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Original article

Synthesis and biological evaluation of novel *C*-indolylxylosides as sodium-dependent glucose co-transporter 2 inhibitors

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ABSTRACT

Sodium-dependent glucose co-transporter 2 (SGLT2) inhibitors are the current focus on the indication for the management of hyperglycemia in diabetes. Here, a novel series of C-linked indolylxyloside-based inhibitors of SGLT2 has been discovered. Structure–activity relationship studies revealed that substituents at the 7-position of the indole moiety and a *p*-cyclopropylphenyl group in the distal position were necessary for optimum inhibitory activity. The pharmacokinetic study demonstrates that the most potent compound **1i** is metabolically stable with a low clearance in rats. In further efficacy study, **1i** is found to significantly lower blood glucose levels of streptozotocin (STZ)-induced diabetic rats.

1. Introduction

The therapeutically beneficial effects of sodium-dependent glucose co-transporter 2 (SGLT2) inhibitors for the treatment of type 2 diabetes mellitus (T2DM) patients has attracted the attention of many researchers in recent years. SGLT2, located on the S1 segment of the proximal tubule in the kidney, mediates the reuptake of the majority (~90%) of glucose filtered by the kidney glomeruli [1,2]. Uptake of the remainder is accomplished by SGLT1, which is mainly expressed in the small intestine but also presents in the S2/S3 segment of the proximal tubule. Accordingly, inhibition of SGLT is considered a feasible approach for the management of hyperglycemia by increasing the urinary glucose excretion and consequently lowering the blood glucose concentration. To avoid the gastrointestinal side effects associated with suppression of SGLT1, inhibitors selective for SGLT2 are most highly desired [3–5].

A number of SGLT2 inhibitors have been developed and submitted to clinical trials [6]. The reported glycoside SGLT2 inhibitors at advanced stages of development are *C*-glucosides, which are metabolically stable and combine higher oral bioavailability and plasma exposure [7,8]. The inherent structural stability of the C-linked glycosidic bond helps ensure these molecules are not degraded by glucosidase enzymes present in the gastrointestinal tract, and obviates any need to derivatize them as prodrugs, which usually require a higher dosage if they are to reach the therapy target.

In the course of our recent investigations into the replacement of glucose with D-xylose coupled with 3-substituted indoles, the resulting *N*-indolylxylosides were found to behave as potent SGLT2 inhibitors (Fig. 1) [9]. A series of *C*-indolylglucosides has also been disclosed as SGLT2 inhibitors with good inhibitory activities [10]. These results suggested to us that the use of C-linked indolylxylosides **1** to inhibit SGLT2 would be feasible. Herein, we describe the synthesis of a novel series of metabolically stable *C*-indolylxylosides and disclose their inhibitory activities against SGLT2 and SGLT1.

2. Results and discussion

2.1. Chemistry

An array of *C*-indolylxylosides **1a**–**1k** was prepared using a five-step synthetic sequence, starting with the coupling of fully protected xylonolactone **2** [11] with a variety of 3-bromo-1-tosyl-1*H*-indoles **3**, Scheme 1. The novel *C*-xylosides **1a**–**1k** used in this work were synthesized according to the well-established chemistry of Kissei, with appropriate modifications [10]. Commercially available

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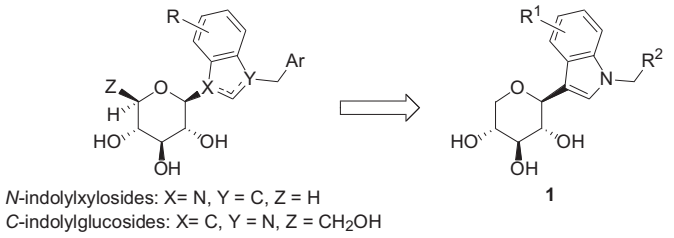


Fig. 1. Design of *C*-indolylxyloside SGLT2 inhibitors.

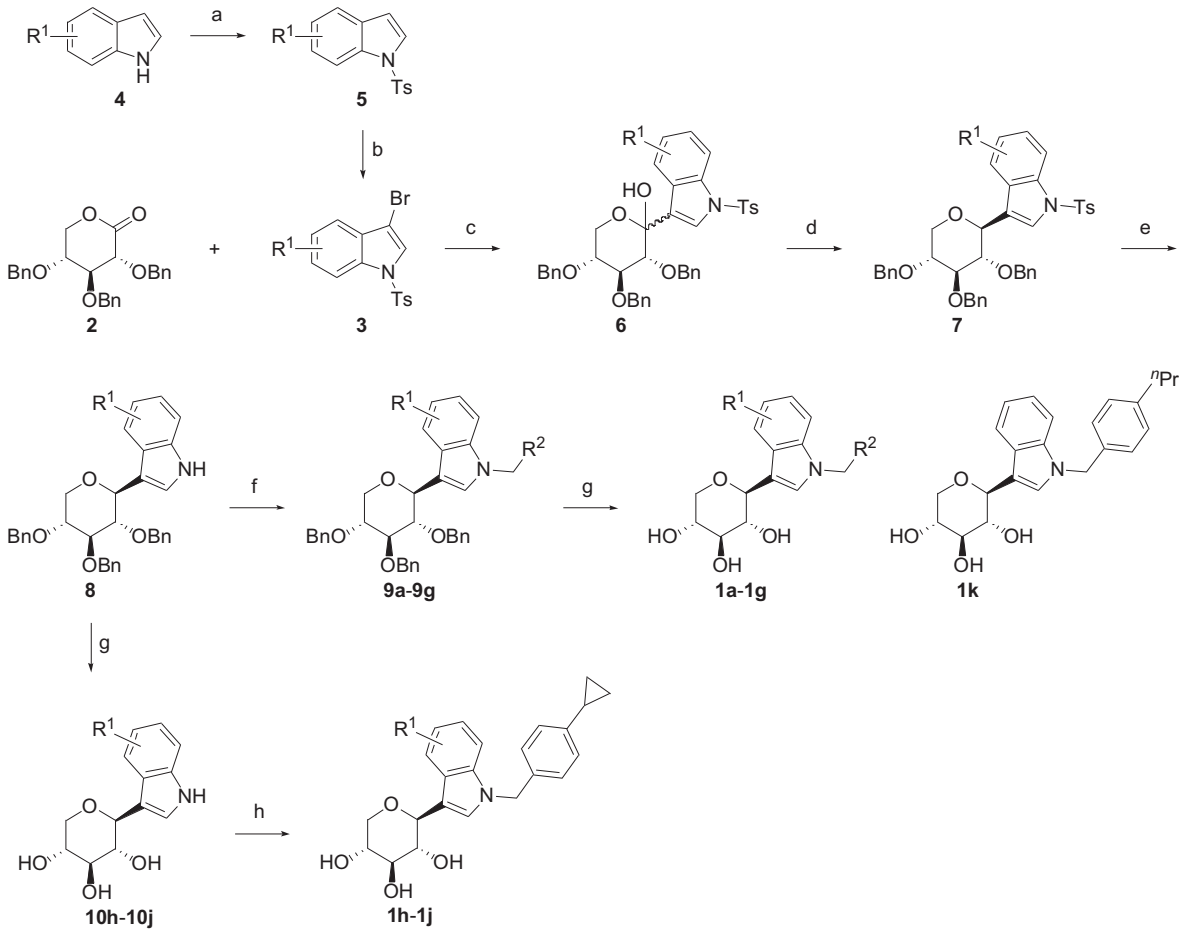
indoles **4** were first subjected to *N*-tosylation with *p*-toluenesulfonyl chloride (*p*TsCl) and sodium hydride (NaH) to afford *N*-tosyl indoles **5**, which were then treated with bromine in dichloromethane at 0 °C for 1 h to provide the coupling precursors 3-bromo-1-tosyl-1*H*-indoles **3**. Lithium-halogen exchange followed by the addition of 2,3,4-tri-*O*-benzyl-D-xylonolactone **2** yielded a mixture of lactols **6**, which were reduced with triethylsilane (Et_3SiH) and boron trifluoride etherate ($BF_3 \cdot OEt_2$) to give the C-linked β-xylosides **7**. Efficient indole *N*-detosylation using potassium hydroxide (KOH) in a mixture of THF and EtOH at 60 °C gave the free indoles **8**.

Two routes to the target *C*-indolylxylosides **1a**–**1k** were developed. Alkylation of **8** with benzyl halides or naphthyl bromide gave the corresponding diarylmethanes **9a**–**9g**, which in turn underwent hydrogenolysis of the benzyl ethers over 10% Pd/C in MeOH/THF to provide the desired β-linked *C*-indolylxylosides **1a**–**1g**. Attempts to synthesize compound **1h** using this strategy were unsuccessful; the *n*-propyl product **1k** was obtained after hydrogenolysis instead. Accordingly, an alternative strategy was pursued for compounds bearing a *p*-cyclopropyl group on the distal phenyl ring. First, the benzyl ether groups of tri-benzyl-xylopyranosyl indole were removed under hydrogenolysis to furnish compounds **10h**–**10j**. Then, N-alkylation of **10h**–**10j** with *p*-cyclopropylbenzyl bromide **11** in the presence of cesium carbonate (Cs_2CO_3) provided the desired products **1h**–**1j**.

2.2. Biological evaluations

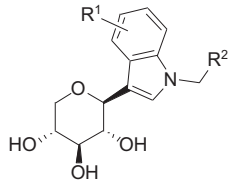
Biological evaluation of these synthesized *C*-indolylxylosides could now take place; the results are presented in Table 1. EC_{50} values were calculated by measuring inhibition of the sodium-dependent uptake of [^{14}C]-labeled α-methyl-D-glucopyranoside (AMG) into Chinese hamster ovary (CHO) cells stably expressing human SGLT2 (hSGLT2) or hSGLT1 [12,13]. Because differences in protein expression levels of the cells will result in differences of EC_{50} values, the SGLT2 inhibitor **12**, dapagliflozin [14], was used as reference compound in this *in vitro* activity evaluation system.

Our previous studies suggested that *N*-indolylxylosides bearing a *para*-substituent at the distal phenyl ring would impart increased inhibitory activity against hSGLT2 [9]. In this study, we began the structure–activity relationship (SAR) studies by investigating the effect of the *para*-substituent at the distal phenyl ring by fixing the

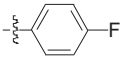
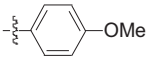
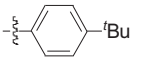
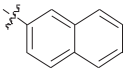
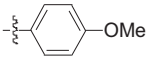
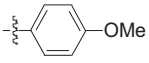
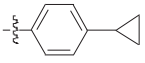
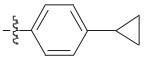
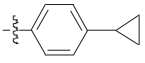
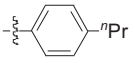


Scheme 1. Reagents and conditions: (a) NaH, *p*TsCl, DMF, rt, 4 h, 67–98%; (b) Br_2, CH_2Cl_2, 0 °C, 1 h, 55–98%; (c) ^{n}BuLi, THF/toluene (2/1), −78 °C → rt, 2.5 h; (d) Et_3SiH, $BF_3 \cdot OEt_2$, CH_3CN, 0 °C → rt, 1 h, 15–54% over two steps; (e) KOH, THF/EtOH (1/2), 60 °C, 20 h, 63–88%; (f) NaH, $RPhCH_2Cl$ or 2-(bromomethyl)naphthalene, DMF, rt, 16 h, 88–100%; (g) 10% Pd/C, $H_{2(g)}$, MeOH/THF (1/1), rt, 3 h, 28–77% (for **1a**–**1g**, **1k**); (h) Cs_2CO_3, 4-cyclopropylbenzyl bromide **11**, DMF, rt, 24 h, 10–51% over 2 steps (for **1h**–**1j** from **8**).

Table 1
Effect of *C*-indolylxylosides **1a**–**1k** on human SGLT inhibitory activity and selectivity.



1a-1k

Compd	R^1	R^2	EC_{50} [nM][a,b] hSGLT2	EC_{50} [nM][a,c] hSGLT1	Selectivity hSGLT1/hSGLT2
1a	H		4153 ± 499	9523 ± 1229	2.3
1b	H		25,505 ± 4726	20,937 ± 9982	0.8
1c	H		736 ± 1636	2491 ± 772	3.4
1d	H		3632 ± 7386	23,063 ± 2662	6.3
1e	H		722 ± 166	4962 ± 354	6.9
1f	4-F		1110 ± 205	6427 ± 2200	5.8
1g	7-F		151 ± 25	550 ± 143	3.6
1h	H		87 ± 19	884 ± 234	10.2
1i	7-F		47 ± 3	282 ± 11	6.0
1j	7-Me		50 ± 11	55 ± 5	1.1
1k	H		588 ± 35	3208 ± 949	5.5
12[d]			2.4 ± 0.6	593 ± 176	247.1
13[d]			205 ± 20	364 ± 152	1.8

[a] These data were obtained by at least of two independent experiments, each experiment performed in triplicates.
[b] Inhibition of uptake of [^{14}C]-AMG in CHO-K1 cells stably transfected with human SGLT2.
[c] Inhibition of uptake of [^{14}C]-AMG in CHO-K1 cells stably transfected with human SGLT1.
[d] Dapagliflozin (**12**); 4-chloro-3-(4-cyclopropylbenzyl)-1-(β-D-xylopyranosyl)-1*H*-indole (**13**).

indole moiety. The unsubstituted phenyl compound **1a** (EC_{50} = 4153 nM) was used as template, to which all structural modifications were compared. When the electron-withdrawing fluoro group was introduced into the *para*-position, the resulting compound **1b** showed a drastic loss of activity (EC_{50} = 25505 nM). Replacing the fluoro group with an electron-donating methoxy group gave xyloside **1c** (EC_{50} = 736 nM), which was a significantly more potent inhibitor than **1a**. Regarding weakly electron-donating substituents, the *n*-propyl analogue **1k** showed slightly improved potency (EC_{50} = 588 nM) compared to **1c**, and xyloside **1d** bearing a bulky *t*-butyl group (EC_{50} = 3632 nM) was found to impart similar activity to the unsubstituted phenyl analogue **1a**. It is noteworthy that when the *p*-cyclopropyl substituent was introduced, the potency of the resulting molecule **1h** against hSGLT2 (EC_{50} = 87 nM) improved 48-fold compared with that of the unsubstituted compound **1a**. Replacement of the phenyl group with a benzofused ring, naphthalene gave compound **1e** with an EC_{50} value of 722 nM; an inferior inhibitor of hSGLT2 than **1h**, and approximately as potent as **1c**.

Next, we turned our attention to the effect of the indole moiety. Previous studies suggested that 4-substituted *N*-indolyl glycosides and *C*-indolylglucosides bearing a 7-substituted or 5,7-disubstituted indole moiety would possess good hSGLT2 inhibition [9,10], and therefore the effect of the substituents incorporated onto the 4- or 7-position of indole was explored. Substitution of **1c** with the fluoro group gave compounds **1f** (4-fluoro) and **1g** (7-fluoro); the former was found to impart an EC_{50} of 1110 nM, about 7 times less potent than the latter (EC_{50} = 151 nM), in which the distal position was occupied with *p*-methoxyphenyl ring. Incorporating a *p*-cyclopropyl phenyl group gave **1i** (EC_{50} = 47 nM), the inhibitory activity of which was significantly enhanced relative to the parent compounds **1h** and **1g**. Similar activity was observed when the electron-donating methyl group (**1j**, EC_{50} = 50 nM) was attached in place of the fluoro group.

The selectivity of hSGLT2 over hSGLT1 is the other important consideration. Unfortunately, none of the *C*-indolylxyloside SGLT2 inhibitors tested showed significant specificity toward hSGLT1 (hSGLT1/hSGLT2 = 0.8–10.2), as shown in Table 1. Although suppressing the reuptake of hSGLT1 may be implicated in gastrointestinal side effects, our previous *in vivo* animal study showed that mice treated with 4-chloro-3-(4-cyclopropylbenzyl)-1-(β-D-xylopyranosyl)-1*H*-indole **13** (hSGLT1/hSGLT2 = 1.8) were not observed to have diarrhea within 8 h of oral administration of a single dose at a range of 10, 50, and 200 mg/kg [9]. Therefore, compounds with good potency against hSGLT2 need to be subjected to a diarrheogenic activity test, to determine whether any adverse effects are associated with the lack of selectivity, and the results used to guide their future development.

In comparison with our previously reported *N*-indolylxylosides [9], *C*-indolylxylosides were found to be superior inhibitors against hSGLT2. Of *N*-indolylxylosides, the most potent compound **13**, exhibiting an EC_{50} value of 205 nM, showed a 4.4-fold loss in potency compared to *C*-indolylxyloside **1i** (EC_{50} = 47 nM). SARs studies indicated that the *p*-cyclopropylphenyl group in the distal position was necessary for optimum inhibitory activity against hSGLT2 in these two series of compounds. And the 7-substituents instead of the 4-substituents, the essential element for potent *N*-xylosides, of the indole moiety are required for C-linked glycosides. Nevertheless, no matter the combination of D-xyloside with 1- or 3-substituted indoles, the resulting compounds showed no significant selectivity for hSGLT2 *versus* hSGLT1.

2.3. Pharmacokinetic profile of *1i*

Among the aforementioned *C*-indolylxylosides, the most potent SGLT2 inhibitor **1i** was chosen for further pharmacokinetic study.

Administration of a single 1 mg/kg intravenous dose to rats revealed that **1i** has the low total body clearance, suggesting that **1i** is metabolically stable (Table 2). After oral administration of a 1 mg/kg dose of **1i** to rats, the C_{max} and T_{max} of **1i** were 161.7 ng/mL and 0.8 h, respectively. The elimination half-life ($t_{1/2}$) of **1i** was 5.2 h. The results indicated that **1i** has favorable pharmacokinetic property in rats.

2.4. Antihyperglycemic effect of *1i*

As shown in Fig. 2, the antihyperglycemic effect of **1i** was assessed in streptozotocin (STZ) induced (STZ at 65 mg/kg, ip) diabetic Sprague–Dawley (SD) rats (blood glucose of >400 mg/dL). Blood samples were obtained from the tail vein at 0 (predose), 0.5, 1, 2, 3, 4, and 5 h after a single oral administration of **1i** (20 mg/kg), **12** (20 mg/kg), or vehicle for blood glucose analysis. *C*-indolylxyloside **1i** at 20 mg/kg was found to cause a 37% reduction in blood glucose level compared with the control.

3. Conclusion

In conclusion, a novel class of *C*-indolylxylosides found to have promising biological activity against hSGLT2 has been designed and synthesized. SAR studies indicated that 7-substituted indolylxlyosides bearing a distal *p*-cyclopropylphenyl group, such as **1i** and **1j**, exhibited the best SGLT2 inhibition. Further pharmacokinetic and animal studies demonstrated that **1i** was metabolically stable along with significant efficacy on lowering blood glucose levels of streptozotocin (STZ)-induced diabetic rats, and therefore hold promise as candidates for the treatment of T2DM – provided that the gastrointestinal side effects associated with the suppression of SGLT1 are not observed in *in vivo* studies.

4. Experimental section

4.1. Chemistry

All chemicals were purchased as reagent grade and used without further purification unless otherwise stated. Column chromatography was performed with silica gel (Merck Kieselgel 60, 230–400 mesh). All reactions were monitored with thin-layer chromatography (TLC) using Merck 60 F254 silica gel glass-backed plates. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury-300 or Mercury-400 spectrometers. Chemical shifts are reported in parts per million (ppm, δ) relative to the internal standard signal of CD_3OD. LC/MS data was obtained on an Agilent MSD-1100 ESI-MS/MS system. Purity of the final compounds was determined on a Hitachi 2000 series HPLC system with a reverse phase C_{18} column (Agilent ZORBAX Eclipse XDB-C18 5 μm, 4.6 mm × 150 mm, 0.5 mL/min flow rate). Mobile phase A: acetonitrile; mobile phase B: 10 mM NH_4OAc aqueous solution containing 0.1% formic acid. The gradient system started from A:B (10%:90%) at 0 min to A:B (90%:10%) at 45 min.

Table 2
Pharmacokinetic profile of *C*-xyloside **1i** in rats.

Parameter	Unit	IV	PO
Dose	mg/kg	1.0	1.0
$t_{1/2}$	h	5.9	5.2
Clearance	mL/min/kg	16.8	
Vss	L/kg	5.0	
C_{max}	ng/mL		161.7
T_{max}	h		0.8
$AUC_{(0\text{-inf.})}$	ng/mL*h	930	764

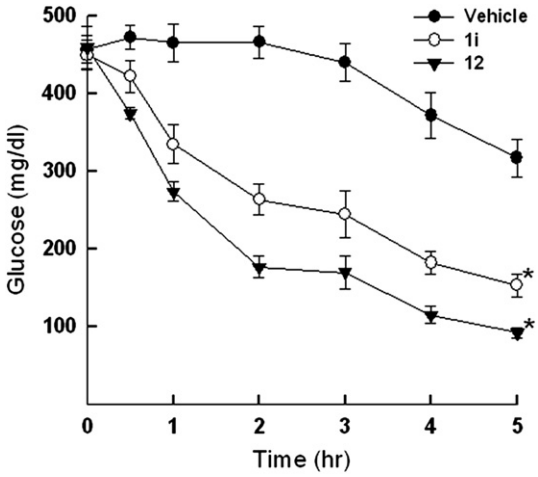


Fig. 2. Antihyperglycemic effect of single oral dosing (20 mg/kg) of **1i** and dapagliflozin **12** in STZ-induced diabetic rats. Data were expressed as the mean ± SEM ($n = 6$/group). Area under the curve of testing agents were significant different from vehicle: $^*P < 0.05$ vs vehicle.

4.1.1. General procedure for the synthesis of compound *5*

NaH (1.1 equiv) was added to a stirred solution of indole (1.0 equiv) in DMF at 0 °C. After being stirring at 0 °C for 10 min, *p*TsCl (1.1 equiv) was added, and the reaction was allowed to stir at room temperature for 4 h. The reaction was quenched by the addition of H_2O at 0 °C and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to afford the desired product **5**.

4.1.2. General procedure for the synthesis of compound *3*

A solution of bromine (1.2 equiv) in CH_2Cl_2 was added to a stirred solution of compound **5** (1.0 equiv) in CH_2Cl_2 at 0 °C under nitrogen. After being stirred at the same temperature for 1 h, the solvent was removed under reduced pressure. The residue was purified by column chromatography to provide the desired bromide **3**.

4.1.3. General procedure for the synthesis of compound *6*

A 1.6 M solution of ^{n}BuLi in hexanes (1.4 equiv) was added to a stirred solution of **3** (1.4 equiv) in THF/toluene (2/1) at −78 °C under nitrogen. After 0.5 h, a solution of fully protected xylonolactone **2** in THF/toluene (2/1) was added to the reaction; the mixture was then warmed to room temperature gradually and stirred for another 2 h. The reaction was poured into saturated aqueous ammonium chloride and the mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to give **6**.

4.1.4. General procedure for the synthesis of compound *7*

Et_3SiH (5.0 equiv) and then $BF_3{\cdot}OEt_2$ (0.5 equiv) were added to a stirred solution of **6** in CH_3CN at 0 °C under argon and then the reaction was warmed to room temperature gradually. After 1 h, the reaction was quenched by the addition of saturated aqueous sodium bicarbonate at 0 °C and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to give **7**.

4.1.5. General procedure for the synthesis of compound *8*

KOH (20 equiv) was added to a stirred solution of **7** (1.0 equiv) in THF/EtOH (1/2) at room temperature. The reaction was warmed to

60 °C and further stirred for 20 h. The reaction was cooled to 0 °C and neutralized by the addition of 1 N HCl(aq). The mixture was extracted with EtOAc and the organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to afford the desired product **8**.

*4.1.6. General procedure for the synthesis of compounds **9a**–**9g***

NaH (1.2 equiv) and aryl bromides or aryl chlorides (1.2 equiv) were sequentially added to a stirred solution of **8** (1.0 equiv) in DMF at 0 °C. The reaction was slowly warmed to room temperature and stirred for 16 h. The reaction was quenched by the addition of H_2O at 0 °C and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to afford the desired products **9a**–**9g**.

*4.1.7. General procedure for the synthesis of compounds **1a**–**1g***

A mixture of **9a**–**9g** and 10% Pd/C in MeOH/THF (1/1) was stirred at room temperature under 1 atm $H_{2(g)}$ for 1 ~ 3 h. The reaction was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was then purified by column chromatography ($MeOH/CH_2Cl_2$) to provide the final products **1a**–**1g**.

*4.1.7.1. 1-Benzyl-3-(β-D-xylopyranosyl)-1H-indole (**1a**).* ^{1}H NMR (400 MHz, CD_3OD): δ 7.71–7.69 (m, 1H), 7.34 (s, 1H), 7.30–7.17 (m, 6H), 7.11 (ddd, *J* = 8.0, 7.2, 1.2 Hz, 1H), 7.04 (ddd, *J* = 8.0, 7.2, 1.2 Hz, 1H), 5.36 (s, 2H), 4.42 (d, *J* = 9.2 Hz, 1H), 3.98 (dd, *J* = 11.2, 5.2 Hz, 1H), 3.75 (t, *J* = 9.2 Hz, 1H), 3.68 (ddd, *J* = 10.4, 9.2, 5.2 Hz, 1H), 3.45 (t, *J* = 9.2 Hz, 1H), 3.38 (dd, *J* = 11.2, 10.4 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 139.5, 138.4, 129.8, 129.1, 128.8, 128.6, 128.2, 123.0, 121.1, 120.5, 114.2, 111.2, 80.2, 79.0, 75.1, 71.8, 71.4, 51.0; ESMS *m*/*z*: 340 (MH^+), 362 (MNa^+); HPLC purity 89.5%.

*4.1.7.2. 1-(4-Fluorobenzyl)-3-(β-D-xylopyranosyl)-1H-indole (**1b**).* ^{1}H NMR (400 MHz, CD_3OD): δ 7.69 (d, *J* = 8.0 Hz, 1H), 7.33 (s, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.21–7.17 (m, 2H), 7.12–7.08 (m, 1H), 7.05–6.97 (m, 3H), 5.34 (s, 2H), 4.41 (d, *J* = 9.2 Hz, 1H), 3.97 (dd, *J* = 10.8, 5.2 Hz, 1H), 3.74 (t, *J* = 9.2 Hz, 1H), 3.67 (ddd, *J* = 10.8, 9.2, 5.2 Hz, 1H), 3.44 (t, *J* = 9.2 Hz, 1H), 3.37 (t, *J* = 10.8 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ 165.3, 162.1, 138.3, 135.6, 135.5, 130.2, 130.1, 129.0, 128.8, 123.0, 121.2, 120.5, 116.6, 116.3, 114.4, 111.2, 80.2, 79.0, 75.1, 71.8, 71.4, 50.2; ESMS *m*/*z*: 358 (MH^+), 380 (MNa^+); HPLC purity 91.5%.

*4.1.7.3. 1-(4-Methoxybenzyl)-3-(β-D-xylopyranosyl)-1H-indole (**1c**).* ^{1}H NMR (400 MHz, CD_3OD): δ 7.67 (d, *J* = 8.0 Hz, 1H), 7.32–7.30 (m, 2H), 7.14–7.08 (m, 3H), 7.04–7.00 (m, 1H), 6.84–6.81 (m, 2H), 5.27 (s, 2H), 4.40 (d, *J* = 9.2 Hz, 1H), 3.96 (dd, *J* = 10.8, 5.2 Hz, 1H), 3.75–3.71 (m, 1H), 3.73 (s, 3H), 3.67 (ddd, *J* = 10.8, 9.2, 5.2 Hz, 1H), 3.44 (t, *J* = 9.2 Hz, 1H), 3.37 (t, *J* = 10.8 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ 160.8, 138.4, 131.4, 129.7, 128.9, 128.8, 122.9, 121.1, 120.4, 115.1, 114.0, 111.3, 80.2, 79.0, 75.1, 71.8, 71.4, 55.8, 50.5; ESMS *m*/*z*: 370 (MH^+), 392 (MNa^+); HPLC purity 99.0%.

*4.1.7.4. 1-(4-tert-Butylbenzyl)-3-(β-D-xylopyranosyl)-1H-indole (**1d**).* ^{1}H NMR (400 MHz, CD_3OD): δ 7.68 (d, *J* = 8.0 Hz, 1H), 7.32–7.30 (m, 4H), 7.12–7.08 (m, 3H), 7.04–7.01 (m, 1H), 5.31 (s, 2H), 4.41 (d, *J* = 9.6 Hz, 1H), 3.96 (dd, *J* = 10.8, 5.2 Hz, 1H), 3.74 (dd, *J* = 9.6, 8.8 Hz, 1H), 3.67 (ddd, *J* = 10.8, 8.8, 5.2 Hz, 1H), 3.44 (t, *J* = 8.8 Hz, 1H), 3.37 (t, *J* = 10.8 Hz, 1H), 1.26 (s, 9H); ^{13}C NMR (75 MHz, CD_3OD): δ 151.7, 138.4, 136.5, 129.0, 128.7, 128.1, 126.7, 122.9, 121.1, 120.4, 114.1, 111.3, 80.2, 79.0, 75.1, 71.8, 71.4, 50.6, 35.5, 31.9; ESMS *m*/*z*: 396 (MH^+), 418 (MNa^+); HPLC purity 95.4%.

*4.1.7.5. 1-(2-Naphthylmethyl)-3-(β-D-xylopyranosyl)-1H-indole (**1e**).* ^{1}H NMR (300 MHz, CD_3OD): δ 7.80–7.70 (m, 4H), 7.61 (s, 1H), 7.45–7.39 (m, 3H), 7.35–7.30 (m, 2H), 7.12–7.01 (m, 2H), 5.51 (s, 2H), 4.43 (d, *J* = 9.3 Hz, 1H), 3.98 (dd, *J* = 11.1, 5.4 Hz, 1H), 3.76 (t, *J* = 9.3 Hz, 1H), 3.68 (ddd, *J* = 10.5, 9.3, 5.4 Hz, 1H), 3.48–3.35 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 138.5, 137.0, 135.0, 134.4, 129.6, 129.2, 129.0, 128.81, 128.80, 127.4, 127.1, 126.8, 126.3, 123.0, 121.2, 120.5, 114.3, 111.3, 80.2, 79.0, 75.1, 71.8, 71.4, 51.1; ESMS *m*/*z*: 390 (MH^+), 412 (MNa^+); HPLC purity 100%.

*4.1.7.6. 4-Fluoro-1-(4-methoxybenzyl)-3-(β-D-xylopyranosyl)-1H-indole (**1f**).* ^{1}H NMR (300 MHz, CD_3OD): δ 7.34 (s, 1H), 7.16–7.01 (m, 4H), 6.85–6.80 (m, 2H), 6.75–6.69 (m, 1H), 5.27 (s, 2H), 4.51 (dd, *J* = 9.6, 1.2 Hz, 1H), 3.93 (dd, *J* = 10.8, 5.1 Hz, 1H), 3.79–3.73 (m, 1H), 3.73 (s, 3H), 3.64 (ddd, *J* = 10.8, 9.0, 5.1 Hz, 1H), 3.43 (t, *J* = 9.0 Hz, 1H), 3.36 (t, *J* = 10.8 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ 160.8, 159.7, 156.5, 141.1, 140.9, 130.8, 129.7, 123.4, 123.3, 117.6, 117.3, 115.2, 112.61, 112.57, 107.72, 107.67, 106.0, 105.7, 80.3, 78.5, 75.2, 75.1, 71.7, 71.2, 55.8, 50.8; ESMS *m*/*z*: 388 (MH^+), 410 (MNa^+); HPLC purity 98.7%.

*4.1.7.7. 7-Fluoro-1-(4-methoxybenzyl)-3-(β-D-xylopyranosyl)-1H-indole (**1g**).* ^{1}H NMR (300 MHz, CD_3OD): δ 7.46 (d, *J* = 7.8 Hz, 1H), 7.30 (s, 1H), 7.11 (d, *J* = 8.7 Hz, 2H), 6.95 (td, *J* = 7.8, 4.5 Hz, 1H), 6.85–6.78 (m, 3H), 5.39 (s, 2H), 4.37 (d, *J* = 9.9 Hz, 1H), 3.96 (dd, *J* = 11.1, 5.1 Hz, 1H), 3.73 (s, 3H), 3.73–3.61 (m, 2H), 3.45–3.32 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ 160.7, 153.2, 150.0, 133.0, 132.9, 132.0, 130.5, 129.5, 125.9, 125.7, 120.8, 120.7, 117.3, 117.2, 115.4, 115.3, 115.1, 108.5, 108.2, 80.1, 78.6, 75.1, 71.7, 71.4, 55.8, 52.7, 52.6; ESMS *m*/*z*: 388 (MH^+), 410 (MNa^+); HPLC purity 99.0%.

*4.1.8. General procedure for the synthesis of compounds **10h**–**10j***

A mixture of **8** and 10% Pd/C in MeOH/THF (1/1) was stirred at room temperature under 1 atm $H_{2(g)}$ for 3 h. The reaction was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was then purified by column chromatography ($MeOH/CH_2Cl_2$) to provide the final products **10h**–**10j**.

*4.1.9. General procedure for the synthesis of compounds **1h**–**1j***

Cs_2CO_3 (5 equiv) was added to a stirred solution of **10h**–**10j** (1.0 equiv) and 4-cyclopropylbenzyl bromide (1.2 equiv) in DMF at room temperature. After 24 h, the reaction was quenched by the addition of H_2O, and the resulting mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to afford the desired products **1h**–**1j**.

*4.1.9.1. 1-(4-Cyclopropylbenzyl)-3-(β-D-xylopyranosyl)-1H-indole (**1h**).* ^{1}H NMR (400 MHz, CD_3OD): δ 7.67 (d, *J* = 8.0 Hz, 1H), 7.30–7.28 (m, 2H), 7.11–6.96 (m, 6H), 5.28 (s, 2H), 4.40 (d, *J* = 9.6 Hz, 1H), 3.96 (dd, *J* = 10.8, 5.2 Hz, 1H), 3.73 (dd, *J* = 9.6, 8.8 Hz, 1H), 3.67 (ddd, *J* = 10.8, 8.8, 5.2 Hz, 1H), 3.43 (t, *J* = 8.8 Hz, 1H), 3.37 (t, *J* = 10.8 Hz, 1H), 1.86–1.82 (m, 1H), 0.93–0.88 (m, 2H), 0.62–0.58 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ 144.9, 138.4, 136.4, 129.0, 128.8, 128.3, 126.9, 122.9, 121.1, 120.4, 114.1, 111.3, 80.2, 79.0, 75.1, 71.8, 71.4, 50.7, 16.0, 9.7; ESMS *m*/*z*: 380 (MH^+), 402 (MNa^+); HPLC purity 96.6%.

*4.1.9.2. 1-(4-Cyclopropylbenzyl)-7-fluoro-3-(β-D-xylopyranosyl)-1H-indole (**1i**).* ^{1}H NMR (400 MHz, CD_3OD): δ 7.46 (d, *J* = 8.0 Hz, 1H), 7.30 (s, 1H), 7.05–6.92 (m, 5H), 6.83–6.78 (m, 1H), 5.41 (s, 2H), 4.37 (d, *J* = 9.6 Hz, 1H), 3.96 (dd, *J* = 10.8, 5.2 Hz, 1H), 3.69–3.62 (m, 2H), 3.42 (t, *J* = 8.8 Hz, 1H), 3.36 (t, *J* = 10.8 Hz, 1H), 1.90–1.80 (m, 1H), 0.94–0.88 (m, 2H), 0.62–0.58 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 152.9, 150.5, 144.9, 137.0, 133.0, 132.9, 130.6, 128.1, 126.9, 120.82, 120.75, 117.3, 117.2, 115.4, 108.5, 108.3, 80.2, 78.7, 75.1, 71.8, 71.4, 53.0, 52.9, 16.0, 9.8; ESMS *m*/*z*: 398 (MH^+), 420 (MNa^+); HPLC purity 100%.

*4.1.9.3. 1-(4-Cyclopropylbenzyl)-7-methyl-3-(β-D-xylopyranosyl)-1H-indole (**1j**).* ^{1}H NMR (400 MHz, CD_3OD): δ 7.54 (d, J = 8.0 Hz, 1H), 7.23 (s, 1H), 6.96–6.89 (m, 3H), 6.81–6.78 (m, 3H), 5.54 (s, 2H), 4.41 (d, J = 9.6 Hz, 1H), 3.97 (dd, J = 10.8, 5.2 Hz, 1H), 3.75 (dd, J = 9.6, 8.8 Hz, 1H), 3.67 (ddd, J = 10.8, 8.8, 5.2 Hz, 1H), 3.44 (t, J = 8.8 Hz, 1H), 3.38 (t, J = 10.8 Hz, 1H), 2.45 (s, 3H), 1.86–1.79 (m, 1H), 0.93–0.85 (m, 2H), 0.61–0.57 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 144.5, 138.6, 137.1, 131.1, 129.9, 127.0, 126.6, 125.8, 122.6, 120.8, 119.1, 113.9, 80.3, 78.8, 75.0, 71.8, 71.4, 53.0, 19.8, 15.9, 9.7; ESMS m/z: 394 (MH^+), 416 (MNa^+); HPLC purity 91.9%.

*4.1.10. 1-(4-propylbenzyl)-3-(β-D-xylopyranosyl)-1H-indole (**1k**)*

NaH (1.2 equiv) and 4-cyclopropylbenzyl bromide (1.2 equiv) were sequentially added to a stirred solution of **8** (1.0 equiv) in DMF at 0 °C. The reaction was slowly warmed to room temperature and stirred for 16 h. The reaction was quenched by the addition of H_2O at 0 °C and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to afford the alkylated intermediate.

A mixture of the alkylated intermediate and 10% Pd/C in MeOH/THF (1/1) was stirred at room temperature under 1 atm $H_{2(g)}$ for 3 h. The reaction was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was then purified by column chromatography ($MeOH/CH_2Cl_2$) to provide the final product **1k**. ^{1}H NMR (400 MHz, CD_3OD): δ 7.69–7.67 (m, 1H), 7.31–7.29 (m, 2H), 7.12–7.00 (m, 6H), 5.31 (s, 2H), 4.41 (d, J = 9.6 Hz, 1H), 3.96 (dd, J = 10.8, 5.2 Hz, 1H), 3.74 (dd, J = 9.6, 9.2 Hz, 1H), 3.67 (ddd, J = 10.8, 9.2, 5.2 Hz, 1H), 3.43 (t, J = 9.2 Hz, 1H), 3.37 (t, J = 10.8 Hz, 1H), 2.52 (t, J = 7.6 Hz, 2H), 1.58 (sext, J = 7.6 Hz, 2H), 0.89 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 143.3, 138.4, 136.7, 129.9, 129.0, 128.8, 128.3, 122.9, 121.1, 120.4, 114.1, 111.3, 80.2, 79.0, 75.1, 71.8, 71.4, 50.8, 38.8, 25.9, 14.2; ESMS m/z: 382 (MH^+), 404 (MNa^+); HPLC purity 90.6%.

*4.1.11. p-Cyclopropylbenzyl bromide (**11**)*

To a stirred solution of *p*-cyclopropylbenzaldehyde [15] (1.14 g, 7.81 mmol) in a mixture of solvents (MeOH/THF = 1/1, 30 mL) was added sodium borohydride (450 mg, 7.81 mmol) at room temperature. After 0.5 h, the solution was concentrated *in vacuo* and the residue redissolved in CH_2Cl_2, and washed with H_2O and $NaCl_{(aq)}$. The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc/Hex = 1/3) to provide *p*-cyclopropylbenzyl alcohol (1.12 g, 97%). Phosphorus tribromide (1.0 mL, 11.3 mmol) was added to a stirred solution of *p*-cyclopropylbenzyl alcohol (1.12 g, 7.56 mmol) in CH_2Cl_2/Et_2O (1/1, 25 mL) at 0 °C under argon. The reaction was warmed up to room temperature gradually and stirred for 1 h. The reaction was quenched by the addition of H_2O and extracted with Et_2O. The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc/Hex = 1/15) to afford *p*-cyclopropylbenzyl bromide (1.19 g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27 (d, J = 8.0 Hz, 2H), 7.03 (d, J = 8.0 Hz, 2H), 4.82 (s, 2H), 1.84–1.93 (m, 1H), 0.89–1.00 (m, 2H), 0.67–0.72 (m, 2H).

4.2. In vitro human SGLT inhibition assays [9,12,13]

Stably transfected CHO-K1 cells were used for transporter studies. SGLT was determined by uptake of [^{14}C]-α-methyl-D-glucopyranoside ([^{14}C]-AMG, specific radioactivity 310 mCi/mmol) purchased from Perkin Elmer (Boston, USA). For the purpose of this study, Krebs-Ringer-Henseleit (KRH) solution containing 120 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2$, 2.2 mM $CaCl_2$, and 10 mM Hepes (pH 7.4 with Tris) was used for SGLT uptake assays. All chemicals were purchased from Sigma (Deisenhofen, Germany). Briefly, hSGLT2/CHO-K1 cells or hSGLT1/CHO-K1 cells were seeded into a white-walled 96-well culture plate (Corning, NY, USA) at a density of 30,000 cells/well (hSGLT2) or 20,000 cells/well (hSGLT1) and incubated for 48 h at 37 °C in a 5% CO_2 atmosphere in growth medium. After 48 h, the culture medium in the wells was removed and wells were washed three times with 280 μL of KRH solution and then incubated in KRH solution containing 3 uM [^{14}C]-AMG in the absence or presence of inhibitors for up to 120 min at 37 °C. At the end of the uptake period, the KRH solution was removed and the uptake of [^{14}C]-AMG was stopped by adding ice-cold KRH solution (stop solution). The wells were rinsed three times with 150 μL stop buffer using the microplate washer (TEcan, Männedorf, Switzerland). After the third rinse, the stop solution was completely removed from the wells and the cells were solubilized by adding 0.1% sodium dodecyl sulfate (Sigma). After 24 h, the microtiter plate was taken for scintillation counting of radioactive [^{14}C]-AMG using a TopCount (Perkin Elmer). The percent of inhibition of inhibitors was calculated by comparing count per minute (CPM) in inhibitor-containing well with CPM in wells containing only DMSO vehicle. Dapagliflozin was evaluated in parallel in every assay. A dose–response curve was fitted to a sigmoidal dose–response model using GraphPad software to determine the inhibitor concentration at half-maximal response (EC_{50}).

4.3. In vivo pharmacokinetics evaluation of **1i** in rats [9]

The animal study was approved by Institutional Animal Care and Use Committee of National Health Research Institutes. A solution of test compound (1 mg/mL) was prepared by dissolving appropriate amount compound in a mixture of PEG 400/DMA (80:20, v/v). Male Sprague–Dawley rats, weighing 250–350 g each (8–10 weeks old), were obtained from BioLASCO, Ilan, Taiwan. **1i** (powder form obtained from column purification) was administered to three male rats each intravenously by a bolus injection to the jugular vein or orally at 1 mg/kg dose. At 0 (prior to dosing), 2, 5 (IV only), 15, and 30 min and at 1, 2, 4, 6, 8 and 24 h after dosing, a blood sample (~150 μL) was collected from each animal via the jugular-vein cannula and stored in ice (0–4 °C). Plasma was separated from the blood by centrifugation (14,000 × *g* for 15 min at 4 °C in a Beckman Model AllegraTM 6R centrifuge) and stored in a freezer (−80 °C). All samples were analyzed for the test compound by LC-MS/MS (ABI 4000Q Trap). Plasma concentration data were analyzed with standard non-compartmental method.

4.4. Blood glucose lowering effect of **1i** in STZ-Induced diabetic rats [9,14a]

Adult male Sprague–Dawley rats (BioLasco, Ilan, Taiwan) received three intraperitoneal injections of streptozotocin from Sigma (catalog no. S0130, St. Louis, MO) at 65 mg/kg freshly prepared in 0.01 M citrate buffer at one injection every other day. The animals were then monitored by using blood glucometer (ACCU-CHEK from Roche, Basel, Switzerland), for the glucose levels in blood collected via stabbing the tail vein with 25G needle once a week. A streptozotocin-induced diabetes was confirmed when the blood glucose levels were up more than 400 mg/dL. The diabetic rats were divided into experimental groups at six rats each and orally administered a single dose of **1i**, **12**, or vehicle as control. Blood samples were obtained from the tail vein at 0 (predose), 0.5, 1, 2, 3, 4, and 5 h after the oral administration and measured for blood glucose levels with the glucometer. A P value of less than 0.05 was considered statistically significant.

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