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Novel L-Xylose Derivatives as Selective Sodium-Dependent Glucose Cotransporter 2 (SGLT2) Inhibitors for the Treatment of Type 2 Diabetes

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Abstract: The prevalence of diabetes throughout the world continues to increase and has become a major health issue. Recently there have been several reports of inhibitors directed toward the sodium-dependent glucose cotransporter 2 (SGLT2) as a method of maintaining glucose homeostasis in diabetic patients. Herein we report the discovery of the novel *O*-xyloside **7c** that inhibits SGLT2 in vitro and urinary glucose reabsorption in vivo.

The prevalence of diabetes has become an increasing concern to the world's population. In 2007, approximately 246 million people were affected by the disease, with an additional 7 million people developing the disease each year. It is estimated that by 2025, 380 million people will have diabetes. Diabetes is a metabolic syndrome characterized by hyperglycemia, which results from an absolute deficiency in insulin secretion (type 1 diabetes) or from resistance to insulin action combined with an inadequate compensatory increase in insulin secretion (type 2 diabetes). Chronic hyperglycemia is a major risk factor for microvascular complications such as retinopathy, nephropathy, and neuropathy. If attempts to adopt a healthier lifestyle fail to achieve and maintain target glycemic levels, additional therapies are required.

Attention has recently focused on the potential of sodium-glucose cotransporters (SGLTs[a]) as new drug targets for the treatment of diabetes.[1] The SGLT family consists of several isoforms that actively transport glucose and galactose across intestinal and renal membranes, a process that is coupled with sodium ion transport.[2] SGLT2 is a low affinity, high capacity sodium-glucose cotransporter located mainly in the S1 segment of the proximal tubule of the kidney.[3] In a healthy person, greater than 99% of the plasma glucose filtered in the kidney is reabsorbed. SGLT2 facilitates approximately 90% of this reabsorption. The remaining 10% is likely mediated by SGLT1, a high-affinity cotransporter located in the intestines and the renal proximal tubule. Using our knockout technology,[4,5] we generated mice lacking SGLT2. These mice exhibited significant glucosuria but were otherwise healthy, and their glucose tolerance was markedly improved after an oral glucose challenge. Likewise, humans with inactivating SGLT2 mutations exhibit persistent renal glucosuria but are otherwise healthy.[6,7] However, humans with inactivating SGLT1 mutations are unable to transport glucose or galactose normally across the intestinal wall, resulting in the potentially life-threatening condition known as glucose-galactose malabsorption.[8,9] Therefore, inhibition of SGLT2 appears to be an attractive way to improve glucose homeostasis. SGLT2 inhibition is expected to clear glucose from the bloodstream by increasing urinary glucose excretion, a mechanism that does not require insulin secretion from marginally functioning pancreatic beta cells.

The first SGLT inhibitor studied was the natural product phlorizin (**1**), which is an *O*-arylglycoside derived from D-glucose attached to a chalcone derivative.[10] Its ability to induce glucosuria when ingested was observed over 120 years ago by von Mering.[11] While phlorizin served as the standard to study the mechanism of action of renal glucose transporters, it was not developed further as a drug because of its poor intestinal absorption and its propensity to hydrolyze to the aglycon, phloretin, in the presence of lactase-phlorizin hydrolase.

In the past few years, several different compounds have been reported as inhibitors of SGLTs (Figure 1) and studied in clinical trials.[12,13] The small molecule inhibitors generally fall into two classes, *O*-glycosides and *C*-glycosides. The *O*-glycosides that have advanced furthest in the clinic are sergliflozin (**2**) and remogliflozin (**3**). Sergliflozin was disclosed by Kissei in 2003 as a potential treatment for diabetes and obesity.[14−18] In 2007, Kissei began developing remogliflozin, a related *O*-glycoside.[19] Like phlorizin, both of these compounds are D-glucose derivatives. They both require relatively large doses in the clinic perhaps because of their inherent metabolic instability in the presence of glucosidases.

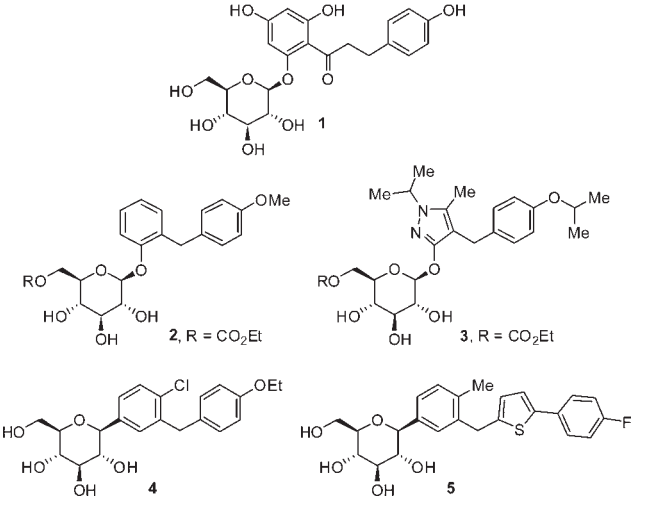

Figure 1. Representative SGLT2 inhibitors.

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[a] Abbreviations: SGLT, sodium-dependent glucose cotransporter; SAR, structure−activity relationship; AMG, α-methylglucopyranoside; IC_{50}, half maximal (50%) inhibitory concentration; mSGLT, mouse SGLT; hSGLT, human SGLT; DIO, diet-induced obese.

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Bristol-Myers Squibb recently disclosed the structure and clinical data of their SGLT2 inhibitor dapagliflozin (**4**),[20,21] a *C*-arylglycoside currently in phase III clinical trials.[22−24] Dapagliflozin was found to have improved in vitro and in vivo potencies compared to the *O*-glycosides. This observed increase in in vivo potency has been attributed to the metabolic stability of the C1 aryl linker to glucosidases present in the intestines, liver, and kidneys.[12] Canagliflozin (**5**), also a *C*-arylglycoside SGLT2 inhibitor, codeveloped by Mitsubishi Tanabe Pharma and Johnson & Johnson, is currently in phase II clinical trials.[25,26]

Herein we report the discovery of a novel class of SGLT inhibitors derived from L-xylose. These compounds are highly potent and have been shown to be stable in both in vitro and in vivo models.

To assess the in vitro potency of SGLT2 inhibitors and their selectivity against SGLT1, cell-based assays were established. When stably expressed in cells, SGLT2 and SGLT1 mediate the sodium-coupled uptake of α-methylglucopyranoside (AMG), a metabolically stable glucose analogue specific for SGLT.[27] The inhibition levels were determined by measuring SGLT2- and SGLT1-mediated [^{14}C]AMG uptake in the presence of increasing compound concentration. Phlorizin (**1**) was used as a reference compound.

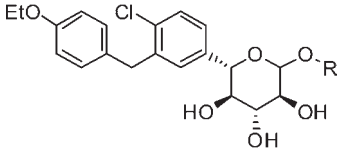
In our efforts to find potent inhibitors of SGLT2, we were attracted to the idea of using L-xylose instead of D-glucose as the sugar core. We believed that converting the scaffold from a D-glucose to an L-xylose would provide advantages from being a non-natural sugar and a non-glucose scaffold. We hoped this would render the compound more stable in the presence of glucosidases and avoid cross-reactivity with other glucose-binding enzymes. In addition, L-xylose would have the same relative configuration of the secondary alcohols as found in D-glucose.

Investigation into the structure−activity relationships (SAR) began by surveying various 1-substituted *O*-xylosides (**6**−**12**). It was quickly discovered that these compounds exhibited low nanomolar inhibition of SGLT2 in vitro (Table 1). Smaller groups at the anomeric position provided more potent compounds. For example, methyl xyloside **7a** was more active against mSGLT2 and hSGLT2 than its ethyl analogue **8**. While the mixture of anomers **7a** had an in vitro activity of 25 nM at hSGLT2, separation of the anomers revealed the β anomer **7c** to be over 300-fold more potent than the alpha anomer **7b**. This preference for the β anomer was also observed for the isopropyl analogues (**9b** versus **9a**). Variation of the R group revealed that small polar groups such as 2-hydroxyethanol **10** were tolerated, but the presence of a larger substituent such as a benzyl ether (**11**) or a more polar group such as 2-dimethylaminoethanol (**12**) diminished activity at SGLT2.

We next made various changes to the aromatic region keeping the methyl *O*-xyloside core of **7a** constant. Compared to **7a**, all changes to the central aromatic ring led to a decrease in potency (Table 2). Chloride substitution proved to be optimal. Replacement of chlorine with methyl (**13**) or hydrogen (**14**) decreased potency. Modifications to the methylene linker were also detrimental to potency (**15**−**17**).[28] However, a linker between the phenyl moieties was necessary, as biphenyl **18** led to a sharp decrease in activity against SGLT2.

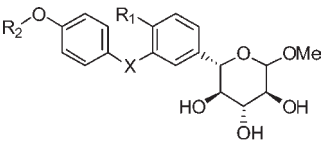
After the central aromatic moiety and linker were optimized, it remained to explore the SAR at the distal aromatic ring, particularly at the phenol (Table 3). A variety of different functionalities were well-tolerated at this position with low nanomolar inhibition of hSGLT2, including the more soluble hydroxyethyl and methoxyethyl moieties (**20** and **21**) and *N*-methylpyrrolidine **22**. The incorporation of aromatic rings,

Table 1. In Vitro Potency of C1 Analogues



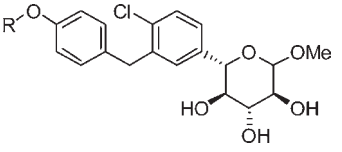
Cmpd	R	anomers α/β	mSGLT2 IC_{50} (nM)	hSGLT2 IC_{50} (nM)
6	H	1:1	100	280
7a	Me	1.2:1	7.1	25
7b	Me	> 20:1	280	4800
7c	Me	1: > 20	6.3	14
8	Et	1.8:1	19	58
9a	*i*-Pr	10:1	190	420
9b	*i*-Pr	1: > 20	15	33
10	$(CH_2)_2OH$	1: > 20	23	36
11	Bn	1: > 20	330	620
12	$(CH_2)_2NMe_2$	1: > 20	2200	3600

Table 2. In Vitro Potency of Compounds with Changes to the Central Aromatic Ring



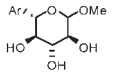
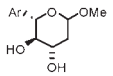
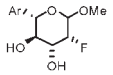
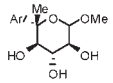
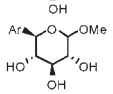
Cmpd	R_1	X	R_2	mSGLT2 IC_{50} (nM)	hSGLT2 IC_{50} (nM)
7a	Cl	CH_2	Et	7.1	25
13	Me	CH_2	Et	23	55
14	H	CH_2	Et	220	1100
15	Cl	S	Me	100	180
16	Cl	O	Et	1900	2400
17	Cl	C(O)	Et	57	100
18	H	none	Et	1900	4600

Table 3. In Vitro Potency of Phenol Analogues



Cmpd	R	mSGLT2 IC_{50} (nM)	hSGLT2 IC_{50} (nM)
7a	Et	7.1	25
19	H	8.0	32
20		9.5	36
21		16	45
22		92	170
23		60	150
24		330	740

Table 4. Changes to the L-Xylose Core

Cmpd	Core[a]	mSGLT2 IC_{50} (nM)	hSGLT2 IC_{50} (nM)
6a		7.1	25
25		14000	>30000
26[b]		220	600
27		220	590
28		9200	>30000

[a] Ar = 4-chloro-3-(4-ethoxybenzyl)phenyl. [b] 2:1 ratio of diastereomers at C2.

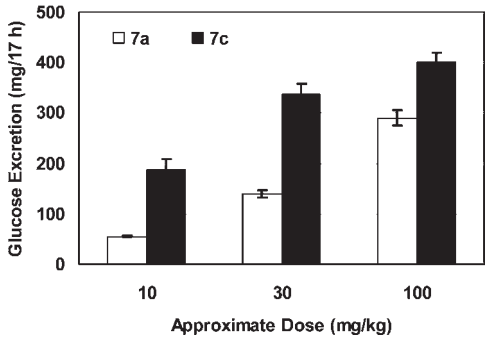


Figure 2. Effect of single oral dose of SGLT2 inhibitor **7a** or **7c** on glucose excretion in DIO mice during the first 17 h following administration (n = 6, vehicle = 1.4 ± 0.1 mg/17 h glucose excretion).

heterocycles (**23**), or thioamides (**24**) resulted in a decrease of activity. Interestingly, the free phenol **19** was a very active inhibitor; however, the metabolic susceptibility of phenols made the original phenetole **7a** a more attractive candidate.

With the SAR at the 2 and 5 positions of the L-xylose moiety developed, modification of the xylose core itself was examined (Table 4). The intact xylose core of **7a** was found to be optimal. Any changes to this core sustained a marked decrease in inhibition of SGLT2 (**25**−**27**). Changing the core from the L- to the D-xylose (**28**) abolished the activity against SGLT2.

The in vitro potencies of the anomeric mixture **7a** and β anomer **7c** against hSGLT1 and hSGLT2 compared to phlorizin (**1**) and dapagliflozin (**4**) are summarized in Table 5. Compound **7c** exhibited a 134-fold selectivity for SGLT2, comparable to the 169-fold selectivity observed for dapagliflozin (**4**).[29]

Dose-dependent glucosuria was observed following oral administration of inhibitor **7a** and **7c** at doses ranging from 10 to 100 mg/kg in adult c57 diet-induced obese (DIO) mice (Figure 2). Compound **7c** was confirmed as the more active anomer in vivo, since glucosuria induced by **7c** was significantly greater than that by anomeric mixture **7a**.

Single dose administration of **7c** by oral gavage or as dietary admixture resulted in sustained glucosuria beyond 24 h. The prolonged pharmacodynamic effect suggests that dosing once per day would be sufficient for chronic administration (Figure 3).

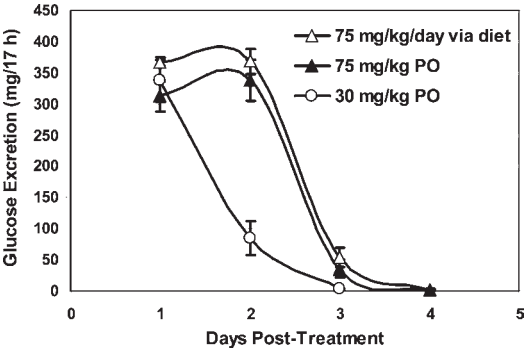


Figure 3. Extended glucosuria of L-xyloside **7c** following single dose administration by oral gavage (po) or as dietary admixture (via diet) (n = 6).

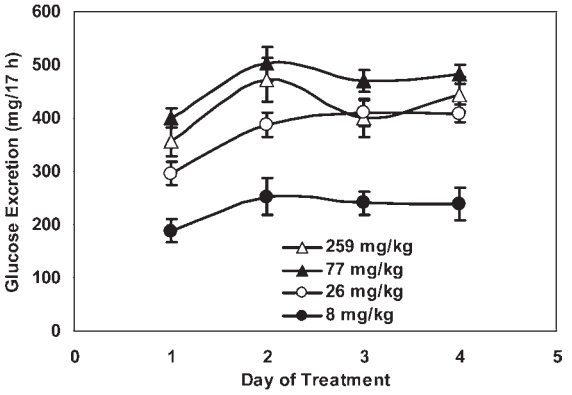


Figure 4. Effect of repeated daily dosing of **7c** via dietary admixture on daily glucose excretion of DIO mice (n = 6). Average daily doses were 8 ± 0.2, 26 ± 0.9, 77 ± 2.6, and 259 ± 6.0 mg/kg.

Table 5. hSGLT2 and hSGLT1 Inhibition for Compounds **1**, **4**, **7a**, and **7c**

Cmpd	hSGLT2 IC_{50} (nM)	hSGLT1 IC_{50} (nM)	hSGLT1/ hSGLT2
1	36	210	5.6
4	4.8	810	169
7a	25	2600	105
7c	14	1900	134

To assess in vivo efficacy of the β anomer **7c** in a repeated dose study, an 11-step diastereoselective synthesis was devised to provide multigram quantities of analytically pure β anomer.[30] DIO mice were administered compound for 4 consecutive days as a dietary admixture. Consistent with data presented in Figure 3, mice receiving daily doses of 26 mg/kg and above exhibited maximal daily urinary glucose losses beginning on day 1 (Figure 4).

In summary, we have reported herein the discovery of a novel series of inhibitors against SGLT2, a potential therapeutic target for the treatment of diabetes. These L-xylosides are potent in vitro and in vivo. Further results of the refinement and development of these compounds for therapeutic use will be reported in subsequent publications.

Acknowledgment. We thank Lisa Imbrogno for development and execution of chromatographic separation methods to access anomerically pure xylosides **7b** and **7c**.

Supporting Information Available: Synthesis details, analytical data of the compounds, in vitro and in vivo methods, and PK properties of **7a** and **7c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(29) The published selectivity of dapagliflozin for hSGLT1/hSGLT2 is 1200-fold (see ref 19). However, in our assays, we observed a 169-fold selectivity for hSGLT2. See Supporting Information for further details on the experimental methods used in this report.

(30) The full diastereoselective synthesis starting from commercially available L-xylose is provided in the Supporting Information.