone **9** and conversion to compounds of formula **I**. Reagents and conditions: (a) *n*-BuLi, *m*-dibromobenzene, THF, −78 °C; (b) BF_3OEt_2, Et_3SiH, CH_3CN, −40 °C (59%, two steps). (c) $RC_6H_4B(OH)_2$, $Pd(PPh_3)_4$, Toluene/EtOH (3:1), Na_2CO_3, 80 °C, (90%). (d) BCl_3, CH_2Cl_2, −78 °C (21–35%). (e) $Me_3SnSnMe_3$, $Pd(PPh_3)_4$, toluene, 80 °C (63–75%). (f) $RC_6H_4CH_2Br$, $Pd(PPh_3)_4$, THF (49–63%). (g) H_2, $Pd(OH)_2$/C (33–60%).



Scheme 3. Construction of aglycones and conversion to compounds of formula **I**. Reagents and conditions: (a) KHMDS, THF, RC_6H_4CHO (45–99%). (b) *n*-BuLi, THF, −78 °C, then **8**. (c) BF_3OEt_2, *i*-Pr_3SiH, CH_3CN, −40 °C (28–77% two steps). (d) H_2, $Pd(OH)_2/C$, EtOAc (40–70%). (e) NaOH, MeOH, RC_6H_4CHO (100%). (f) $NaBH_4$, MeOH (99%). (g) BF_3OEt_2, Et_3SiH, CH_3CN, −40 °C (73%). (h) 4-$CH_3C_6H_4Br$, $Cu(OAc)_2$, pyridine, TEA, 4 Å MS, CH_2Cl_2 (40%). (i) BBr_3, CH_2Cl_2, −78 °C. (j) *n*-BuLi, $(4\text{-MePhS})_2$, THF, −78 °C (51%). (k) BF_3OEt_2, EtSH (30%).

Table 1
[26]*C*-Aryglucoside (**I**) SAR exploration of the aglycone spacing element (A) and distal ring substituent (R)



I

Compound	A	R	*h*SGLT2 EC_{50} (nM)	Select. vs. *h*SGLT1	Synthetic method Scheme # (overall yield, %)
7a	CH_2	4-Me	22	>600	**1** (42)[27]
7b	CH_2	3-Me	510	ND	**2B** (12)
7c	CH_2	H	190	>50	**2B** (9)
10a	Bond	H	623	>13	**1** (11)
10b	Bond	3-Me	1200	ND	**2A** (19)
10c	Bond	4-Me	290	>30	**2A** (11)
11a	$(CH_2)_2$	H	710	ND	**3A** (50)
11b	$(CH_2)_2$	3-Me	970	ND	**3A** (11)
11c	$(CH_2)_2$	4-Me	430	>20	**3A** (7)
12a	$(CH_2)_3$	H	480	ND	**3A** (6)
12b	$(CH_2)_3$	3-Me	1200	ND	**3B** (14)
12c	$(CH_2)_3$	4-Me	630	>13	**3A** (20)
13	O	4-Me	540	>15	**3C** (2)
14	S	4-Me	69	>100	**3D** (7)
1			35	10	
4			8	350	
1[a]			160	1	
2[a]			50	4	

Note: [a]EC_{50} data from Oku et al.[28]

advantage conferred by the single methylene of **7a** is further confirmed by the respective 3- and 25-fold reduction in affinity upon replacement with a sulfur (**14**) or oxygen (**13**) bridging atom. Significant inhibition of human SGLT1 was not observed for any of the *C*-arylglucosides tested. In particular, the demonstrably high level of selectivity of **7a** is expected to preclude gastrointestinal side effects.

Upon iv administration to rats and mice at 1 and 0.3 mg/kg, respectively, **7a** produced maximum glucosuric levels of 230 and 600 mg/dL.[29] In contrast, upon administration of *O*-glucoside **4** under the same conditions, efficacy in rats was reduced ~50-fold relative to that obtained in mice. *C*-Arylglucoside **7a** was found to be ~100-fold more stable in the presence of rat liver microsomes than the corresponding *O*-glucoside **4**.[30] We attribute the greater in vitro stability of **7a** and the diminished variability in glucosuric response across species to **7a** being impervious to glucosidase cleavage (unlike **4**). Further discussion of the in vitro SAR and in vivo efficacy of **7a** and analogs leading to the discovery of dapagliflozin[31] will be a subject of a subsequent publication.

Conclusions: A combination of the *meta*-aryl presentation of the salient structural elements of compound **6** and SAR considerations of the *o*-benzylaryl-*O*-glucosides represented by **4** led to the discovery of the *C*-arylglucoside **7a** as a potent SGLT2 inhibitor. A comparison of the profile of the *C*-glucoside **7** to that of **4** reveals greater selectivity versus SGLT1, as well as enhanced metabolic stability. *C*-Arylglucosides show enhanced glucosuric activity in rats compared to *O*-arylglucosides that we attribute, in part, to the metabolic stability of the aryl-glucosyl C–C bond. Further exploration of the in vitro SAR around compound **7a** and in vivo results will be reported in due course.

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26. The intracellular accumulation of the SGLT-selective glucose analog [^{14}C]-alpha-methyl glucopyranoside (AMG) by CHO cells expressing the human SGLT2 or the human SGLT1 transporter was quantified in vitro in the presence and absence of inhibitors, using the following conditions: Each inhibitor,

dissolved in DMSO, was tested at 8 concentrations in the presence of 137 mM NaCl and 10 μM [^{14}C] AMG, over a 120-min incubation in protein-free buffer. Percent inhibition of transport activity was calculated based on a comparison of the activity of non-inhibited control cells treated with DMSO alone. The response curve was fitted to an empirical four-parameter model to determine the inhibitor concentration at half-maximal inhibition. The reported EC_{50} is the aggregate result of triplicate dose–response determinations.

27. Characterization of compound **7a**: ^{1}H NMR (400 MHz, CD_3OD) δ 7.27 (s, 1H), 7.23 (d, 2H, J = 4 Hz), 7.1 to 7.0 (m, 5H), 4.08 (d, 1H, J = 9 Hz), 3.91 (s, 2H), 3.90 to 3.85 (m, 1H), 3.70 to 3.65 (m, 1H), 3.37 to 3.30 (m, 4H), 2.27 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 142.7, 140.8, 139.5, 136.5, 130.0, 129.8, 129.6, 129.5, 129.1, 126.6, 83.7, 82.2, 79.8, 76.4, 71.9, 63.2, 42.4, 21.0. HPLC: 99.0% pure, 6.56 min retention time. Gradient 0–100% B over 8 min. Solvent A: 10%MeOH/H_2O + 0.2%H_3PO_4. Solvent B: 10%MeOH/H_2O + 0.2%H_3PO_4. Column: YMC S-5 C-18 4.6 × 50 mm. Flow rate: 2.5 mL/min. Monochrome detection at 220 nm.
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30. The oxidative metabolism of SGLT2 inhibitors was evaluated in liver microsomes using the following conditions: Each BMS compound was dissolved in acetonitrile and tested at a concentration of 10 μM in the presence of 1 mM NADPH and 1 mg/mL microsomal protein in 50 mM potassium phosphate buffer. The final organic solvent content was 1%. Incubations were conducted for various times, up to 60 min. Percent turnover was calculated based on a comparison of the parent peak at time t compared to that at time 0. Rates (nmol/min/mg) were calculated by taking the % turnover and multiplying by the starting nanomoles of substrate in incubation, then dividing by time of incubation and milligrams of protein.
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