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Drug Design

Design, Syntheses, and SAR Studies of Carbocyclic Analogues of Sergliflozin as Potent Sodium-Dependent Glucose Cotransporter 2 Inhibitors**

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Type 2 diabetes mellitus (T2DM) or noninsulin-dependent DM, is the most common type of diabetes.[1] The number of patients suffering from T2DM worldwide is expected to rise to 380 million by 2025.[2] Unfortunately, current therapeutic agents are not effective enough such that only less than 36% of the patients have been treated satisfactorily;[3] in addition many side effects are associated with the medicine.[4] Thus, we set out to develop novel small-molecule carbohydrate mimics as potential antidiabetic agents to supplement the existing medication. Our endeavors in the synthesis of carbohydrate mimics have already produced potent α-glucosidase inhibitors as potential antidiabetic molecules.[5,6] We herein report the design, syntheses, and structure–activity relationship (SAR) studies of another class of potential antidiabetic agents—transporter protein inhibitors.

Sodium-dependent glucose cotransporter 2 (SGLT2), found mainly in the proximal tubule of kidneys, is responsible for about 90% of renal glucose reabsorption.[7] On the other hand, sodium glucose cotransporter 1 (SGLT1) is located not only in kidneys, but also in small intestines and other tissues.[8] Inhibition of SGLT2 can help reduce blood glucose level in patients by promoting urinary glucose excretion,[9] whereas inhibition of SGLT1 may lead to delayed absorption of carbohydrates[9] or diarrhea.[10] Selective inhibition of SGLT2 over SGLT1 is thus highly desirable and SGLT2 has emerged as a promising tool to combat type 2 diabetes.[9–11] Research towards SGLT2 inhibition was initially prompted by the SAR studies[12] of phlorizin (**1**), an aryl β-D-glucopyranoside and a nonselective SGLT inhibitor.[13] Synthetic aryl *O*-glucosides such as Sergliflozin (**2a**) and Remogliflozin (**3a**) (Figure 1)

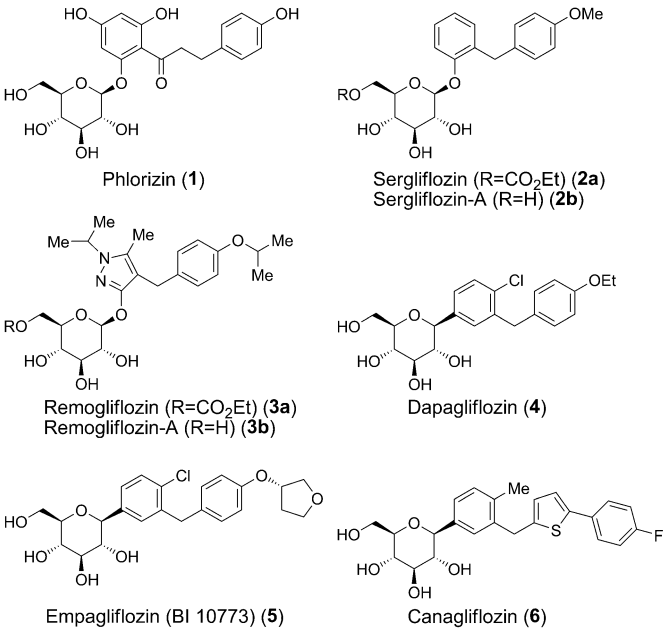

Figure 1. Examples of some SGLT2 inhibitors.

had been developed but were later abandoned during clinical trials due to their poor selectivity/efficacy and metabolic instability.[14] In recent years, the corresponding metabolically stable *C*-glucosides have received great attention.[11] Out of this class of compounds, Dapagliflozin (**4**),[14] Empagliflozin (**5**),[15] and Canaglifozin (**6**)[16] are the three most promising synthetic therapeutic candidates.[10]

However, results from recent clinical trials indicated that none of these SGLT2 inhibitors could inhibit more than 50% of renal glucose reabsorption in humans.[17] Moreover, an increase in cancer risks was reported during the late development phase of the *C*-glucoside **4**.[9b] Thus, the quest for safer SGLT2 inhibitors with increased effectiveness and metabolic stability continues.

Recent research works on SGLT2 inhibitors focused mainly on the modifications of the aglycone unit of *C*-glucoside analogues.[9–11,15–19] Departing from this trend, we envisioned that the metabolic instability of the *O*-glucoside SGLT2 inhibitors can also be greatly improved by modifying the sugar core. In this project, SARs on the sugar core of Sergliflozin-A (**2b**) was performed in view of its high SGLT2 inhibitory potency and selectivity.[20]

The metabolic instability of **2b** was attributed to its acid- and glucosidase-labile glycosidic bond.[21] To address this shortcoming, its endocyclic oxygen atom would be replaced

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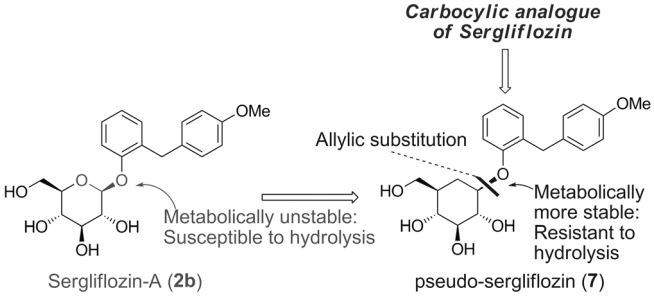
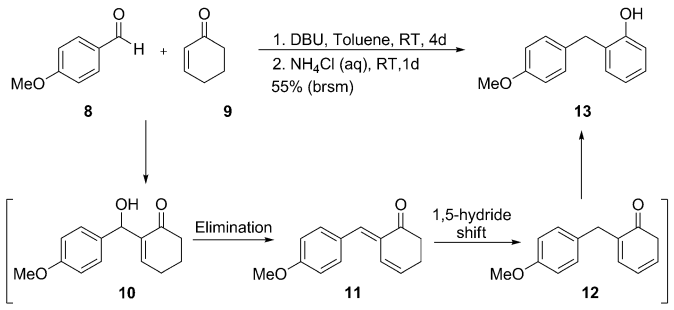
by a methylene unit to render the molecule free from glycosidase degradation, and hence it should be able to exert a more long-lasting blood-glucose-lowering effect (Scheme 1).



Scheme 1. Design of a carbocyclic SGLT2 inhibitor, pseudo-sergliflozin (**7**).

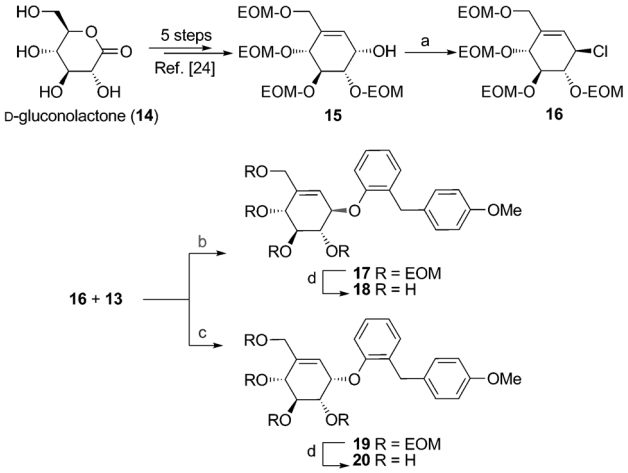
We present herein the syntheses and SARs of seven small-molecule carbohydrate mimics, including a metabolically stable, potent, and selective SGLT2 inhibitor referred to herein as pseudo-sergliflozin (**7**).[22] The present synthetic approach features a stereodivergent transition-metal-catalyzed allylic substitution protocol, in which the unexpected stereochemical outcome of a ruthenium-catalyzed allylic substitution reaction is particularly noteworthy.

To perform the key allylic substitution reaction, the aglycone of **7** and a carba-sugar core were prepared as the coupling precursors. The preparation of aglycone **13** was achieved by a novel one-pot reaction sequence starting with a Baylis–Hillman reaction between *p*-methoxybenzaldehyde (**8**) and cyclohexenone (**9**) (Scheme 2). The Baylis–Hillman adduct **10** was obtained by employing DBU as the base. Subsequent addition of saturated ammonium chloride solution promoted the elimination of a water molecule from the allylic alcohol **10** and gave the diene intermediate **11**. A plausible reaction mechanism is that a 1,5-hydride shift occurred and resulted in the formation of intermediate **12**, which rapidly tautomerized under acidic conditions to give the *ortho*-substituted phenol **13**.[23]



Scheme 2. One-pot synthesis of aglycone of pseudo-sergliflozin (**7**). DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, brsm = based on recovered starting material.

The carba-sugar core of **7** was effectively constructed from inexpensive δ-D-gluconolactone (**14**). On the basis of our recent synthetic endeavors,[24] allylic alcohol **15** was obtained in just five steps from **14** (Scheme 3). It was then mesylated and immediately displaced with chloride anion to give allylic chloride **16** in 84% overall yield.



Scheme 3. Synthesis of the carba-sugar core and allylic substitution reaction between β-allylic chloride **16** and phenol **13**. a) MsCl, Et_3N, 3 Å MS, nBu_4NCl, CH_2Cl_2, 81%; b) $[Pd(dba)_2]$, dppe, K_2CO_3, CH_3CN, RT, 94%; c) $[RuCl_2(cod)]_n$, dppf, K_2CO_3, CH_3CN, 60°C, 72%; d) TFA, H_2O, RT, **18**: 85%; **20**: 84%. cod = 1,5-cyclooctadiene, dba = dibenzylideneacetone, dppe = 1,2-bis(diphenylphosphino)ethane, dppf = 1,1′-bis(diphenylphosphino)ferrocene, EOM = ethoxymethyl, Ms = methanesulfonyl, TFA = trifluoroacetic acid.

With the coupling precursors β-allylic chloride **16** and *ortho*-substituted phenol **13** in hand, allylic substitution reactions were thoroughly studied. This seemingly simple transformation proved very challenging. The use of either monodentate phosphine ligands or bidentate phosphine ligands with a large bite angle led to the formation of a significant amount of diene side products, presumably due to the steric bulk of both the nucleophile and β-elimination-prone electrophile. The difficulty of coupling an *ortho*-substituted phenol and a sterically hindered cyclic allylic electrophile was also reported previously by Trost et al.[25] After extensive screening, we found that dppe, a bidentate phosphine ligand with a small bite angle,[26] effectively suppressed the formation of the diene side products. Thus, the combination of $[Pd(dba)_2]$ and dppe gave β-allylic ether **17** in 94% yield with excellent regio- and stereoselectivities (Scheme 3).

Extensive screening of the experimental conditions also resulted in the discovery of a novel ruthenium-catalyzed allylic substitution reaction. The versatile polymeric ruthenium(II) complex $[RuCl_2(cod)]_n$ catalyzed the reaction regio- and stereospecifically with an overall inversion of configuration at C-1, yielding α-allylic ether **19**. The stereochemical outcome was completely unexpected since an overall retention of configuration at C-1 had been anticipated.[27,28] It is the first ruthenium-catalyzed allylic substitution reaction with

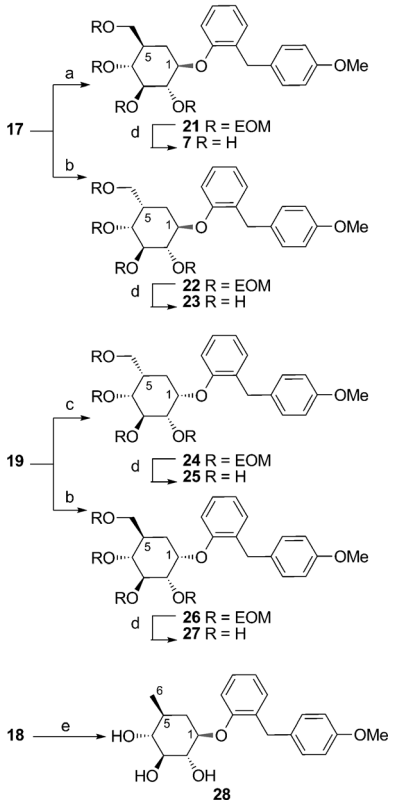
simultaneous inversion of the configuration of a chiral allylic electrophile.[28] This reaction would supplement its corresponding palladium-catalyzed substitution reaction, providing facile access to different diastereomeric compounds during natural product syntheses and drug discovery processes. Research towards the scope and generality of this novel transformation is underway.

Subsequently, the EOM ether protecting groups in allylic ether **17** and **19** were hydrolyzed under acidic conditions. The corresponding tetraol **18** and **20** were obtained as the first two carbocyclic analogues of Sergliflozin. We speculated that the distorted-chair conformation of **18** and **20** could provide us a better understanding of the SAR of this class of SGLT2 inhibitors. The configuration at C-1 was unambiguously confirmed by 1D ^{1}H NMR and 2D ^{1}H–^{1}H COSY experiments.

With allylic ether **17** and **19** in hand, reduction of their alkene moiety was carried out using different hydrogenation methods to yield four diastereoisomeric carbocyclic analogues of Sergliflozin (Scheme 4). Hydrogenation of **17** catalyzed by Pearlman's catalyst[29] followed by acid hydrolysis provided pseudo-sergliflozin (**7**) in 66% overall yield for two steps. Diimide reduction[30] and subsequent acid hydrolysis furnished a mixture of C-5 epimeric tetraols, **7** and **23**, in nearly 1:1 ratio. Similarly, Raney-Nickel catalyzed hydrogenation of **19** followed by global deprotection gave tetraol **25**, whereas diimide reduction stereospecifically gave α-D-glucose-configured cyclohexane **26**. Upon deprotection, tetraol **27** was obtained in 74% overall yield.

Under the action of Pearlman's catalyst, hydrogenolysis followed by hydrogenation of allylic tetraol **18** occurred and yielded the C-6 deoxygenated analogue **28**. The absence of a primary hydroxy group in **28** made it an important candidate for subsequent SAR studies, owing to the replacement of a hydrogen-bond donor with a hydrophobic methyl group.

Thus, seven novel carbocyclic analogues of sergliflozin with diverse conformations were synthesized in a convergent and stereodivergent approach. The stereochemistry of the analogues was verified by 1D ^{1}H NMR and 2D ^{1}H–^{1}H COSY experiments. The structure of **7** was further confirmed by X-ray crystallographic analysis (Figure 2).[31]



Scheme 4. Syntheses of diastereoisomeric carbocyclic analogues of Sergliflozin. a) $Pd(OH)_2/C$, EtOH, H_2, room temperature; b) *p*-$TsNHNH_2$, NaOAc, $THF/H_2O = 1:1$, reflux; c) Raney Ni, H_2O/1,4-dioxane = 1:1, H_2, RT; d) HCl, H_2O, EtOH, 50 °C, **7**: 66% from **17**; **23**: 47% from **17**; **25**: 87% from **19**; **27**: 74% from **19**; e) $Pd(OH)_2/C$, EtOH, RT, 58%. Ts = toluenesulfonyl.

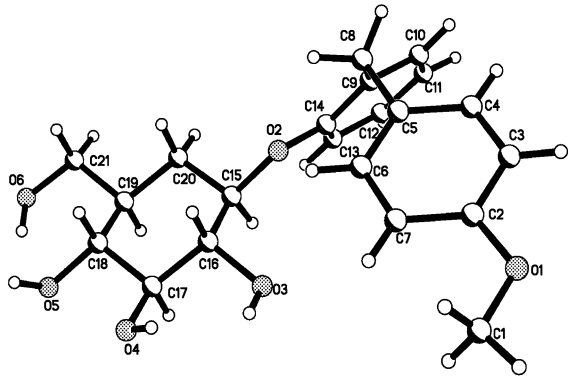


Figure 2. X-ray crystallographic structure of pseudo-sergliflozin (**7**).

In vitro ^{14}C-α-methyl-D-glucopyranoside (^{14}C-AMG) uptake assay was used to determine the SGLT2/SGLT1 inhibitory activities of these newly synthesized compounds. The results are summarized in Figure 3. Pseudo-sergliflozin (**7**) was found to be a very potent and selective SGLT2 inhibitor. It demonstrated an IC_{50} value of 2.45 nM and an over 200000-fold SGLT2/SGLT1 selectivity. It is noteworthy that **7** was seven times more potent than the reference compound Dapagliflozin (**4**), which is one of the most potent SGLT2 inhibitors to date.[15] Thus, the combination of high metabolic stability,[22] potency, and selectivity makes **7** an excellent candidate for further development into an antidiabetic agent.

The SARs on the sugarlike core of other carbocyclic Sergliflozin analogues were further explored. The C-6 hydroxy group was found to be very important for SGLT2/SGLT1 selectivity as this ratio dropped dramatically from over 200000-fold to about 129-fold in the C-6 deoxygenated analogue **28**. The β-stereogenic centers at both C-5 and C-1 were essential for low-nanomolar SGLT2 inhibition as indicated by the significant loss of SGLT2 inhibitory activity in **23**, **25**, and **27**. Allylic analogues **18** and **20** did not show any nanomolar-range inhibition towards either SGLT2 or SGLT1, hinting that a subtle change of the chair conformation of the sugarlike core can lead to a complete loss in inhibitory activity in this class of compounds.

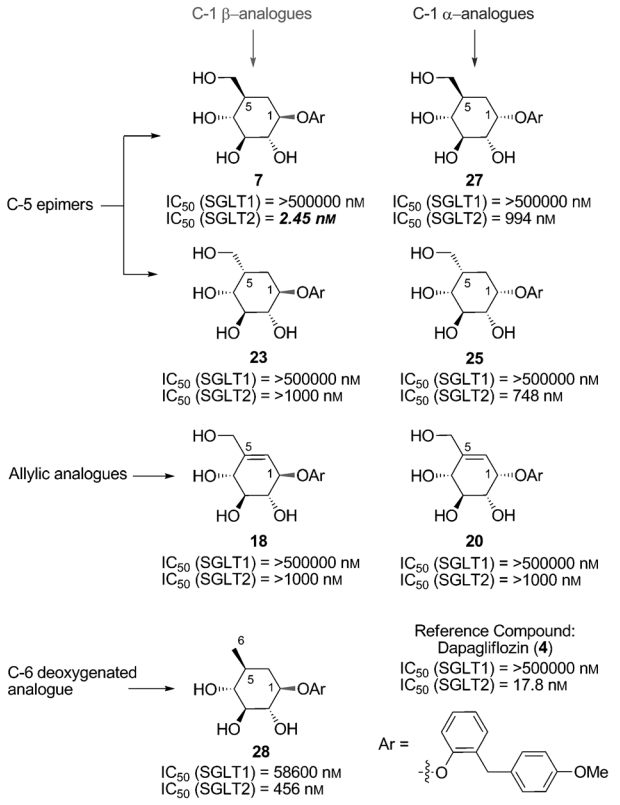


Figure 3. Structures and IC_{50} values of carbocyclic analogues of Sergliflozin.

In conclusion, we have synthesized and evaluated seven small-molecule carbohydrate mimics as SGLT2 inhibitors. The synthetic route towards the most potent compound, pseudo-sergliflozin (**7**), featured a regio- and stereoselective allylic substitution reaction in which the formation of elimination side products was minimized. This short and facile synthetic route, involving only nine steps from δ-D-gluconolactone in 24% overall yield, should facilitate further preclinical trials of the highly selective and low-nanomolar SGLT2 inhibitor **7**.

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[31] CCDC 930197 (**7**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

