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C-Glucosides with heteroaryl thiophene as novel sodium-dependent glucose cotransporter 2 inhibitors

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ABSTRACT

Canagliflozin (**1**), a novel inhibitor for sodium-dependent glucose cotransporter 2 (SGLT2), has been developed for the treatment of type 2 diabetes. To investigate the effect of replacement of the phenyl ring in **1** with heteroaromatics, *C*-glucosides **2** were designed, synthesized, and evaluated for their inhibitory activities against SGLT2. Of these, 3-pyridyl, 2-pyrimidyl or 5-membered heteroaryl substituted derivatives showed highly potent inhibitory activity against SGLT2, while 5-pyrimidyl substitution was associated with slightly reduced activity. In particular, **2g** (TA-3404) had remarkable anti-hyperglycemic effects in high-fat diet fed KK (HF-KK) mice.

1. Introduction

The achievement and maintenance of near-normal glycemia reduces the risk of diabetes complications.[1,2] Despite lifestyle and pharmacological interventions, glucose levels increase over time in type 2 diabetes, probably as a consequence of declining pancreatic β-cell function.[3] The progressive nature of type 2 diabetes makes it difficult to maintain good glycemic control with several glucose-lowering agents,[4] and as a result, it requires the gradual dose escalation, the use of combination therapies or insulin.[5] Therefore, there is a need for agents with a newer and complementary mechanism of action which can be used throughout the life of patients with type 2 diabetes. Inhibitors of sodium-glucose cotransporters (SGLTs) is an attractive approach to such need, as their action of reducing blood glucose is independent from insulin.[6] The kidney contributes to glucose homeostasis by reabsorbing approximately 180 g of glucose from the glomerular filtrate each day, and SGLT2 expressed in the early convoluted segment (S1) of the proximal tubule mediates 80–90% of renal glucose reabsorption.[7–9] The remaining 10–20% of renal glucose reabsorption occurs through SGLT1, which is expressed in the more distal, straight section of the proximal tubule (S3).[9] Besides the kidney, SGLT1 is distributed in the intestine, heart, and trachea, while SGLT2 is expressed solely in the kidney.[10] By enhancing glucose excretion into urine, SGLT2 inhibitors should lead to a significant loss of calories. Their potential advantage would be reducing blood glucose and leading to weight loss; thus, they could be used at any stage of type 2 diabetes.[11] In fact, several specific and potent SGLT2 inhibitors have been developed.[12–15]

Canagliflozin (**1**), one of potent SGLT2 inhibitors, is being studied in clinical trials, and a more thorough understanding of its derivatives is required. To explore the structure–activity relationship (SAR) of the aryl substituent at thiophene ring on **1**, we developed synthetic strategies for *C*-glucosides with heteroaryl thiophene **2** and evaluated these compounds on SGLT2 activity and urinary glucose excretion (UGE). Herein, we report the synthesis and the biological result of **2**.

2. Chemistry

We initially planned to synthesize *C*-glucosides **2** bearing a heteroaromatic ring using a previously reported strategy,[12,13] and compound **2a** was prepared as shown in Scheme 1. 2-(3-Pyridyl) thiophene (**3**) was treated with *n*-butyllithium, followed by addition of 5-bromo-2-methylbenzaldehyde to give diarylcarbinol **4**. One of the reducing agents of diarylcarbinols is a combination of sodium tetrahydroborate and trifluoroacetic acid along with

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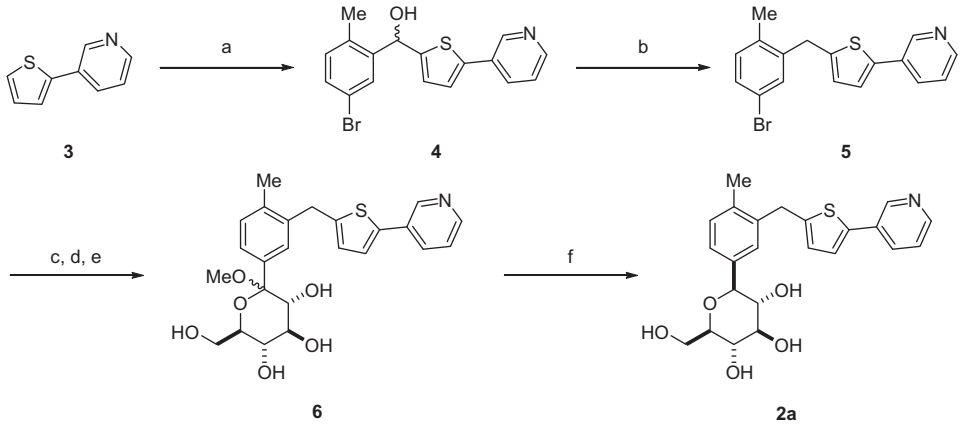
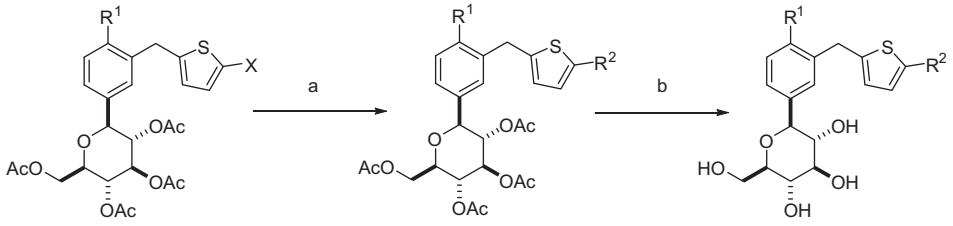


Scheme 1. Synthesis of **2a**. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then 5-bromo-2-methyl-benzaldehyde, −78 °C, 67%; (b) $NaBH(OAc)_3$, TFA, 0 °C to rt, quant.; (c) *n*-BuLi, THF–toluene, −78 °C; (d) 2,3,4,6-tetrakis-*O*-trimethylsilyl-D-gluconolactone, −78 °C; (e) $MeSO_3H$, MeOH, −78 °C to rt, 2.3%; (f) Et_3SiH, $BF_3{\cdot}Et_2O$, $CHCl_3$, 0 °C, 30%.



Scheme 2. Syntheses of **2b**–**2d**, **2f**, **2g**. Reagents and conditions: (a) **7**, $Pd_2(dba)_3$, P(*tert*-Bu)$_3{\cdot}HBF_4$, CsF, 5-tri(*n*-butyl)stannylpyrimidine, dioxane, 100 °C, 41% (for **10b**) or **8**, $PdCl_2(PPh_3)_2$, CuI, 2-tri(*n*-butyl)stannylpyrimidine, NMP, 100 °C, 66% (for **10c**) or **8**, $Pd(PPh_3)_4$, CuI, 5-tri(*n*-butyl)stannylthiazole, dioxane, reflux, 23% (for **10d**) or **9**, $Pd(PPh_3)_4$, CsF, pyridine-3-boronic acid or 6-fluoropyridine-3-boronic acid pinacol ester, DME, reflux, 53–81% (for **10f**, **10g**); (b) NaOMe, MeOH–THF, rt, 55–100%.

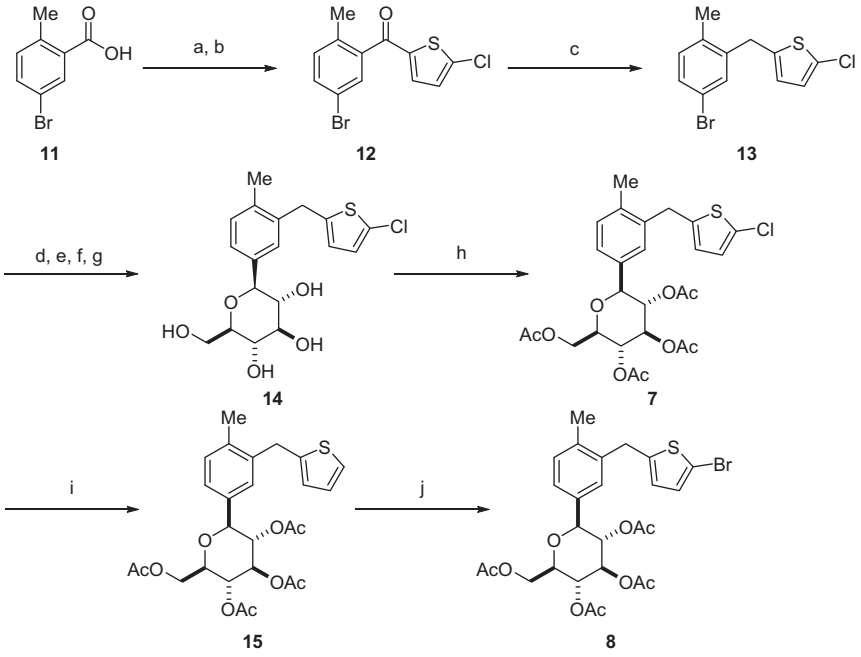
hydrogen gas evolution.[16] To avoid gas evolution, sodium triacetoxyborohydride was used instead of sodium tetrahydroborate. With this combination, compound **4** was rapidly reduced to yield aglycon **5**. Lithium halogen exchange of **5** with *n*-butyllithium followed by addition of 2,3,4,6-tetrakis-*O*-trimethylsilyl-D-gluconolactone[12] generated an anomeric mixture of lactols, which were converted in situ to the desilylated methyl ether **6** by treatment with methanesulfonic acid in methanol. Unfortunately, the lithiation of 3-pyridylthiophene derivative **5** was problematic and this reaction resulted in a low yield (2.3%). Finally, the *C*-glucoside derivative **2a** was obtained by stereoselective reduction of **6** using a combination of triethylsilane and boron trifluoride etherate. The stereochemistry of **2a** was determined as the β-configuration based on the coupling constant between anomeric C–H and adjacent C–H (J = 9.5 Hz) in the ^{1}H NMR spectrum.

To avoid the problematic lithium halogen exchange reaction, we designed new approaches to compounds **2** using palladium catalyzed coupling reactions of *C*-glucosides having halogenothiophene. The coupling reactions of halogenothiophene derivatives **7**, **8** or **9** with the heteroaryl stannanes, boronic acid, or boronate ester proceeded smoothly to give *O*-acetyl protected *C*-glucosides **10** in moderate to good yields as shown in Scheme 2. *C*-glycosides **2b**–**2d**, **2f**, and **2g** were obtained by methanolysis of the corresponding **10b**–**10d**, **10f**, and **10g**.

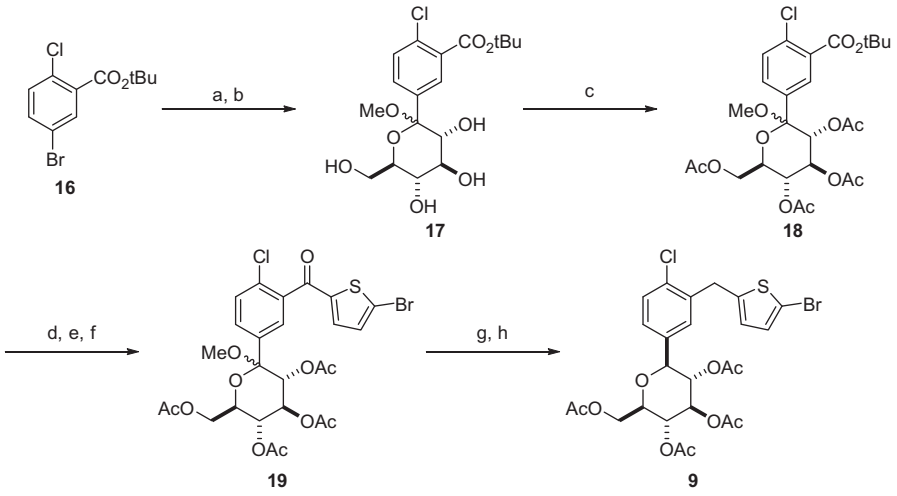
The synthetic route to intermediates **7** and **8** is outlined in Scheme 3. Benzoic acid **11** was treated with oxalyl chloride, followed by addition of 2-chlorothiophene and aluminum trichloride to give ketone **12**. Reduction of **12** with triethylsilane and boron trifluoride etherate in acetonitrile–chloroform afforded thiophene aglycon **13**. The aglycon **13** was converted into β-*C*-glucoside **14** in a manner similar to **2e**. The chlorothiophene derivative **7** was obtained by protection of the hydroxyl groups with acetic anhydride. The bromothiophene derivative **8** was synthesized by hydrogenolysis of **7** with palladium on carbon and triethylamine in methanol–tetrahydrofuran, followed by bromination with bromine.

The synthetic route to **9** is outlined in Scheme 4. While the *tert*-butyl ester group of **16** did not tolerate conditions of lithium halogen exchange with *n*-butyllithium or *tert*-butyllithium, we found that aryllithium was generated from **16** successfully using 2,4,6-trimethylphenyllihium (mesityllithium), which has a less nucleophilic character.[17] In addition, Barbier-type conditions had a good result. In particular, a mixture of **16** and 2,3,4,6-tetrakis-*O*-trimethylsilyl-D-gluconolactone was added dropwise to mesityllithium in tetrahydrofuran, followed by addition of methanesulfonic acid in methanol to generate desilylated methyl ether **17** as an amorphous powder (89.9% pure by HPLC). Acetylation of **17** with acetic anhydride and recrystallization from toluene–*n*-hexane gave **18** with a purity of 98.7% by HPLC. The *tert*-butyl ester **18** was converted into carboxylic acid using formic acid, and treated with oxalyl chloride, then 2-bromothiophene and aluminum trichloride to afford **19**. Finally, intermediate **9** was obtained by reduction of the carbonyl group of **19** to the benzyl alcohol using sodium tetrahydroborate, followed by simultaneous reduction of the resultant hydroxyl group and the anomeric methoxy group with triethylsilane and boron trifluoride etherate in acetonitrile–water.

C-Glucoside **2e** was synthesized according to the method described previously by our group using palladium-catalyzed coupling of aglycon **24** with glucal boronate **25**[18] as shown in Scheme 5. The coupling of 2-bromothiophene (**20**) with pyrazole



Scheme 3. Syntheses of intermediates 7, **8**. Reagents and conditions: (a) $(COCl)_2$, DMF, $CHCl_3$, rt; (b) $AlCl_3$, 2-chlorothiophene, CH_2Cl_2, 0 °C to rt, 81%; (c) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_3CN–$CHCl_3$, 0 °C to rt, 100%; (d) *n*-BuLi, THF–toluene, −78 °C; (e) 2,3,4,6-tetrakis-*O*-trimethylsilyl-D-gluconolactone, −78 °C; (f) $MeSO_3H$, MeOH, −78 °C to rt; (g) Et_3SiH, $BF_3{\cdot}Et_2O$, $CHCl_3$, 0 °C, 57%; (h) Ac_2O, pyridine, 4-dimethylaminopyridine, $CHCl_3$, rt, 75%; (i) H_2, Pd-C, Et_3N, MeOH–THF, rt, 72%; (j) Br_2, pyridine, $CHCl_3$, 0 °C to rt, 93%.

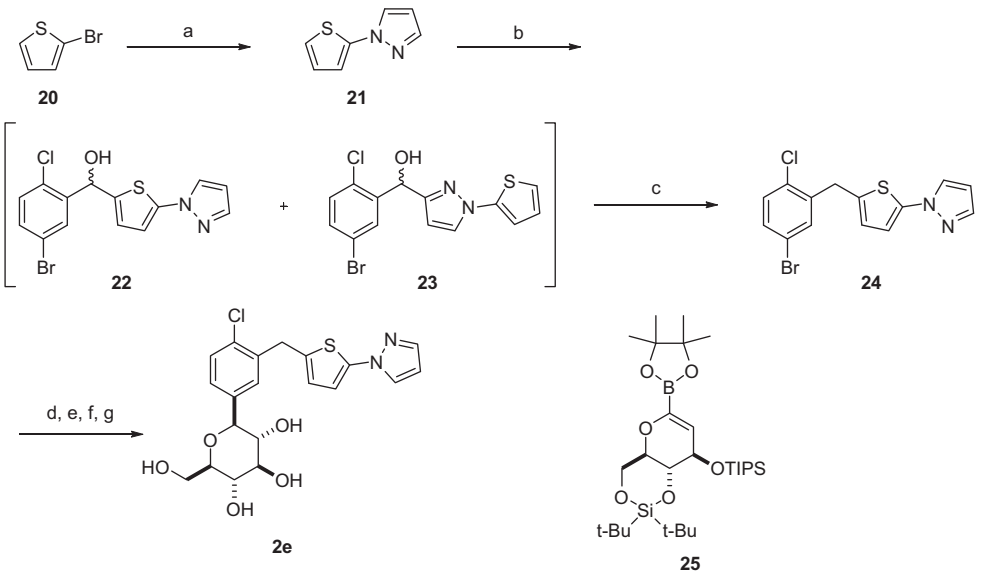


Scheme 4. Synthesis of intermediate **9**. Reagents and conditions: (a) 2,3,4,6-tetrakis-*O*-trimethylsilyl-D-gluconolactone, mesityllithium, THF, −78 °C; (b) $MeSO_3H$, MeOH, −78 °C to rt, 87%; (c) Ac_2O, iPr_2NEt, 4-dimethylaminopyridine, toluene, 0 °C to rt; 73%; (d) HCO_2H, 50 °C; (e) $(COCl)_2$, DMF, CH_2Cl_2, rt; (f) $AlCl_3$, 2-bromothiophene, CH_2Cl_2, −78 °C to 0 °C, 79%; (g) $NaBH_4$, EtOH–THF, 0 °C; (h) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_3CN–H_2O, 0 °C to rt, 66%;

gave 2-(pyrazol-1-yl)thiophene (**21**).[19] Lithiation of **21** with *n*-butyllithium, followed by addition of 5-bromo-2-chlorobenzaldehyde, generated an inseparable mixture of **22** and **23**. Under reaction conditions of triethylsilane and boron trifluoride etherate, only **22** was reduced to afford a mixture of **23** and **24**, which was easily separated by column chromatography to yield desired **24**. Aglycon **24** was coupled with glucal-boronate **25** in the presence of catalytic dichlorobis(triphenylphosphine)palladium, followed by the stereoselective hydroboration, oxidation using hydrogen peroxide in alkaline condition, and the deprotection of *O*-silyl groups with tetra-*n*-butylammonium fluoride to afford *C*-glucoside **2e**.

3. Results and discussion

We evaluated the effects of the heteroaryl thiophene derivatives **2** (Fig. 1) on human SGLT2 (hSGLT2) activity and on UGE in male Sprague–Dawley (SD) rats per 200 g of body weight over 24 h. The SARs of the representative compounds are shown in Table 1. 3-Pyridyl (**2a**) or 2-pyrimidyl (**2c**) derivative showed higher hSGLT2 inhibitory activities than 5-pyrimidyl derivative **2b**. 5-Membered heteroaryl derivatives (**2d** and **2e**) also retained good hSGLT2 inhibitory activities. For R^1 substituents, chlorine atom (**2f**) possessed better in vitro potency than methyl group (**2a**). Next, we were interested in the *C*-glucosides with fluorinated



Scheme 5. Synthesis of **2e**. Reagents and conditions: (a) pyrazole, NaH, CuO, pyridine, reflux, 67%; (b) *n*-BuLi, THF, −78 °C, then 5-bromo-2-chlorobenzaldehyde, −78 °C; (c) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C to rt, 29% from **21**; (d) $PdCl_2(PPh_3)_2$, 25, DME–Na_2CO_3 aq, reflux; (e) $BH_3{\cdot}$THF, THF, 0 °C; (f) H_2O_2 aq–NaOH aq, 0 °C to rt; (g) *n*-Bu_4NF, THF, 60 °C, 39%.

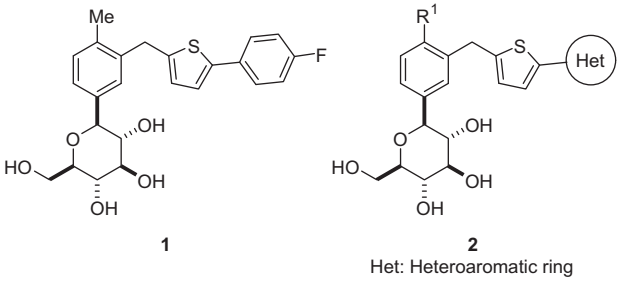


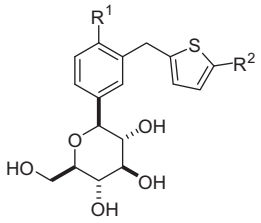
Figure 1. Structures of *C*-glucosides with thiophene ring.

heteroaryl thiophene, considering the structural similarity with **1**. 6-Fluoro-3-pyridyl derivative **2g** possessed comparable hSGLT2 inhibitory activity to **1** and showed the highest UGE effect among **2a**–**2g**.

We next determined the selectivity of **2g** for glucose transporters. hSGLT1 or hSGLT2 were stably expressed in Chinese hamster ovary-K (CHOK) cells and we investigated the inhibitory effects of **2g** on the uptake of [^{14}C]α-methyl-D-glucopyranoside (AMG) in these cell lines.[20] The IC_{50} values were 750 nM for hSGLT1 and 2.0 nM for hSGLT2, respectively. We next evaluated the inhibitory effect of **2g** on facilitated glucose transporter **1** (GLUT1) in L6 myoblast cells. Compound **2g** inhibited [3*H*]-2-deoxy-D-glucose (2-DG) uptake mediated by GLUT1 transporters[21] by only 19% at 10 μM. Taken together, these results identified compound **2g** as a potent and selective inhibitor of hSGLT2.

Oral administration (30 mg/kg) of **2g** to male SD rats induced UGE over 24 h by 2666 mg/200 g body weight. Following intravenous and oral doses of 3 and 10 mg/kg, respectively, to male SD rats, $AUC_{0-inf,po}$, $t_{1/2,po}$, and oral bioavailability were determined to be 10391 ng h/mL, 3.53 h, and 27.4%, respectively (Table 2). These results suggest that the exposure to **2g** is sufficient to inhibit SGLT2 activity, and inhibition of SGLT2 in renal tubules after oral administration of **2g** is likely to continuously suppress glucose reabsorption. Since most of the filtered glucose is reabsorbed by SGLT2 in the renal tubules, this novel compound **2g** would be useful as an anti-diabetic agent.

Table 1
SAR of **2a**–**2g**



Compd	R^1	R^2	hSGLT2 IC50 (nM)	UGE[a] (mg/day)
2a	Me		1.0	2023
2b	Me		7.3	N.D.[b]
2c	Me		1.7	2220
2d	Me		2.4	N.D.[b]
2e	Cl		1.5	1940
2f	Cl		0.8	2140
2g	Cl		2.0	2666
1	Me		2.2	3696

[a] Each compound was orally administered at a dose of 30 mg/kg to male Sprague–Dawley (SD) rats. Urinary glucose excretion (UGE) data over 24 h were normalized per 200 g body weight.
[b] N.D.: not determined.

Single oral administration of **2g** at 3 mg/kg remarkably reduced blood glucose levels without influencing food intake in hyperglycemic high-fat diet fed KK (HF-KK) mice (Fig. 2). There was a 43%

Table 2
Pharmacokinetic (PK) parameters of **2g** in male Sprague–Dawley (SD) rats following intravenous and oral administrations

Compd	**2g**	**2g**
Dose (mg/kg)	3	10
Route	iv	po
C_{max} (ng/mL)		964
t_{max}		3.0
$AUC_{0\text{-inf}}$ (ng h/mL)	11371	10391
$t_{1/2}$ (h)	3.22	3.53
CL_{tot} (mL/h/kg)	264	
Vd_{ss} (mL/kg)	1000	
F (%)		27.4

reduction in blood glucose level versus vehicle at 6 h. Therefore, **2g** (TA-3404) would control hyperglycemia in the therapy of type 2 diabetes.

4. Conclusion

We have demonstrated the synthesis and biological evaluation of *C*-glucosides with heteroaryl thiophene. Among the tested compounds, **2g** showed a highly potent and selective inhibition for hSGLT2 with favorable pharmacokinetic profiles and remarkably increased UGE. In addition, oral administration of **2g** induced anti-hyperglycemic effects in HF-KK mice. The 6-fluoro-3-pyridylthiophene derivative **2g** (TA-3404) has potential for the treatment of type 2 diabetes.

5. Experimental

5.1. Chemistry

All reactions were carried out under inert gas or with a $CaCl_2$ drying tube, and reaction mixtures were stirred magnetically. All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise noted. Reaction products were monitored by TLC using 0.25 mm E. Merck silica gel plates (60 F254) and were visualized using UV light or 5% phosphomolybdic acid in 95% ethanol. Melting points were measured by a BÜCHI model B-545 instrument and were uncorrected. NMR spectra were collected on JEOL JNM-ECX400P and Varian UNITY INOVA500 spectrometers. Chemical shifts are reported in parts per million (ppm) downfield from internal reference tetramethylsilane standard; coupling constants (*J* value) are given in hertz (Hz). MS (APCI) spectra were obtained on Finnigan MAT SSQ7000C or ThermoQuest LCQ Advantage, eluting with 10 mM $AcONH_4$/MeOH. MS (GC) spectra were measured on a Shimadzu GCMS-QP2010. Analytical HPLC spectra were reported using Agilent 1100 with a UV detector measuring absorbance at 210 nm. Elemental analyses were conducted by Medicinal Chemistry Research Laboratories, Mitsubishi Tanabe Pharma.

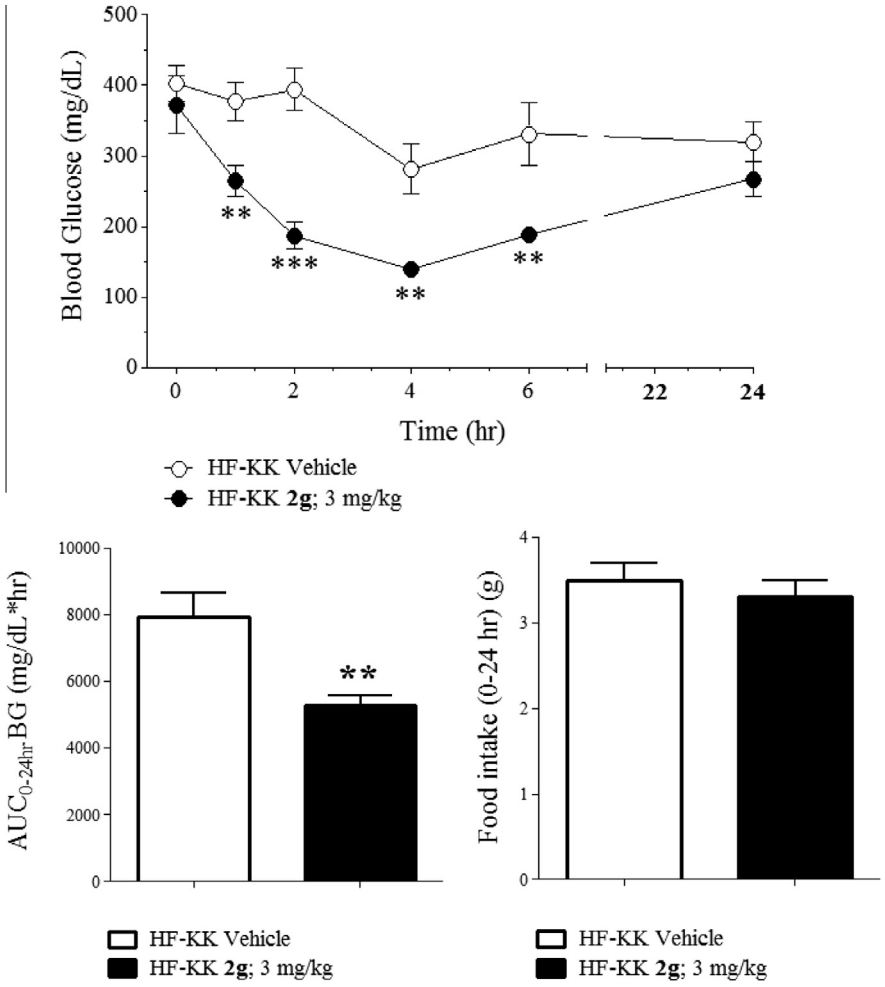


Figure 2. Effects of single oral dosing of 2 g on blood glucose levels and food intake in high-fat diet fed KK (HF-KK). Data are expressed as the mean ± SEM (n = 6): $^{**}P$ <0.01, $^{***}P$ <0.001 versus vehicle.

5.1.1. (5-Bromo-2-methylphenyl)(5-pyridin-3-yl-thiophen-2-yl) methanol (4)

To a stirred solution of 3-pyridylthiophene (**3**) (1.22 g, 7.54 mmol) in tetrahydrofuran (30 mL) at −78 °C under argon atmosphere was added dropwise *n*-butyllithium (2.6 N *n*-hexane solution, 2.91 mL, 7.54 mmol). After being stirred at 0 °C for 10 min, the mixture was cooled again at −78 °C, and to the mixture was added dropwise a solution of 5-bromo-2-methyl-benzaldehyde (1.50 g, 7.54 mmol) in tetrahydrofuran (5 mL). The resultant mixture was stirred at the same temperature for 1 h. The reaction mixture was quenched with saturated aqueous ammonium chloride solution, and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate prior to filtration, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (33% ethyl acetate in *n*-hexane) to give titled compound **4** (1.81 g, 67%) as a yellowish brown caramel. ^{1}H NMR ($CDCl_3$) δ: 2.24 (s, 3H), 2.96 (br s, 1H), 6.15 (br s, 1H), 6.82 (dd, J = 3.7, 0.7 Hz, 1H), 7.02 (d, J = 8.1 Hz, 1H), 7.08 (d, J = 3.7 Hz, 1H), 7.24 (m, 1H), 7.28 (dd, J = 8.1, 2.2 Hz, 1H), 7.80 (m, 1H), 7.82 (d, J = 2.2 Hz, 1H), 8.44 (dd, J = 4.9, 1.6 Hz, 1H), 8.68 (dd, J = 2.4, 0.7 Hz, 1H). Mass (APCI) m/z: 360/362 (M+H).

5.1.2. 3-[5-(5-Bromo-2-methylbenzyl)thiophen-2-yl]pyridine (5)

To a stirred solution of **4** (1.62 g, 4.49 mmol) in trifluoroacetic acid (20 mL) at 0 °C under argon atmosphere was added portionwise sodium triacetoxyborohydride (4.76 g, 22.5 mmol). After being stirred at the same temperature for 5 min, the resultant mixture was allowed to warm to room temperature and stirred for 90 min. The reaction mixture was quenched with 10% aqueous sodium hydroxide at 0 °C, extracted with diethyl ether. The organic layer was washed with brine, dried over magnesium sulfate prior to filtration, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (20% ethyl acetate in *n*-hexane) to give titled compound **5** (1.63 g, quant.) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 2.28 (s, 3H), 4.11 (s, 2H), 6.73 (dt, J = 3.5, 1.1 Hz, 1H), 7.06 (d, J = 8.1 Hz, 1H), 7.18 (d, J = 3.7 Hz, 1H), 7.24–7.36 (m, 3H), 7.78 (m, 1H), 8.47 (dd, J = 4.8, 1.5 Hz, 1H), 8.80 (d, J = 1.7 Hz, 1H). Mass (APCI) m/z: 344/346 (M+H).

5.1.3. 1-(1-Methoxyglucopyranosyl)-4-methyl-3-[5-(3-pyridyl)-2-thienylmethyl] benzene (6)

To a stirred solution of **5** (1.78 g, 5.17 mmol) in tetrahydrofuran (8 mL) and toluene (16 mL) at −78 °C under argon atmosphere was added dropwise *n*-butyllithium (2.6 N *n*-hexane solution, 1.96 mL, 5.07 mmol), and the mixture was stirred at the same temperature for 30 min. To the mixture was added a solution of 2,3,4,6-tetrakis-*O*-trimethylsilyl-D-gluconolactone (2.19 g, 4.70 mmol) in toluene (8 mL) dropwise at −78 °C, and the mixture was stirred at the same temperature for 3 h. Subsequently, to the mixture was added a solution of methanesulfonic acid (0.91 mL, 14.1 mmol) in methanol (20 mL), and the resulting mixture was allowed to warm to room temperature and stirred overnight. Under ice-cooling, to the mixture were added saturated aqueous sodium hydrogen carbonate solution and water, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate prior to filtration, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (5–10% methanol in chloroform) to give titled compound **6** (49 mg, 2.3%) as a pale brown foam. ^{1}H NMR (DMSO-d_6) δ: 2.27 (s, 3H), 2.92 (m, 1H), 2.96 (s, 3H), 3.22 (m, 1H), 3.38 (m, 1H), 3.50–3.64 (m, 2H), 3.74 (m, 1H), 4.13 (d, J = 15.7 Hz, 1H), 4.21 (d, J = 16.1 Hz, 1H), 4.49 (t, J = 5.9 Hz, 1H), 4.64 (d, J = 7.2 Hz, 1H), 4.67 (d, J = 5.1 Hz, 1H), 4.92 (d, J = 5.5 Hz, 1H), 6.84 (d, J = 3.4 Hz, 1H), 7.15 (d, J = 7.9 Hz, 1H), 7.33 (m, 1H), 7.39 (dd, J = 7.7, 4.8 Hz, 1H), 7.42–7.46 (m, 2H), 7.93 (m, 1H), 8.44 (dd, J = 4.7, 1.1 Hz, 1H), 8.79 (d, J = 1.8 Hz, 1H). Mass (APCI) m/z: 458 (M+H).

5.1.4. 1-(β-D-Glucopyranosyl)- 4-methyl-3-[5-(3-pyridyl)-2-thienylmethyl]benzene (2a)

To a stirred solution of **6** (46 mg, 0.10 mmol) in chloroform (5 mL) was added triethylsilane (0.06 mL, 0.40 mmol) followed by boron trifluoride diethyletherate (0.05 mL, 0.40 mmol) at −78 °C. The mixture was allowed to warm to 0 °C, and stirred for 3 h. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate solution, and extracted with ethyl acetate–tetrahydrofuran. The organic layer was washed with brine, dried over magnesium sulfate prior to filtration, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (5–10% methanol in chloroform) followed by trituration with diethyl ether to give tilted compound **2a** (13 mg, 30%) as a pale brown powder. ^{1}H NMR (DMSO-d_6) δ: 2.27 (s, 3H), 3.13–3.40 (m, 4H), 3.44 (m, 1H), 3.69 (m, 1H), 3.97 (d, J = 9.5 Hz, 1H), 4.14 (d, J = 15.9 Hz, 1H), 4.18 (d, J = 15.9 Hz, 1H), 4.41 (t, J = 5.7 Hz, 1H), 4.72 (d, J = 5.6 Hz, 1H), 4.90 (d, J = 4.8 Hz, 2H), 6.86 (d, J = 3.4 Hz, 1H), 7.10–7.17 (m, 2H), 7.23 (s, 1H), 7.39 (dd, J = 7.6, 4.7 Hz, 1H), 7.45 (d, J = 3.5 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 8.44 (d, J = 3.5 Hz, 1H), 8.80 (d, J = 1.8 Hz, 1H). Mass (APCI) m/z: 428 (M+H). HPLC: 97.2% (t_R = 6.8 min, L-column ODS 4.6 × 150 mm, CH_3CN/20 mM phosphate buffer (pH 6.5) (30/70), 1 mL/min of flow rate). Anal. Calcd for $C_{23}H_{25}NO_5S{\cdot}0.2AcOEt{\cdot}0.5H_2O$: C, 62.88; H, 5.86; N, 2.93; S, 7.06. Found: C, 62.94; H, 6.15; N, 3.08; S, 7.00.

5.1.5. 4-Methyl-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-[5-(5-pyrimidyl)-2-thienylmethyl]benzene (10b)

To a mixture of **7** (100 mg, 0.18 mmol) and 5-(tri-*n*-butylstannyl)pyrimidine (100 mg, 0.27 mmol) in 1,4-dioxane (5 mL) were added tris(dibenzylideneacetone)dipalladium(0) (8.3 mg, 0.009 mmol), tri-*tert*-butylphosphine tetrafluoroborate (10.5 mg, 0.036 mmol) and cesium fluoride (69 mg, 0.45 mmol), then the mixture was stirred at 100 °C for 3 h under argon atmosphere. After being cooled to room temperature, the insoluble was filtered off, and the filtrate was extracted with ethyl acetate, washed with brine, and dried over sodium sulfate prior to filtration. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (25–40% ethyl acetate in *n*-hexane) to give titled compound **10b** (266 mg, 41%) as colorless crystals. ^{1}H NMR (DMSO-d_6) δ: 1.72 (s, 3H), 1.93 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.26 (s, 3H), 4.05–4.16 (m, 3H), 4.19 (s, 2H), 4.64 (d, J = 9.6 Hz, 1H), 4.99 (t, J = 9.5 Hz, 1H), 5.06 (t, J = 9.6 Hz, 1H), 5.35 (t, J = 9.5 Hz, 1H), 6.89 (d, J = 3.5 Hz, 1H), 7.17 (s, 2H), 7.20 (s, 1H), 7.60 (d, J = 3.5 Hz, 1H), 9.01 (s, 2H), 9.05 (s, 1H). Mass (APCI) m/z: 597 (M+H).

5.1.6. 4-Methyl-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-[5-(2-pyrimidyl)-2-thienylmethyl]benzene (10c)

To a mixture of **8** (1.20 g, 2.01 mmol) and 2-(tri-*n*-butylstannyl)pyrimidine (1.11 g, 3.01 mmol) in *N*-methyl-2-pyrrolidone (24 mL) were added dichlorobis(triphenylphosphine)palladium (141 mg, 0.20 mmol) and copper(I) iodide (38 mg, 0.20 mmol), then the mixture was stirred at 100 °C for 3.5 h under argon atmosphere. After being cooled to room temperature, to the mixture were added ethyl acetate and water, and the insoluble was filtered off. The organic layer was separated, washed with brine, and dried over sodium sulfate prior to filtration. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (33–50% ethyl acetate in *n*-hexane) to give titled compound **10c** (787 mg, 66%) as a colorless amorphous

powder. ^{1}H NMR (DMSO-d_6) δ: 1.74 (s, 3H), 1.92 (s, 3H), 2.00 (s, 3H), 2.01 (s, 3H), 2.26 (s, 3H), 4.04–4.15 (m, 3H), 4.17 (d, J = 16.2 Hz, 1H), 4.20 (d, J = 16.2 Hz, 1H), 4.65 (d, J = 10.0 Hz, 1H), 4.98 (t, J = 9.5 Hz, 1H), 5.06 (t, J = 9.6 Hz, 1H), 5.35 (t, J = 9.5 Hz, 1H), 6.86 (d, J = 3.5 Hz, 1H), 7.17 (s, 1H), 7.18 (s, 2H), 7.29 (t, J = 5.0 Hz, 1H), 7.76 (d, J = 3.5 Hz, 1H), 8.73 (d, J = 5.0 Hz, 2H). Mass (APCI) m/z: 597 (M+H).

5.1.7. 4-Methyl-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-[5-(5-thiazolyl)-2-thienylmethyl]benzene (10d)

To a mixture of **8** (500 mg, 0.84 mmol) and 5-(tri-*n*-butylstannyl)thiazole (626 mg, 1.67 mmol) in 1,4-dioxane (5 mL) were added tetrakis(triphenylphosphine)palladium (193 mg, 0.17 mmol) and copper(I) iodide (48 mg, 0.26 mmol), then the mixture was stirred at refluxed temperature for 2 h under argon atmosphere. After being cooled to room temperature, to the mixture was added aqueous potassium fluoride solution, and the mixture was stirred at the same temperature for 30 min, and diluted with ethyl acetate. The insoluble was filtered off, and the organic layer was separated, washed with brine, and dried over sodium sulfate prior to filtration. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (33–50% ethyl acetate in *n*-hexane) to give titled compound **10d** (117 mg, 23%) as a colorless amorphous powder. ^{1}H NMR (DMSO-d_6) δ: 1.72 (s, 3H), 1.92 (s, 3H), 2.00 (s, 3H), 2.01 (s, 3H), 2.25 (s, 3H), 4.04–4.13 (m, 3H), 4.14 (s, 2H), 4.65 (d, J = 9.8 Hz, 1H), 4.98 (t, J = 9.8 Hz, 1H), 5.06 (t, J = 9.6 Hz, 1H), 5.35 (t, J = 9.5 Hz, 1H), 6.79 (d, J = 3.4 Hz, 1H), 7.17 (s, 3H), 7.22 (d, J = 3.5 Hz, 1H), 8.00 (s, 1H), 8.99 (s, 1H). Mass (APCI) m/z: 602 (M+H).

5.1.8. 4-Chloro-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-[5-(3-pyridyl)-2-thienylmethyl]benzene (10f)

To a mixture of **9** (300 mg, 0.49 mmol) and 3-pyridylboronic acid (179 mg, 1.46 mmol) in 1,2-dimethoxyethane were added tetrakis(triphenylphosphine)palladium (58 mg, 0.049 mmol) and cesium fluoride (443 mg, 2.91 mmol), then the mixture was stirred at refluxed temperature under nitrogen atmosphere overnight. After being cooled to room temperature, to the mixture were added ethyl acetate and saturated aqueous sodium hydrogen carbonate solution. The organic layer was separated, washed with brine, dried over sodium sulfate prior to filtration. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (50–80% ethyl acetate in *n*-hexane), followed by trituration with methanol to give titled compound **10f** (157 mg, 53%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.72 (s, 3H), 1.92 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 4.05–4.17 (m, 3H), 4.29 (s, 2H), 4.71 (d, J = 9.8 Hz, 1H), 4.97 (t, J = 9.8 Hz, 1H), 5.08 (t, J = 9.3 Hz, 1H), 5.36 (t, J = 9.3 Hz, 1H), 6.90 (d, J = 3.6 Hz, 1H), 7.31 (dd, J = 8.2, 2.1 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H), 7.40 (dd, J = 7.7, 4.6 Hz, 1H), 7.473 (d, J = 3.6 Hz, 1H), 7.474 (d, J = 8.2 Hz, 1H), 7.94 (dd, J = 7.7, 4.6 Hz, 1H), 8.46 (dd, J = 4.6, 1.5 Hz, 1H), 8.81 (d, J = 2.6 Hz, 1H). Mass (APCI) m/z: 616/618 (M+H).

5.1.9. 4-Chloro-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)- 3-(5-(6-fluoro-3-pyridyl)-2-thienylmetyl)benzene (10g)

A suspension of **9** (43.0 g, 69.6 mmol), 2-(2-(6-fluoro)pyridine)-4,4,5,5-tetramethyl-1,3-dioxaborolane (23.3 g, 104 mmol), cesium fluoride (63.4 g, 418 mmol) and tetrakis(triphenylphosphine)palladium(0) (8.04 g, 6.96 mmol) in 1,2-dimethoxyethane (1300 mL) was vigorously stirred at reflux for 3 h under argon atmosphere. The reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution (1200 mL), and the mixture was extracted with ethyl acetate (1000 mL) twice. The combined organic layers were washed with brine (600 mL), dried over magnesium sulfate prior to filtration, and the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (1500 mL), and the mixture was treated with activated carbon and NH–silica gel (450 mL). The insoluble was filtered off and the filtrate was evaporated under reduced pressure. To the residual solid was added ethyl alcohol (1000 mL), and the mixture was refluxed for 30 min and then stirred at room temperature overnight. The pale yellow crystals were collected by filtration. To the crystals was added methanol (1000 mL), and the mixture was refluxed for 1 h and then stirred at room temperature overnight. The colorless crystals were collected by filtration, washed with methanol and dried under reduced pressure to give **10g** (35.8 g, 81%). Mp 161–162 °C. ^{1}H NMR (DMSO-d_6) δ: 1.75 (s, 3H), 1.93 (s, 3H), 2.00 (s, 3H), 2.01 (s, 3H), 4.07–4.15 (m, 3H), 4.26 (d, J = 15.8 Hz, 1H), 4.30 (d, J = 15.8 Hz, 1H), 4.72 (d, J = 9.8 Hz, 1H), 4.97 (t, J = 9.7 Hz, 1H), 5.08 (t, J = 9.6 Hz, 1H), 5.36 (t, J = 9.5 Hz, 1H), 6.92 (d, J = 3.5 Hz, 1H), 6.99 (dd, J = 8.0, 2.3 Hz, 1H), 7.33 (dd, J = 8.3, 2.0 Hz, 1H), 7.38 (d, J = 1.9 Hz, 1H), 7.48 (d, J = 8.3 Hz, 1H), 7.69 (d, J = 3.7 Hz, 1H), 7.77 (dd, J = 7.5, 2.3 Hz, 1H), 7.97 (q, J = 8.2 Hz, 1H). Mass (APCI) m/z: 634/636 (M+H). HPLC: 99.2% (t_R = 12.5 min, L-column ODS 4.6 × 150 mm, CH_3CN/20 mM phosphate buffer (pH 6.5) (60/40), 1 mL/min of flow rate). Anal. Calcd for $C_{30}H_{29}ClFNO_9S$: C, 56.83; H, 4.61; Cl, 5.59; F, 3.00; N, 2.21; S, 5.06. Found: C, 56.80; H, 4.47; Cl, 5.60; F, 2.91; N, 2.29; S, 4.93.

5.1.10. 1-(β-D-Glucopyranosyl)-4-methyl-3-[5-(5-pyrimidyl)-2-thienylmethyl]benzene (2b)

To a solution of compound **10b** (260 mg, 0.44 mmol) in methanol (5 mL) and tetrahydrofuran (2 mL) was added sodium methoxide (28% methanol solution, 6 drops), and the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (0–10% methanol in chloroform) to give titled compound **2b** (160 mg, 86%) as a colorless powder. ^{1}H NMR (DMSO-d_6) δ: 2.27 (s, 3H), 3.12–3.30 (m, 4H), 3.44 (dt, J = 11.8, 6.2 Hz, 1H), 3.70 (ddd, J = 11.8, 5.7, 2.1 Hz, 1H), 3.97 (d, J = 9.3 Hz, 1H), 4.17 (d, J = 15.9 Hz, 1H), 4.20 (d, J = 15.9 Hz, 1H), 4.42 (t, J = 5.7 Hz, 1H), 4.73 (d, J = 5.7 Hz, 1H), 4.91 (d, J = 5.1 Hz, 1H), 4.92 (d, J = 4.6 Hz, 1H), 6.93 (d, J = 3.6 Hz, 1H), 7.13 (d, J = 7.7 Hz, 1H), 7.16 (dd, J = 7.7, 1.5 Hz, 1H), 7.24 (s, 1H), 7.58 (d, J = 3.6 Hz, 1H), 9.02 (s, 2H), 9.05 (s, 1H). Mass (APCI) m/z: 429 (M+H). HPLC: 98.6% (t_R = 3.4 min, Sumipax ODS F210SLP 4.6 × 50 mm, 0.05% TFA in CH_3CN/H_2O (25/75), 1 mL/min of flow rate). Anal. Calcd for $C_{22}H_{24}N_2O_5S{\cdot}1.0H_2O$: C, 59.18; H, 5.87; N, 6.27; S, 7.18. Found: C, 59.31; H, 5.59; N, 6.19; S, 7.20.

5.1.11. 1-(β-D-Glucopyranosyl)-4-methyl-3-[5-(2-pyrimidyl)-2-thienylmethyl]benzene (2c)

Compound **2c** (313 mg, 55%) was prepared according to the method described for the synthesis of **2b** using **10c** (787 mg, 1.32 mmol). ^{1}H NMR (DMSO-d_6) δ: 2.26 (s, 3H), 3.13–3.29 (m, 4H), 3.41–3.47 (dt, J = 11.7, 5.8 Hz, 1H), 3.69 (dd, J = 11.6, 5.3 Hz, 1H), 3.97 (d, J = 9.5 Hz, 1H), 4.15 (d, J = 16.1 Hz, 1H), 4.19 (d, J = 16.1 Hz, 1H), 4.43 (t, J = 5.9 Hz, 1H), 4.73 (d, J = 5.6 Hz, 1H), 4.92 (d, J = 4.7 Hz, 2H), 6.89 (d, J = 3.7 Hz, 1H), 7.13 (d, J = 7.9 Hz, 1H), 7.15 (dd, J = 7.9, 1.3 Hz, 1H), 7.24 (s, 1H), 7.29 (t, J = 4.8 Hz, 1H), 7.76 (d, J = 3.7 Hz, 1H), 8.73 (d, J = 4.8 Hz, 2H). Mass (APCI) m/z: 429 (M+H). HPLC: 99.6% (t_R = 5.4 min, L-column ODS 4.6 × 150 mm, CH_3CN/20 mM phosphate buffer (pH 6.5) (30/70), 1 mL/min of flow rate). Anal. Calcd for $C_{22}H_{24}N_2O_5S$: C, 61.67; H, 5.65; N, 6.54; S, 7.48. Found: C, 61.56; H, 5.44; N, 6.50; S, 7.36.

5.1.12. 1-(β-D-Glucopyranosyl)-4-methyl-3-[5-(5-thiazolyl)-2-thienylmethyl]benzene (2d)

Compound **2d** (84 mg, quantitative) was prepared according to the method described for the synthesis of **2b** using **10d** (115 mg,

0.19 mmol). ^{1}H NMR (DMSO-d_6) δ: 2.25 (s, 3H), 3.12–3.29 (m, 4H), 3.44 (dt, J = 11.6, 5.9 Hz, 1H), 3.67–3.72 (dd, J = 11.6, 6.6 Hz, 1H), 3.96 (d, J = 9.5 Hz, 1H), 4.12 (d, J = 16.1 Hz, 1H), 4.15 (d, J = 16.1 Hz, 1H), 4.43 (t, J = 5.8 Hz, 1H), 4.73 (d, J = 5.8 Hz, 1H), 4.92 (d, J = 4.8 Hz, 2H), 6.82 (d, J = 3.5 Hz, 1H), 7.13 (d, J = 7.7 Hz, 1H), 7.15 (dd, J = 7.9, 1.1 Hz, 1H), 7.20 (d, J = 3.5 Hz, 1H), 7.22 (s, 1H), 8.01 (s, 1H), 8.99 (s, 1H). Mass (APCI) m/z: 434 (M+H). HPLC: 90.7% (t_R = 1.8 min, Sumipax ODS D-210SLP 4.6 × 50 mm, 0.05% TFA in CH_3CN/H_2O (35/65), 1 mL/min of flow rate). Anal. Calcd for $C_{21}H_{23}NO_5S_2{\cdot}0.12CHCl_3{\cdot}0.8H_2O$: C, 54.87; H, 5.39; Cl, 2.76; N, 3.03; S, 13.87. Found: C, 54.90; H, 5.13; Cl, 2.82; N, 2.96; S, 13.31.

5.1.13. 4-Chloro-1-(β-D-glucopyranosyl)-3-[5-(3-pyridyl)-2-thienylmethyl]benzene (2f)

Compound **2f** (45 mg, 86%) was prepared according to the method described for the synthesis of **2b** using **10f** (72 mg, 0.12 mmol). ^{1}H NMR (DMSO-d_6) δ: 3.07–3.30 (m, 4H), 3.45 (dt, J = 11.8, 6.2 Hz, 1H), 3.70 (ddd, J = 11.8, 5.7, 1.5 Hz, 1H), 4.03 (d, J = 9.3 Hz, 1H), 4.26 (d, J = 15.9 Hz, 1H), 4.30 (d, J = 15.9 Hz, 1H), 4.44 (t, J = 6.2 Hz, 1H), 4.85 (d, J = 6.2 Hz, 1H), 4.95 (t, J = 5.1 Hz, 2H), 6.93 (d, J = 3.6 Hz, 1H), 7.28 (dd, J = 8.2, 2.1 Hz, 1H), 7.40 (ddd, J = 8.2, 4.6, 1.0 Hz, 1H), 7.42 (d, J = 8.2 Hz, 1H), 7.45 (s, 1H), 7.46 (d, J = 3.6 Hz, 1H), 7.95 (ddd, J = 7.7, 2.6, 1.5 Hz, 1H), 8.46 (dd, J = 5.1, 1.5 Hz, 1H), 8.81 (d, J = 1.5 Hz, 1H). Mass (APCI) m/z: 448/450 (M+H). HPLC: 98.2% (t_R = 3.7 min, Sumipax ODS D-210SLP 4.6 × 50 mm, 0.05% TFA in CH_3CN/H_2O (20/80), 1 mL/min of flow rate). Anal. Calcd for $C_{22}H_{22}ClNO_5S{\cdot}0.5H_2O$: C, 57.83; H, 5.07; Cl, 7.76; N, 3.07; S, 7.02. Found: C, 57.63; H, 4.81; Cl, 7.88; N, 3.08; S, 7.02.

5.1.14. 4-Chloro-3-(5-(6-fluoro-3-pyridyl)-2-thienylmethyl) -1-(β-D-glucopyranosyl)benzene (2g)

Compound **10g** (35.5 g, 56.0 mmol) was dissolved in methanol (350 mL)—tetrahydrofuran (700 mL). To the mixture was added sodium methoxide (28% methanol solution, 0.11 mL, 0.56 mmol), and the mixture was stirred at room temperature for 17 h under argon atmosphere. To the mixture was added silica gel (260 mL), and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (0–10% methanol in chloroform) to give a colorless solid. The solid was dissolved in tetrahydrofuran (1000 mL), and the mixture was treated with activated carbon. The insoluble was filtered off and the filtrate was evaporated under reduced pressure. The residue was recrystallized from ethyl acetate (1000 mL) to give titled compound **2g** (23.3 g, 89%) as colorless crystals. ^{1}H NMR (DMSO-d_6) δ: 3.12 (td, J = 9.03, 5.08 Hz, 1H), 3.18 (dd, J = 8.91, 5.22 Hz, 1H), 3.22–3.27 (m, 2H), 3.45 (ddd, J = 11.8, 6.10, 5.98 Hz, 1H), 3.70 (ddd, J = 12.0, 5.38, 1.44 Hz, 1H), 4.03 (d, J = 9.47 Hz, 1H), 4.24 (d, J = 15.5 Hz, 1H), 4.29 (d, J = 15.5 Hz, 1H), 4.45 (t, J = 5.86 Hz, 1H), 4.85 (d, J = 5.94 Hz, 1H), 4.95 (d, J = 4.81 Hz, 1H), 4.96 (d, J = 4.49 Hz, 1H), 6.94 (d, J = 3.69 Hz, 1H), 6.99 (dd, J = 8.10, 2.17 Hz, 1H), 7.29 (dd, J = 8.26, 2.01 Hz, 1H), 7.42 (d, J = 8.34 Hz, 1H), 7.45 (d, J = 1.77 Hz, 1H), 7.68 (d, J = 3.69 Hz, 1H), 7.76 (dd, J = 7.70, 2.41 Hz, 1H), 7.97 (q, J = 8.18 Hz, 1H). Mass (APCI) m/z: 483/485 (M + NH_4). HPLC: 99.9% (t_R = 10.7 min, L-column ODS 4.6 × 150 mm, CH_3CN/20 mM phosphate buffer (pH 6.5) (35/65), 1 mL/min of flow rate). Anal. Calcd for $C_{22}H_{21}ClFNO_5S$: C, 56.71; H, 4.54; Cl, 7.61; F, 4.08; N, 3.01; S, 6.88. Found: C, 56.60; H, 4.49; Cl, 7.34; F, 4.09; N, 2.97; S, 6.56.

5.1.15. (5-Bromo-2-methylphenyl)-(5-chlorothiophen-2-yl)methanone (12)

To a stirred suspension of 5-bromo-2-methylbenzoic acid (**11**) (39 g, 181 mmol) in chloroform (390 mL) was added oxalyl chloride (20.7 mL, 218 mmol) dropwise at room temperature, followed by *N*,*N*-dimethylformamide (1 drop). The resultant mixture was stirred at the same temperature overnight. The mixture was concentrated in vacuo, dissolved in dichloromethane (650 mL), and to the mixture was added 2-chlorothiophene (21.8 mL, 236 mmol). To the mixture was added portion-wise aluminum chloride (26.6 g, 200 mmol) at 0 °C. The resultant mixture was allowed to warm to room temperature, and stirred at the same temperature overnight. The reaction mixture was poured into ice–water, and extracted with chloroform. The organic layer was separated, washed with saturated aqueous sodium hydrogen carbonate solution, and brine, and dried over sodium sulfate prior to filtration. The solvent was evaporated under reduced pressure, and the residue was dissolved in methanol on heating, and treated with activated carbon. The insoluble was filtered off, and the filtrate was evaporated under reduced pressure. The residue was recrystallized from methanol to give titled compound **12** (52 g, 81%) as pale yellow powder. ^{1}H NMR (DMSO-d_6) δ: 2.22 (s, 3H), 7.30 (d, J = 4.2 Hz, 1H), 7.33–7.36 (m, 2H), 7.66–7.68 (m, 2H). Mass (APCI) m/z: 314/316/318 (M+H).

5.1.16. 2-(5-Bromo-2-methylbenzyl)-5-chlorothiophene (13)

To a solution of **12** (47.3 g, 150 mmol) in chloroform (470 mL) and acetonitrile (470 mL) was added triethylsilane (52.3 g, 450 mmol) and then dropwise boron trifluoride diethyletherate (63.9 g, 450 mmol) at 0 °C. The resultant mixture was stirred at the same temperature for 1 h, allowed to warm to room temperature and stirred overnight. The reaction mixture was basified with saturated aqueous sodium hydrogen carbonate solution at 0 °C, and extracted with chloroform. The organic layer was washed with brine, and dried over sodium sulfate prior to filtration. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane) to give tilted compound **13** (45 g, quantitative) as a pale yellow oil. ^{1}H NMR (DMSO-d_6) δ: 2.21 (s, 3H), 4.09 (s, 2H), 6.72 (br d, J = 3.6 Hz, 1H), 6.95 (d, J = 3.6 Hz, 1H), 7.15 (d, J = 8.2 Hz, 1H), 7.35 (dd, J = 8.2, 2.6 Hz, 1H), 7.39 (d, J = 2.6 Hz, 1H). Mass (GC) m/z: 300/302/304 (M+). HPLC: 98.0% (t_R = 4.2 min, Sumipax ODS D-210SLP 4.6 × 50 mm, 0.05% TFA in CH_3CN/H_2O (75/25), 1 mL/min of flow rate). Anal. Calcd for $C_{12}H_{10}BrClS$: C, 47.78; H, 3.34; Br, 26.49; Cl, 11.75; S, 10.63. Found: C, 47.64; H, 3.34; Br, 25.76; Cl, 11.62; S, 10.52.

5.1.17. 3-[5-Chloro-2-thienylmethyl]-1-(1-β-D-glucopyranosyl)-4-methylbenzene (14)

To a stirred solution of **13** (15.4 g, 51.1 mmol) in tetrahydrofuran (150 mL) and toluene (300 mL) at −78 °C under argon atmosphere was added *n*-butyllithium (1.6 N *n*-hexane solution, 32.3 mL, 51.1 mmol) dropwise over a period of 5 min, and the mixture was stirred at the same temperature for 20 min. To the mixture was added a solution of 2,3,4,6-tetrakis-*O*-trimethylsilyl-D-gluconolactone (23.8 g, 51.1 mmol) in toluene (150 mL) dropwise over a period of 20 min at −78 °C, and the mixture was stirred at the same temperature for 1 h. Subsequently, to the mixture was added a solution of methanesulfonic acid (9.94 mL, 153 mmol) in methanol (300 mL), and the resulting mixture was allowed to warm to room temperature and stirred overnight. Under ice-cooling, to the mixture were added saturated aqueous sodium hydrogen carbonate solution (200 mL) and water (150 mL), and the mixture was extracted with ethyl acetate (600 mL) twice. The combined organic layers were washed with brine, dried over sodium sulfate prior to filtration, and the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane (200 mL), and to the mixture was added triethylsilane (24.4 mL, 153 mmol) and then dropwise boron trifluoride diethyletherate (19.4 mL g, 153 mmol) at −78 °C under argon atmosphere. The resultant mixture was allowed to warm to 0 °C and stirred for 2 h. The reaction mixture was basified with saturated aqueous sodium hydrogen carbonate solution (250 mL) at 0 °C, and extracted with dichloromethane (150 mL). The organic layer was washed with brine (150 mL), and dried over sodium sulfate prior to filtration. The solvent was evaporated under reduced

pressure, and the residue was purified by silica gel column chromatography (0–10% methanol in chloroform) to give tilted compound **14** (11.2 g, 57%) as a colorless amorphous powder. ^{1}H NMR (DMSO-d_6) δ: 2.22 (s, 3H), 3.11–3.28 (m, 3H), 3.40–3.47 (m, 1H), 3.66–3.72 (m, 1H), 3.95 (d, J = 9.5 Hz, 1H), 4.04 (d, J = 15.9 Hz, 1H), 4.09 (d, J = 15.9 Hz, 1H), 4.42 (t, J = 5.8 Hz, 1H), 4.70 (d, J = 5.8 Hz, 1H), 4.91 (d, J = 4.7 Hz, 1H), 4.91 (d, J = 5.1 Hz, 1H), 6.66 (d, J = 3.7 Hz, 1H), 6.91 (d, J = 3.7 Hz, 1H), 7.11 (d, J = 7.7 Hz, 1H), 7.14 (dd, J = 7.7, 1.5 Hz, 1H), 7.18 (br s, 1H). Mass (APCI) m/z: 402/404 (M+NH_4).

5.1.18. 3-[5-Chloro-2-thienylmethyl]-4-methyl-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)benzene (7)

To a stirred solution of **14** (11 g, 28.6 mmol), acetic anhydride (27 mL, 286 mmol), and pyridine (23.1 mL, 286 mmol) in chloroform (110 mL) was added 4-(dimethylamino)pyridine (349 mg, 2.86 mmol) at room temperature. The resultant mixture was stirred at the same temperature for 1 h. The solvent was evaporated under reduced pressure, and the residue was poured into water (330 mL). The mixture was extracted with ethyl acetate (220 mL) twice. The combined organic layers were washed with 10% copper sulfate solution (280 mL) twice, and brine (280 mL), and dried over magnesium sulfate prior to filtration. The solvent was concentrated in vacuo, and the residue was recrystallized from ethanol (150 mL) to give titled compound **7** (11.9 g, 75%) as a colorless solid. Mp 157–158 °C. ^{1}H NMR (DMSO-d_6) δ: 1.93 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.22 (s, 3H), 4.06 (m, 4H), 4.12 (m, 1H), 4.63 (d, J = 9.8 Hz, 1H), 4.97 (t, J = 9.6 Hz, 1H), 5.05 (t, J = 9.6 Hz, 1H), 5.35 (t, J = 9.5 Hz, 1H), 6.64 (d, J = 3.9 Hz, 1H), 6.92 (d, J = 3.7 Hz, 1H), 7.12 (s, 1H), 7.16 (s, 2H). Mass (APCI) m/z: 570/572 (M + NH_4). HPLC: 97.8% (t_R = 9.2 min, L-column ODS 4.6 × 150 mm, CH_3CN/20 mM phosphate buffer (pH 6.5) (65/35), 1 mL/min of flow rate). Anal. Calcd for $C_{26}H_{29}ClO_9S$: C, 56.47; H, 5.29; Cl, 6.41; S, 5.80. Found: C, 56.43; H, 5.24; Cl, 6.32; S, 5.65.

5.1.19. 4-Methyl-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-[2-thienylmethyl]benzene (15)

A vigorous stirred suspension of **7** (11.9 g, 21.5 mmol), 10% palladium on carbon (3.5 g), and triethylamine (24 mL, 172 mmol) in tetrahydrofuran (120 mL) and methanol (360 mL) was hydrogenated at room temperature for 18 h. The insoluble was removed by filtration, washed with tetrahydrofuran (500 mL). The filtrate and the washing were combined, and the solvent was evaporated under reduced pressure. The residue was dissolved in chloroform (150 mL), washed with 5% aqueous citric acid solution (100 mL), saturated aqueous sodium hydrogen carbonate solution (100 mL), and water (100 mL), and dried over sodium sulfate prior to filtration. The solvent was concentrated under reduced pressure, and the residue was recrystallized from methanol to give titled compound **15** (8.03 g, 72%) as a colorless solid. Mp 124 °C. ^{1}H NMR (DMSO-d_6) δ: 1.71 (s, 3H), 1.92 (s, 3H), 2.00 (s, 3H), 2.01 (s, 3H), 2.22 (s, 3H), 4.04–4.09 (m, 2H), 4.10–4.16 (m, 3H), 4.62 (d, J = 9.8 Hz, 1H), 4.96 (t, J = 9.7 Hz, 1H), 5.05 (t, J = 9.6 Hz, 1H), 5.35 (t, J = 9.5 Hz, 1H), 6.75 (dd, J = 3.4, 1.0 Hz, 1H), 6.93 (dd, J = 5.1, 3.4 Hz, 1H), 7.12 (br s, 1H), 7.15 (s, 2H), 7.32 (dd, J = 5.1, 1.1 Hz, 1H). Mass (APCI) m/z: 536 (M+NH_4). HPLC 98.0% (t_R = 8.5 min, L-column ODS 4.6 × 150 mm, CH_3CN/20 mM phosphate buffer (pH 6.5) (60/40), 1 mL/min of flow rate). Anal. Calcd for $C_{26}H_{30}O_9S$: C, 60.22; H, 5.75; S, 6.18. Found: C, 60.17; H, 5.75; S, 6.15.

5.1.20. 3-[5-Bromo-2-thienylmethyl]-4-methyl-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)benzene (8)

To a stirred 0 °C solution of **15** (8.03 g, 15.5 mmol) in chloroform (70 mL) was added a solution of bromine (385 mg, 2.41 mmol) in chloroform (7 mL) dropwise over a period of 10 min. The mixture was poured into a mixture of 10% aqueous sodium pyrosulfate solution (90 mL) and saturated aqueous sodium hydrogen carbonate solution (90 mL), extracted with chloroform (100 mL) twice. The combined organic layers were washed with brine, and dried over magnesium sulfate prior to filtration. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (10–40% ethyl acetate in *n*-hexane) followed by recrystallization from methanol to give titled compound **8** (8.63 g, 93%) as a colorless solid. Mp 155–157 °C. ^{1}H NMR (DMSO-d_6) δ: 1.72 (s, 3H), 1.93 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.22 (s, 3H), 4.08 (s, 2H), 4.09 (m, 3H), 4.63 (d, J = 9.8 Hz, 1H), 4.97 (t, J = 9.6 Hz, 1H), 5.05 (t, J = 9.6 Hz, 1H), 5.25 (t, J = 9.5 Hz, 1H), 6.62 (d, J = 3.7 Hz, 1H), 7.03 (d, J = 3.7 Hz, 1H), 7.12 (s, 1H), 7.16 (s, 2H). Mass (APCI) m/z: 614/616 (M+NH_4). HPLC 98.0% (t_R = 14.4 min, L-column ODS 4.6 × 150 mm, CH_3CN/20 mM phosphate buffer (pH 6.5) (60/40), 1 mL/min of flow rate). Anal. Calcd for $C_{26}H_{29}BrO_9S$: C, 52.27; H, 4.89; Br, 13.37; S, 5.37. Found: C, 52.29; H, 4.69; Br, 13.14; S, 5.35.

5.1.21. *tert*-Butyl 2-chloro-5-(1-methoxy-D-glucopyranosyl)benzoate (17)

Mesityl bromide (64.43 g, 323.62 mmol) was dissolved in tetrahydrofuran (1100 mL), and the mixture was cooled in a dry ice–acetone bath under argon atmosphere. To the mixture was added dropwise *n*-butyllithium (2.71 N *n*-hexane solution, 119.4 mL, 323.62 mmol) over a period of 15 min below −64 °C (internal temperature), and the resultant suspended mixture was stirred at the same temperature for 1 h. Then, a solution of *tert*-butyl 5-bromo-2-chlorobenzoate **16** (67.4 g, 231.16 mmol) and 2,3,4,6-tetrakis-*O*-trimethylsilyl-D-gluconolactone (110 g, 235.78 mmol) in tetrahydrofuran (540 mL) was added dropwise to the reaction mixture over a period of 1 h below −63 °C (internal temperature), and the orange mixture was further stirred at the same temperature for 30 min. A solution of methanesulfonic acid (66 mL, 1017.10 mmol) in methanol (660 mL) was added dropwise to the reaction mixture over a period of 5 min, and the mixture was stirred at room temperature for 40 h. Under ice-water cooling, to the mixture was added saturated aqueous sodium hydrogen carbonate solution (1500 mL). The organic layer was concentrated to about half volume under reduced pressure, and the residue was extracted with ethyl acetate (1200 mL, 800 mL and 500 mL) three times. The combined organic layers were washed with brine (1000 mL), dried over magnesium sulfate, and the solvent was evaporated under reduced pressure. The resultant residue was dissolved in toluene (250 mL), and the mixture was added dropwise to *n*-hexane (3000 mL) under vigorous stirring. After being stirred for 30 min, the precipitate was collected by filtration, washed with *n*-hexane and soon dried under reduced pressure at room temperature to give **17** (81.59 g, 87%) as a colorless powder. ^{1}H NMR (DMSO-d_6) δ: 1.56 (s, 9H), 2.87 (dd, J = 9.0, 7.4 Hz, 1H), 2.93 (s, 3H), 3.22 (m, 1H), 3.38 (m, 1H), 3.53 (m, 1H), 3.59 (m, 1H), 3.75 (m, 1H), 4.61 (t, J = 5.9 Hz, 1H), 4.79 (d, J = 5.1 Hz, 1H), 4.90 (d, J = 7.2 Hz, 1H), 5.00 (d, J = 5.0 Hz, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.62 (dd, J = 8.4, 2.0 Hz, 1H), 7.76 (d, J = 1.9 Hz, 1H). Mass (APCI) m/z: 422/424 (M + NH_4). HPLC 89.9% (t_R = 9.7 min, L-column ODS 4.6 × 150 mm, CH_3CN/20 mM phosphate buffer (pH 6.5) (30/70), 1 mL/min of flow rate). Anal. Calcd for $C_{18}H_{25}ClO_8$: C, 53.40; H, 6.22; Cl, 8.76. Found: C, 52.69; H, 6.12; Cl, 8.05.

5.1.22. *tert*-Butyl 2-chloro-5-(1-methoxy-2,3,4,6-tetra-O-acetyl-D-glucopyranosyl)benzoate (18)

To a mixture of **17** (81.3 g, 200.82 mmol) in toluene (1300 mL) were added successively acetic anhydride (133 mL, 1405 mmol), *N*,*N*-diisopropylethylamine (245 mL, 1405 mmol) and 4-(dimethylamino)pyridine (4.89 g, 40 mmol) at 0 °C. The resultant mixture was stirred at room temperature for 1 h. The mixture was poured into water (1300 mL) and extracted with ethyl acetate (500 mL)

twice. The combined organic layers were successively washed with 20% aqueous phosphoric acid solution (700 mL), water (300 mL) and saturated aqueous sodium hydrogen carbonate solution (600 mL), dried over magnesium sulfate and treated with activated carbon. The insoluble was filtered off and the filtrate was evaporated under reduced pressure. The residue was dissolved in toluene (150 mL), and to the mixture were added successively *n*-hexane (600 mL) and seed crystals under stirring. After being stirred overnight, the crystals were collected by filtration, washed with toluene–*n*-hexane (40–160 mL) and dried under reduced pressure to give titled compound **18** (83.75 g, 73%) as colorless crystals. Mp 116–118 °C. ^{1}H NMR (DMSO-d_6) δ: 1.56 (s, 9H), 1.91 (s, 3H), 1.92 (s, 3H), 2.03 (s, 3H), 2.06 (s, 3H), 3.07 (s, 3H), 4.05 (m, 1H), 4.22–4.34 (m, 2H), 4.84 (d, J = 10.1 Hz, 1H), 5.20 (t, J = 9.8 Hz, 1H), 5.44 (t, J = 9.8 Hz, 1H), 7.52 (dd, J = 8.5, 2.1 Hz, 1H), 7.60 (d, J = 8.5 Hz, 1H), 7.65 (d, J = 2.1 Hz, 1H). Mass (APCI) m/z: 590/592 (M+NH$_4$). HPLC 98.7% (t_R = 10.0 min, L-column ODS 4.6 × 150 mm, CH$_3$CN/20 mM phosphate buffer (pH 6.5) (60/40), 1 mL/min of flow rate). Anal. Calcd for $C_{26}H_{33}ClO_{12}$: C, 54.50; H, 5.81; Cl, 6.19. Found: C, 54.23; H, 5.79; Cl, 6.08.

5.1.23. 2-Chloro-5-(1-methoxy-2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl)phenyl 5-bromo-2-thienyl ketone (19)

A solution of **18** (83.4 g, 145.5 mmol) in formic acid (300 mL) was stirred at 50 °C for 30 min. The solvent was evaporated and removed by azeotropic distillation with toluene under reduced pressure to give 2-chloro-5-(1-methoxy-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)benzoic acid (ESI-Mass m/z 515/517 (M−H)) as a colorless foam. The benzoic acid was dissolved in dichloromethane (600 mL), and to the mixture were added oxalyl chloride (16.5 mL, 189.15 mmol) and *N*,*N*-dimethylformamide (5 drops). The mixture was stirred at room temperature for 2 h. The solvent was evaporated and removed by azeotropic distillation with toluene under reduced pressure to give 2-chloro-5-(1-methoxy-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)benzoyl chloride as a pale yellow syrup. This benzoyl chloride and 2-bromothiophene (30.84 g, 189.15 mmol) were dissolved in dichloromethane (700 mL), and the mixture was cooled in a dry ice-acetone bath, and to the mixture was added aluminum chloride (97 g, 727.5 mmol). After being stirred for 10 min, the resultant mixture was allowed to warm gradually to ice-water temperature and stirred at same temperature for 1 h. The reaction mixture was poured into ice-water (1500 mL), and extracted with chloroform (800 mL). The organic layer was washed with brine and saturated aqueous sodium hydrogen carbonate solution, and dried over magnesium sulfate prior to filtration. The solvent was evaporated under reduced pressure, and the residue was dissolved in chloroform (300 mL)–ethanol (400 mL) and the mixture was treated with activated carbon. The insoluble was filtered off and the filtrate was evaporated under reduced pressure. The residue was recrystallized from ethanol (400 mL) to give titled compound **19** (75.7 g, 79%) as pale yellow crystals. Mp 162–163 °C. ^{1}H NMR (DMSO-d_6) δ: 1.87 (s, 3H), 1.92 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 3.13 (s, 3H), 4.04 (m, 1H), 4.20–4.30 (m, 2H), 4.92 (d, J = 10.1 Hz, 1H), 5.21 (t, J = 9.8 Hz, 1H), 5.44 (t, J = 9.8 Hz, 1H), 7.16 (d, J = 4.0 Hz, 1H), 7.46 (d, J = 4.1 Hz, 1H), 7.53–7.60 (m, 2H), 7.67 (d, J = 8.9 Hz, 1H). Mass (APCI) m/z: 680/682 (M + NH$_4$). HPLC: 87.2% (t_R = 11.6 min, L-column ODS 4.6 × 150 mm, CH$_3$CN/20 mM phosphate buffer (pH 6.5) (60/40), 1 mL/min of flow rate). Anal. Calcd for $C_{26}H_{26}BrClO_{11}S$: C, 47.18; H, 3.96; Br, 12.07; Cl, 5.36; S, 5.39. Found: C, 47.75; H, 3.93; Br, 11.31; Cl, 5.39; S, 4.53.

5.1.24. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-chloro-3-(5-bromo-2-thienylmethyl)benzene (9)

A solution of **19** (75.5 g, 114 mmol) in ethanol (755 mL) and tetrahydrofuran (450 mL) was cooled in an ice–water bath, and to the mixture was added sodium tetrahydroborate (6.47 g, 171 mmol). The mixture was stirred at the same temperature for 2 h. Furthermore, to the mixture was added sodium tetrahydroborate (1.3 g, 34.2 mmol), and the resultant mixture was stirred at the same temperature for 30 min. The mixture was poured into hydrochloric acid (0.5 N, 900 mL), diluted with water (1000 mL) and extracted with ethyl acetate (1500 mL, 500 mL) twice. The combined organic layers were washed with brine and saturated aqueous sodium hydrogen carbonate solution, and dried over magnesium sulfate prior to filtration. The solvent was evaporated under reduced pressure to give yellow foam. The resultant foam was dissolved in acetonitrile (1000 mL) and water (4.1 mL, 228 mmol), and the mixture was cooled in an ice-water bath under argon atmosphere. To the mixture were added triethylsilane (109 mL, 684 mmol) and then dropwise boron trifluoride diethyletherate (58 mL, 456 mmol) over a period of 10 min. After being stirred at the same temperature for 30 min, the mixture was allowed to warm to room temperature and stirred overnight. The resultant mixture was poured into saturated aqueous sodium hydrogen carbonate solution (1400 mL)–ice, and extracted with ethyl acetate (1500 mL, 500 mL) twice. The combined organic layers were washed with brine (1000 mL), dried over magnesium sulfate and treated with activated carbon and silica gel (300 mL). The insoluble was filtered off and the filtrate was evaporated under reduced pressure. The residue was recrystallized from methanol (400 mL) to give titled compound **9** (46.53 g, 66%) as colorless crystals. Mp 142–143 °C. ^{1}H NMR (DMSO-d_6) δ: 1.73 (s, 3H), 1.93 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 4.10 (m, 3H), 4.20 (s, 2H), 4.70 (d, J = 9.8 Hz, 1H), 4.95 (t, J = 9.6 Hz, 1H), 5.08 (t, J = 9.6 Hz, 1H), 5.36 (t, J = 9.6 Hz, 1H), 6.69 (d, J = 3.7 Hz, 1H), 7.04 (d, J = 3.7 Hz, 1H), 7.31 (dd, J = 8.3, 1.9 Hz, 1H), 7.34 (d, J = 1.4 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H). Mass (APCI) m/z: 634/636 (M+NH$_4$). HPLC: 95.1% (t_R = 10.9 min, L-column ODS 4.6 × 150 mm, CH$_3$CN/20 mM phosphate buffer (pH 6.5) (65/35), 1 mL/min of flow rate). Anal. Calcd for $C_{25}H_{26}BrClO_9S$: C, 48.60; H, 4.24; Br, 12.93; Cl, 5.74; S, 5.19. Found: C, 48.87; H, 4.15; Br, 12.80; Cl, 5.75; S, 5.11.

5.1.25. 1-Thiophen-2-yl-1*H*-pyrazole (21)

Sodium hydride (60% in mineral oil, 4.0 g, 100 mmol) was washed with *n*-hexane three times, and treated with pyridine (20 mL). To the mixture was added a solution of pyrazole (6.81 g, 100 mmol) in pyridine (20 mL) at 0 °C under argon atmosphere. When the evolution of hydrogen gas was ceased, 2-bromothiophene **20** (16.3 g, 100 mmol) and copper oxide (500 mg, 6.29 mmol) were added to the mixture, and the resultant mixture was stirred at refluxed temperature for 17 h. After being cooled to room temperature, the reaction mixture was diluted with diethyl ether (200 mL), and filtered through a Celite pad. The filtrate was washed with water (100 mL) and 10% copper sulfate solution (150 mL) twice. The organic layer was dried over magnesium sulfate prior to filtration, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (17% ethyl acetate in *n*-hexane) to give titled compound **21** (10.0 g, 67%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ: 6.43 (dd, J = 2.4, 1.8 Hz, 1H), 6.93–7.05 (m, 3H), 7.67 (br d, 1H), 7.80 (dd, J = 2.4, 0.6 Hz, 1H). Mass (APCI) m/z: 151 (M+H).

5.1.26. 1-[5-(5-Bromo-2-chlorobenzyl)thiophen-2-yl]-1*H*-pyrazole (24)

To a stirred solution of **21** (3.0 g, 20 mmol) in tetrahydrofuran (200 mL) was added a solution of *n*-butyllithium (1.58 N *n*-hexane solution, 12.6 mL, 19.9 mmol) at −78 °C dropwise over a period of 10 min under argon atmosphere, and the mixture was stirred at the same temperature for 1 h. To the mixture was added dropwise a solution of 5-bromo-2-chlorobenzaldehyde (4.37 g, 19.9 mmol) in tetrahydrofuran (15 mL). The resultant mixture was stirred at the same temperature for 30 min. The reaction mixture was

quenched with saturated aqueous ammonium chloride solution (300 mL), and extracted with ethyl acetate (200 mL) twice. The organic layer was washed with brine (100 mL), dried over sodium sulfate prior to filtration, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (10–40% ethyl acetate in *n*-hexane) to give an inseparable 3:2 mixture of **22** and **23** (5.55 g) as a pale yellow oil which was taken onto the next step. The obtained pale yellow oil (5.04 g, 19.6 mmol) was treated with dichloromethane (100 mL), and cooled to 0 °C. To the mixture was added triethylsilane (6.50 mL, 40.8 mmol), followed by boron trifluoride diethyletherate dropwise over a period of 5 min. The resultant mixture was allowed to warm to room temperature, and stirred for 3 h, and quenched with saturated aqueous sodium hydrogen carbonate solution at 0 °C. The mixture was extracted with dichloromethane (80 mL) twice, and the combined organic layers were washed with water (50 mL), dried over sodium sulfate prior to filtration. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (0–40% ethyl acetate in *n*-hexane) to give titled compound **24** (1.85 g, 29% in 2 steps) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 4.21 (s, 2H), 6.50 (t, J = 2.3 Hz, 1H), 6.81 (d, J = 3.9 Hz, 1H), 7.12, (d, J = 3.9 Hz, 1H), 7.44 (d, J = 8.5 Hz, 1H), 7.51 (dd, J = 8.5, 2.4 Hz, 1H), 7.64 (d, J = 2.3 Hz, 1H), 7.69 (d, J = 2.3 Hz, 1H), 8.33 (d, J = 2.4 Hz, 1H). Mass (APCI) m/z: 353/355/357 (M+H). HPLC: 99.8% (t_R = 3.2 min, Sumipax ODS D-210SLP 4.6 × 50 mm, 0.05% TFA in CH_3CN/H_2O (70/30), 1 mL/min of flow rate). Anal. Calcd for $C_{14}H_{10}BrClN_2S$: C, 47.55; H, 2.85; Br, 22.59; Cl, 10.02; N, 7.92; S, 9.07. Found: C, 47.39; H, 2.72; Br, 22.01; Cl, 9.97; N, 7.84; S, 8.90.

5.1.27. 4-Chloro-1-(β-D-glucopyranosyl)-3-(5-(2-pyrazol-1-yl)-2-thienylmethyl)benzene (2e)

To a mixture of **24** (800 mg, 2.40 mmol) and boronic acid ester **25** (2.65 g, 3.60 mmol) in 1,2-dimethoxyethane (24 mL) were added dichlorobis(triphenylphosphine)palladium (84 mg, 0.12 mmol) and 2 M aqueous sodium carbonate solution (6 mL), then the mixture was stirred at refluxed temperature for 1.5 h under argon atmosphere. The mixture was cooled to room temperature and extracted with ethyl acetate, washed with water, brine, and dried over sodium sulfate prior to filtration. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (0–7% ethyl acetate in *n*-hexane), and the obtained material was further purified by NH–silica gel column chromatography (0–5% ethyl acetate in *n*-hexane) to give a colorless viscous oil (1.25 g). The obtained oil was treated with tetrahydrofuran (20 mL), and to the mixture was added borane–tetrahydrofuran complex (1 M solution in tetrahydrofuran, 4.5 mL) dropwise at 0 °C. After being stirred at 0 °C overnight, to the mixture were added 30% aqueous hydrogen peroxide solution (6 mL) and 3 N aqueous sodium hydroxide solution (6 mL), and the resultant mixture was allowed to warm to room temperature, and stirred for 2 h, and poured into saturated aqueous ammonium chloride solution. The mixture was extracted with ethyl acetate, and the organic layer was washed with 10% aqueous sodium thiosulfate solution, brine, and dried over sodium sulfate prior to filtration. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (0–5% ethyl acetate in *n*-hexane) to give colorless amorphous powder (788 mg). The obtained powder was treated with tetrahydrofuran (16 mL), and to the mixture was added tetra-*n*-butylammonium fluoride (1 M solution in tetrahydrofuran, 5.35 mL) dropwise under argon atmosphere, and the mixture was stirred at 60 °C for 2 h. After being cooled to room temperature, the mixture was poured into 1% hydrochloric acid solution, and extracted with ethyl acetate. The organic layer was washed with brine, and dried over sodium sulfate prior to filtration. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (0–10% methanol in chloroform) followed by recrystallization from ethyl acetate–diethylether to give titled compound **2e** (383 mg, 39% in 3 steps) as colorless crystals. ^{1}H NMR (DMSO-d_6) δ: 3.07–3.19 (m, 2H), 3.21–3.28 (m, 2H), 3.45 (m, 1H), 3.69 (m, 1H), 4.02 (d, J = 9.5 Hz, 1H), 4.20 (m, 2H), 4.45 (t, J = 5.9 Hz, 1H), 4.85 (d, J = 5.8 Hz, 1H), 4.95–4.96 (m, 2H), 6.49 (dd, J = 2.3, 1.9 Hz, 1H), 6.76 (d, J = 3.7 Hz, 1H), 7.10 (d, J = 3.7 Hz, 1H), 7.28 (dd, J = 8.2, 2.4 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 7.44 (d, J = 1.9 Hz, 1H), 7.64 (d, J = 2.3 Hz, 1H), 8.31 (d, J = 2.4 Hz, 1H). Mass (APCI) m/z: 437/439 (M+H). HPLC 99.8% (t_R = 10.2 min, L-column ODS 4.6 × 150 mm, CH_3CN/20 mM phosphate buffer (pH 6.5) (30/70), 1 mL/min of flow rate). Anal. Calcd for $C_{20}H_{21}N_2O_5ClS$: C, 54.98; H, 4.84; Cl, 8.11; N, 6.41; S, 7.34. Found: C, 54.90; H, 4.63; Cl, 8.19; N, 6.40; S, 7.42.

5.2. Pharmacology

5.2.1. Sodium-dependent glucose uptake in CHO cells expressing hSGLT1 and hSGLT2

Parental CHOK cells expressing hSGLT1 and hSGLT2 were used in these experiments. For the uptake assay, cells were seeded into 24-well plates, and were post-confluent on the day of assay.

Cells were rinsed one time with 400 μL Assay Buffer (137 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 50 mM HEPES, 20 mM Tris Base, pH 7.4), and were pre-incubated with the solutions of compounds (250 μL) for 10 min at 37 °C. The transport reaction was initiated by addition of 50 μL AMG/^{14}C-AMG solution (16.7 μCi; final concentration, 0.3 mM for CHOK-hSGLT1 and 0.5 mM for CHOK-hSGLT2, respectively) and incubated for 120 min at 37 °C. After the incubation, the AMG uptake was halted by aspiration of the incubation mixture followed by immediate washing three times with PBS. The cells were solubilized in 0.3 N NaOH of 300 μL and the radioactivity associated with the cells was monitored by a liquid scintillation counter (Quantasmart™ (Packard, Boston, MA, USA)). Inhibitory concentration of 50% (IC_{50}) was calculated by nonlinear least squares analysis using a four-parameter logistic model (Prism version 4; GraphPad Software, San Diego, CA, USA).

5.2.2. 2-DG uptake in L6 myoblast cells

The rat skeletal muscle cell line, L6 (JCRB9081), was obtained from Health Science Research Resources Bank (HSRRB, Osaka, Japan). L6 myoblast cells were maintained in Dulbecco's modified Eagle's medium containing 5.6 mM glucose supplemented with 10% FBS. Cells were seeded in 24-well plates at a density of 3.0×10^5 cells/well and cultured for 24 h in an atmosphere of 5% CO_2 at 37 °C before the experiment. Prior to the transport experiment, cells were rinsed twice with KRPH buffer (pH 7.4, 150 mM NaCl, 5 mM KCl, 1.25 mM $MgSO_4$, 1.25 mM $CaCl_2$, 10 mM HEPES, 2.9 mM Na_2HPO_4), and were pre-incubated with compounds (250 μl) for 5 min at room temperature. The transport reaction was initiated by addition of 50 μl [^{3}H]-2-DG solution (0.625 μCi; final concentration, 750 μM) and incubated for 15 min at room temperature. After the incubation, the 2-DG uptake was halted by aspiration of the incubation mixture. Cells were immediately washed three times with ice-cold PBS and were solubilized in 300 μL of 0.3 N NaOH. The radioactivity associated with the cells was determined by a liquid scintillation counter (Quantasmart™ (Packard, Boston, MA, USA)).

5.2.3. UGE Study

Male SD rats aged 4–5 weeks were obtained from Japan SLC (Shizuoka, Japan) and were used for experiments at 6 weeks of age after acclimation period. The animals were divided into experimental groups matched for body weight (n = 3). The compounds

were prepared in vehicles as suspension or solution. UGE studies were performed after two-day acclimation period in metabolic cages. The compounds or vehicle were orally administered at a dose of 30 mg/kg in 0.2% CMC/0.2% Tween 80. Urine samples were collected for 24 h using metabolic cages to measure urinary glucose excretion. Urine glucose contents were determined by an enzymatic assay kit (UGLU-L, Serotec, Hokkaido, Japan). All animals were allowed free access to a standard pellet diet (CRF1; Oriental Yeast Co., Ltd, Tokyo, Japan) and tap water.

5.2.4. Single oral dosing study

Male KK/Ta Jcl mice aged 9 weeks were obtained from CLEA Japan Inc. (Tokyo, Japan) and kept on a standard diet (CRF-1; 5.7% (w/w) fat, 3.59 kcal/g, Oriental Yeast Co., Ltd, Tokyo, Japan), 20-week-old mice were fed with a high-fat diet (60 kcal %, Research Diets, Inc., New Brunswick, NJ) for 4 weeks. The experiment was carried out at the age of 24 weeks. Male C57BL/6N mice aged 11 weeks were obtained from Charles River Laboratories Japan, INC. (Yokohama, Japan) and were also used in this study. The animals were divided into experimental groups matched for body weight and blood glucose levels, which were measured in the fed state on the day of the experiment.

The compounds (3 mg/kg) or vehicle (0.2% CMC/0.2% Tween 80) were orally administered at a volume of 10 mL/kg. The blood samples were collected from the tail vein before and at 1, 2, 4, 6 and 24 h after the administration.

The blood glucose level was determined using commercially available kits based on the glucose oxidase method (Glucose CII-Test Wako; Wako Pure Chemical Industries, Osaka, Japan). Data are expressed as means ± SEM. Area under the curve for blood glucose levels ($AUC_{0-24\ h}BG$) was calculated by the trapezoidal rule. Differences between groups were analyzed by repeated measurement ANOVA followed by Student's *t*-test (EXSAS, Arm Systex Co. Ltd). Probabilities less than 5% ($P < 0.05$) were considered to be statistically significant.

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