Discovery of a Potent, Selective Renal Sodium-Dependent Glucose Cotransporter 2 (SGLT2) Inhibitor (HSK0935) for the Treatment of Type 2 Diabetes

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Supporting Information

ABSTRACT: A new class of potent and highly selective SGLT2 inhibitors is disclosed. Compound 31 (HSK0935) demonstrated excellent hSGLT2 inhibition of 1.3 nM and a high hSGLT1/hSGLT2 selectivity of 843-fold. It showed robust urinary glucose excretion in Sprague–Dawley (SD) rats and affected more urinary glucose excretion in Rhesus monkeys. Finally, an efficient synthetic route has been developed featuring a ring-closing cascade reaction to incorporate a double ketal 1-methoxy-6,8-dioxabicyclo[3.2.1]octane ring system.

INTRODUCTION

The incidence of diabetes has been increasing at an alarming rate worldwide. About 90% of diabetes is type 2 diabetes, which is characterized by chronically increased glycemic levels, insulin resistance (IR), and β-cell dysfunction. Currently, a wide range of antidiabetic agents are prescribed for tight glycemic control, but many of them cannot achieve and maintain satisfactory target glycemic levels. Thus, additional therapies with novel molecular mechanisms are still in great demand. It is well-known that the kidneys play an important role in glucose homeostasis via gluconeogenesis, glucose uptake from the circulation and glucose recovery from the urine. Sodium-dependent glucose cotransporter 1 (SGLT1) are a family of glucose transporters and contribute to glucose reabsorption. The two most well-known members of SGLT family are SGLT1 and SGLT2, which are members of the SLCSA gene family. In the kidney of rats, approximately 90% of glucose reabsorption has been shown to occur in the site expressing the high-affinity, low-capacity SGLT2 (Figure 1). The remaining 10% of glucose is thought to be recovered during passage of the filtrate through the site expressing the high-affinity, low-capacity SGLT1. Some recent studies indicate that SGLT2 is responsible for 97% of renal glucose recovery. The case for selective inhibition of SGLT2 is bolstered by the fact that SGLT2 appears to be expressed only in the kidney, while SGLT1 is mainly present in the small intestine (the transporter responsible for absorption of both glucose and galactose) and in the heart (function unknown). Moreover, individuals with a defective SGLT2 are marked only with significant glycosuria (as much as 140 g daily) with no other ill effects, while individuals expressing a defective SGLT1 are unable to transport glucose or galactose normally across the intestinal wall, resulting in the potentially life-threatening condition known as glucose–galactose malabsorption. Therefore, selective SGLT2 inhibitors provide an attractive strategy for the treatment of type 2 diabetes. Since 2013, leading SGLT2 inhibitors, such as dapagliflozin (1), empagliflozin (2), and canagliflozin (3), have been approved by the FDA. Ipragliflozin (4), luseogliflozin (5), and tofogliflozin (6) were approved in Japan in 2014. The glucose-lowering effect of SGLT2 inhibitors is independent of insulin secretion or insulin action. These marketed SGLT2 inhibitors exhibit a modest reduction in body weight as well as blood pressure, and they are generally well tolerated and have low risk of hypoglycemia. It is noteworthy that empagliflozin was reported to show a significant reduction in both cardiovascular risk and cardiovascular death in a dedicated outcome trial which has rarely been reported with other antidiabetic agents. Thus, in recent years, although there are some concerns on the risks of ketoacidosis, SGLT2 inhibitors are considered to have more benefits than risks for diabetic patients and have become more and more commonly prescribed.

Over the past decade, many compounds have been reported as SGLT inhibitors. They are generally divided into two classes, O-glucosides and C-glucosides. The natural product, phlorizin (4), is the first nonselective SGLT1/2 dual inhibitor. It was not clinically developed as a drug candidate due to its poor metabolic stability to β-glucosidases. Optimization of phlorizin led to the identifi- cation of several selective O-aryl glucoside SGLT2 inhibitors such as T-1095A (5), sergli- fozin-A (6), remogli- fozin (7), and remogli- fozin etabonate (7a). However, these O-glucosides are usually associated with degradation by β-glucosidases found in the gut as well. 5a, 6a, and 7a were all discontinued after phase II trials for treating type 2 diabetes (7a is still active in phase II trials for the treatment of nonalcoholic steatohepatitis (NASH)). In contrast, C-glucosides are generally more metabolically stable.

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and exhibit better oral bioavailability and plasma exposure.\textsuperscript{28} The leading C-glucosides, dapagli
fl ozin and canagli fl ozin, show good in vitro and in vivo potencies against SGLT2. Herein we report the discovery of compound 31 (HSK0935), a potent and selective SGLT2 inhibitor.

\section*{RESULTS AND DISCUSSION}

\textbf{Chemistry.} The design of SGLT inhibitors began with combination of the rigid and SGLT2 selective dioxabicyclo[3.2.1]octane motif of ertugliflozin\textsuperscript{29} (8) and the thiomethyl xyloside core of sotagli fl ozin\textsuperscript{30} (9). To our knowledge, when this project was initiated, ertugliflozin and sotagli flozin were in phase II clinical trials for type 2 diabetes (now both are in phase III clinical trials). Both compounds showed good results and possessed novel structures. On the basis of the skeleton of the dual SGLT1/2 inhibitor sotagli flozin, a new class of highly selective SGLT2 inhibitors incorporating a structurally new 1-methoxy-6,8-dioxabicyclo[3.2.1]octane ring system featured by a cascade ring-closing reaction to form the double ketal O-glycoside were designed by introducing the dioxabicyclo[3.2.1]octane motif of ertugliflozin (Figure 2). The synthetic methods are described as follows.

The known carboxylic acid 10\textsuperscript{31} was first converted to the corresponding acid chloride (Scheme 1) and then directly reacted with sodium 2-thioxopyridin-1(2\textsubscript{H})-olate to furnish the Barton ester 11. Compound 11 then underwent photochemical decarboxylation with a 500 W halogen lamp, and the radical intermediate was either trapped with MeSSMe to give methylthioether 12 or trapped with its pyridyl-2-thio radical intermediate to provide pyridylthioether 13.\textsuperscript{32} Finally debenzylation reactions of compounds 12,13 were carried out in the presence of BCl\textsubscript{3} to furnish the thioether compounds 14,15, respectively.

Target compounds 31–42 were synthesized from key intermediates 21a–d by one of three strategies. As shown in route 1 (Scheme 2), the known C-glycosides\textsuperscript{33} 16a–b were first iodinated with triphenylphosphine and iodine to give the iodo intermediates 17a–b, which underwent dehydroiodination to provide alkenes 18a–b.\textsuperscript{34} Epoxidation of compounds 18a–b with mCPBA led to epoxides 19a–b,\textsuperscript{35} which underwent epoxide opening in the presence of trifluoroacetic acid to provide the hydroxyl diketone intermediates 20a–b.\textsuperscript{36} The crude hydroxyl diketone intermediates underwent a ring closure cascade in 2 M NaOH to produce the key 1-hydroxy-6,8-dioxabicyclo[3.2.1]octane skeleton of intermediates 21a,b.\textsuperscript{36}

Alternatively, as shown in route 2 (Scheme 3), hydroxyl diketone 20a was also prepared in three simple steps. The dihydroxyl compound 24a was prepared by reacting the freshly prepared Grignard reagent of bromide 23a with 3,4,5,6-tetra-O-benzyl-D-glucopyranose 22. The resulting dihydroxy compound 24a was oxidized using trifluoroacetic anhydride in DMSO to provide diketone 25a. Because benzyl-protected primary alcohols are more susceptible to deprotection under acidic conditions, selective debenzylation of 25a with BCl\textsubscript{3} produced alcohol 20a in acceptable yield.

Furthermore, as shown in route 3 (Scheme 4), selective debenzylation and acetylation of the dihydroxy intermediates 24a,c,d led to the triacetoxy intermediates 26a,c,d. These were hydrolyzed, selectively silylated, and oxidized to diketone compounds 29a,c,d. Treatment of 29a,c,d with TBAF produced the desired intermediates 21a,c,d. The chemistry as described in route 3 allowed a more scalable synthesis for kilogram production.\textsuperscript{37}
With key intermediates 21a–d ready in hand, compounds 31–42 were synthesized (Scheme 5) from compounds 30a–k, respectively, by debenzylation. Compounds 30a–k could be obtained by alkylation of intermediates 21a–d.

As shown in Scheme 6, compounds 45 and 49 were prepared from the common intermediate ethyl ester 43, which was prepared by reacting the key intermediate 21a with ethyl 2-bromoacetate. Reduction of compound 43 with LiBH₄ gave hydroxyethyl compound 44, which was debenzylated to produce compound 45. The cyclopropyl compound 49 was prepared from alcohol 44 in four standard steps: iodination, elimination, cyclopropanation, and debenzylation.

**Biological Evaluation.** As shown in Table 1, compounds 14 and 15, where X = S and R₁ = methyl or 2-pyridyl, were found to have only moderate inhibitory activity (IC₅₀ = 13.9 and 28.4 nM, respectively). However, when the sulfur atom of...
compound 14 was replaced by an oxygen atom, compound 31 exhibited 10-fold stronger inhibitory activity (IC$_{50}$ = 1.3 nM) than compound 14. On the basis of this observation, the X group was kept as an oxygen atom and the R1 group was varied to explore the SAR. Initially, the small R1 groups such as ethyl, cyanomethyl, difluoromethyl, and cyclopropyl (compounds 32, 38, 40, and 49) were all well tolerated (IC$_{50}$ = 2.5, 1.1, 1.0, and 4.4 nM, respectively). However, when R1 was elongated one more atom than ethyl, such as hydroxethyl (compound 45), the inhibitory potency was found to decrease to 31.0 nM. After exploration of R1, the R2, R3, and R4 substitutions on the bisaryl aglycon were further investigated.
chlorine atom with a hydrogen atom at the R3 position decreased the SGLT2 inhibitory potency from 1.3 nM (compound 31) to 5.0 nM (compound 39). Introduction of a fluorine atom at the R3 position was found to decrease the potencies as well (compounds 33–35). Finally, when R4 group was modified from the smaller ethyl group (compound 31) to larger groups, such as tetrahyrofuran-3-yl (compound 37) and 2-cyclopropoxethyl group (compound 36), the inhibitory potencies decreased to 5.9 and 10.8 nM, respectively. A similar trend was observed for compounds 40–42 as well.

Compounds 14, 15, 31, and 40 were further evaluated for selectivity against hSGLT1 by measuring their in vitro hSGLT1 inhibitory activities (Table 2). Compounds 31 and 40 showed better selectivity profiles. It is noteworthy that the selectivity of compound 31 was even better than that of compound 1 (dapagliflozin). However, compounds 14 and 15 showed moderate selectivities (32- and 39-fold, respectively). This observation indicated that the replacement of the sulfur atom at the X position with an oxygen atom could improve not only the inhibitory potency of hSGLT2 but also the selectivity of hSGLT2 over hSGLT1.

Compounds 31 and 40 with their better in vitro activities and greater selectivity for SGLT2 were further investigated for pharmacokinetic (PK) studies in Sprague-Dawley (SD) rats. After single oral or intravenous doses of compounds 31 and 40, blood samples were taken at planned time points and the unchanged drugs in plasma were quantified using a LC-MS/MS method. Major PK parameters were calculated using Winnolin software, version 6.3. As shown in Table 3, compounds 31 and 40 presented acceptable oral PK profiles. Compound 31 showed better systemic exposure and oral bioavailability than compound 40 (76% and 46%, respectively), and the low CL demonstrated that the test compounds were metabolized and excreted very slowly.

As shown in Figure 3a, single oral administrations of compounds 31 and 40 of 1, 3, and 10 mg/kg to SD rats induced dose-dependent urinary glucose excretion, resulting in a more than 1000-fold elevation in glucosuria relative to the vehicle control. The ability of compound 31 to increase urinary glucose excretion was similar to that of canagliflozin. Compound 40 was found to be more efficacious than compound 31 and canagliflozin in this experiment. Afterward, a single dose of compounds 31 or 40 of 25 mg/kg was orally administrated to Rhesus monkeys. As indicated in Figure 3b, compounds 31 and 40 could increase urinary glucose excretion robustly, which was superior to that of canagliflozin. In addition, both compounds 31 and 40 could maintain a comparatively high urinary glucose excretion level for 4 to 5 days, similar to dapagliflozin, while canagliflozin could maintain a high level of excretion for less than 3 days. These results indicated that both compounds 31 and 40 are potent SGLT2 inhibitors and exhibit a long duration of action.

Furthermore, streptozotocin (STZ) and high-fat diet (HFD) induced Institute of Cancer Research (ICR) mice were used to assess the antihyperglycemic effect in an oral glucose tolerance test (OGTT). A single oral dose of compounds 31 or 40 (10 mg/kg) reduced the blood glucose excursion when the compounds were administrated half an hour before an oral glucose challenge. The area under the curve of blood glucose–time (AUC0−120 min) showed that compounds 31 and 40 had statistically significant antihyperglycemic efficacies (Figure 4) compared with vehicle treatment (P < 0.001).

Because of the good in vivo profiles, compounds 31 and 40 were selected for preclinical development. They were tested for their ability to inhibit the hERG channel as part of the evaluation of these compounds. Compounds 31 and 40 were not found to block the hERG channel at concentrations up to 30 μM in the manual whole-cell patch-clamp assay. In a single oral dose toxicity study in SD rats, the maximum tolerated doses (MTDs) of the two compounds were over 1000 mg/kg. Compound 31 was also evaluated in a 28-day repeat-dose toxicity study in beagle dogs. It was found that compound 31 was well tolerated up to 300 mg/kg without any mortality or severe untoward effects being noted.

**CONCLUSION**

We have disclosed a new class of potent and highly selective SGLT2 inhibitors that feature a novel double ketal dioxabicyclo[3.2.1]octane scaffold. Compound 31, which is currently undergoing further preclinical development for the treatment of type 2 diabetes, increased urinary glucose excretion efficaciously and exhibited a long duration of action in Rhesus monkeys.
In Vitro hSGLTs Uptake Assays. The CHO cells stably expressing human SGLT2 or SGLT1 were seeded into 96-well plates (Corning, NY) at a density of 30000 cells/well and incubated for 48 h in a 5% CO₂ atmosphere at 37 °C in growth medium (1:1 F-12/DMEM media and 10% FBS). The culture medium was removed, and the cells were washed three times with 200 μL of KRH solution (120 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl₂, 2.2 mM CaCl₂, and 10 mM HEPES, pH 7.4), then incubated in KRH solution containing 3 μM methyl-α-D-[U-14C]-glucopyranoside ([14C]AMG) in the absence or presence of compounds.

Table 1. In Vitro Inhibitory Activity of hSGLT2

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“In vitro human SGLT2 inhibition activities of compounds were determined at the same laboratory by evaluating the sodium-dependent uptake of methyl-α-D-[U-14C]-glucopyranoside in Chinese hamster ovary (CHO) cells stably expressing human SGLT2.

EXPERIMENTAL SECTION

In Vitro hSGLTs Uptake Assays. The CHO cells stably expressing human SGLT2 or SGLT1 were seeded into 96-well plates (Corning, NY) at a density of 30000 cells/well and incubated for 48 h in a 5% CO₂ atmosphere at 37 °C in growth medium (1:1 F-12/DMEM media and 10% FBS). The culture medium was removed, and the cells were washed three times with 200 μL of KRH solution (120 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl₂, 2.2 mM CaCl₂, and 10 mM HEPES, pH 7.4), then incubated in KRH solution containing 3 μM methyl-α-D-[U-14C]-glucopyranoside ([14C]AMG) in the absence or presence of compounds.

Table 2. In Vitro Inhibitory Activity and Selectivity

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<tr>
<th>Compd</th>
<th>hSGLT2 IC₅₀ (nM)</th>
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<th>Selectivity hSGLT1/hSGLT2</th>
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<td>453</td>
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“In vitro human SGLT1 and SGLT2 inhibition activities of compounds were determined at the same laboratory by evaluating the sodium-dependent uptake of methyl-α-D-[U-14C]-glucopyranoside in Chinese hamster ovary (CHO) cells stably expressing human SGLT1 or SGLT2.

Table 3. Pharmacokinetic Parameters of Compounds 31 and 40 in SD Rats

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Figure 3. Effects of oral administration of selected compounds on urinary glucose excretion in SD rats and Rhesus monkeys. Animals were fasted overnight and orally administrated vehicle or selected compounds at 1, 3, and 10 mg/kg for SD rats (n = 3) and 25 mg/kg for Rhesus monkeys (n = 2).
presence of inhibitors for up to 120 min at 37 °C. After that, the KRH solution was removed and the wells were rinsed three times with 200 μL of ice-cold KRH solution. The cells were lysed in 0.1% sodium dodecyl sulfate (Sigma). After 24 h, plates were quantitated in a TopCount (PerkinElmer) for counting of radioactive [14C]AMG. The percent effect of compounds to inhibit AMG uptake was calculated by comparing counts per minute (CPM) in inhibitor-containing wells with in DMSO wells. The IC50 values were fitted to a sigmoidal dose–response model using Origin 8.0 software.

Oral Glucose Tolerance Test in STZ and HFD Induced Mice
Male ICR mice of 4 weeks of age were obtained from Vital River (Beijing, China), fed with high fat diet (15% fat by weight). All mice were given access to food and water ad libitum. At 7 weeks of age, all mice were injected with a single dose of 50 mg/kg of streptozotocin (STZ). The diet induced obesity (DIO) mice at 8 weeks of age (n = 10/group) were randomly assigned to treatment groups and fasted overnight. Mice were then treated orally with vehicle (0.5% methylcellulose) or 10 mg/kg of SGLT2 inhibitors (10 mg/kg). All animals received an oral glucose load (1 g/kg) half an hour later; areas under the curve of blood glucose–time (0–120 min) are shown. Values are means ± SDs (n = 10). ***, P < 0.001.

Figure 4. Effects of SGLT2 inhibitors during an OGTT in STZ and HFD induced ICR mice. Animals were fasted overnight and orally given vehicle or SGLT2 inhibitors (10 mg/kg). All animals received an oral glucose load (1 g/kg) half an hour later; areas under the curve of blood glucose–time (0–120 min) are shown. Values are means ± SDs (n = 10). ***, P < 0.001.

General Procedures. All purchased starting materials were used without further purification. 1H NMR, 13C NMR, and 19F NMR spectra were acquired on a Bruker Avance-400 spectrometer (400 MHz) or a Bruker Avance-300 spectrometer (300 MHz), with tetramethylsilane (TMS) as an internal standard; chemical shifts are expressed in parts per million (ppm, δ units). Mass spectra were obtained on a Finnigan LCQad instrument (ESI) and Agilent 6120 (APCI). Most masses were reported as those of the protonated parent ions. Preparative column chromatography was performed using Yantai Daltonics, Inc. (U.S.). High-resolution liquid chromatography (HPLC) was performed on an Agilent1260 chromatographic column (Agilent Zorbax SB-C18 4.6 mm × 100 mm, 3.5 μm). Rotation was performed on automatic polarimeter SGP-3. Melting point was obtained on an OptiMelt MPA100. All compounds submitted for in vitro testing were >95% purity (HPLC), and those for in vivo testing were >98% purity (HPLC).

General Procedure A. For the preparation of compounds 14, 15, and 38, taking the preparation for compound 14 as an example.

(1R,2S,3R,4R,5S)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-(pyridin-2-ylthio)-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol (14). To a solution of 12 (440.0 mg, 0.89 mmol) in DCM (5 mL) was added boron trichloride (17.7 mL, 1 M in DCM, 17.70 mmol) at −78 °C. Upon completion of the addition, the mixture was stirred for 2 h. The resulting mixture was quenched by the dropwise addition of a solution of DCM and MeOH (20 mL, v/v = 1:1). Water (20 mL) was then added. The water layer was extracted with DCM (20 mL × 2). The combined organic extracts were dried (Na2SO4), filtered, and concentrated. The crude product was purified by column chromatography (DCM/MeOH (v/v) = 4:1–3:1, Rf = 0.1 to 0.05) to afford compound 14 as a white solid (55.0 mg, yield 14%; HPLC 95.72% (210 nm)). 1H NMR (400 MHz, CD3OD): δ 7.90−7.84 (m, 2 H, ArH), 7.54 (d, J = 8.3 Hz, 1 H, ArH), 7.12 (d, J = 8.5 Hz, 2 H, ArH), 6.85 (d, J = 8.3 Hz, 2 H, ArH), 5.63 (d, J = 6.6 Hz, 1 H, CH), 4.67 (dd, J = 6.6, 4.5 Hz, 1 H, CH), 4.41 (dd, J = 5.7, 1.6 Hz, 1 H, CH), 4.10 (s, 2 H, CH2), 4.04−3.95 (m, 3 H, CH2, CH3), 3.74 (d, J = 12.2 Hz, 1 H, CH2), 2.02 (s, 3 H, CH3), 1.37 (t, J = 7.0 Hz, 3 H, CH3). 13C NMR (101 MHz, CD3OD): δ 196.4, 157.7, 139.9, 139.4, 133.5, 131.5, 130.1, 129.5, 129.1, 129.0, 126.9, 125.7, 114.3, 93.1, 80.9, 79.1, 78.7, 63.1, 57.7, 13.8, 8.3. LC-MS m/z (ESI): 475.0 [M + Na]+. HRMS: calcd C22H29ClNO6S (M + Na)+ 676.1842, found 676.1841. Rotation was performed on automatic polarimeter SGP-3. Melting point was obtained on an OptiMelt MPA100. All compounds submitted for in vitro testing were >95% purity (HPLC), and those for in vivo testing were >98% purity (HPLC).
H3. LC-MS (d, ArH), 7.06 (d, 2 H, ArH), 6.75 (d, J = 9 Hz, 2 H, ArH), 6.75 (d, J = 6 Hz, 2 H, ArH), 4.75–4.95 (m, 4 H, CH2), 4.27 (d, J = 9 Hz, 1 H, CH2), 4.20 (d, J = 9 Hz, 1 H, CH2), 3.80–4.01 (m, 8 H, CH2), 3.62 (m, 1 H, CH3), 3.45 (s, 3 H, OCH3), 1.30 (s, f = 6 Hz, 3 H, CH3). LC-MS m/z (APCI): 707.2 [M + H]+.

(3R,2R,5R,5S)-2,3,4-Tetakis(benzylxoy)-1-(4-chloro-3-(4-ethoxybenzylo)phenyl)hexane-1,2,6-triol (26a). Crude 26a (2.18 kg) was dissolved in acetic anhydride (5 L), and 2-propanol (1.2 L) was added. The resulting mixture was cooled to room temperature and poured into water (3 L). The mixture was stirred at room temperature and quenched with water (100 mL × 3). The combined organic extracts were washed with saturated NaHCO3 solution (150 mL × 3) and water (25 mL) was added potassium hydroxide (12.1 g, 216.07 mmol) at −5°C in an ice–water bath. The resulting mixture was quenched with water (100 mL) and extracted with EA (200 mL × 3). The combined organic extracts were washed with sodium bicarbonate solution (2 L × 2) and water (2 L × 1), dried (Na2SO4), and concentrated. The residue (2.28 kg) was directly used for the next step without purification. 1H NMR (400 MHz, CDCl3): δ 7.39–7.27 (m, 11 H, ArH), 7.25–7.10 (m, 5 H, ArH), 7.05 (t, J = 8.6 Hz, 2 H, ArH), 6.83 (m, 2 H, ArH), 6.78–6.71 (m, 2 H, ArH), 6.71–6.37 (m, 1 H, CH), 4.95 (d, J = 11 Hz, 1 H, CH2), 4.80 (d, J = 3.1, 11.0 Hz, 3 H, CH3CH2), 4.45 (d, J = 8.7 Hz, 1 H, CH), 4.26 (d, J = 10.9 Hz, 1 H, CH), 4.09–3.82 (m, 7 H, CH2), 3.78 (d, J = 9.1 Hz, 1 H, CH), 3.66 (d, J = 7.8 Hz, 1 H, CH), 3.18 (t, J = 7.0 Hz, 3 H, CH3). LC-MS m/z (APCI): 743.2 [M + H]+.

General Procedure B. The preparation of compounds 31–37, 40–42, 45, and 49 followed the method for compound 31.

(15Z,25,3R,4R,4S)-2,3,4-Tris(benzylxoy)-6-[(tetr-butyldimethylsiloxy)-1-(4-chloro-3-(4-ethoxybenzylo)phenyl)hexane-1,5-diol (27a). Crude 27a (1.5 kg, 2.10 mol) was treated under a nitrogen atmosphere with TBSO (3660 g, 2.31 mol), imidazole (2660 g, 3.15 mol), and DCM (4 L) at room temperature. After completion of reaction, the mixture was poured into water (4 L). The mixture was extracted with DCM (2 L × 2). The organic layer was dried (Na2SO4) and concentrated, and the residue was directly used for the next step without purification.

(15Z,25,3R,4R,4S)-2,3,4-Tris(benzylxoy)-6-[(tetr-butyldimethylsiloxy)-1-(4-chloro-3-(4-ethoxybenzylo)phenyl)hexane-1,5-dione (28a). A solution of dimethyl sulfoxide (971 mL) in DCM (2.8 L) was added dropwise to a solution of trifluoroacetic anhydride (1424 mL) in DCM (1.4 L) at −78°C. 28a in DCM (2.3 L) was then added. After stirring for 1 h at −78°C, triethylamine (2530 mL, 18.20 mol) in DCM (1.4 L) was added, the temperature was allowed to reach room temperature, and the mixture was poured into water (3 L). The mixture was extracted with DCM (4 L × 2). The organic layer was dried (Na2SO4), and concentrated, and the residue was directly used for the next step without purification.

(15Z,25,3R,4R,4S)-2,3,4-Tris(benzylxoy)-5-(4-chloro-3-(4-ethoxybenzylo)phenyl)-1-methoxy-6,8-dioxybicyclo[3.2.1]octane (30a). To a solution of 21a (330.0 g, 0.484 mol) in DMF (1.5 L) was added iodomethane (1010.0 g, 0.72 mol) under nitrogen atmosphere at 0°C. Then NaH (140.0 g, 0.61 mol) was added in batches, and the mixture was stirred for 1 h at room temperature under nitrogen atmosphere. The resulting mixture was adjusted to pH = 7 with saturated NH4Cl solution. The water phase was extracted with EA (2 L × 3). The combined organic extracts were washed with water (5 L × 1) and water (5 L × 1), dried (Na2SO4), and concentrated. The crude product was purified by column chromatography (PE/EA (v/v) = 15:1, Rf = 0.2) to give compound 30a as a light-yellow oil (303.0 g, yield 90%). 1H NMR (300 MHz, CDCl3): δ 7.13–7.35 (m, 16 H, ArH), 7.06 (d, J = 6 Hz, 2 H, ArH), 6.85 (d, J = 6 Hz, 2 H, ArH), 6.75 (d, J = 9 Hz, 2 H, ArH), 4.74–4.95 (m, 4 H, CH2), 4.27 (d, J = 9 Hz, 1 H, CH2), 4.20 (d, J = 9 Hz, 1 H, CH2), 3.80–4.01 (m, 8 H, CH2), 3.62 (m, 1 H, CH3), 3.45 (s, 3 H, OCH3), 1.38 (t, f = 6 Hz, 3 H, CH3). LC-MS m/z (APCI): 707.2 [M + H]+.

(3R,2R,5R,5S)-2,3,4-Tetakis(benzylxoy)-5-(4-chloro-3-(4-ethoxybenzylo)phenyl)-1-(difluoromethoxy)-6,8-dioxybicyclo[3.2.1]octane (30b). A solution of dimethyl sulfoxide (971 mL) in DCM (2.8 L) was added dropwise to a solution of trifluoroacetic anhydride (1424 mL) in DCM (1.4 L) at −78°C. 28a in DCM (2.3 L) was then added. After stirring for 1 h at −78°C, triethylamine (2530 mL, 18.20 mol) in DCM (1.4 L) was added, the temperature was allowed to reach room temperature, and the mixture was poured into water (3 L). The mixture was extracted with DCM (4 L × 2). The organic layer was dried (Na2SO4), and concentrated, and the residue was directly used for the next step without purification.
(1S,2S,3R,4R,5S)-5-(4-Chloro-3-(4-ethoxy-3-fluorophenyl)-1-ethoxy-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol (32). Prepared from compound 30b following general procedure B to afford compound 32 as a white solid (0.2 g, yield 86%; HPLC 95.17% (210 nm)). H NMR (400 MHz, CD3OD): δ 7.40 (s, 1 H, ArH), 7.38–7.36 (m, 2 H, ArH), 6.99–6.87 (m, 3 H, ArH), 4.11–4.02 (m, 5 H, CH2,CH2), 3.84 (m, 1 H, CH), 3.68–3.56 (m, 2 H, CH2), 3.53 (d, 1 H, CH3), 3.46 (s, 3 H, CH3, OCH3), 1.38 (s, 3 H, CH3). 13C NMR (101 MHz, CD3OD): δ 157.5–153.1 (ArCF), 145.2, 137.7, 136.3, 132.7, 128.9, 128.7, 125.8, 124.5, 113.4, 112.9, 114.9, 110.6, 106.7, 78.0, 75.9, 72.9, 67.5, 65.3, 53.0, 37.9, 4.5. LC-MS m/z (ESI): 405.0 [M – EtO]+. HRMS: calcld C16H22ClNO5 (M + NH4)+ 468.1789, found 468.1788.

(15,25,3R,4R,5S)-5-(4-Chloro-3-(4-ethoxy-3-fluorophenyl)-1-ethoxy-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol (33). Prepared from compound 30d following general procedure B to afford compound 33 as a white solid (0.2 g, yield 86%; HPLC 98.98% (210 nm)). H NMR (400 MHz, CD3OD): δ 7.42–7.36 (m, 2 H, ArH), 6.99–6.87 (m, 3 H, ArH), 4.11–4.02 (m, 5 H, CH2,CH2), 3.84 (m, 1 H, CH), 3.68–3.56 (m, 2 H, CH2), 3.53 (d, 1 H, CH3), 3.46 (s, 3 H, CH3, OCH3), 1.38 (s, 3 H, CH3). 13C NMR (101 MHz, CD3OD): δ 163.8–157.3 (129 ArCF), 145.2, 137.7, 136.3, 132.7, 128.9, 128.7, 125.8, 124.3, 113.4, 112.9, 114.9, 110.6, 106.7, 78.0, 75.9, 72.9, 67.5, 65.3, 64.7, 50.1, 37.8, 13.8. LC-MS m/z (ESI): 423.0 [M – MeO]+. HRMS: calcld C17H23ClNO6 (M + Na)+ 472.1538, found 472.1527.

(15,25,3R,4R,5S)-5-(4-Chloro-3-(4-ethoxy-3-fluorophenyl)-1-ethoxy-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol (34). Prepared from compound 30e following general procedure B to afford compound 34 as a white solid (0.2 g, yield 86%; HPLC 98.98% (210 nm)). H NMR (400 MHz, CD3OD): δ 7.42 (s, 1 H, ArH), 7.41–7.35 (m, 2 H, ArH), 6.96 (t, J = 8.3 Hz, 1 H, ArH), 6.90 (d, J = 10.4 Hz, 2 H, ArH), 4.12–4.08 (m, 1 H, CH), 4.07 (m, 4 H, CH2), 3.85–3.80 (m, 2 H, CH2), 3.75–3.66 (m, 1 H, CH), 3.64–3.57 (m, 2 H, CH2), 3.52 (d, J = 7.9 Hz, 1 H, CH), 1.38 (s, J = 7.0 Hz, 3 H, CH3). 13C NMR (101 MHz, CD3OD): δ 157.3–151.3 (210 ArCF), 145.2, 137.7, 136.3, 132.7, 128.9, 128.7, 125.8, 124.3, 113.4, 112.9, 114.9, 110.6, 106.7, 78.0, 75.9, 72.9, 67.5, 65.3, 64.7, 50.1, 37.8, 13.8. LC-MS m/z (ESI): 423.0 [M – MeO]+. HRMS: calcld C17H23ClNO6 (M + Na)+ 472.1538, found 472.1527.
Experimental, analytical details and spectra data for compounds 10–49 (PDF)
Molecular formula strings (CSV)

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Notes
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■ ABBREVIATIONS USED
AMG, methyl-α-D-glucopyranoside; AUC, area under the plasma concentration time curve; BCl3, boron trichloride; Bu4NF, BrCH2CO2Et, ethyl 2-bromooacetate tetrabutylammonium fluoride; BrF2CPO3(Et)2, diethyl (bromodiethylphosphonate); CH3ONa, sodium methoxide; CH3J2, diiodomethane; Cl2, clearance; (COC13)2 oxalyl chloride; DCM, dichloromethane; DIO, diet induced obesity; DMP, N,N-dimethyleformamide; DMSO, dimethyl sulfoxide; EA, ethyl acetate; Et3N, triethylamine; EtZn, zinc diethyl; F, oral bioavailability; FDA, U.S. Food and Drug Administration; H2, hydrogen; HDF, high-fat diet; I2, iodine; IC50, half-maximal inhibitory concentration; K2CO3, potassium carbonate; KOH, potassium hydrate; KRH, Krebs–Ringer HEPES buffer; LiBH4, lithium borohydride; mCPBA, 3-chlorobenzperoxycarboxic acid; MeCN, methyl cyanide; Mg, magnesium; MeOH, methanol; MeSSMe, 1,2-dimethyldisulfane; MTD, maximum tolerated doses; NaH, sodium hydride; NaHCO3, sodium hydrogen carbonate; Na2SO4, sodium sulfate; OGT, oral glucose tolerance test; PE, petroleum ether; PPh3, triphenylphosphine; PK, pharmacokinetics; p-TsOH, p-toluene sulfonic acid; RP, retention factor value; rt, room temperature; SGLT2, sodium-glucose co-transporter; SD, Sprague–Dawley; SAR, structure–activity relationship; STZ, streptozotocin; t-BuMe2SiCl, tert-butyldimethyl chlorosilane; TFA, trifluoroacetic acid; TFAA, trifluoroacetic anhydride; THF, tetrahydrofuran; toluene, methylbenzene; T2DM, type 2 diabetes mellitus; UGE, urinary glucose excretion; WHO, World Health Organization

■ REFERENCES


(37) The cost for compound 22 is no more than $250 per kilogram, and the cost for compound 23a is less than $1400 per kilogram in P. R. China.

(38) This study was designed according to the NDA data of canagliflozin.