Na^+-Glucose Cotransporter Inhibitors as Antidiabetics. I. Synthesis and Pharmacological Properties of 4′-Dehydroxyphlorizin Derivatives Based on a New Concept

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Based on our new concept that inhibitors of the Na^+-glucose cotransporter (SGLT) would be useful as antidiabetics, 4′-dehydroxyphlorizin derivatives 1a—f were designed, synthesized, and examined for various pharmacological properties related to antidiabetic activity. In normal rats, 1a, e and phlorizin showed a strong SGLT-inhibitory effect and significantly increased urinary glucose on intraperitoneal administration at 10 mg/kg, though only 1a resulted in excretion of large quantities of urinary glucose on oral administration at 100 mg/kg. Compounds 1a, e, and phlorizin markedly inhibited glucose uptake in the small intestine during enteric perfusion in normal rats. Compound 1a had a significant reducing effect on blood glucose in the glucose tolerance test in mice when administered orally and also lowered blood glucose in streptozotocin-induced diabetic rats. The aglycons 2a, e of 1a, e, and 1a showed weak inhibitory effects on the facilitated glucose transporter-1 (GLUT-1) in human erythrocytes, while phloretin had a strong inhibitory effect on GLUT-1. Compound 1a caused no apparent renal damage in rats when administered orally at 1 g/kg for 4 successive weeks. Thus, 1a was considered to be a promising candidate as a lead compound for antidiabetics of a new type, and was selected for further pharmacological evaluation.

Key words antidiabetic; Na^+-glucose cotransporter inhibitor; phlorizin; 4′-dehydroxyphlorizin

The incidence of diabetes mellitus, especially non-insulin-dependent diabetes mellitus (NIDDM), has been increasing markedly in the developed countries. One of the main reasons for this phenomenon is thought to be excess energy intake due to hyperphagia. Therefore, anti-obesity drugs such as appetite suppressants,[1] absorption inhibitors,[2] and thermogenic stimulators[3] have been developed as antidiabetics. Blood glucose produces various glycated proteins in tissues non-enzymatically, and in the state of hyperglycemia, these glycated proteins give rise to the diabetes complications.[4,5] Therefore, a category of drug that would enable patients to escape from the state of chronic hyperglycemia is expected to be useful for the prophylaxis or treatment of diabetes. We have hypothesized that inhibitors of the Na^+-glucose cotransporter (SGLT) would be suitable for this purpose. SGLT, which exists on the chorionic membrane of the intestine and the kidney, actively transports glucose by coupling with Na^+.[6]

Phlorizin was reported to be a selective inhibitor of SGLT,[7] but there has been no attempt to use phlorizin as an antidiabetic. We considered that compounds with an inhibitory effect on SGLT might be able not only to inhibit glucose uptake at the intestine, but also to stimulate blood glucose excretion into urine directly at the kidney, and they might be useful in the treatment of diabetes, in preventing chronic hyperglycemia.

In this paper, we describe the design of SGLT inhibitors as candidate antidiabetic drugs. 4′-Dehydroxyphlorizin derivatives were synthesized and their pharmacological properties, such as SGLT-inhibitory effect, promotion of urinary glucose excretion, blood glucose reduction, inhibition of glucose uptake in the small intestine, *etc.*, were examined in normal and diabetic rats and normal mice.

Drug Design Phlorizin was found in the last century and studied as a promoter of urinary glucose excretion. It is an inhibitor of SGLT, preventing renal tubular glucose reabsorption and promoting excretion of glucose, so that the blood glucose level is controlled.[7] Based on this action of phlorizin, it has been used as a blood glucose-lowering reagent to verify the glucose toxicity theory.[5] Namely, when the blood glucose level in diabetic animals is controlled to a normal level for a long period by subcutaneous daily administration of phlorizin without using insulin, the condition of the animals is normalized.

Phlorizin, however, is considered to have little effect when administered orally, because it is hydrolyzed into

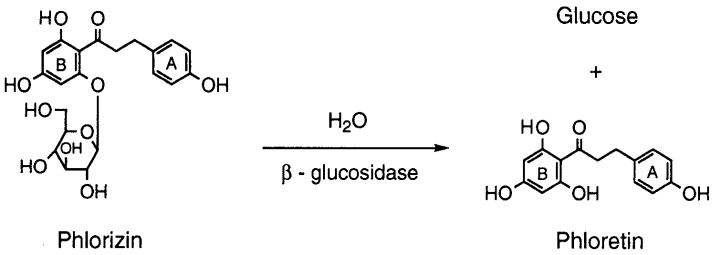

Chart 1

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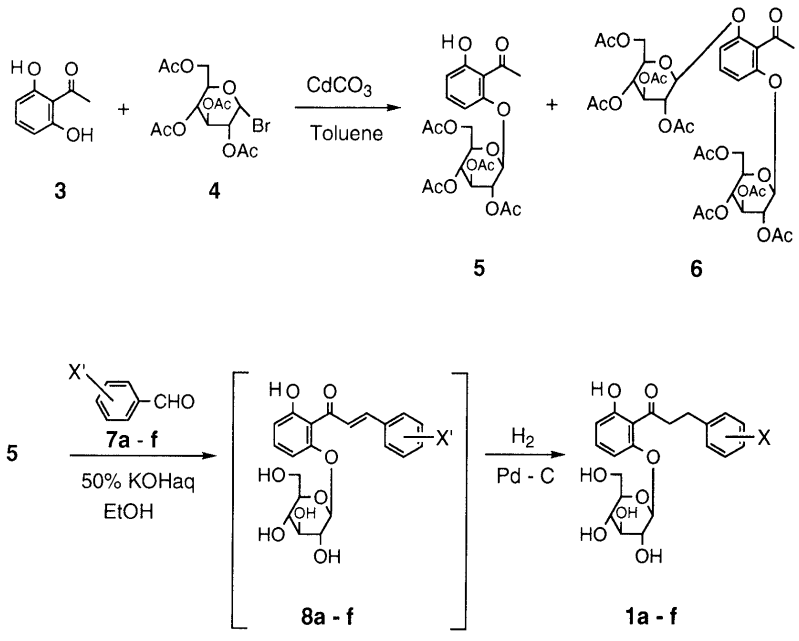
glucose and phloretin, the aglycon of phlorizin, by β-glucosidase in the intestine.[8] Moreover, phloretin inhibits the facilitated glucose transporters (GLUT) very strongly.[9] So, when phloretin is intravenously administered to rats, the glucose concentration in the rat brain is decreased abruptly.[10] Long-term inhibition of GLUT by phloretin may induce injuries in various organs, because GLUT is distributed in almost all tissues.

Therefore, to use a phlorizin derivative as an antidiabetic, it must meet the following criteria. (i) It must inhibit SGLT selectively, reversibly and strongly. (ii) It must induce urinary excretion of glucose when administered orally. (iii) It must lower high blood glucose levels when administered orally. (iv) Its aglycon produced by hydrolysis must not inhibit GLUT. (v) It must have low toxicity, especially to the kidney.

Phlorizin itself meets only criterion 1. Concerning phlorizin derivatives, it has been reported[11] that, the 4′-OH group on the B ring is not essential for SGLT inhibition and the 4-OH group on the A ring is exchangeable for other groups. Based on the study of phloretin, the four hydroxy groups on the A and B rings were essential to inhibit GLUT in human erythrocytes.[9a] In addition, Hase *et al.*[12] reported that phlorizin strongly inhibited the Na^+, K^+-activated ATPase and the 4′-OH group on the B ring was important for this activity. Therefore, the 4′-OH group on the B ring may be responsible for the toxicity. Based on these findings, we designed 4′-dehydroxyphlorizin derivatives **1a**—**f** as candidate SGLT inhibitors.

Chemistry

The synthesis of **1a**—**f** was carried out as shown in Chart 2. Diedrich[13] reported that the reaction of acetophenone **3** with acetylated bromoglucose **4** in aqueous acetone containing KOH gave the glycoside **5** in 32% yield. Although we tried this method under modified conditions, the yield of **5** did not exceed 38% and the purification was very difficult. After several attempts, Dick's method[14] was found to be suitable for this glycosylation in greatly superior yield. Thus, **3** and **4** (2 eq mol) were reacted with $CdCO_3$ (4 eq mol) as a base in refluxing toluene with removal of generated water to afford the desired **5** in 71% yield and the diglycoside **6** as a by-product in 8% yield. According to Winget's procedure,[15] the glycoside **5** was condensed with the aldehyde **7a** (1.2 eq mol) in a mixture of 50% aqueous KOH solution and EtOH at room temperature to provide the chalcone **8a** as yellow needles in 74% yield, and this was converted into the dihydrochalcone **1a** by catalytic hydrogenation over 10% Pd on carbon in EtOH in 90% yield (2 steps, 67% yield). Conveniently, catalytic hydrogenation of the alkaline reaction mixture containing crude **8a** was found to proceed smoothly (2 steps, 78% yield). Therefore, **1b**—**f** were prepared similarly by condensation of **5** with **7b**—**f** followed by catalytic hydrogenation without isolation of **8b**—**f**. On the other hand, the aglycons **2a**, **e** of **1a**, **e**, which exhibited potent SGLT-inhibitory activity, were prepared



7, 8	X′
a	4 - OMe
b	3,4 - $(OMe)_2$
c	2,4 - $(OMe)_2$
d	3,4,5 - $(OMe)_3$
e	4 - OBn
f	3,4 - $(OBn)_2$

1	X
a	4 - OMe
b	3,4 - $(OMe)_2$
c	2,4 - $(OMe)_2$
d	3,4,5 - $(OMe)_3$
e	4 - OH
f	3,4 - $(OH)_2$

Chart 2

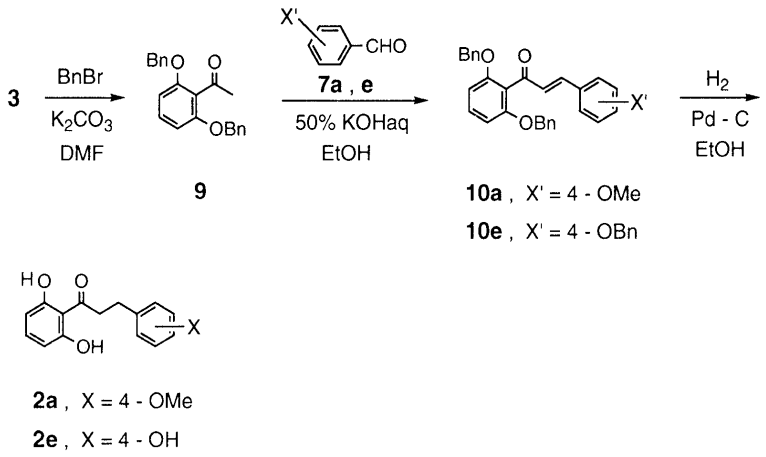


Chart 3

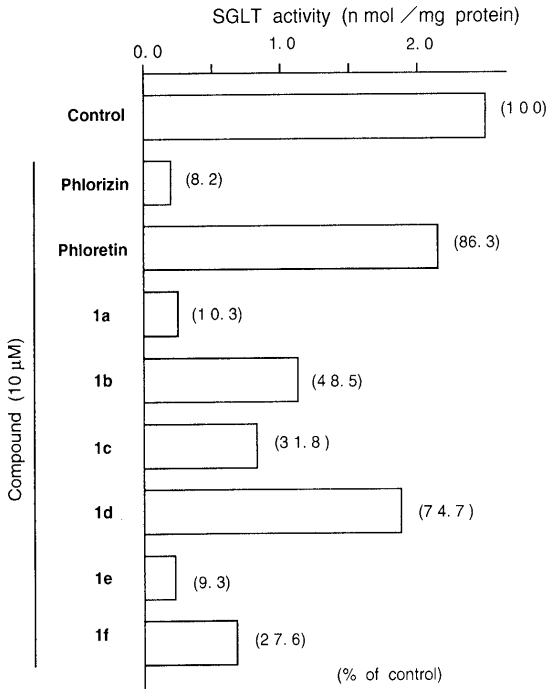


Fig. 1. Effect of 4'-Dehydroxyphlorizin Derivatives on Rat Kidney SGLT Activity

as shown in Chart 3. When the condensations of **3** with aldehydes were performed in aqueous EtOH containing KOH, none of the desired chalcone was obtained and **3** was decomposed. However, **9**, the dibenzyl ether of **3**, was condensed with **7a** or **7e** in an aqueous KOH solution and EtOH to give the chalcone **10a** (94%) or **10e** (91%), respectively. They were then hydrogenated to provide the dihydrochalcones **2a** (85%) and **2e** (82%), respectively.

Biological Results and Discussion

Using rat renal brush border membrane vesicles (BBMV), the inhibitory effects of the 4'-dehydroxyphlorizin derivatives **1a**—**f** on SGLT were studied, and the results are shown in Fig. 1. Compounds **1a** (4-OMe derivative) and **1e** (4-OH derivative) showed the most potent inhibitory effects (around 90% inhibition), comparable to that of phlorizin. Compounds **1c** (2,4-$(OMe)_2$ derivative) and **1f** (3,4-$(OH)_2$ derivative) produced about 70% inhibition. Compound **1b** (3,4-$(OMe)_2$ derivative) showed about 50% inhibition and **1d** (3,4,5-$(OMe)_3$ derivative) was less potent. Therefore, it became clear that the substituent groups on the B-ring contributed to the inhibitory effect.

Table 1. Effect of 4'-Dehydroxyphlorizin Derivatives on Urinary Glucose Excretion in Rats

Compound	Urinary glucose (mg/24 h)	
	10 mg/kg, i.p.	100 mg/kg, *p.o.*
Control	3± 1	3± 1
Phorizin	320±25	11± 6
1a	204± 8	380±52
1b	7± 2	2± 1
1c	5± 0	3± 1
1d	3± 0	2± 1
1e	329± 7	60± 9
1f	43±14	2± 1

Each value represents the mean ± S.E. ($n=3$).

Next, the effect of these derivatives on urinary glucose excretion was investigated. Normal rats were given the compounds orally or intraperitoneally and glucose in urine collected for 24 h was measured. The results are shown in Table 1. When given intraperitoneally at 10 mg/kg, **1a**, **e** and phlorizin induced marked urinary excretion of glucose, while **1f** showed a weak effect, and **1b**—**d** were inactive. The effects of these derivatives on urinary glucose excretion correlate with the SGLT-inhibitory activities, except in the case of **1c**. This result strongly suggests that urinary glucose excretion is induced by the inhibition of SGLT in the kidney. On the other hand, when the compounds were given orally at 100 mg/kg, phlorizin had little effect on urinary glucose level. This is reasonable, because phlorizin is hydrolyzed to glucose and phloretin by β-glucosidase in the small intestine. However, **1a** markedly increased urinary glucose and hence **1a** was considered to be more stable to β-glucosidase than phlorizin. Compound **1e** also moderately induced urinary glucose excretion, but the polysubstituted compounds **1b**—**d**, **f** had no effect.

The inhibitory effects of those derivatives on glucose uptake in the small intestine were studied using an enteric perfusion technique in normal rats. The results are shown in Table 2. Compounds **1a**, **e** had strong activities equivalent to that of phlorizin, and **1c**, **f** were less active.

Table 2. Inhibitory Effects of 4′-Dehydroxyphlorizin Derivatives on Glucose Uptake in Rat Small Intestine

Compound	Inhibition (%)
Phorizin	61.2±7.9**
1a	50.5±5.6**
1b	16.7±6.8
1c	42.8±3.6*
1d	11.4±5.2
1e	64.5±4.1*
1f	39.7±1.7**

Each value represents the mean±S.E. ($n=4$). * $p<0.05$, ** $p<0.01$ *vs.* control.

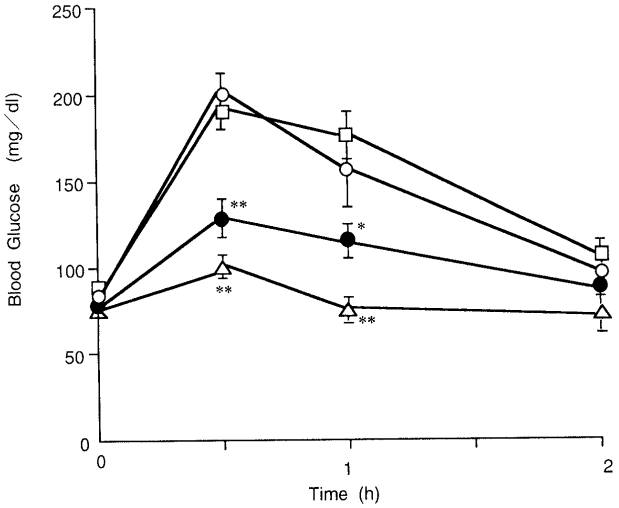


Fig. 2. Effect of 4′-Dehydroxyphlorizin Derivatives on Glucose Tolerance in Mice

Each sample was administered immediately before a subcutaneous load of glucose (1 g/kg). Points and bars represent the means and S.E. ($n=6$). ○, control; ●, **1a** (100 mg/kg, *p.o.*); □, **1d** (100 mg/kg, *p.o.*); △, phlorizin (30 mg/kg, i.p.). Significantly different from control (* $p<0.05$, ** $p<0.01$).

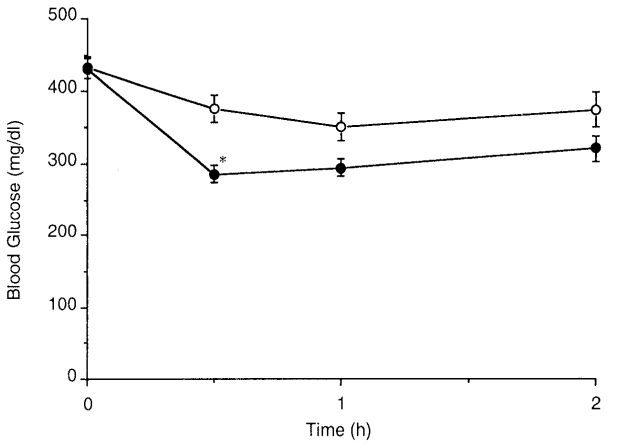


Fig. 3. Effect of Compound **1a** on Blood Glucose Level in Streptozotocin-Induced Diabetic Rats

Points and bars represent the means and S.E. ($n=5$). ○, control; ●, **1a** (100 mg/kg, *p.o.*). Significantly different from control (* $p<0.01$).

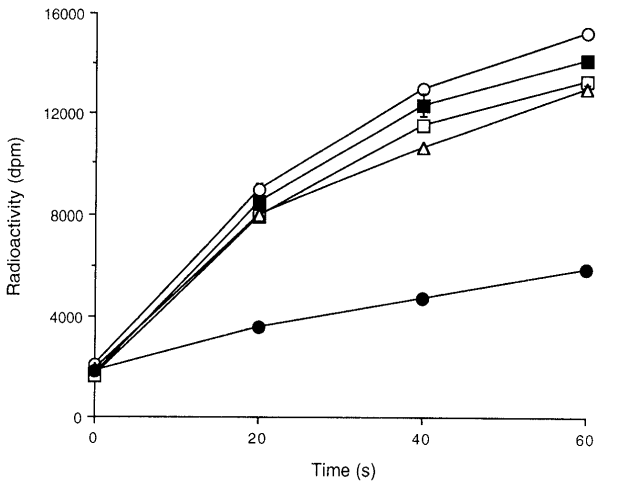


Fig. 4. Effect of Phloretin and Aglycons on Glucose Transport in Erythrocytes

○, control; ●, phloretin (10 μM); □, **2a** (10 μM); △, **2e** (10 μM); ■, **1a** (10 μM).

These results showed a good correlation to SGLT-inhibitory activity in the kidney and also to urinary glucose excretion after intraperitoneal administration, except in the case of **1c**.

Next, the effects of **1a, d** on glucose tolerance in mice were examined and the results are shown in Fig. 2. Phlorizin, the positive control, strongly lowered the blood glucose level at 30 mg/kg on intraperitoneal administration. On the other hand, **1a** which markedly induced urinary excretion of glucose, lowered the blood glucose level to a normal level on oral administration at 100 mg/kg. Compound **1d**, which had no effect on urinary glucose, showed no effect. These results suggest that **1a** lowered blood glucose level through enhancing urinary glucose excretion. Next, the effect of **1a** on the blood glucose concentration in streptozotocin (STZ)-induced diabetic rats was examined. The results are shown in Fig. 3. The oral administration of **1a** at 100 mg/kg lowered blood glucose to about 100 mg/dl 30 min after administration, and the effect lasted at least 2 h in STZ-induced diabetic rats.

The inhibitory effects of **2a, e** and phloretin, aglycons of **1a, e** and phlorizin, and **1a** itself on GLUT-1 in human erythrocytes were also studied. As shown in Fig. 4, **2a, e** and **1a** itself had only a weak GLUT-1-inhibitory effect but phloretin inhibited GLUT-1 very strongly. Rosenberg and Wilbrandt[9a] reported that the four hydroxy groups of phloretin were necessary to inhibit the glucose transporter in human erythrocytes; the activity was reduced markedly when even one of four hydroxy groups was methylated. Therefore, it is reasonable that **2a** and **2e**, as 4′-dehydroxyphloretin derivatives, had only weak activities.

It must be considered that these compounds, as inhibitors of SGLT on the renal tubule, might induce kidney damage. Phlorizin has been reported to induce kidney injury,[7a] though no details were given. Recently, Gouvea[16] studied the effect of phlorizin on gentamicin-induced renal damage and reported that phlorizin induced marked excretion of urinary glucose, but showed no adverse influence on renal function or morphology in rats when subcutaneously administered at massive doses for 15 successive days. In our study, **1a** produced no sign of renal damage when administered orally at 1 g/kg for 4 successive weeks in rats (data not shown). The inhibitory effect of phlorizin on SGLT has been reported to be reversibly antagonistic with respect to glucose.[7] Compounds that antagonistically and reversibly inhibit SGLT

may thus have no adverse effects on the kidney.

In conclusion, **1a** inhibited glucose uptake in the small intestine and glucose reuptake in the kidney through inhibition of SGLT on these organs when administered orally, so that **1a** was able to lower high blood glucose levels in normal mice and STZ-induced diabetic rats directly without mediation of its action by insulin. It did not induce either hypoglycemia or toxic signs, including renal damage in rats, and the GLUT-1-inhibitory effects of the aglycon **2a** and **1a** itself were very weak. Therefore, **1a** seems to meet the five criteria for SGLT inhibitors to be usable as antidiabetics (see Drug Design section). Compound **1a** is considered to be a suitable lead compound for new-type antidiabetics, and has been selected for further pharmacological evaluation.

Experimental

All melting points were determined on a Büchi 535 digital melting point apparatus and are uncorrected. Infrared (IR) spectra were taken on a Hitachi IR-215 or an Analect FX-6200 FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded on a Hitachi R-90H or a JEOL JNM-FX-200 spectrometer. Mass spectra were recorded on a Hitachi RMU-6 or a JEOL JMS-HX100 mass spectrometer. Microanalyses were performed on a Perkin–Elmer 2400 C, H, N analyzer.

2′,6′-Dihydroxyacetophenone 2′-*O*-(2,3,4,6-*O*-Tetraacetyl-*β*-D-glucopyranoside) (5) A mixture of 2′,6′-dihydroxyacetophenone **3** (50 g, 0.33 mol) and $CdCO_3$ (227 g, 1.32 mol) in toluene (4 l) was refluxed for 2 h with removal of the generated water in a Dean–Stark apparatus. Then acetobromoglucose **4** (270 g, 0.66 mol) was added and the whole was heated at reflux for 18 h. The hot mixture was filtered through a plug of Celite and the solid was washed with hot $CHCl_3$. The filtrate and the washing were combined and evaporated. The resultant residue was triturated in MeOH to provide a pale yellow solid, which was subjected to column chromatography to afford **5** (113 g, 71%) from the first eluate and the diglycoside **6** (21 g, 8%) from the second eluate. Compound **5**: colorless needles, mp 200.5—201.5 °C (lit.[13] mp 201—203 °C). IR (Nujol): 1760, 1740, 1630 cm^{-1}, ^{1}H-NMR (DMSO-d_6) δ: 1.96 (3H, s), 2.01 (6H, s), 2.02 (3H, s), 2.35 (3H, s), 4.05—4.30 (3H, m), 4.99 (1H, dd, J=8.9, 9.5 Hz), 5.05 (1H, dd, J=8.0, 9.7 Hz), 5.41 (1H, t, J=9.6 Hz), 5.55 (1H, d, J=8.1 Hz), 6.61 (1H, d, J=8.3 Hz), 6.63 (1H, d, J=8.3 Hz), 7.26 (1H, t, J=8.3 Hz), 10.81 (1H, s). FAB-MS m/z: 483 $(M+H)^+$. Compound **6**: colorless needles, mp 188—189.5 °C. IR (Nujol): 1750, 1740, 1710 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.96 (6H, s), 2.00 (6H, s), 2.01 (6H, s), 2.02 (6H, s), 2.21 (3H, s), 4.08 (2H, m), 4.22 (4H, m), 4.99 (4H, m), 5.39 (2H, t, J=9.6 Hz), 5.53 (2H, d, J=8.1 Hz), 6.90 (2H, d, J=8.5 Hz), 7.41 (1H, t, J=8.5 Hz). FAB-MS m/z: 835 $(M+Na)^+$.

2′,6′-Dihydroxy-4-methoxychalcone 2′-*O*-*β*-D-Glucopyranoside (8a) A solution of KOH (10 g) in H_2O (10 ml) was added to a suspension of **5** (9.65 g, 20 mmol) in EtOH (100 ml) and the mixture was stirred at room temperature for 10 min. Then *p*-anisaldehyde **7a** (3.27 g, 24 mmol) was added and the whole was stirred at room temperature for 18 h. The reaction mixture was acidified with 10% HCl to pH 5 under ice-cooling. The resulting yellow needles were collected by filtration, washed with water and then Et_2O, and dried to give **8a** (6.36 g, 74%), mp 136—140 °C (lit.[15] mp around 126 °C). IR (Nujol): 3540, 3480, 3320, 3240, 1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.10—3.40 (4H, m), 3.47 (1H, m), 3.69 (1H, ddd, J=1.7, 5.3, 11.5 Hz), 3.81 (3H, s), 4.58 (1H, t, J=5.7 Hz), 5.03 (1H, d, J=6.9 Hz), 5.04 (1H, d, J=5.5 Hz), 5.06 (1H, d, J=5.2 Hz), 5.09 (1H, d, J=5.3 Hz), 6.59 (1H, d, J=8.3 Hz), 6.72 (1H, d, J=8.1 Hz), 6.98 (2H, d, J=8.8 Hz), 7.29 (1H, t, J=8.3 Hz), 7.40 (1H, d, J=15.9 Hz), 7.47 (1H, d, J=16.0 Hz), 7.71 (2H, d, J=8.8 Hz), 11.02 (1H, s). FAB-MS m/z: 433 $(M+H)^+$.

2′,6′-Dihydroxy-4-methoxydihydrochalcone 2′-*O*-*β*-D-Glucopyranoside (1a) A solution of **8a** (7.30 g, 16.90 mmol) in EtOH (150 ml) was hydrogenated over 10% Pd–C (2.00 g) under H_2 (1 atm) at room temperature for 1 h. The catalyst was removed by filtration and the filtrate was concentrated to dryness. The residue was crystallized from EtOH–H_2O to give **1a** (6.57 g, 90%) as pale yellow needles, mp 105—107 °C (lit.[15] mp 106—108 °C). IR (Nujol): 3440, 3400, 3240, 1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.84 (2H, t, J=7.3 Hz), 3.19—3.49 (7H, m), 3.70 (1H, m), 3.71 (3H, s), 4.56 (1H, t, J=5.4 Hz), 4.91 (1H, d, J=7.3 Hz), 5.03 (1H, d, J=4.9 Hz), 5.10 (1H, d, J=4.4 Hz), 5.22 (1H, d, J=4.9 Hz), 6.55 (1H, d, J=8.3 Hz), 6.67 (1H, d, J=8.3 Hz), 6.81 (2H, d, J=8.8 Hz), 7.17 (2H, d, J=8.8 Hz), 7.24 (1H, t, J=8.3 Hz), 10.99 (1H, s). FAB-MS m/z: 435 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{26}O_9 \cdot 1/4H_2O$: C, 60.20; H, 6.08. Found: C, 60.36; H, 6.14.

Synthesis of 1a without Isolation of 8a **5** (3.31 g, 6.86 mmol) was condensed with **7a** (1.12 g, 8.23 mmol) by the same procedure as described for the synthesis of **8a**. The reaction mixture containing **8a** was diluted with water and washed with toluene to remove excess **7a**. The aqueous layer was hydrogenated over 10% Pd–C (0.15 g) at room temperature for 2 h. The catalyst was removed by filtration and the filtrate was neutralized with 10% HCl and extracted with AcOEt. The organic layer was washed with water and dried over $MgSO_4$. The solvent was removed and the residue was crystallized from EtOH–H_2O to give **1a** (2.31 g, 78%), which was identical with the sample obtained as described above.

Compounds **1b—f** were prepared in the same manner as described above.

1b: Yield 51% as pale yellow needles, mp 176—178.5 °C (MeOH). IR (Nujol): 3560, 3490, 3460, 1620 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.84 (2H, t, J=7.6 Hz), 3.10—3.55 (7H, m), 3.70 (3H, s), 3.71 (1H, m), 3.73 (3H, s), 4.56 (1H, t, J=5.6 Hz), 4.92 (1H, d, J=6.8 Hz), 5.03 (1H, d, J=4.9 Hz), 5.09 (1H, d, J=4.4 Hz), 5.22 (1H, d, J=4.9 Hz), 6.55 (1H, d, J=8.3 Hz), 6.68 (1H, d, J=8.3 Hz), 6.74 (1H, dd, J=2.0, 8.3 Hz), 6.78 (1H, d, J=8.3 Hz), 6.84 (1H, d, J=2.0 Hz), 7.25 (1H, t, J=8.3 Hz), 11.06 (1H, s). FAB-MS m/z: 465 $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{28}O_{10}$: C, 59.48; H, 6.08. Found: C, 59.54; H, 6.17.

1c: Yield 69% as pale yellow needles, mp 86—89 °C (AcOEt–iso-Pr_2O). IR (Nujol): 3400, 1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.78 (2H, t, J=7.3 Hz), 3.10—3.55 (7H, m), 3.70 (1H, m), 3.72 (3H, s), 3.76 (3H, s), 4.55 (1H, t, J=5.6 Hz), 4.91 (1H, d, J=7.3 Hz), 5.02 (1H, d, J= 4.9 Hz), 5.08 (1H, d, J=3.9 Hz), 5.12 (1H, d, J=4.9 Hz), 6.41 (1H, dd, J=2.0, 7.8 Hz), 6.50 (1H, d, J=2.0 Hz), 6.55 (1H, d, J=8.3 Hz), 6.67 (1H, d, J=8.3 Hz), 7.06 (1H, d, J=7.8 Hz), 7.24 (1H, t, J=8.3 Hz), 11.01 (1H, s). FAB-MS m/z: 465 $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{28}O_{10} \cdot 1/2H_2O$: C, 58.35; H, 6.17. Found: C, 58.56; H, 6.13.

1d: Yield 54% as a white powder, mp 136.5—139 °C (Et_2O). IR (Nujol): 3420, 1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.81 (2H, t, J=7.1 Hz), 3.10—3.55 (7H, m), 3.70 (1H ,m), 3.73 (3H, s), 3.75 (3H, s), 3.77 (3H, s), 4.54 (1H, t, J=5.6 Hz), 4.91 (1H, d, J=6.8 Hz), 5.02 (1H, d, J=4.9 Hz), 5.08 (1H, d, J=3.9 Hz), 5.14 (1H, d, J=4.9 Hz), 6.55 (1H, d, J=7.8 Hz), 6.66 (1H, d, J=2.4 Hz), 6.70 (1H, d, J=2.9 Hz), 6.90 (1H, d, J=8.3 Hz), 7.24 (1H, t, J=8.3 Hz), 10.94 (1H, s). FAB-MS m/z: 495 $(M+H)^+$. *Anal.* Calcd for $C_{24}H_{30}O_{11}$: C, 58.29; H, 6.12. Found: C, 58.22; H, 6.11.

1e: Yield 62% as pale yellow needles, mp 133—135 °C (EtOH–H_2O) (lit.[13] mp 134—136 °C). IR (Nujol): 3520, 3400, 1620 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.78 (2H, t, J=7.6 Hz), 3.20 (2H, t, J=7.6 Hz), 3.10—3.55 (5H, m), 3.70 (1H, dd, J=4.6, 11.0 Hz), 4.56 (1H, t, J=5.6 Hz), 4.91 (1H, d, J=6.8 Hz), 5.03 (1H, d, J=4.9 Hz), 5.09 (1H, d, J=3.9 Hz), 5.22 (1H, d, J=4.9 Hz), 6.54 (1H, d, J=8.3 Hz), 6.64 (2H, d, J=8.8 Hz), 6.67 (1H, d, J=8.3 Hz), 7.03 (2H, d, J=8.3 Hz), 7.24 (1H, t, J=8.3 Hz), 9.09 (1H, br), 11.00 (1H, br). FAB-MS m/z: 421 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{24}O_9 \cdot 1/2H_2O$: C, 58.74; H, 5.87. Found: C, 58.93; H, 5.87.

1f: Yield 52% as a pale yellow powder, mp 78—80 °C (AcOEt–iso-Pr_2O). IR (Nujol): 3380, 1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.72 (2H, t, J=7.6 Hz), 3.10-3.55 (7H, m), 3.70 (1H, d, J=11.7 Hz), 4.55 (1H, br), 4.90 (1H, d, J=7.3 Hz), 4.80-5.40 (3H, br), 6.45—6.62 (4H, m), 6.67 (1H, d, J=8.3 Hz), 7.24 (1H, t, J=8.3 Hz), 8.61 (2H, br), 11.02 (1H, br). FAB-MS m/z: 437 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{24}O_{10} \cdot 1/4H_2O$: C, 57.21; H, 5.60. Found: C, 57.38; H, 5.90.

2′,6′-Dibenzyloxyacetophenone (9) A mixture of **3** (15.22 g, 100 mmol), benzyl bromide (68.42 g, 400 mmol) and K_2CO_3 (138 g, 1 mol) in *N*,*N*-dimethylformamide (DMF) (300 ml) was stirred at 80 °C for 2.5 h. The mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was dissolved in AcOEt, and the solution was washed with water and brine, dried, and evaporated *in vacuo*. The residue was crystallized from toluene–hexane to afford **9** (29.63 g, 89%) as colorless needles, mp 68.5—70 °C. IR (Nujol): 1700 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.49 (3H, s), 5.08 (4H, s), 6.59 (2H, dd, J=0.4, 8.3 Hz), 7.35 (11H, m). MS m/z: 332 (M^+).

2′,6′-Dibenzyloxy-4-methoxychalcone (10a) A mixture of **9** (3.32 g, 10 mmol), **7a** (1.63 g, 12 mmol), 50% aqueous KOH (5 ml) and EtOH (50 ml) was stirred at room temperature for 3 h. The mixture was evaporated and diluted with water. The precipitated solid was collected

by filtration, washed with water, dried, and recrystallized from EtOH–Et_2O to give **10a** (4.26 g, 94%) as yellow crystals, mp 120—121 °C. IR (Nujol): 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.82 (3H, s), 5.08 (4H, s), 6.61 (2H, d, J=8.4 Hz), 6.78—7.48 (17H, m). MS m/z: 450 (M^+).

2′,4,6′-Tribenzyloxychalcone (10e) **10e** was prepared by the condensation of **9** (2.02 g, 6.08 mmol) with **7e** (1.55 g, 7.30 mmol) in the same manner as described for the synthesis of **10a**. Yellow crystals (2.91 g, 91%, recrystallized from AcOEt–Et_2O), mp 122—125 °C. IR (Nujol): 1670 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.07 (4H, s), 5.08 (2H, s), 6.61 (2H, d, J=8.6 Hz), 6.68 (1H, d, J=16.0 Hz), 6.95 (2H, d, J=9.0 Hz), 7.12—7.47 (19H, m). FAB-MS m/z: 527 $(M+H)^+$.

2′,6′-Dihydroxy-4-methoxydihydrochalcone (2a) A solution of **10a** (3.30 g, 7.32 mmol) in EtOH (50 ml) and AcOEt (50 ml) was hydrogenated over 10% Pd–C (0.83 g) under H_2 (1 atm) at room temperature for 3 h. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was crystallized from toluene–hexane to give **2a** (1.69 g, 85%) as pale yellow prisms, mp 128—131 °C. IR (Nujol): 3260, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.97 (2H, t, J=7.6 Hz), 3.43 (2H, t, J=7.6 Hz), 3.79 (3H, s), 6.38 (2H, d, J=8.3 Hz), 6.83 (2H, ddd, J=2.0, 2.7, 8.8 Hz), 7.16 (2H, dd, J=2.4, 8.3 Hz), 7.22 (1H, t, J=8.3 Hz), 9.51 (2H, s). MS m/z: 272 (M^+). *Anal.* Calcd for $C_{16}H_{16}O_4$: C, 70.58; H, 5.92. Found: C, 70.51; H, 5.83.

2′,4,6′-Trihydroxydihydrochalcone (2e) A solution of **10a** (2.87 g, 5.46 mmol) in tetrahydrofuran (THF) (50 ml) was hydrogenated over 10% Pd–C (0.5 g) under H_2 (1 atm) at room temperature for 2.5 h. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was crystallized from iso-Pr_2O–toluene to give **2e** (1.15 g, 82%) as pale yellow crystals, mp 169—172.5 °C (lit.[17] mp 155.5—156 °C, from aqueous EtOH). IR (Nujol): 3280, 1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.79 (2H, t, J=7.6 Hz), 3.29 (2H, t, J=7.8 Hz), 6.37 (2H, d J=8.3 Hz), 6.67 (2H, d, J=8.8 Hz), 7.03 (2H, d, J=8.3 Hz), 7.23 (1H, t, J=8.3 Hz), 9.14 (1H, s), 11.67 (2H, s). MS m/z: 258 (M^+). *Anal.* Calcd for $C_{15}H_{14}O_4$: C, 69.76; H, 5.46. Found: C, 69.79; H, 5.64.

Inhibition on Rat Kidney SGLT Activity Male Sprague–Dawley (SD) rats (10—13 weeks old) were used. Brush border membrane vesicles (BBMV) from the rat kidney were prepared according to the method of Nagasawa *et al.*[18] The protein concentration was measured by use of a Coomassie protein assay kit (Pierce) and diluted with buffer A (10 mM Hepes/Tris (pH 7.4), 100 mM mannitol) to 4 mg/ml protein concentration.

Assay tubes containing 50 μl of BBMV suspension (0.2 mg, protein) and 100 μl of buffer A containing 1% (v/v) of DMSO or DMSO solution of the test compounds (final concentration, 10 μM) were preincubated at 37 °C for 2 min. Then 50 μl of buffer A containing 0.4 mM D-glucose (final concentration, 0.1 mM), 400 mM NaSCN (final concentration, 100 mM), and 20 μCi/ml [^{3}H]glucose (final radioactivity, 1 μCi) was added. The assay tubes were incubated at 37 °C for 5 s. The incubation was terminated by addition of 1.5 ml of ice-cold stopping solution (10 mM Hepes/Tris (pH 7.4), 150 mM NaCl, 0.3 mM phlorizin) followed by rapid filtration through a membrane filter (nitrocellulose, 25 mm ϕ, pore size 0.45 μm, Advantec). The filter was washed with 4.5 ml of the stopping solution. Then the radioactivity of the pellet was measured with a liquid scintillation counter (Tricarb 2200CA, Packard).

Measurement of Urinary Glucose Excretion Male SD rats (6 weeks old) were used. Test compounds were administered twice, with an 8 h interval, at 10 mg/kg, i.p. or at 100 mg/kg, *p.o.* The volume of injection was kept at 5 ml/kg and an equal volume of the vehicle was given to the control group. Urine was collected for 24 h after first administration and urinary glucose was measured by use of a glucose analyzer (Apec).

Inhibition of Glucose Uptake in the Small Intestine Male SD rats (6—7 weeks old) were starved overnight. The glucose absorption in the small intestine was measured by the circulating method described by Kawaguchi.[19] Rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and the abdomen was opened lengthwise. The small intestine at 2 cm from Treitz' ligament was cannulated to introduce the circulating solution and the tube was connected with a peristaltic pump. The small intestine at 25 cm from that position toward the anus was cannulated to remove the circulating solution and the abdomen was closed. The circulating solution (5 mM glucose containing 0.9% NaCl and 1% DMSO, 20 ml) with or without a test compound (0.5 mM) was kept at 38 °C and circulated at 4 ml/min after flushing with 0.9% saline for 30 min. One drop of circulating solution was sampled at 0, 15, 30, 45, and 60 min, and the concentration of glucose was measured by glucose oxidase assay (new blood sugar test, Boehringer Mannheim). The inhibition (%) was calculated from the following equation.

$$\text{inhibition } (\%) = (A - B)/A \times 100$$

where A is the mean rate of glucose absorption from the circulating solution without test compound and B is that with test compound.

Blood Glucose-Reducing Effect in Glucose Tolerance Test Male ddY mice (8 weeks old) were starved overnight. Test compounds (100 mg/kg, *p.o.*) or phlorizin (30 mg/kg, i.p.) were administered immediately before subcutaneous loading of glucose (1 g/kg). The control group received glucose only. Blood was sampled from the tail vein at 0.5, 1, and 2 h later. Blood glucose levels were measured with the new blood sugar test (Boehringer Mannheim).

Effect of Compound 1a on Blood Glucose Level in STZ-Induced Diabetic Rats Male SD rats (6 weeks old) were starved overnight. Streptozotocin was administered at 50 mg/kg, i.v. After 1 week, compound **1a** (100 mg/kg, *p.o.*) was administered and blood was sampled from the vein at 0.5, 1, and 2 h later. Blood glucose levels were measured with the new blood sugar test (Boehringer Mannheim).

Glucose Transport in Erythrocytes Fresh human blood from healthy donors was used. The erythrocytes were made substantially glucose-free by washing them four times with 10 volumes of phosphate-buffered saline (PBS; Dulbecco's formula without magnesium and calcium) and then resuspended in PBS to give a hematocrit value of 20%. The cell suspension (100 μl) was placed in a centrifuge tube and 100 μl of PBS containing 1 mM D-[^{3}H]glucose (18.5 kBq), 2 mM D-glucose and test compound (20 μM) was added with mixing. After incubation of the tube for 0—60 s at 4 °C, 1 ml of ice-cold stopping solution (0.3 mM phloretin/PBS) was added. Erythrocytes were sedimented by centrifugation and washed once with the stopping solution. The pellet was lysed with 100 μl of H_2O and deproteinized with 0.6 ml of 5% trichloroacetic acid. An aliquot (0.4 ml) of supernatant was neutralized with 3 M NaOH and the radioactivity was measured by liquid scintillation counting.

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