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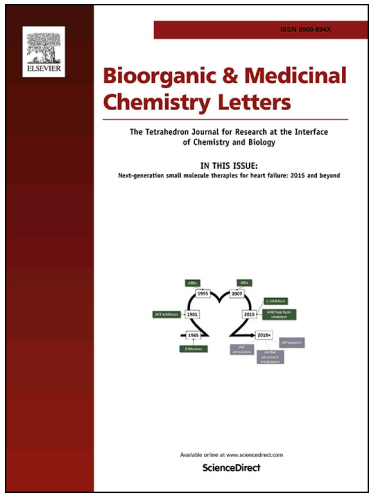

Design, Synthesis and Biological Evaluation of (2S,3R,4R,5S,6R)-5-Fluoro-6-(hydroxymethyl)-2-aryltetrahydro-2H-pyran-3,4-diols as Potent and Orally Active SGLT Dual Inhibitors

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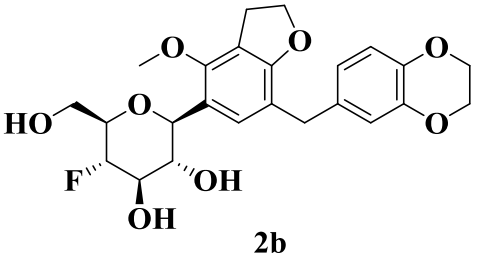
ABSTRACT: A new series of (2S,3R,4R,5S,6R)-5-fluoro-6-(hydroxymethyl)-2-aryltetrahydro-2H-pyran-3,4-diols as dual inhibitors of sodium glucose co-transporter proteins (SGLTs) were disclosed. Two methods were developed to efficiently synthesize C_5-fluoro-lactones **3** and **4**, which are key intermediates to the C_5-fluoro-hexose based C-aryl glucosides. Compound **2b** demonstrated potent hSGLT1 and hSGLT2 inhibition (IC_{50} = 43 nM for SGLT1 and IC_{50} = 9 nM for SGLT2). It showed robust inhibition of blood glucose excursion in oral glucose tolerance test (OGTT) in Sprague Dawley (SD) rats and exerted pronounced antihyperglycemic effects in *db/db* mice and high-fat diet-fed ZDF rats when dosed orally at 10 mg/kg.



2b

IC_{50} (hSGLT2) = 9 nM
IC_{50} (hSGLT1) = 43 nM

Keywords
Diabetes
Glucose transporter
SGLT1 inhibitor
SGLT2 inhibitor
Bioisostere
C-Aryl Glucoside

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Design, Synthesis and Biological Evaluation of (2S,3R,4R,5S,6R)-5-Fluoro-6-(hydroxymethyl)-2-aryltetrahydro-2H-pyran-3,4-diols as Potent and Orally Active SGLT Dual Inhibitors

Guozhang Xu*, Michael D. Gaul, Gee-Hong Kuo, Fuyong Du, June Zhi Xu, Nathaniel Wallace, Simon Hinke, Thomas Kirchner, Jose Silva, Norman D. Huebert, Seunghun Lee, William Murray, Yin Liang, Keith Demarest

Persistent hyperglycemia is a defining feature of diabetes mellitus (DM), which falls into two broad etiopathgenic categories-type 1 and type 2 diabetes.[1-2] The United States Centers for Disease Control (CDC) states that in 2015 diabetes affected 30.3 million people, nearly 9.4% of the total U.S. population.[3] The primary focus for management of T2DM is to achieve recommended levels of glucose control and thereby slow the progression of microvascular and macrovascular complications.[4] Despite the availability of insulin and many oral antidiabetic agents, therapeutic efficacy is also offset by side effects such as weight gain and hypoglycemia.[5-6] Therefore, the search for additional therapeutic agents with an improved benefit-risk profile continues.

It is well-known that the human kidney plays a significant role in maintaining glucose homeostasis of the body.[7-8] Sodium-dependent glucose cotransporters (SGLTs) are a family of glucose transporters that contribute to glucose reabsorption. Two major isoforms of SGLT family are SGLT1 and SGLT2, among which SGLT2 is a low affinity, high capacity glucose transporter that is predominately expressed in the S1 and S2 segments of the early proximal tubule of the kidney.[9-10] Studies indicated that SGLT2 is responsible for 90% of renal glucose recovery.[11] Sodium glucose transporter 1 (SGLT1) is a high affinity, low capacity glucose transporter and highly expressed in the proximal portion of the small intestine. It is also expressed in the S3 segment of the proximal tubule of the kidney as well as the heart.[12-14]

There has been a tremendous effort over the past decades to develop selective SGLT2 inhibitors as a new treatment for diabetes via disposal of excess glucose in the urine through inhibition of glucose reabsorption in the kidney.[15] Several SGLT2 selective inhibitors have been approved by the FDA for the treatment of diabetes. Canagliflozin (Invokana®) was approved in 2013,[16] followed by dapagliflozin[17] (Farxiga®) and empagliflozin[18] (Jardiance®). In addition, ipragliflozin,[19] luseogliflozin,[20] and tofogliflozin [21]have all been approved in Japan. Due to the progressive nature of T2DM, a potent SGLT1 and SGLT2 dual inhibitor will reduce calorie intake and achieve a more systematic normoglycemia via two distinct SGLT mediated events: reducing dietary glucose absorption in the intestine and increasing urinary glucose excretion from the kidney. Sotagliflozin, a dual SGLT1 and SGLT2 inhibitor, is currently in late-stage clinical development for diabetes.[22-23]

Herein, we designed, synthesized, and evaluated the human SGLT1/2 inhibitory potential of a new series of (2S,3R,4R,5S,6R)-5-fluoro-6-(hydroxymethyl)-2-

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aryltetrahydro-2H-pyran-3,4-diols. Further investigation of the structure-activity relationships of this series resulted in the discovery of **2b**, as a potent dual SGLT inhibitor with IC_{50} of 43 nM for SGLT1 and IC_{50} of 9 nM for SGLT2. Compound **2b,** when dosed orally, caused a dose-dependent inhibition of glucose excursion in oral glucose tolerance test (OGTT) in Sprague Dawley (SD) rats and exerted significant and long-lasting antihyperglycemic effects in *db/db* mice and high-fat diet-fed Zucker Diabetic Fatty (ZDF) rats at 10 mg/kg dosing.

At the beginning of our SGLT1/2 dual inhibitor program, we realized that most of the reported SGLT inhibitors were derived from C-aryl glucosides (shown as **1** in Figure 1 with a diarylmethane structure as aglycone) due to the relative ease of synthesis. It is well documented that fluorine can act as an isostere of hydroxy group since the C-F bond length (1.39 Å) is quite close to the C-O bond length (1.43 Å).[24] In addition, early SAR studies indicated that the C_5 site of the C-aryl glucosides is tolerate for structural modification.[25] Our design strategy was therefore directed to the bioisosteric replacement of C_5-hydroxy group with a fluorine atom with the same equatorial configuration as original hydroxy group to construct a C_5-fluoro-hexose-based SGLT inhibitor such as **2** (**Ar** shown in the blue box represents a generic aglycone moiety). Interestingly, there were very few literature reports of orally active SGLT1 and SGLT2 dual inhibitors based on this chemotype.

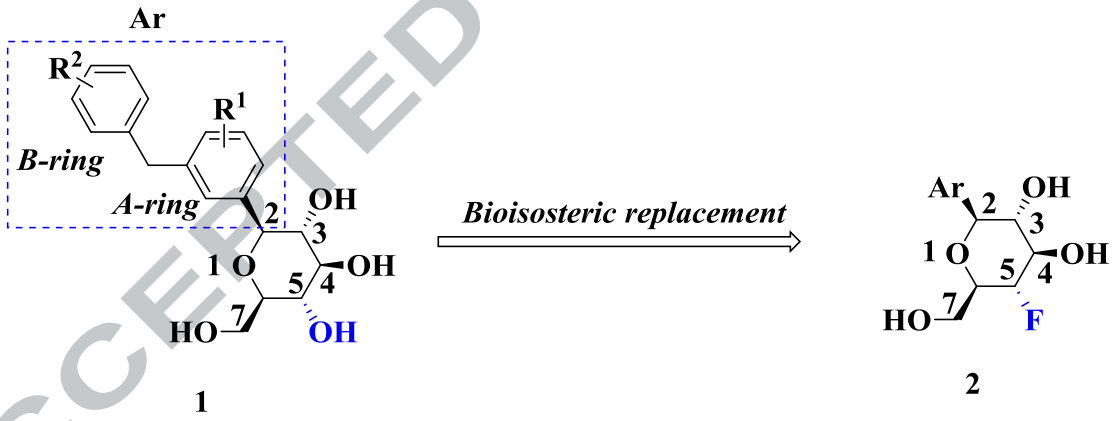

Figure 1. Design of new SGLT1 and SGLT2 dual inhibitors

A retrosynthetic analysis of target molecule **2** (Figure 2) indicated that the C_5-fluorolactone **3** or **4** with appropriate protecting group such as TBDMS or benzyl (Bn) was highly desirable as a key intermediate for the target synthesis.

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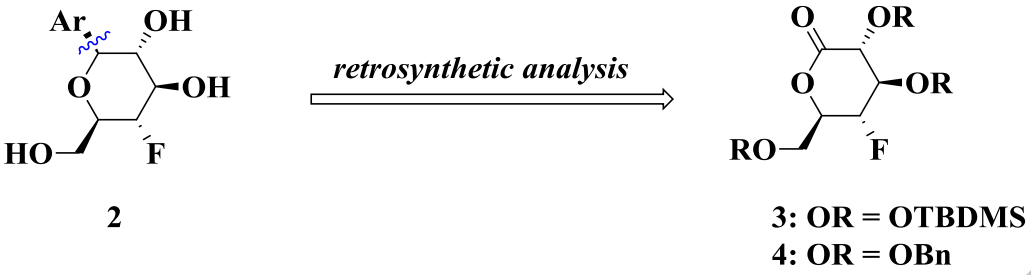
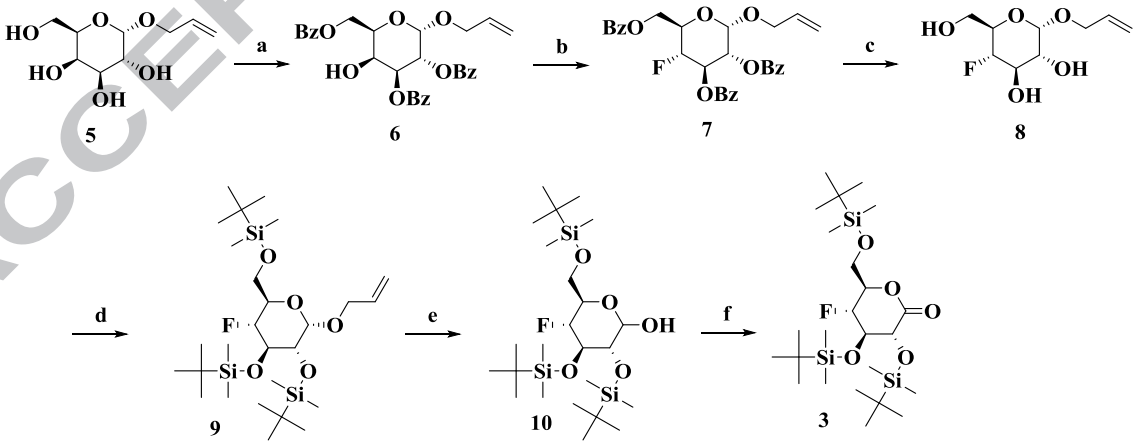


Figure 2. Retrosynthetic analysis of C5-fluoro-hexose-based aryl glucosides

To that end, two methods were developed for the syntheses of these C_5-fluoro-lactone intermediates. As shown in Scheme 1 for the first approach, treatment of commercially available (2S,3R,4S,5R,6R)-2-(allyloxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol **5** with 3.1 equivalents of benzoyl chloride in pyridine at -35°C afforded C-2,3,6-tribenzoyl-protected **6** in 73% yield. Conversion of the axial C_5-hydroxyl group to the corresponding equatorial fluoride was accomplished in the presence of diethylaminosulfur trifluoride (DAST)/dichloromethane (DCM) at -40°C in 70% yield. The benzoyl-protected **7** was hydrolyzed in NaOMe/MeOH to provide **8**, which subsequently reacted with excess TBDMSOTf in 2,6-lutidine/DCM to afford **9** in excellent yield over two steps. Removal of the allyl protecting group of **9** was achieved using a two-step process:[26] (1) carbon-carbon double isomerization in the presence of $(PPh_3)_2RhCl$/DABCO under refluxing condition; (2) bond cleavage in the presence of NMO/OsO_4 at room temperature to afford **10** in 87% yield. The resulting lactol **10** was oxidized in Ac_2O/DMSO to provide a key intermediate **3** as a stable white solid in high yield. To our knowledge, this is the first-time report for the synthesis of this TBDMS-protected C_5-fluoro-lactone. This six-step process also allowed us to synthesize the key intermediate **3** in large quantity.



Scheme 1. Reagents and conditions: (a) 3.1 eq. BzCl, pyridine, 35°C to room temperature, 73%; (b) DAST/DCM, -40°C to room temperature, 70% yield; (c) NaOMe, MeOH, room temperature, quantitative yield; (d) TBDMSOTs, 2,6-lutidine/DCM, 0°C to 4 °C, 96%; (e) cat. $(PPh_3)_3RhCl$, DABCO, EtOH/water (10:1 v/v), 80-100°C, NMO/cat. OsO_4, acetone/water (10:1 v/v), 87%: (f) DMSO/Ac_2O, 84-88%.

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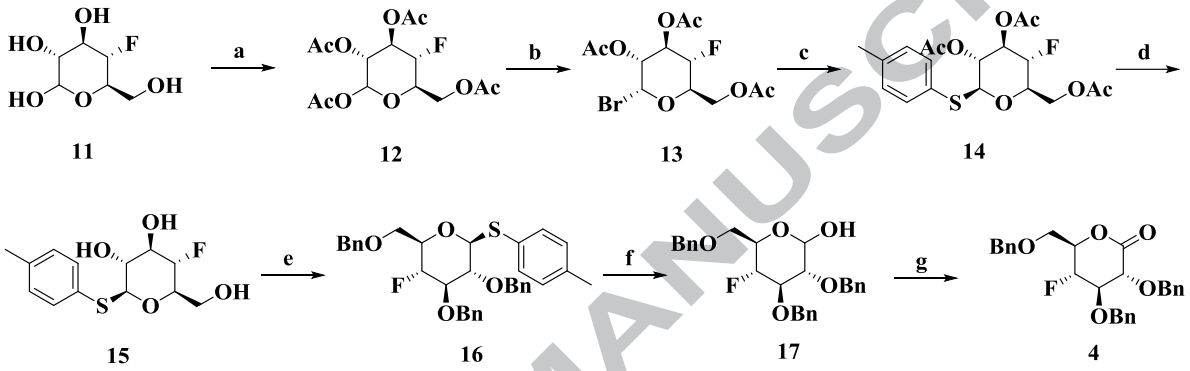
Our second approach was to synthesize benzyl-protected C_5-fluoro-lactone **4**, which started with C_5-fluoro-substituted glucose **11** (Scheme 2). All the free hydroxyl groups were acetylated in the presence of $NaOAc/Ac_2O$. Treatment of **12** with excess HBr in HOAc afforded α–bromo analog **13**. Nucleophilic substitution of **13** with *p*-tolylthiol yielded **14** in 47% yield over three steps, which underwent hydrolysis (**15**), benzylation (**16**), and oxidative removal of *p*-tolylthio with NBS in acetone/water to afford lactol **17**. The resulting lactol **17** was then oxidized under Swern oxidation condition to provide lactone intermediate **4** in 87%.



Scheme 2. Reagents and conditions: (a) NaOAc, Ac_2O 100%; (b) HBr in HOAc; (c) 4-methylbenzenethiol/Et_3N, DCM 47% over two steps; (d) NaOMe, MeOH; (e) NaH, BnBr, n-Bu_4NI 95%: (f) NBS, acetone/water 86%; g) DMSO, Ac_2O 87%.

With both C_5-fluoro-lactones **3** and **4** in hand, we first synthesized targets **2a-2f** containing a fused dihydrobenzofuran as the central moiety of aglycone (Scheme 3). Bromination of known 2,3-dihydrobenzofuran-4-ol (shown in the box) with $PyBr_3$, followed by iodination with NIS provided 5-bromo-7-iodo-2,3-dihydrobenzofuran-4-ol **18** in 60% yield over two steps. Interestingly, reversing the order of additions led to the formation of 7-bromo-5-iodo-2,3-dihydrobenzofuran-4-ol **22**. Benzylation of either **18** or **22** with benzyl bromide in the presence of K_2CO_3 produced **19** or **23** respectively, which reacted with Turbo Grignard reagent (i-PrMgCl.LiCl) at low temperature to undergo selective metal/iodo exchange. The resulting dihydrobenzofuran-derivatized lithium species reacted with aryl aldehyde, followed by reduction with triethylsilane and boron trifluoride diethyl etherate to generate the corresponding aglycone **20** or **24** in 70% overall yield. The addition of the aryl lithium species generated by lithium/halogen exchange of aglycone **20** or **24** to C_5-fluoro-lactone **3** generated the corresponding lactol, which was subjected to further triethylsilane/$BF_3.Et_2O$ reduction in DCM/acetonitrile at -30^{o}C to room temperature to provide the corresponding β-C-aryl glucoside **21** or **25**. Removal of the benzyl groups of **21** or **25** under the hydrogenolysis condition employing 20% $Pd(OH)_2$ as catalyst afforded the corresponding **2a** or **2d**. Further methylation gave the target compounds **2b-c** as well as **2e-f.**

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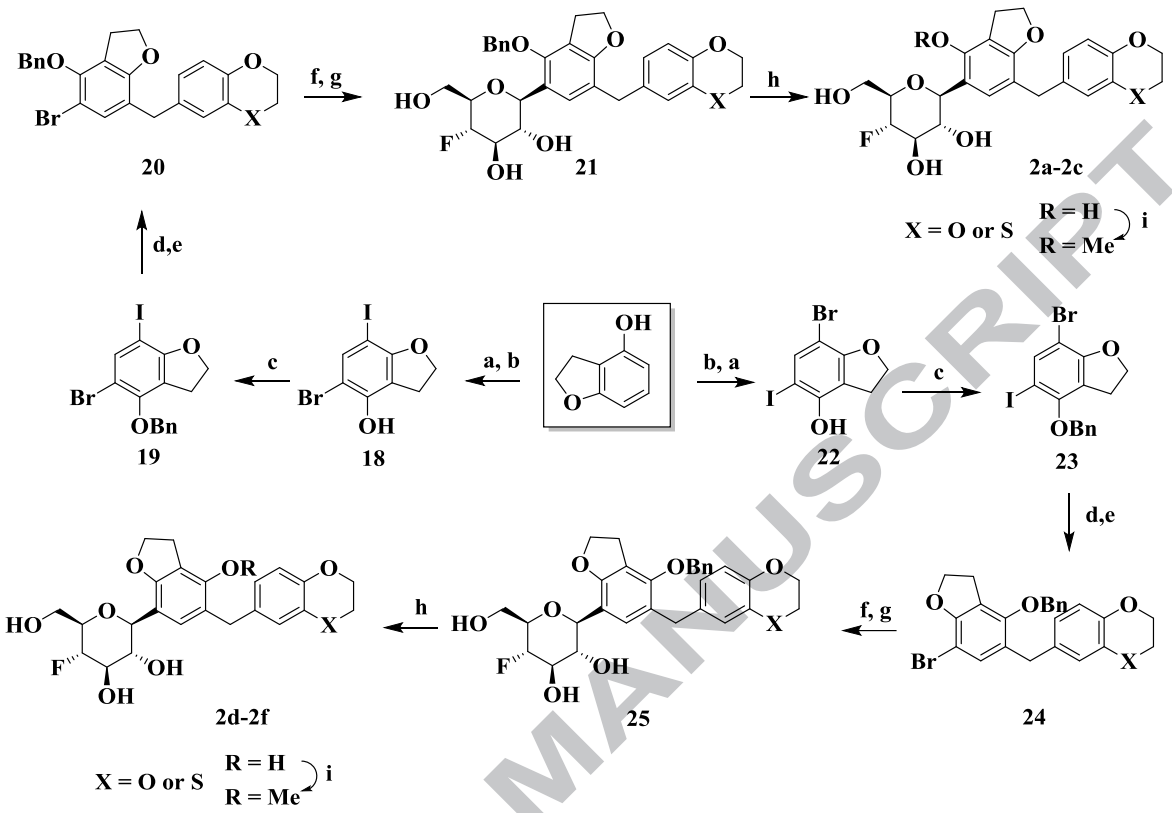

Scheme 3. Reagents and conditions: (a) $PyBr_3$, MeOH 60%; (b) NIS, acetonitrile 100%; (c) Benzylbromide/K_2CO_3, acetone/reflux, 86%; (d) Aryl aldehyde such as 2,3-dihydrobenzo[b][1,4]dioxine-6-carbaldehyde, i-PrMgCl.LiCl, 70%; (e) Triethylsilane/$BF_3.Et_2O$, DCM, 98%: f) BuLi, C5-fluorolactone **3**/THF, -78°C; g) Triethylsilane, $BF_3.Et_2O$, DCM, acetonitrile, -30°C-0°C, 50% over two steps; h) 20% $Pd(OH)_2$, H_2, EtOAc, MeOH, 94%; i) MeI, K_2CO_3, acetone, 70%.

Scheme 4 illustrated the construction of bicyclic 6,7-dihydrothieno[3,2-c]pyridine-derived C_5-fluoro-glucosides **2g-o**. Starting from 1-(benzyloxy)-2-bromo-4-(dimethoxymethyl)-5-methylbenzene **26**, lithium/bromide exchange with *n*-butyllithium, generated aryllithium, which reacted with benzyl-protected C_5-fluoro-lactone **4**, followed by acid hydrolysis provided aldehyde **27** in 68% yield over two steps. A second lithium/bromide exchange of *tert*-butyl 2-bromo-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate with *n*-butyllithium, followed by the addition of the lithiated thiophene to intermediate **27** provided the corresponding **28**, which was a mixture of diastereomers. Without further separation, compound **28** was reduced using triethylsilane and $BF_3.Et_2O$ to furnish 2-substituted-4,5,6,7-tetrahydrothieno[3,2-c]pyridine **29** in 65% isolated yield. Derivatization of **29** with either cyanic bromide or various carbonylchloride yield benzyl-protected **30**. Removal of the benzyl groups with BCl_3 in the presence of pentamethylbenzene gave the desired target molecules such as **2h**, **2k**, **2m-n** respectively. Methylation of either **2h** or **2k** with MeI and K_2CO_3 in acetone afforded the corresponding analog **2i** or **2l**. Similarly, analog **2g** or **2o** (not shown in the scheme 4) was made starting from 4-bromo-2-(dimethoxymethyl)-1-methylbenzene.

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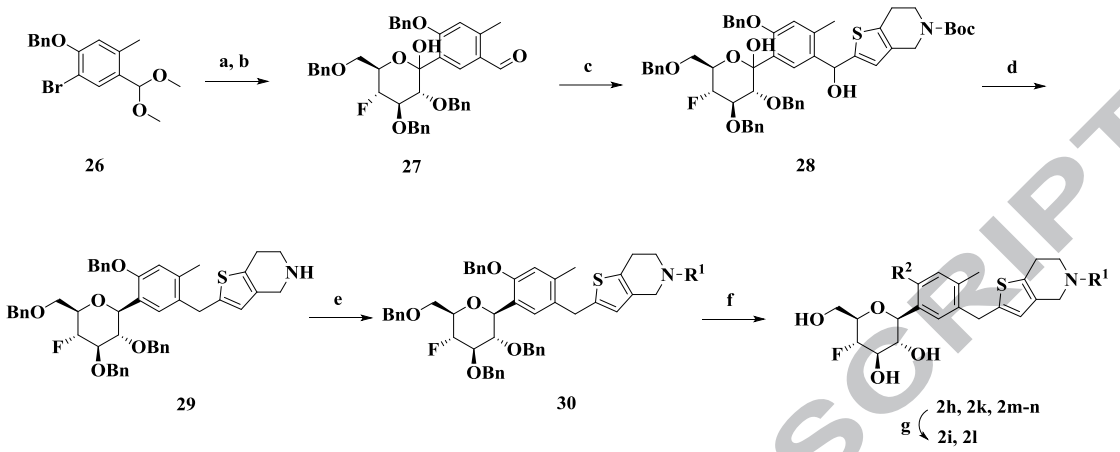

Scheme 4. Reagents and conditions: (a) n-Buli,, then C_5-fluoro-lactone **4,** THF at -78ºC, 2 h; b) 1N HCl, THF, rt, 2 h, 68% yield two steps; (c) n-BuLi,, THF, then tert-butyl 2-bromo-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate, at -78ºC, 2h, 87% (d) Triethylsilane/$BF_3.Et_2O$, DCM, at 0ºC for 1 h, 65%; e) R_1Br such as cyanic bromide or R_1Cl such as ethoxy carbonylchloride/DIEA, DCM; f) BCl_3, pentylmethylbenzene, DCM, at -78ºC for 30 mins, 42%; g) MeI, K_2CO_3, acetone.

The *in vitro* inhibitory activities of newly synthesized compounds on SGLT1 and SGLT2 were assessed by monitoring the inhibition of accumulating methyl-α-D-[U-^{14}C]-glucopyranoside (AMG) in CHO-K1 cells stably expressing human SGLT1 or SGLT2 (See details in *supporting material*). Triplicate determinations were made for each test compound and the IC_{50} values or inhibition activities of the test compounds against SGLT1 and SGLT2 were determined.

We first investigated a series of 2,3-dihydrobenzo[b][1,4]dioxines and oxathiines with 2,3-dihydrobenzofuran as the central moiety and the data were summarized in Table 1. The potency of dapagliflozin was also included for comparison. Compound **2a** with *ortho*-hydroxy group was very potent for both SGLT1 (IC_{50}= 14 nM) and SGLT2 (IC_{50} = 3 nM) while methylation of the phenol group (**2b**) resulted in 3-fold loss of activity for both SGLT1 and SGLT2. Replacement of distal 2,3-dihydrobenzo[b][1,4]dioxine with 2,3-dihydrobenzo[b][1,4]oxathiine (**2c**) reduced SGLT1 inhibitory activity (IC_{50} = 162.7 nM) while SGLT2 activity remained unchanged. Surprisingly, reverse the dihydrofuran attachment (**2d**) significantly diminished the inhibitory activity against both SGLT1 and SGLT2. Methylation of the phenol (**2e**) improved both SGLT1 (IC_{50} = 96.4 nM) and SGLT2 (IC_{50} = 7.5 nM) activities. Replacement of distal 2,3-dihydrobenzo[b][1,4]dioxine with 2,3-dihydrobenzo[b][1,4]oxathiine (**2f**) led to a loss of inhibitory activity, especially for SGLT2 (IC_{50} = 62.3 nM). Compounds (**2a-c**, and **2e**) were also tested in liver microsomes (human, mouse, and rat), **2c** and **2e** showed poor *in vitro* stability in rat liver microsomes with only 12 and 2% remaining respectively.

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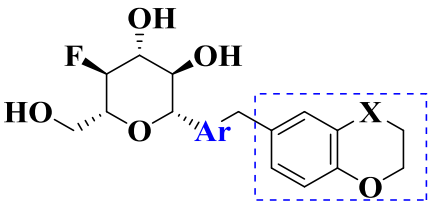
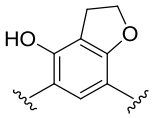
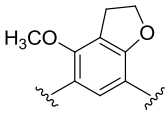
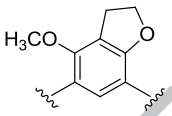
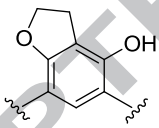
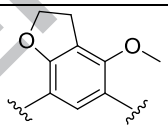
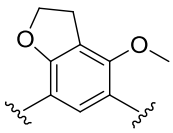
Table 1. In Vitro Potency at SGLT1 and SGLT2 assays



Distal aryl group

Cpd	X	Ar	SGLT1 IC_{50} (nM)	SGLT2 IC_{50} (nM)	Microsomal stability (%)[a] human/mouse/rat
Dapa			ND[b]	2	
2a	O		14	3	97/97/64
2b	O		43	9	95/82/66
2c	S		162.7	8.3	73/61/12
2d	O		31% at 0.3 uM	26% at 0.3 uM	ND[b]
2e	O		96.4	7.5	91/94/2
2f	S		186.6	62.3	ND[b]

[a] Liver microsomal stability assay conditions: 1 µM compound concentration; liver microsomal protein content: 0.5 mg/ml, cofactor NADPH added, compound remaining at 10 mins time point; solvents: 0.01% DMSO, 0.05% acetonitrile; positive controls: verapamil, cerivaststin, and warfarin. [b] ND, not determined.

Since both the substituted-thiophene and benzothiophene have been explored in the design of SGLT inhibitors and are key structural features in Canagliflizon and Ipragliflozin,[16, 19] we decided to explore a novel bicyclic 6,7-dihydrothieno[3,2-c]pyridine as the distal aryl group and investigated the effects of modifications of both central benzene ring and distal aryl motif. The results were presented in Table 2. Compound **2h** with *ortho*–hydroxy group (R^2 = OH) was the most active SGLT2

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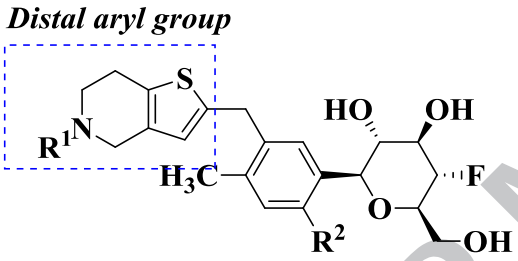
inhibitor with IC_{50} = 2.6 nM and also showed significant SGLT1 inhibitory activity among the first three 6,7-dihydrothieno[3,2-c]pyridine-5-(4H)-carbonitriles (**2g-i**). Methylation of this phenol group resulted in a 17-fold loss of SGLT1 activity while slightly lost its SGLT2 potency (**2i** with SGLT1 IC_{50} = 1421 nM and SGLT2 IC_{50} = 5.3 nM). Similar trend was also observed for the 2-thiazol-amide derivatives (**2j-l**) although all these compounds were less active against SGLT2. In addition, the distal aryl motif also tolerated other functional groups. Urea **2m** and carbamate **2n** exhibited modest activity for both SGLT1 and SGLT2, however, amide **2o** without *ortho*-hydroxy group on the central benzene moiety resulted in significant loss of hSGLT1 and SGLT2 activities. Compounds (**2h**, **2k**, **2m-n**) were also tested in liver microsomes (human, mouse, and rat), all of which showed good *in vitro* stability across species with 66~95% remaining after 10 mins incubation.

Table 2. In Vitro Potency at SGLT1 and SGLT2 assays



Cpd	R^1	R^2	SGLT1 IC_{50} (nM)	SGLT2 IC_{50} (nM)	Microsomal stability (%)[a] human/mouse/rat
2g	-CN	H	29% at 0.3 uM	76% at 0.3 uM	ND[b]
2h	-CN	OH	86	2.6	95/91/79
2i	-CN	OCH_3	1421	5.3	ND[b]
2j	2-Thiazol-(CO)-	H	39% at 0.3 uM	73% at 0.3 uM	ND[b]
2k	2-Thiazol-(CO)-	OH	100.9	36	69/66/91
2l	2-Thiazol-(CO)-	OCH_3	376.1	23.2	ND[b]
2m	1-Pyrrolidino-(CO)-	OH	40.4	13.6	71/74/82
2n	C_2H_5O-(CO)-	OH	156	36	80/86/78
20	Cyclic-C_5H_9-(CO)-	H	49% at 0.3 uM	59% at 0.3 uM	ND[b]

[a] Liver microsomal stability assay conditions: 1 µM compound concentration; liver microsomal protein content: 0.5 mg/ml, cofactor NADPH added, compound remaining at 10 mins time point; solvents: 0.01% DMSO, 0.05% CAN; positive controls: verapamil, cerivaststin, and warfarin. [b] Not determined.

Since complete inhibition of intestinal SGLT1 might not be desirable due to the concern of glucose-galactose malabsorption,[27] we select compounds (**2a-b**, and **2h**) with a balanced SGLT1 and SGLT2 inhibitory profile (IC_{50} of less than 10 nM for SGLT2 and IC_{50} of 100 nM for SGLT1) as well as good *in vitro* liver microsomal stability across multiple species (50% remaining after 10 mins incubation) for further *in vivo*

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pharmacokinetic studies in SD rats. Administration of a single 2.0 mg/kg dose intravenously or a single 10 mg/kg dose orally revealed that **2a** and **2h** had low C_{max} (168 ng/ml for **2a** and 13.7 ng/ml for **2h** respectively) and very poor oral bioavailability in SD rats (Table 3). Compound **2h** also had very high clearance, which could be due to the phase 2 metabolism of phenol moiety in the central benzene ring. Unlike **2a** and **2h**, compound **2b** was notable for rapid absorption and had higher C_{max} of 990 ng/ml with an oral bioavailablity of 48.2% in SD rats. The high clearance for **2b** in rat seems to be species-related. In higher species such as monkey (Table 3), **2b** had a high volume of distribution at steady-state about (four times total body water considering monkey total body water of 0.693 L/kg) and a very moderate systemic clearance of 15.5 ml/min/kg given monkey's heptatic blood flow is 43.6 ml/min/kg. After 10 mg/kg oral administration, plasma concentration of **2b** reached C_{max} of 1487 ng/ml after 1.33 hours and had an oral bioavailability of 56%.

Table 3. Pharmacokinetic properties of three SGLT dual inhibitors after oral and intravenous administration to rats and monkeys[a]

Parameters	**2a**	**2a**	**2b**	**2b**	**2h**	**2h**	**2b**	**2b**
	rat		rat		rat		monkey	
	iv	po	iv	po	iv	po	iv	po
Dose (mg/kg)	2	10	2	10	2	10	2	10
$T_{1/2}$ (h)	0.31	ND[b]	0.41	1.44	0.99	1.44	7.9	5.6
CL (ml/min/kg)	28.1		50.9		149		15.5	
Vd_{ss} (L/kg)	1.22		1.07		3.79		3.05	
C_{max} (ng/ml)		168		990		13.7		1487
T_{max} (h)		0.25		0.30		2.20		1.33
$AUC_{(0\text{-}inf)}$ (ng.h/ml)		307		1600		44.6		6089
F (%)		4.0		48.2		5.0		56

[a] PK experiments were conducted in male Sprague-Dawley rats or male cynomolgus monkeys (n = 3 per group). iv formulation: 20% HPbCD.; po formulation: 0.5% Methocel in saline. [b] ND not determined.

To understand more on the species-related metabolic stability of **2b** and potential implication to its human blood clearance, we further investigated it in different cryopreserved hepatocytes and the data were summarized in Table 4.

Table 4. In vitro clearance studies of 2b in cryopreserved hepatocytes[a]

Compound	Parameters	human	SD-rat	dog	monkey
2b	$T_{1/2}$ (h)	5.4	1.2	8.0	2.2
2b	CL (ml/min/kg)	9.76	51.42	9.29	25.30
Diclofenac	$T_{1/2}$ (h)	0.7	0.8	5.2	1.0

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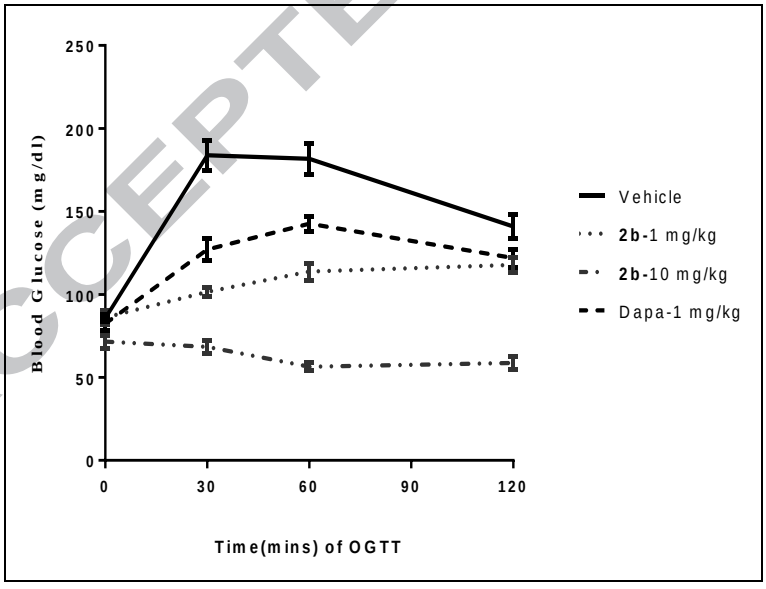
Diclofenac	CL (ml/min/kg)	20.88	61.41	13.13	36.90
Midazolam	$T_{1/2}$ (h)	1.0	0.5	0.5	1.4
Midazolam	CL (ml/min/kg)	20.24	67.32	30.85	43.45

[a] Cryopreserved hepatocytes stability assay conditions: 1 µM compound concentration; liver hepatocytes: 0.5 million cells/ml; timepoints: 0, 0.5, 1, 2, 3, 4 h; positive controls: diclofenac (control for phase 2 metabolism), midazolam (control for phase 1 metabolism) at 1 µM.

In the studies, Diclofenac and midazolam were chosen as internal standards, **2b** had high clearance in rat with calculated clearance of 51.42 ml/min/kg, which is consistent with its *in vivo* clearance of 50.9 ml/min/kg. Compound **2b** had a modest clearance in monkey hepatocytes (CL = 25.30 ml/min/kg), which was slightly higher than the *in vivo* finding (CL = 15.5 ml/min/kg). In both dog and human hepatocytes, **2b** had low clearance.

Compound **2b** was further evaluated in cell-based SGLT functional assays in CHO-K1 cells expressing rat SGLT1 and SGLT2 and its IC_{50} values for SGLT1 and SGLT2 were 68 nM and 16 nM respectively, which are slightly less potent than its human SGLT1 and SGLT2 values. With its good *in vitro* potency and in *vivo* PK profiles, compound **2b** was next studied in an oral glucose tolerance test (OGTT) in SD rats.[28] As shown in Figure 3, a single oral dose of **2b** (1 and 10 mg/kg) resulted in a dose-dependent inhibition of glucose excursion (Figure 3, A). The reduction of glucose AUC reached 32.4 % versus vehicle by the administration of **2b** at 1 mg/kg dosing, which was more pronounced than that of Dapagliflozin at the same dose (22.3%) (Figure 3, B).

A)



B)

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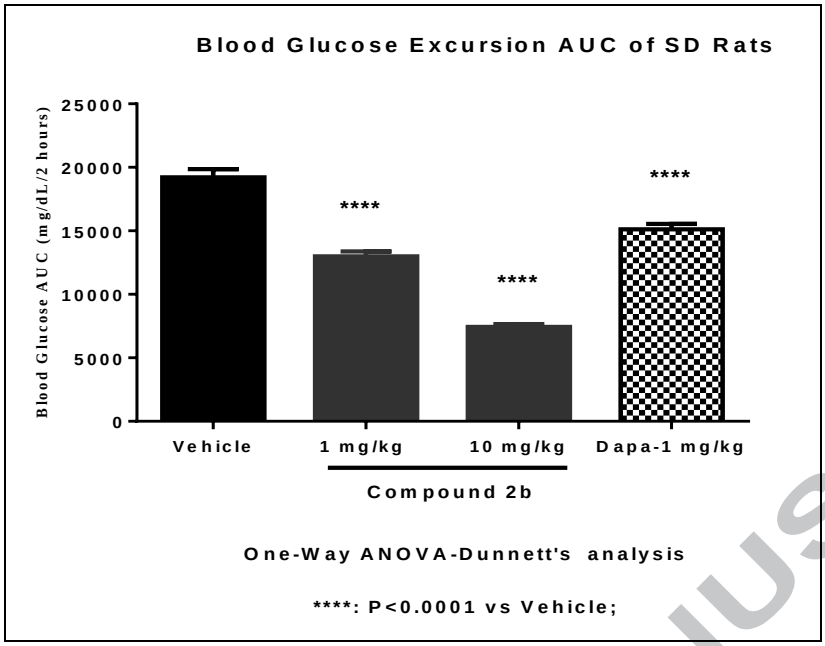

Figure 3. Effect of compound **2b** on post-prandial glucose control in SD rats (n=8). SD rats were treated with either vehicle, Dapa, or compound **2b** 60 min prior to OGTT (2 g/kg glucose challenge). Blood glucose levels were determined at 0, 30, 60, and 120 min after glucose administration (A) and blood glucose excursion AUC (B) was calculated based on the blood glucose levels at timepoints of OGTT. Compared with vehicle group, compound **2b** treatments significantly reduced blood glucose levels in a dose-dependent fashion (****: P<0.0001, one-way ANOVA followed by Dunnett's multiple comparison test).

Furthermore, *db/db* mice and ZDF rats, both of which are rodent models of diabetes, were used to assess the antihyperglycemic effect of compound **2b**.[28] As shown in Figure 2, a single oral dose (10 mg/kg) of compound **2b** in *db/db* mice significantly lowered the fed blood glucose levels up to 24 h and total 24-hrs blood glucose AUC compared with vehicle treatment (Figure 4, A and B) indicating its remarkable antihyperglycemic efficacy. The SGLT2 inhibitory effect of **2b** was evidenced by increased urine glucose excretion (UGE) over 24 h in *db/db* mice (Figure 4, C). It is interesting to observe that **2b** induced $UGE_{0\text{-}24h}$ is lower than with a selective SGLT2 inhibitor (data not shown) due to decreased glucose absorption by SGLT1 inhibition at intestinal lumen.

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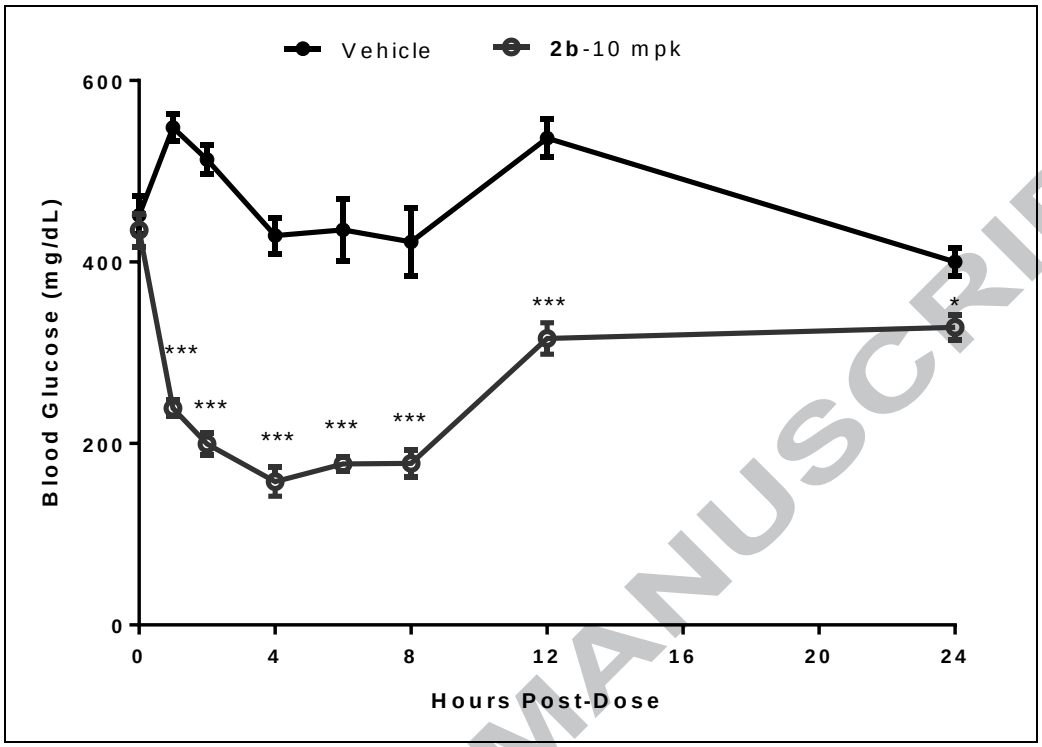
A)



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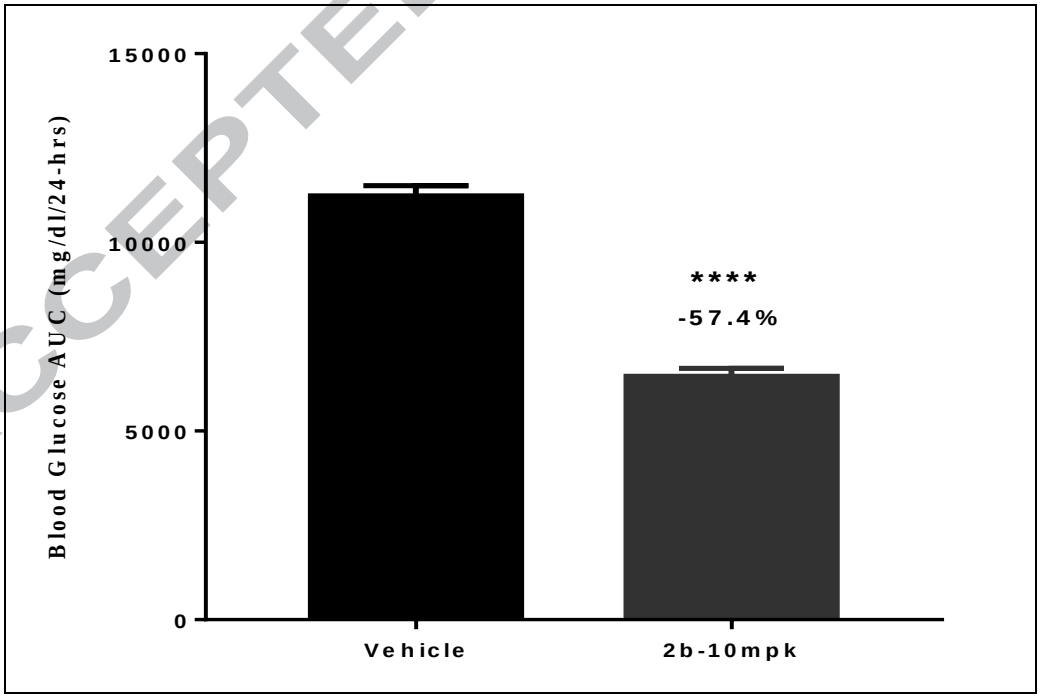

C)

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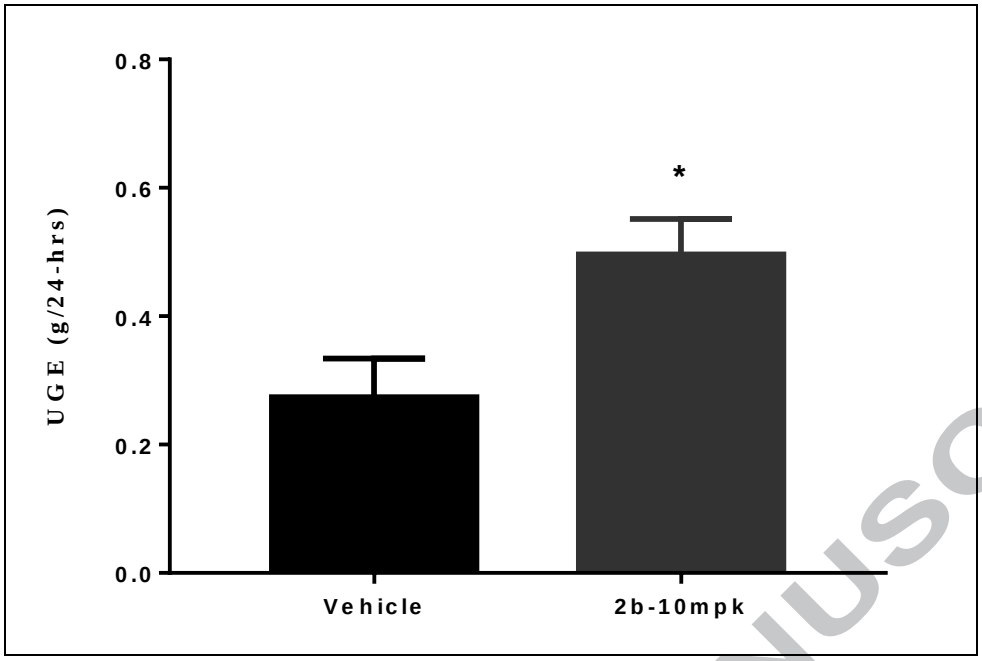

Figure 4. Anti-hyperglycemic effect of compound **2b** in *db/db* mice (n=8). The mice were treated with either vehicle or compound **2b** at 10 mg/kg dose. Fed blood glucose levels were determined at multiple timepoints after the animals were treated (A). Total blood glucose AUC of 24-hrs was calculated based on the fed blood glucose levels at multiple timepoints (B). Total urine glucose excretion (UGE) was calculated based on urine glucose concentrations and urine volumes at multiple timepoints (C). Compared with vehicle group, a single dose of compound **2b** treatment significantly reduced the fed blood glucose levels and increased urine glucose excretion in *db/db* mice (mean±se, *: P<0.05, ***: P<0.001, ****: P<0.0001. T test).

As indicated in Figure 5, a single dose of **2b** at 10 mg/kg was orally administrated to ZDF rats and their blood glucose level was monitored for 24 hours.[28] It significantly decreased fed blood glucose levels of ZDF rats at all timepoints including at 24 h (Figure 5, A). It also significantly reduced total fed blood glucose AUC (Figure 5, B).

A)

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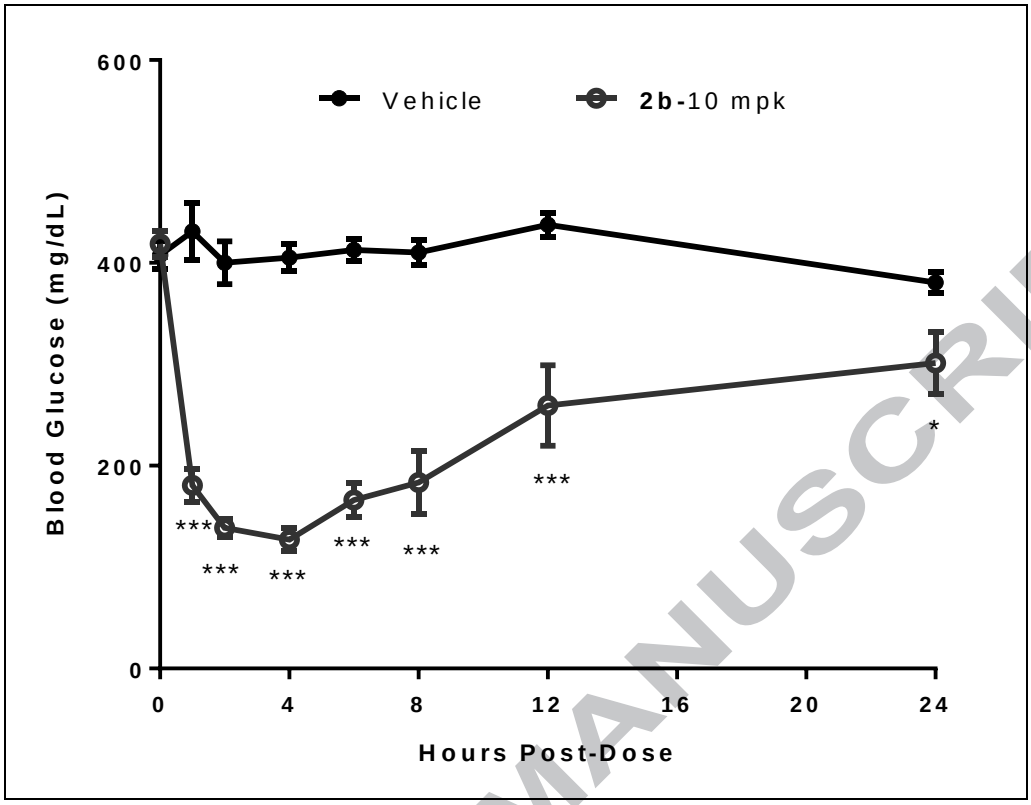

B)

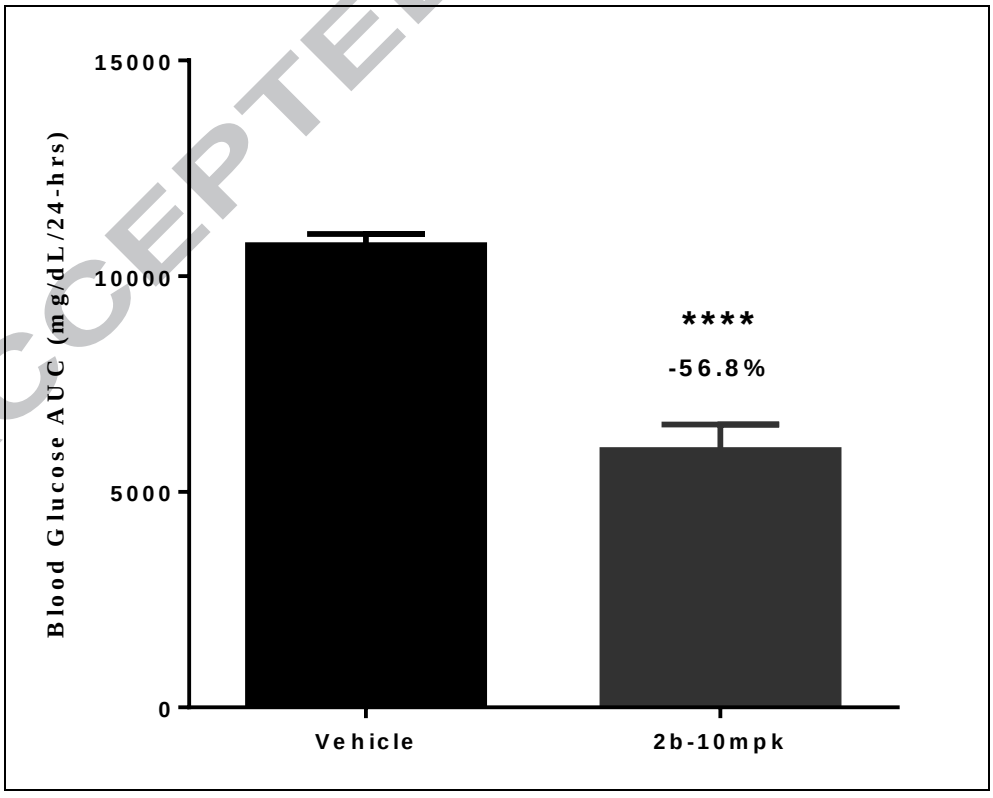

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Figure 5. Anti-hyperglycemic effect of compound **2b** in ZDF rats (n=8). ZDF rats were treated with either vehicle or compound **2b** at 10 mg/kg dose. Fed blood glucose levels were determined at multiple timepoints after the animals were treated (A). Total fed blood glucose AUC was calculated based on the fed blood glucose levels measured at multiple timepoints (B) and 24-hrs UGE was measured based on the urine glucose concentrations and urine volumes at multiple timepoints post dosing (mean±se, *: P<0.05, ***: P<0.001, ****: P<0.0001. T test).

Because of its excellent *in vivo* PD profiles, compound **2b** was assessed against 55 different GPCRs, kinases, enzymes, and ion channels and it showed minimal activity (less than 21% inhibition) at a concentration of 10 μM; No activity was observed against the hERG channel at 50 μM. It was also tested in AMES II mutagenicity assay (TA98 strains) and *in vitro* micronucleus assay. Unlike some compounds from C-aryl glucoside class,[29] compound **2b** was negative under both assays.

In summary, we successfully designed and synthesized a new series of (2S,3R,4R,5S,6R)-5-fluoro-6-(hydroxymethyl)-2-aryltetrahydro-2H-pyran-3,4-diols as SGLT dual inhibitors. More importantly, two methods were developed to efficiently synthesize C_5-fluoro-lactones **3** and **4**, key intermediates for diverse C_5-fluoro-hexose based C-aryl glucosides. The study of structure-activity relationships of these novel SGLT dual inhibitors resulted in the identification of **2b** with potent SGLT1 and SGLT2 activities (hSGLT1 IC_{50} = 43 nM, hSGLT2 IC_{50} = 9 nM), which is orally active in *db/db* mouse and ZDF rat models of diabetes and has several encouraging preclinical data profiles.

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References and notes

1. Stumvoll, M.; Goldstein, B. J.; van Haeften, T. W. *Lancet* **2005**, 365, 1333.
2. American Diabetes Association. *Diabetes Care* **2004**, 27 (suppl. 1), 5.
3. Diabetes Fact Sheet. https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf (accessed December 21, 2017)
4. Fowler, M. J. Clin. *Diabetes* **2008**, 26, 77.
5. Davidson, M. B.; Peter, A. L. *Am. J. Med.* **1997**, 102, 99.
6. Davis, S. N. *J. Diabetes Complications* **2004**, 18, 367.
7. Marsenic, O. *Am. J. Kidney Dis.* **2009**, 53, 875.
8. DeFronzo, R. A.; Davidson, J. A.; Del Prato, S. *Diabetes Obes. Metab.* **2012**, 14, 5.
9. Kanai, Y.; Lee, W. S.; You, G.; Brown, D.; Hediger, M. A. *J. Clin. Invest.* **1994**, 93, 397.
10. Wright, E. M.; Hirayama, B. A.; Loo, D. F. *J. Intern. Med.* **2007**, 261, 32.
11. Wang, X. X.; Levi, J.; Luo, Y.; Myakala, K.; Herman-Edelstein, M.; Qiu, L.; Wang, D.; Peng, Y.; Grenz, A.; Lucia, S.; Dobrinskikh, E.; D'Agati, V. D.; Koepsell, H.; Kopp, J. Rosenberg, A. Z.; Levi, M. *J. Biol. Chem.* **2017**, 292, 5335.
12. Turk, E.; Zabel, B.; Mundlos, S.; Dyer, J.; Wright, E. M. *Nature* **1991**, 350, 354.
13. Martin, M. G.; Turk, E.; Lostao, M. P.; Kerner, C.; Wright, E. M. *Nat. Genet.* **1996**, 12, 216.
14. Lee, W. S.; Kanai, Y.; Wells, R. G.; Hediger, M. A. *J. Biol. Chem.* **1994**, 269, 12032.
15. Chao, E. C.; Henry, R. R. *Nat Rev Drug Discov.* **2010**, 9, 551.
16. Nomura, S.; Sakamaki, S.; Hongu, M.; Kawanishi, E.; Koga, Y.; Sakamoto, T.; Yamamoto, Y.; Ueta, K.; Kimata, H.; Nakayama, K.; Tsuda-Tsukimoto, M. *J. Med. Chem.* **2010**, 53, 6355.
17. Meng, W.; Ellsworth, B. A.; Nirschl, A. A.; McCann, P. J.; Patel, M.; Girotra, R. N.; Wu, G.; Sher, P. M.; Morrison, E. P.; Biller, S. A.; Zahler, R.; Deshpande, P. P.; Pullockaran, A.; Hagan, D. L.; Morgan, N.; Taylor, J. R.; Obermeier, M. T.; Humphreys, W. G.; Khanna, A.; Discenza, L.; Robertson, J. G.; Wang, A.; Han, S.; Wetterau, J. R.; Janovitz, E. B.; Flint, O. P.; Whaley, J. M.; Washburn, W. N. *J. Med. Chem.* **2008**, 51, 1145.
18. Grempler, R.; Thomas, L.; Eckhard, M.; Himmelsbach, F.; Sauer, A.; Sharp, D. E.; Bakker, R. A.; Mark, M.; Klein, T.; Eickelmann, P. *Diabetes, Obes. Metab.* **2012**, 14, 83.

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19. Imamura, M.; Nakanishi, K.; Suzuki, T.; Ikegai, K.; Shiraki, R.; Ogiyama, T.; Murakami, T.; Kurosaki, E.; Noda, A.; Kobayashi, Y.; Yokota, M.; Koide, T.; Kosakai, K.; Ohkura, Y.; Takeuchi, M.; Tomiyama, H.; Ohta, M. *Bioorg. Med. Chem.* **2012**, 20, 3263.
20. Kakinuma, H.; Oi, T.; Hashimoto-Tsuchiya, Y.; Arai, M.; Kawakita, Y.; Fukasawa, Y.; Iida, I.; Hagima, N.; TTakeuchi, H.; Chino, Y.; Asami, J.; Okumura-Kitajima, L.; Io, F.; Yamamoto, D.; Miyata, N.; Takahashi, T.; Uchida, S.; Yamamoto, K. *J. Med. Chem.* **2010**, 53, 3247.
21. Suzuki, M.; Honda, K.; Fukazawa, M.; Ozawa, K.; Hagita, H.; Kawai, T. Takeda, M.; YYata, T.; Kawai, M.; Fukuzawa, T.; Kobayashi, T.; Sato, T.; Kawabe, Y.; Ikeda, S. *J. Pharmacol. Exp. Ther.* **2012**, 341, 692.
22. Goodwin, N. C.; Mabon, R.; Harrison, B. A.; Shadoan, M. K.; Almstead, Z. Y.; Xie, Y.; Healy, J.; Buhring, L. M.; DaCosta, C. M.; Bardenhagen, J.; Mseeh, F.; Liu, Q.; Nouraldeen, A.; Wilson, A. G. E.; Kimball, S. D.; Powell, D. R.; Rawlins, D. B. *J. Med. Chem.* **2009**, 52, 6201.
23. Sands, A. T.; Zambrowicz, B. P.; Rosenstock, J. *Diabetes Care* **2015**, 38, 1181.
24. Lima, L. M.; Barreiro, E. J. *Curr. Med. Chem.* **2005**, 12, 23.
25. Robinson, R. P.; Mascitti, V.; Boustany-Kari, C. M.; Carr, C. L.; Foley, P. M.; Kimoto, E.; Leiniger, M. T.; Lowe, A.; Klenotic, M. K.; MacDonald, J. I.; Maguire, R. J.; Masterson, V. M.; Maurer, T. S.; Miao, Z.; Patel, J. D.; Preville, C.; Reese, M. R.; She, L.; Steppan, C. M.; Thuma, B. A.; Zhu, T. *Bioorg. Med. Chem. Lett.* **2010**, 20, 1569.
26. a) Corey, E. J.; Suggs, W. J. *J. Org. Chem.* **1973**, 38, 3224; b) Lamberth, C.; Bednarski, M. D. *Tetrahedron Lett.* **1991**, 32, 7369
27. Wright, E. M. *Am. J. Physiol.* **1998**, 275, G879.
28. All reported studies on rats and mice were carried out in accordance with the recommendations in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocols for all studies performed at Janssen Pharmaceuticals were approved by the Johnson & Johnson Animal Care and Use Committee. Rats or mice were housed 2 animals per cage in a temperature-controlled room with 12-hour light/dark cycle. They are allowed *ad libitum* access to water and food. For details, please see the supporting material.
29. Washburn, W.; Meng, W. *Chem. Abstr.* **2006**, 144, 305154.

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Highlights

- A new series of SGLT1 and SGLT2 dual inhibitors were disclosed.
- Two methods were developed to efficiently synthesize C_5-fluoro-lactones **3** and **4**.
- Lead compound **2b** demonstrated potent hSGLT1 and hSGLT2 inhibition.
- Lead compound **2b** showed robust *in vivo* efficacy in rodent models of diabetes.

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