Bioorganic & Medicinal Chemistry Letters 19 (2009) 5632–5635

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

O-Spiro *C*-aryl glucosides as novel sodium-dependent glucose co-transporter 2 (SGLT2) inhibitors

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ARTICLE INFO

Article history:
Received 11 May 2009
Revised 28 July 2009
Accepted 7 August 2009
Available online 12 August 2009

Keywords:
SGLT
Spiro
Glucosides

ABSTRACT

Two series of *O*-spiro *C*-aryl glucosides were synthesized and tested for inhibition of hSGLT1 and hSGLT2. 6′-*O*-Spiro *C*-aryl glucosides exhibited potent in vitro hSGLT2 inhibitory activity but 2′-*O*-spiro *C*-aryl glucosides showed no in vitro hSGLT2 inhibitory activity at a screening concentration of 1 μM.

According to the World Health Organization, more than 180 million people worldwide have diabetes mellitus. This number is likely to more than double by 2030. Diabetes is characterized by failing to produce enough insulin from pancreatic β-cells (type 1 diabetes),[1] or failing to respond properly to the insulin produced by the pancreas (type 2 diabetes).[2] The essential characteristic of diabetes is hyperglycemia, which is considered to be the major pathogenic factor for the development of serious complications including retinopathy,[3] cardiovascular disease,[4] nephropathy,[5] neuropathy,[6] ulcers[7] and heart disease.[8]

Cellular glucose transport is conducted by two classes of glucose transporters, the facilitative glucose transporters (GLUTs) and sodium-dependent glucose co-transporters (SGLTs).[9] Two important isoforms of SGLT, SGLT1 and SGLT2, were identified, the former is found predominantly in the intestinal brush border, while the latter is localized in the renal proximal tubule and is responsible for the majority of glucose re-absorption by the kidney.[10] Recent studies suggest that inhibition of SGLT at the kidney may be a useful approach to decrease glucose absorption and this could result in the increase of the amount of glucose excreted in the urine.[11]

Dapagliflozin **1**, a *C*-aryl glucoside SGLT2 inhibitor developed by Bristol-Myers Squibb Company, exhibits powerful ability of urinary glucose excretion and can dramatically lower fasting and postprandial blood glucose level in hyperglycemic streptozotocin (STZ)-induced diabetic rats.[12] BMS' biological results with dapagliflozin prompted us to design two novel types of *O*-spiro *C*-aryl glucosides, 6′-*O* and 2′-*O*-spiro *C*-aryl glucosides (Fig. 1). Chugai Seiyaku Kabushiki Kaisha disclosed a patent for application of 6′-*O*-spiro *C*-aryl glucosides without the 4-chloro substituent as SGLT inhibitors prior to us.[13] Herein, we report our initial design, synthesis, and biological evaluation of the *O*-spiro *C*-aryl glucoside SGLT2 inhibitors.

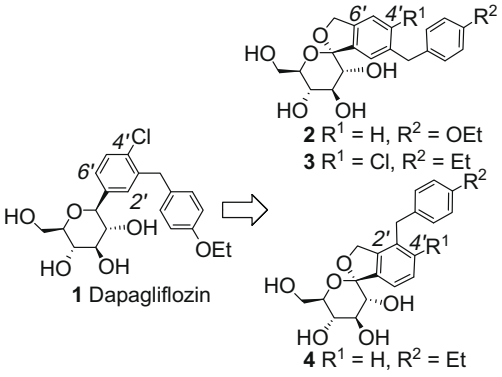

Figure 1.

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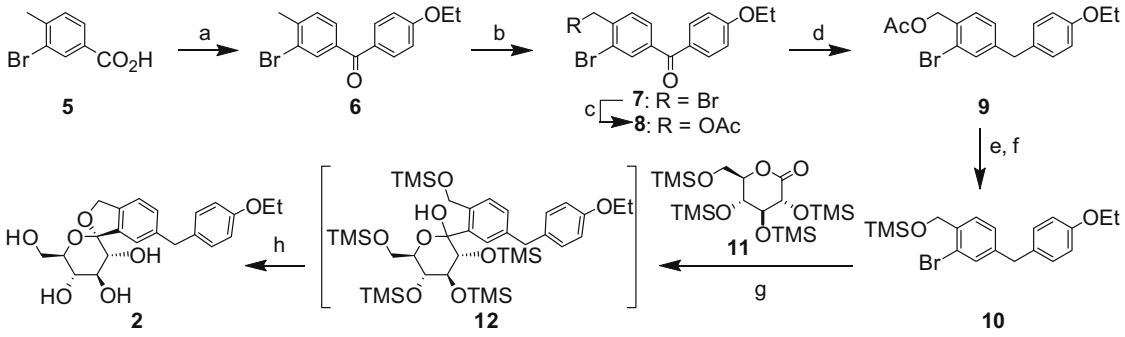
doi:10.1016/j.bmcl.2009.08.030

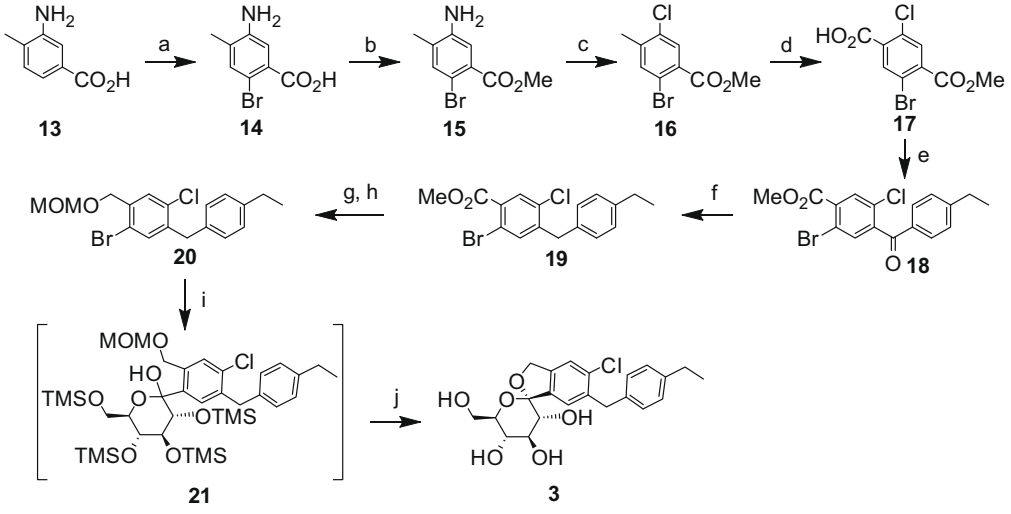
Two possible kinds of spiro compounds at the 6′-position and the 2′-position of the proximal phenyl ring can be constructed connecting at the C-1 position of the glucose ring. Accordingly, we synthesized 6′-*O*-spiro compounds **2** and **3**, and 2′-*O*-spiro compound **4** (Fig. 1). Beginning with the synthesis of 6′-*O*-spiro glucoside **2**, as shown in Scheme 1, commercially available 3-bromo-4-methylbenzoic acid **5** was converted to acid chloride with oxalyl chloride, which subsequently underwent Friedel–Crafts acylation reaction with phenetole to afford diaryl ketone **6**. Bromination of the latter ketone with *N*-bromosuccinimide followed by displacement with sodium acetate provided acetate **8**. Selective reduction of the carbonyl group of **8** with triethylsilane and boron trifluoride etherate gave diarylmethane **9**. Hydrolysis of **9** followed by silylation of the ensuing alcohol with trimethylsilyl chloride furnished silyl ether **10**. Treatment of the silyl ether with *n*-BuLi at −78 °C, followed by the addition of 2,3,4,6-tetra-*O*-trimethylsilyl-D-gluconolactone **11**, prepared from corresponding gluconolactone in the presence of trimethylsilyl chloride and *N*-methylmorpholine,[14] afforded corresponding coupling intermediate **12**, which finally underwent deprotection and cyclization in one-pot with methylsulfonic acid to provide the desired 6′-*O*-spiro glucoside **2** as a single anomer[15] in moderate yield.[16]

In view of that the 4′-position of the proximal phenyl ring of dapagliflozin is substituted by a chloro group, we thus introduced a same group in the corresponding position of 6′-*O*-spiro glucoside **2** to obtain 6′-*O*-spiro glucoside **3**, as shown in Scheme 2. Bromination of commercially available 3-amino-4-methylbenzoic acid **13** with *N*-bromosuccinimide afforded bromide **14**, which subsequently underwent esterification to give ester **15**. Chlorination of the ester under standard Sandmeyer reaction conditions provided chloride **16**, which was oxidized by $KMnO_4$ to generate benzoic acid **17**. Friedel–Crafts acylation of ethylbenzene with benzoyl chloride, prepared from benzoic acid **17** using oxalyl chloride, gave diaryl ketone **18**. Selective reduction of the latter ketone with triethylsilane in the presence of catalytic amount of trifluoromethanesulfonic acid gave the corresponding diarylmethane **19**, which was reduced with sodium borohydride, followed by protection of the resulting benzyl alcohol with chloromethoxymethane to afford methoxymethyl ether **20**. Treatment of this ether using a similar approach that was described for synthesis of compound **2** provided the desired 6′-*O*-spiro glucoside **3**.[16]

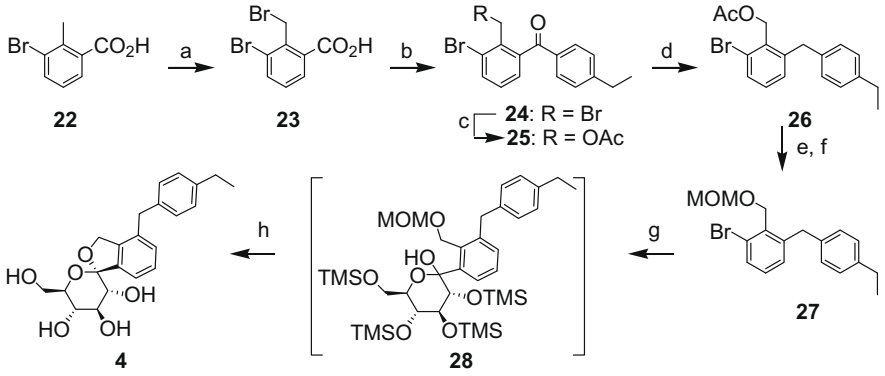
Scheme 3 depicts the synthesis of 2′-*O*-spiro glucoside **4**. Bromination of commercially available 3-bromo-2-methylbenzoic acid **22** with *N*-bromosuccinimide gave benzylbromide **23**. Our initial attempts to displace the bromide in **23** with sodium acetate failed to yield the desired acetate, probably due to intramolecular lactonization. Thus, benzylbromide **23** was converted to the acid chloride with oxalyl chloride and this material was subsequently



Scheme 1. Reagents and conditions: (a) oxalyl chloride, DMF, $AlCl_3$, phenetole, CH_2Cl_2 (75%); (b) NBS, AIBN, CCl_4, reflux (50%); (c) AcONa, DMF, 68 °C (95%); (d) $BF_3{\cdot}Et_2O$, Et_3SiH, $CH_3CN/ClCH_2CH_2Cl$ (2:1), rt (61%); (e) $LiOH{\cdot}H_2O$, THF/MeOH/H_2O (2:3:1), rt (92%); (f) TMSCl, *N*-methylmorpholine, THF (95%); (g) *n*-BuLi, 2,3,4,6-tetra-*O*-trimethylsilyl-D-gluconolactone **11**, THF/toluene (1:2), −78 °C, then H_2O; (h) $MeSO_3H$, THF, −78 °C to rt (37% two steps).



Scheme 2. Reagents and conditions: (a) NBS, DMF, 5 °C (87%); (b) $SOCl_2$, MeOH, reflux (99%); (c) CuCl, $NaNO_2$, conc. HCl, 1,4-dioxane, H_2O, 0 °C (93%); (d) KMnO4, 18-crown-6, $MgSO_4$, *t*-BuOH/H_2O (1:2), reflux (32%); (e) oxalyl chloride, DMF, $AlCl_3$, ethylbenzene, CH_2Cl_2 (87%); (f) Et_3SiH, CF_3SO_3H, TFA, rt (100%); (g) $NaBH_4$, MeOH, THF (quantitative); (h) MOMCl, DIPEA, CH_2Cl_2, rt (90%); (i) *n*-BuLi, 2,3,4,6-tetra-*O*-trimethylsilyl-D-gluconolactone **11**, THF/toluene (1:2), −78 °C, then H_2O; (j) $MeSO_3H$, THF, −78 °C to rt (63% two steps).



Scheme 3. Reagents and conditions: (a) NBS, AIBN, CCl_4, reflux (95%); (b) oxalyl chloride, DMF, $AlCl_3$, ethylbenzene, CH_2Cl_2; (c) AcONa, DMF, 68 °C; (d) $BF_3{\cdot}Et_2O$, Et_3SiH, $CH_3CN/ClCH_2CH_2Cl$ (2:1), rt (36% three steps); (e) $LiOH{\cdot}H_2O$, THF/MeOH/H_2O (2:3:1), rt; (f) MOMCl, DIPEA, CH_2Cl_2, rt (44% two steps); (g) *n*-BuLi, 2,3,4,6-tetra-*O*-trimethylsilyl-D-gluconolactone **11**, THF/toluene (1:2), −78 °C, then H_2O; (h) $MeSO_3H$, THF, −78 °C to rt (41% two steps).

Table 1
In vitro data for hSGLT inhibitory activity and selectivity

Compds	hSGLT2 IC_{50} (nM)	hSGLT1 IC_{50} (nM)	Selectivity (hSGLT1/hSGLT2)
1	6.7 (4.8–9.0)[a]	885 (528–1480)[a]	132
2	71 (52–96)[a]	10,000–100,000	141–1410
3	6.6 (3.4–12.8)[a]	620 (450–853)[a]	94
4	0%[b]	38%[c]	—

[a] Numbers in parentheses indicate 95% confidence intervals.
[b] Inhibition at a screening concentration of 1 μM.
[c] Inhibition at a screening concentration of 100 μM.

subjected to Friedel–Crafts acylation reaction with ethylbenzene to afford diaryl ketone **24**. The bromide **24** was substituted with sodium acetate, followed by selective reduction of the carbonyl with triethylsilane, to yield acetate **26**. Saponification of acetate **26**, and protection of the resulting alcohol with chloromethoxymethane provided methoxymethyl ether **27**. Treatment of **27** using a similar method used for synthesis of analogue **2** gave target 2′-*O*-spiro glucoside **4** in moderate yield.[16]

All compounds were screened in a cell-based SGLT functional assay,[17] and hSGLT inhibitory activity (IC_{50}) and selectivity (hSGLT1/hSGLT2) are presented in Table 1. Using dapagliflozin **1** as the reference compound we identified 6′-*O*-spiro *C*-aryl glucoside **2** as our lead compound because of its good inhibitory activity toward hSGLT2 (IC_{50} = 71 nM). Introduction of a chloro group at the 4′-position of the proximal phenyl ring led to analogue **3** with a 10-fold elevation of the hSGLT2 inhibitory activity with an IC_{50} value of 6.6 nM, which was similar to that of dapagliflozin **1** (6.7 nM) in the same assay. This result suggested that introducing a substituent in the 4′-position of the phenyl ring was very important for improvement of the inhibitory activity.[12] However, 6′-*O*-spiro C-aryl glucoside **3** was less selective for hSGLT2 versus hSGLT1 than dapagliflozin. On the other hand, 2′-*O*-spiro *C*-aryl glucoside **4** showed no inhibitory activity toward hSGLT2 at a screening concentration of 1 μM. All above results demonstrated that 6′-*O*-spiro was the preferred conformation for the binding site.

In summary, we have identified a novel and potent hSGLT2 inhibitor series 6′-*O*-spiro *C*-aryl glucosides. Glucoside **3** had similar in vitro hSGLT2 inhibitory activity and a little less selectivity as compared to dapagliflozin **1**. Further modification of this series will be reported in due course.

Acknowledgments

We gratefully acknowledge Ying Chen for activity testing, the analytical group of Egret Pharma (Shanghai) Limited for measurements of all spectra and Dr. Jacques Y. Roberge for thoughtful review of this Letter.

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Plasmid containing human SGLT2 was linearized and stably transfected into HEK293.ETN cells. SGLT2-expressing clones were selected based on resistance to puromycin (Invitrogen Corporation) and activity in AMG uptake assay. Cells expressing SGLT1 or SGLT2 were seeded on 96-well ScintiPlates (PerkinElmer, Inc.) in DMEM containing 10% FBS (1×10^5 cells per well in 100 μL medium) incubated at 37 °C under 5% CO_2 for 48 h prior to the assay. Cells were washed twice with 150 μL of either sodium buffer (137 mM NaCl, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM tris(hydroxymeth-yl)aminomethane/*N*-2-hydroxyethylpiperazine-*N*′-ethanesulfonic acid [Tris/Hepes], pH 7.2) or sodium-free buffer (137 mM *N*-methyl-glucamine, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM Tris/Hepes, pH 7.2). Test compound in 50 μL each of sodium or sodium-free buffer containing 40 μCi/mL methyl-α-D-[U-^{14}C]glucopyranoside (Amersham Biosciences/GE Healthcare) was added per well of a 96-well plate and incubated at 37 °C with shaking for either 2 h (SGLT1 assay) or 1.5 h (SGLT2 assay). Cells were washed twice with 150 μL of wash buffer (137 mM *N*-methylglucamine, 10 mM Tris/Hepes, pH 7.2) and methyl-α-D-[U-^{14}C]glucopyranoside uptake was quantitated using a TopCount scintillation counter (PerkinElmer, Inc.). Inhibitors were assayed at 8 concentrations in triplicates. Sodium-dependent glucopyranoside uptake was calculated by subtracting the values obtained with sodium-free buffer from those obtained using sodium buffer. In general, ratios of sodium-dependent to sodium-independent AMG uptake in SGLT1 and SGLT2 expressing cells were 10–15 and 15–20, respectively. Results of AMG uptake were analyzed using GraphPad Prism (Intuitive Software for Science). IC_{50} calculations were performed using nonlinear regression with variable slope. As a reference standard, a derivative of dapagliflozin was routinely included in the assays. In 26 independent evaluations, the reference compound inhibited SGLT2 activity by 69.7 ± 9.6% and SGLT1 by 72.7 ± 6.7% at 10 nM and 10 μM, respectively.