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Glycosylated dihydrochalcones as potent and selective sodium glucose co-transporter 2 (SGLT2) inhibitors

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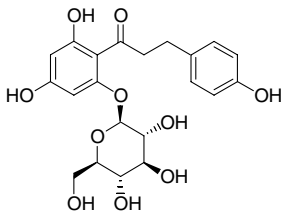
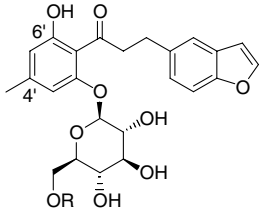
Abstract—A series of glucose conjugates was synthesized and tested for inhibition of SGLT1 and SGLT2. The core structure was derived from compound **1a**. Modification of the benzofuran moiety and 4′-substituent of the phenyl ring in compound **1a** improved selectivity at SGLT2. Select compounds were compared to **1a** in metabolic stability and in vivo efficacy studies.

Diabetes mellitus is a polygenic disorder characterized by chronic hyperglycaemia associated with a deficiency in insulin action. In diabetic patients, one mechanism for protection against the adverse effects of high plasma glucose levels is the compensatory increase in urinary glucose excretion.[1] In the kidney, plasma glucose is continuously filtered in the glomerulus and then reabsorbed in the proximal tubules by a class of transporters called the sodium-glucose co-transporters (SGLTs).[2] Renal reabsorption of glucose is mediated by SGLT1 and SGLT2,[1] while intestinal absorption of glucose is primarily mediated by SGLT1 and the facilitative glucose transporter GLUT5.[3]

Phlorizin, a natural SGLTs specific inhibitor, provided proof of concept in vivo by promoting glucose excretion and lowering fasting and postprandial blood glucose without hypoglycemic side effects in several animal models.[4] Compound **1a** and its carbonate prodrug **1b**, which are synthetic analogues of Phlorizin, have been shown to inhibit glucose transport in brush border membrane vesicles by inhibition of both SGLT1 and SGLT2.[5] Other synthetic SGLT inhibitors have also been published as potential treatments for diabetes.[6] Considering that SGLT1 is a high affinity/low capacity transporter and SGLT2 is a low affinity/high capacity transporter, selective inhibition of SGLT2 should produce a therapeutically useful enhancement of urinary glucose excretion. In addition, inhibition of SGLT1 may produce unwanted side effects.[7] We report herein a series of Phlorizin analogues with increased selectivity towards SGLT2.



Phlorizin



1a. R = H
1b. R = CO_2CH_3

Our goal was to synthesize compounds that would address the structure–activity relationships of three regions in compound **1a**: the benzofuran portion, the 4′-position of the phenyl ring, and the 6′-hydroxyl group of the phenyl ring. As shown in Scheme 1,[5a] the appropriately substituted resorcinol **2** was treated with acetic anhydride and then subjected to Fries rearrangement conditions to give the ketone **3**. For analogues where R_1 = Cl and R_2 = H, the ketone **4** was commercially available. For analogues where $R_1 = NBn_2$ and R_2 = Me, the ketone **5** was prepared from pentane-2,4-dione and 2,2,6-trimethyl-[1,3]dioxin-4-one as reported in the literature.[8] Monoglycosylation of **3**–**5** proceeded under phase transfer conditions with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide to give the peracetylated sugar derivatives **6**–**8**. Aldol condensation with the desired aldehyde under saponifying conditions, followed by

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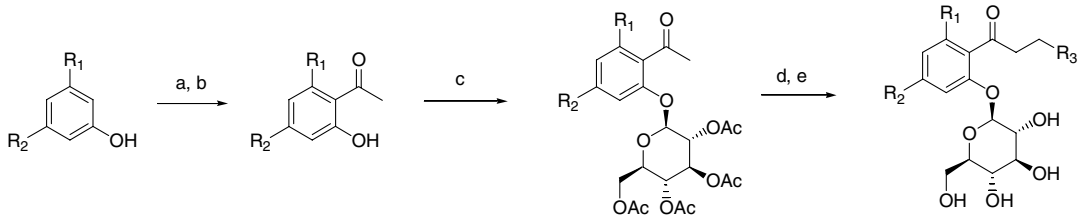


2a. R_1 = OH, R_2 = H
2b. R_1 = OH, R_2 = Me
2c. R_1 = OH, R_2 = Et
2d. R_1 = OH, R_2 = iPr
2e. R_1 = OH, R_2 = Cl
2f. R_1 = OH, R_2 = F

3a. R_1 = OH, R_2 = H
3b. R_1 = OH, R_2 = Me
3c. R_1 = OH, R_2 = Et
3d. R_1 = OH, R_2 = iPr
3e. R_1 = OH, R_2 = Cl
3f. R_1 = OH, R_2 = F
4. R_1 = Cl, R_2 = H
5. R_1 = NBn_2, R_2 = Me

6a. R_1 = OH, R_2 = H
6b. R_1 = OH, R_2 = Me
6c. R_1 = OH, R_2 = Et
6d. R_1 = OH, R_2 = iPr
6e. R_1 = OH, R_2 = Cl
6f. R_1 = OH, R_2 = F
7. R_1 = Cl, R_2 = H
8. R_1 = NBn_2, R_2 = Me

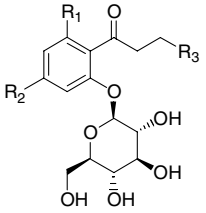
9a - 9q
10a - 10c
11a - 11c

Scheme 1. Reagents: (a) acetic anhydride, pyridine; (b) $AlCl_3$, chlorobenzene, 90 °C; (c) 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide, benzyltributylammonium chloride, K_2CO_3, $CHCl_3$, H_2O; (d) R_3CHO, KOH, EtOH; (e) H_2, Pd/C, DAMP, EtOH.

hydrogenation of the ensuing olefin gave the target compounds **9**–**11**.[9]

All compounds were screened in the SGLT cell base functional assay.[10] Modification of the benzofuran moiety of **1a** was explored first (Table 1). Changing from benzofuran to other bicyclic ring systems such as naphthalene (**9a**) or benzodioxane (**9b**) led to analogues with similar SGLT2 inhibitory activity to **1a** but with increased selectivity for SGLT2 versus SGLT1. Other bicyclic heterocycles gave analogues with similar potencies (data not shown). The monocyclic 4-ethoxyphenyl

Table 1. In vitro screening data for SGLT inhibitory activity and selectivity



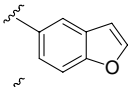
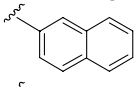
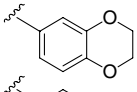
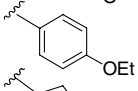
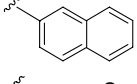
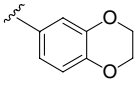
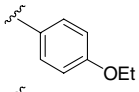
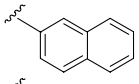
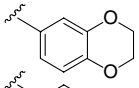
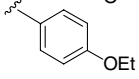
Entry	R_1	R_2	R_3	SGLT2 K_i[a] (μM)	SGLT1 K_i[a] (μM)	Ratio (SGLT1/SGLT2)
1a	OH	Me		0.004 ± 0.001	0.058 ± 0.004	14.5
9a	OH	Me		0.010 ± 0.004	1.325 ± 0.034	132.5
9b	OH	Me		0.009 ± 0.001	1.461 ± 0.432	162.2
9c	OH	Me		0.017 ± 0.007	1.573 ± 0.395	92.5
9d	OH	Me		0.235 ± 0.019	4.383 ± 0.705	18.7
9e	OH	Me		0.070 ± 0.004	1.384 ± 0.006	19.8
9f	OH	Me		0.045 ± 0.002	1.080 ± 0.273	24.1
10a	Cl	H		>1.7	>45	—
10b	Cl	H		>1.7	>45	—

Table 1 (*continued*)

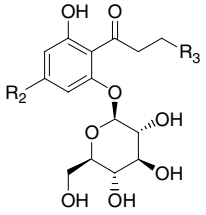
Entry	R_1	R_2	R_3	SGLT2 K_i[a] (μM)	SGLT1 K_i[a] (μM)	Ratio (SGLT1/SGLT2)
10c	Cl	H		>1.7	>45	—
11a	NH_2	Me		0.520 ± 0.008	9.897 ± 2.626	19.1
11b	NH_2	Me		0.297 ± 0.020	16.15 ± 3.791	54.3
11c	NH_2	Me		1.283 ± 0.300	13.52 ± 1.348	10.5

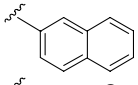
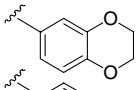
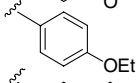
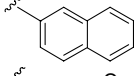
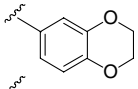
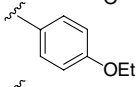
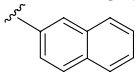
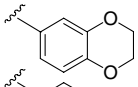
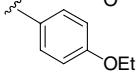
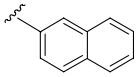
[a] K_i values are mean ± SEM.

analogue (**9c**) gave a similar result. However, the monocyclic furan-(**9d**), thiophene-(**9e**) and cyclohexane-(**9f**) analogues were all less potent and less selective than **9b**. Thus, bicyclic or 4-substituted phenyl rings found in compounds **9a**–**c** were the optimal replacements for the benzofuran moiety in **1a**. In this set, the best results were obtained with the benzodioxane analogue **9b**. This compound is a potent SGLT2 inhibitor and 162-fold selective for SGLT2 over SGLT1.

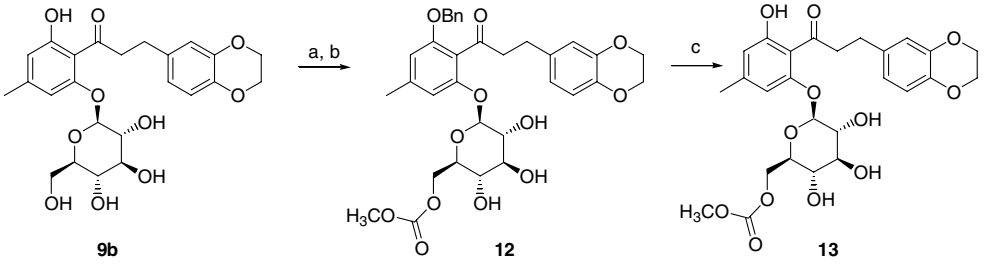
The importance of the 6′-hydroxyl group was investigated next. The hydroxyl group can act as either a hydrogen bond donor (via intermolecular interactions or intramolecular interaction with the ketone) or as a

Table 2. In vitro screening data for SGLT inhibitory activity and selectivity



Entry	R_2	R_3	SGLT2 K_i[a] (μM)	SGLT1 K_i[a] (μM)	Ratio (SGLT1/SGLT2)
9g	Et		0.018 ± 0.005	6.50 ± 1.42	361.1
9h	Et		0.015 ± 0.004	6.28 ± 0.99	418.6
9i	Et		0.034 ± 0.007	8.39 ± 2.01	246.7
9j	*i*-Pr		0.150 ± 0.021	8.59 ± 0.21[b]	57.3[b]
9k	*i*-Pr		0.029 ± 0.004	16.1 ± 3.27	554
9l	*i*-Pr		0.110 ± 0.009	33.1 ± 4.40	301
9m	Cl		0.031 ± 0.018	4.78 ± 0.05	154.2
9n	Cl		0.029 ± 0.010	3.75 ± 0.45	129.3
9o	Cl		0.087 ± 0.008	4.64 ± 0.25	53.3
9p	F		0.071 ± 0.011	3.69 ± 0.21	51.9

[a] K_i values are mean ± SEM.
[b] Maximal efficacy could not be achieved for compound **9j** in this assay due to poor substrate solubility at higher concentrations.



Scheme 2. Reagents: (a) BnBr, K_2CO_3, acetone; (b) methyl chloroformate, collidine; (c) Pd/C, H_2 (30 psi), EtOH.

hydrogen bond acceptor. It has been reported that conversion of the hydroxyl group to a methoxy group reduced the inhibitory activity in vivo, while removal of the hydroxyl group eliminated all in vivo activity.[5c] Introduction of a chloro-group, which has no hydrogen bond donor character but greater hydrogen bond acceptor character than a methoxy-group, led to analogues **10a–c** with potency in the micromolar range. Introduction of an amino-group at the 6′-position maintained the hydrogen bond donor character and added greater hydrogen bond acceptor character. This modification led to analogues **11a–c** that were less potent and less selective than compounds **9a–c**. Additional analogues that are designed to address the role of substituents at this position are in progress.

The role of substitution at the 4′-position was examined with a series of compounds that encompassed alkyl and halogen groups (Table 2). Increasing the size of the substituent from methyl to ethyl or isopropyl gave compounds **9g–l**, which were uniformly less potent than **9a–c** but more selective for inhibition of SGLT2. The benzodioxane analogue **9k** was the most selective with a subtype K_i ratio of 554, and it should be noted that the benzodioxane ring system maintained potency in this series across a range of changes in other regions.

The electronic character of the 4′-substituent was also investigated. The electron-withdrawing chloro-group was introduced in analogues **9m–o**. These compounds were comparable in potency to the isopropyl series **9j–l**, but were not as selective, perhaps due to the decrease in steric bulk. Following this trend, the smaller 4′-fluoro-substituent **9p** had comparable potency to the ethyl analogue **9g**, but showed even less selectivity for inhibition of SGLT2.

Select compounds were tested for human liver microsomal stability, indicative of a compound's first-pass liability.[11] While compound **1a** showed a metabolic half-life of 24 min, compounds **9b**, **9c** and **9i** all exhibited microsomal stability greater than 45 min. Compound **9b** and its methyl carbonate prodrug **13** (Scheme 2) were evaluated for their ability to induce urinary glucose excretion in male Zucker Diabetic Fatty (ZDF) rats.[12] When a 3 mg/kg dose was administered intravenously, compound **9b** induced excretion of 608 mg of urinary glucose, indicating this compound had inhibitory activity at SGLT2 in vivo. However, compound **1a** induced excretion of 1189 mg of urinary glucose at the same dose by intravenous administration, which was about 2-fold more efficacious than compound **9b**. Both compounds **9b** and **13** induced only marginal urinary glucose excretion at doses of 100 mg/kg with oral administration, probably due to poor drug exposure, as both compounds **9b** and **13** showed no bioavailability in standard rat PK studies.

In summary, we have developed a series of SGLT inhibitors to further evaluate the SAR of compound **1a**. Modification of the benzofuran moiety and 4′-substituent of the phenyl ring improved selectivity at SGLT2 with a slight decrease in potency, and increased human microsomal stability compared to compound **1a**. In addition, these studies show that the 6′-hydroxyl group cannot be replaced with a chloro- or amino-group. The SAR derived from these studies could be used in the search for a novel series of SGLT2 inhibitors. Progress will be reported in due course.

References and notes

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WO02/068439; *Chem. Abstr*. **2002**, *137*, 232854; (d) Fujikura, H.; Fushimi, N.; Nishimura, T.; Tatani, K.; Katsuno, K.; Hiratochi, M.; Tokutake, Y.; Isaji, M. WO01/68660; *Chem. Abstr*. **2003**, *135*, 242456; (e) Washburn, W. N.; Ellsworth, B.; Meng, W.; Wu, G.; Sher, P. M. US03/0114390; *Chem. Abstr*. **2003**, *139*, 36736.

7. Unwanted side effects due to SGLT1 inhibition may include flatulence and bloating, a consequence of altered carbohydrate absorption from the intestine. It is also possible that SGLT1 inhibition may have unwanted effects in target tissues other than the intestine. SGLT1 expression has been reported to be very high in the heart. The functional consequence of SGLT1 expression in the heart remains unknown. See: Zhou, L.; Cryan, E. V.; D'Andrea, M. R.; Belkowski, S.; Conway, B. R.; Demarest, K. T. *J. Cell. Biochem.* **2003**, *90*, 339.
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9. All compounds provided satisfactory spectral data (^{1}H NMR, LCMS) and were homogeneous by TLC.
10. Cell-based assay for sodium-dependent glucose transport: Cell-based functional screens were conducted to assess SGLT inhibitors. CHO-K1 cells overexpressing human SGLT2 or SGLT1 were used. Cells were treated with compound in the absence or presence of NaCl for 15 min. Cells were then labelled with ^{14}C-α-methylglucopyranoside (AMG)—a nonmetabolizable glucose analogue specific for sodium-dependent glucose transporters. After 2 h the labelled cells were washed three times with ice-cold PBS. Cells were then solubilized and Na-dependent ^{14}C-AMG uptake was quantified by measuring radioactivity.
11. Tests were conducted at Absorption Systems Exton PA. Incubation at 37 °C with test compound at 5 μM and 1 mg protein/mL human liver microsomal prep. Half life was determined by measuring the percent parent compound remaining. In this system, values above 30 min were considered acceptable.
12. Male Zucker Diabetic Fatty (ZDF) rats (7–8 weeks) were obtained from Charles River. Animals were maintained on a 12-h light/dark cycle in a temperature-controlled room. Animals were given ad libitum access to food (standard rodent diet Purina 5008) and water. Animals were fasted for 12 h prior to initiation of the experiment. On the morning of the experiment, animals were administered vehicle (0.5% methylcellulose) or compound by oral gavage (2 mL/kg). For intravenous dosing, animals received either vehicle (10% Solutol) or compound (2 mL/kg). After 1 h, animals received an oral glucose challenge (4 mL/kg of 50% solution) and were immediately placed in metabolism cages. Animals were given free access to water and urine was collected for 4 h. Urinary glucose was quantified using the Trinder reagent (Sigma).