Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 3445–3449
Bioorganic & Medicinal Chemistry

Na^+-Glucose cotransporter (SGLT) inhibitory flavonoids from the roots of *Sophora flavescens*

Seizo Sato,* Jiro Takeo, Chihiro Aoyama and Hiroyuki Kawahara

Central Research Laboratory, Nippon Suisan Kaisha, Ltd, 559-6 Kitano-machi, Hachioji, Tokyo 192-0906, Japan

Received 6 February 2007; revised 5 March 2007; accepted 5 March 2007
Available online 12 March 2007

Abstract—The methanol extract of *Sophora flavescens*, which is used in traditional Chinese medicine (sophorae radix), showed potent Na^+-glucose cotransporter (SGLT) inhibitory activity. Our search for active components identified many well-known flavonoid antioxidants: kurarinone, sophoraflavanone G, kushenol K, and kushenol N.

1. Introduction

During our search for the primary compounds of antidiabetic medicines from natural sources, we focused our attention on traditional Chinese medicine. Current antidiabetic medicines may be subdivided into six groups: insulin, sulfonylureas, α-glucosidase inhibitors, biguanides, meglitinides, and thiazolidinediones. However, these groups are insufficient, given the great diversity of patients. Thus, we sought inhibitors which would inhibit a novel site, the Na^+-glucose cotransporter (SGLT).

SGLT is a membrane protein that plays an important role in the reabsorption of glucose in the kidneys. SGLT is known to have three isoforms (SGLT1, SGLT2, and SGLT3).[1–3] SGLT1 is expressed primarily in the brush border membrane of mature enterocytes in the small intestine, where it absorbs dietary glucose and galactose from the gut lumen.[4] SGLT2 is only expressed in the renal cortex, where it is assumed to be present in the brush border membrane of the S1 and S2 segments of the proximal tubule, and to be responsible for the reabsorption of glucose from the glomerular filtrate.[4] It is expected that the inhibition of SGLT could decrease glucose reabsorption and that this could thus result in an increase in urinary sugar excretion, and a decrease in blood glucose level. Thus, SGLT inhibitors have therapeutic potential for type 2 diabetes.[5]

Our screening study on SGLT inhibitors in traditional Chinese medicine discovered that the methanol extract of *Sophora flavescens* shows potent SGLT inhibitory activity. The roots of *S. flavescens* are used as a traditional Chinese medicine (sophorae radix), and have various physiological activities including antioxidant, antibacterial, anti-inflammatory, antipyretic, antiarrhythmic, antiasthmatic, anti-ulcerative, and antineoplastic effects. It is used to treat jaundice, leucorrhea, carbuncles, pyogenic infections of the skin, scabies, enteritis, and dysentery.[6] In previous studies, quinolizidine alkaloids, flavonoids, benzofuran, and triterpenoid have been isolated from the roots of *S. flavescens*.[7–17] Additionally, a recent study by Kim et al. found that some lavandulyl flavanones from this species inhibit α-glucosidase and β-amylase.[18] Thus, many researchers have examined the secondary metabolites of this species and have reported intriguing results.

In the present study, we found that some lavandulyl flavanones inhibit SGLT1 and SGLT2. In the present paper, we describe the isolation of SGLT inhibitors, specifically, lavandulyl flavanones and isoflavonoids, and report their inhibitory activity.

2. Results and discussion

2.1. Extraction and isolation

The dried and chopped roots (1.0 kg) of *S. flavescens* were extracted with MeOH for three days at room temperature. The combined extracts were concentrated in

Keywords: *Sophora flavescens*; Na^+-Glucose cotransporter; SGLT; Lavandulyl flavanone.
* Corresponding author. Tel.: +81 42 656 5192; fax: +81 42 656 5188; e-mail: s-satou@nissui.co.jp

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.03.011

vacuo, and the resulting aqueous suspension was partitioned between H_2O and EtOAc. The EtOAc extract (22 g) was divided into ten fractions (Fr. 1–Fr. 10) by reverse-phase column chromatography (eluent: 50% MeOH/H_2O to MeOH). The 10 fractions were further divided into several subfractions, after which we monitored the TLC spots and ^{1}H NMR signals. This procedure, that is, fractionation followed by estimation of the ^{1}H NMR signals, was repeated until the isolation of four pure compounds (**1**–**4**) was achieved from Fr. 3: maackiain (**1**, 14.7 mg), pterocarpin (**2**, 2.2 mg), variabilin (**3**, 1.4 mg), and formononetin (**4**, 13.8 mg). Additionally, five pure compounds (**5**–**9**) were isolated from Fr. 5–Fr. 9: (−)-kurarinone (**5**, 488.2 mg), kushenol N (**6**, 30.2 mg), kushenol K (**7**, 21.5 mg), sophoraflavanone G, (**8**, 372.4 mg), and kuraridin (**9**, 19.9 mg). Compounds **1**–**9** (Fig. 1) were identified by spectroscopic analyses such as NMR and MS, and their optical rotations were found to be identical to those given in previously published data.[19–23]

2.2. Biological activity

The SGLT inhibitory activities of nine isolated compounds (**1**–**9**) against [^{14}C]methyl-α-D-glucopyranoside uptake in COS-1 cells expressing hSGLT1 or hSGLT2 were evaluated. Four compounds (**5**, **6**, **8**, and **9**) showed strong inhibitory activity against SGLT1 and SGLT2 at 50 μM (Table 1). The IC_{50} values of two of these, (−)-kurarinone (**5**) and sophoraflavanone G (**8**), on SGLT inhibitory activities are shown in Table 2.

As shown in Table 1, isoflavonoids tended to be more potent in their inhibition of SGLT2 than SGLT1. This result suggests that good inhibitory activity against SGLT2 requires a hydroxyl group in the structures.

As shown in Tables 1 and 2, flavonoids show inhibitory activity against SGLT1 and SGLT2. Furthermore, flavonoid inhibitors also tended to be more potent in their inhibition of SGLT2 than SGLT1. The replacement of C-5 in **5** by the hydroxyl group did not affect inhibitory activity **5** [(SGLT1: IC_{50} = 10.4 μM; SGLT2: IC_{50} = 1.7 μM), **8** (SGLT1: IC_{50} = 18.7 μM; SGLT2: IC_{50} = 4.1 μM)], while a saturated sample of C-4a/C-5a (**7**) and a hydroxylation of C-3 (**6** and **7**) led to a decrease in inhibitory activity [**6** (SGLT1: inhibition rate, 58.4%, 50 μM; SGLT2: inhibition rate, 87.3%, 50 μM), **7** (SGLT1: inhibition rate, 29.7%, 50 μM; SGLT2: inhibition rate, 43.7%, 50 μM)]. These results indicate that the lavandulyl group and the dehydroxylation of C-3 in the flavanone skeleton assume a crucial

Table 1. Effects of flavonoids on SGLT inhibitory activity

Compound	Inhibition rate[a] (%)	
	SGLT-1	SGLT-2
1	NI[b]	66.5
2	NI	NI
3	NI	49.9
4	NI	75.4
5	98.8	99.7
6	58.4	87.3
7	29.7	43.7
8	99.2	100
9	71.1	100

[a] All compounds were evaluated at 50 μM.
[b] No inhibition, or less than 25% inhibition.

Table 2. IC_{50} values of active compounds

Compound	IC_{50}	
	SGLT1 (μM)	SGLT2 (μM)
(−)-Kurarinone (**5**)	10.4	1.7
Sophoraflavanone G (**8**)	18.7	4.1
Phlorizin[a]	0.2	0.1

[a] A well-known SGLT inhibitor.

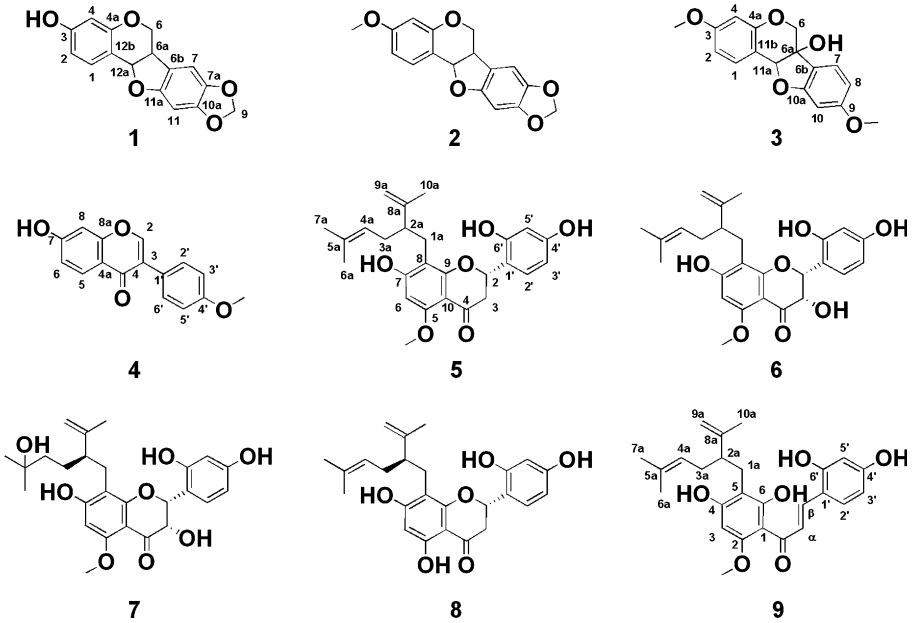

Figure 1. Flavonoids isolated from *Sophora flavescens*.

role in SGLT inhibitory activities. Furthermore, the expression of good inhibitory activity against SGLTs requires a methoxy group of C-5 in the flavanone skeleton.

In a recent report, it was found that the glucosidase-inhibitory activities of lavandulyl flavanoids indicated that the lavandulyl group in the flavanone skeleton assumed a crucial role in glucosidase-inhibitory activities.[13] These results are very interesting in terms of compounds that may be appropriate for an antidiabetes model.

In conclusion, we have identified seven flavonoids (**1**, **3**–**8**), and a 5-lavandulyl chalcone (**9**) from *S. flavescens* that potently inhibited SGLT1 and SGLT2. These flavonoids more potently inhibited SGLT2 than SGLT1. Furthermore, SGLT1-inhibitory flavanones (**5**, **6**, **8**) and the chalcone of **5** share a common functional group in their structures, the lavandulyl group of C-8 or C-5 (chalcone). These results suggest that the lavandulyl group in the flavanone skeleton plays a crucial role in SGLT1-inhibitory activity. In addition, SGLT2-inhibitory isoflavonoids require a hydroxyl group in their structures, and lavandulyl flavanones require a dehydroxy group of C-3. Furthermore, the methoxy group in the flavanone skeleton of C-5 may augment inhibitory activities against SGLTs. Therefore, it may be useful to investigate these compounds for the possible development of novel inhibitors of SGLTs as an antidiabetic medicine.

3. Experimental

3.1. Chemistry

NMR spectra were recorded with a DPX400 (Bruker BioSpin K.K., Ibaraki, Japan) instrument (^{1}H, 400 MHz; ^{13}C, 100 MHz). Chemical shift data were calculated from the residual solvent signals of δ_H 3.30 and δ_C 49.0 ppm in methanol-d_4. MS spectra were recorded with a GCMS-QP2010 (Shimadzu Co., Kyoto, Japan) instrument (ionization, electron impact). Optical rotations were recorded with a DIP-370 (JASCO Co., Tokyo, Japan) instrument. The following experimental conditions were used for chromatography: reverse-phase column chromatography, Chromatorex DM1020T (Fuji Silysia Chemical, Ltd, Aichi, Japan); TLC, precoated TLC plates with silica gel 60 F_{254} (Merck, 0.25 mm, 0.5 mm thickness) and RP-18 F_{254S} (Merck, 0.2 mm thickness). TLC spots were detected by a UV lamp (254 nm). The mobile phase of chromatography was carried out using high quality reagents from Kanto Chemical Co., Inc. (Tokyo, Japan).

3.1.1. Maackiain (1). ^{1}H NMR (400 MHz, δ methanol-d_4 ppm, J in Hz): 3.43 (1H, m), 3.52 (1H, m), 4.19 (1H, m), 5.42 (1H, br s), 5.85 (1H, br s), 6.29 (1H, d, J = 2.5), 6.36 (1H, s), 6.47 (1H, dd, J = 8.5, 2.5), 6.78 (1H, s), 7.24 (1H, d, J = 8.5). ^{13}C NMR (100 MHz, methanol-d_4 ppm): 41.6 (C-6a), 67.4 (C-6), 80.1 (C-12a), 94.2 (C-11), 102.5 (C-9), 104.1 (C-4), 106.0 (C-7), 110.8 (C-2), 112.7 (C-12b), 119.8 (C-6b), 133.1 (C-1), 143.1 (C-7a), 149.5 (C-10a), 155.6 (C-11a), 158.1 (C-4a), 160.4 (C-3). EI-MS (m/z): 284 (M^+), 267, 197, 162, 134.

3.1.2. Pterocarpin (2). ^{1}H NMR (400 MHz, δ methanol-d_4 ppm, J in Hz): ^{1}H NMR (400 MHz, δ methanol-d_4 ppm, J in Hz): 3.54 (1H, m), 3.57 (1H, m), 3.84 (3H, s), 4.31 (1H, m), 5.50 (1H, br s), 5.86 (1H, br s), 6.37 (1H, s), 6.68 (1H, d, J = 8.9), 6.81 (1H, s), 6.90 (1H, d, J = 8.9). ^{13}C NMR (100 MHz, methanol-d_4 ppm): 41.6 (C-6a), 55.8 (C-3 –OCH_3), 67.4 (C-6), 80.1 (C-12a), 94.2 (C-11), 102.5 (C-9), 103.9 (C-4), 106.0 (C-7), 110.2 (C-2), 112.7 (C-12b), 119.8 (C-6b), 133.2 (C-1), 143.1 (C-7a), 149.5 (C-10a), 155.6 (C-11a), 158.1 (C-4a), 162.1 (C-3). EI-MS (m/z): 298 (M^+), 267, 197, 162, 134.

3.1.3. Variabilin (3). $[\alpha]_D^{23}$ −435.6° (c 0.10, methanol). ^{1}H NMR (400 MHz, δ methanol-d_4 ppm, J in Hz): 3.74 (3H, s), 3.75 (3H, s), 3.95 (1H, d, J = 11.4), 4.13 (1H, d, J = 11.4), 5.23 (1H, s), 6.37 (1H, d, J = 2.3), 6.42 (1H, d, J = 2.3), 6.52 (1H, dd, J = 8.2, 2.2), 6.63 (1H, dd, J = 8.6, 2.2), 7.24 (1H, d, J = 8.2), 7.36 (1H, d, J = 8.6). ^{13}C NMR (100 MHz, methanol-d_4 ppm): 55.8 (C-3 –OCH_3), 55.9 (C-9 –OCH_3), 70.9 (C-6), 77.2 (C-6a), 86.0 (C-11a), 97.6 (C-10), 102.4 (C-4), 108.2 (C-8), 110.2 (C-2), 114.1 (C-11b), 122.4 (C-6b), 125.1 (C-7), 133.2 (C-1), 157.4 (C-4a), 157.5 (C-10a), 162.2 (C-3), 163.8 (C-9). EI-MS (m/z): 300 (M^+), 285, 272, 255, 241.

3.1.4. Formononetin (4). ^{1}H NMR (400 MHz, δ methanol-d_4 ppm, J in Hz): 3.81 (3H, s), 6.63 (1H, d, J = 2.3), 6.79 (1H, dd, J = 2.3, 9.0), 6.96 (2H, dd, J = 8.9, 2.0), 7.44 (2H, dd, J = 8.9, 2.0), 7.93 (1H, d, J = 9.0), 8.05 (1H, s). ^{13}C NMR (100 MHz, δ methanol-d_4 ppm): 55.7 (C-7 –OCH_3), 104.0 (C-8), 114.8 (C-3′, 5′), 125.1 (C-4a), 125.2 (C-3), 127.6 (C-5), 131.4 (C-2′, 6′), 154.1 (C-2), 160.9 (C-7), 178.1 (C-4). EI-MS (m/z): 268 (M^+), 253, 225, 132, 117.

3.1.5. (−)-Kurarinone (5). $[\alpha]_D^{23}$ −45.7° (c 0.10, methanol). ^{1}H NMR (400 MHz, δ methanol-d_4 ppm, J in Hz): 1.46 (3H, s), 1.55 (3H, s), 1.62 (3H, s), 1.99 (2H, m), 2.48 (1H, m), 2.61 (2H, m), 2.68 (1H, dd, J = 16.8, 2.8), 2.86 (1H, dd, J = 16.8, 13.3), 3.79 (3H, s), 4.50 (1H, s), 4.56 (1H, s), 4.94 (1H, m), 5.53 (1H, dd, J = 13.3, 2.8), 6.32 (1H, d, J = 2.4), 6.34 (1H, dd, J = 8.0, 2.4), 7.29 (1H, d, J = 8.0). ^{13}C NMR (100 MHz, δ methanol-d_4 ppm): 17.6 (C-7a), 18.9 (C-10a), 25.7 (C-6a), 27.9 (C-1a), 32.1 (C-3a), 45.3 (C-3), 48.4 (C-2a), 55.7 (C-5 –OCH_3), 75.2 (C-2), 93.0 (C-6), 103.1 (C-5′), 105.5 (C-10), 107.4 (C-3′), 109.3 (C-8), 111.0 (C-9a), 118.2 (C-1a), 124.6 (C-4a), 128.3 (C-2′), 131.8 (C-5a), 149.5 (C-8a), 156.4 (C-6′), 159.2 (C-4′), 161.6 (C-5), 164.5 (C-9), 164.6 (C-7), 193.7 (C-4). EI-MS (m/z): 438 (M^+), 422, 299, 179, 153.

3.1.6. Kushenol N (6). $[\alpha]_D^{23}$ −3.3° (c 0.10, methanol). ^{1}H NMR (400 MHz, δ methanol-d_4 ppm, J in Hz): 1.47 (3H, s), 1.54 (3H, s), 1.56 (3H, s), 1.99 (1H, m), 2.48 (1H, m), 2.61 (2H, m), 3.79 (3H, s), 4.49 (1H, d, J = 12.0), 4.50 (1H, s), 4.56 (1H, s), 4.94 (1H, m), 5.29 (1H, d, J = 12.0), 6.03 (1H, s), 6.32 (1H, d, J = 2.4), 6.34 (1H, dd, J = 8.0, 2.4), 7.27 (1H, d, J = 8.0). ^{13}C

NMR (100 MHz, δ methanol-d_4 ppm): 17.9 (C-7a), 19.1 (C-10a), 25.7 (C-6a), 25.9 (C-1a), 32.4 (C-3a), 48.1 (C-2a), 55.8 (C-5 –OCH_3), 73.4 (C-3), 78.6 (C-2), 94.6 (C-6), 103.6 (C-5′), 105.5 (C-10), 107.7 (C-3′), 110.3 (C-8), 111.0 (C-9a), 116.2 (C-1a), 124.6 (C-4a), 128.3 (C-2′), 131.8 (C-5a), 149.5 (C-8a), 156.4 (C-6′), 159.2 (C-4′), 161.6 (C-5), 164.5 (C-9), 164.6 (C-7), 193.7 (C-4). EI-MS (*m*/*z*): 454 (M^+), 436, 313, 285, 179.

3.1.7. Kushenol K (7). $[\alpha]_D^{23}$ −32.6° (*c* 0.10, methanol). ^{1}H NMR (400 MHz, δ methanol-d_4 ppm, *J* in Hz): 1.01 (6H, s), 1.21 (1H, m), 1.31 (1H, m), 1.33 (2H, m), 1.57 (3H, s), 2.38 (1H, m), 2.53 (1H, m), 3.79 (3H, s), 4.49 (1H, d, *J* = 12.0), 4.55 (1H, s), 4.60 (1H, s), 5.28 (1H, d, *J* = 12.0), 6.01 (1H, s), 6.32 (1H, d, *J* = 2.4), 6.34 (1H, dd, *J* = 8.0, 2.4), 7.27 (1H, d, *J* = 8.0). ^{13}C NMR (100 MHz, δ methanol-d_4 ppm): 17.9 (C-7a), 19.1 (C-10a), 25.7 (C-6a), 25.9 (C-1a), 32.4 (C-3a), 48.1 (C-2a), 55.8 (C-5 –OCH_3), 73.4 (C-3), 78.6 (C-2), 94.6 (C-6), 103.6 (C-5′), 105.5 (C-10), 107.7 (C-3′), 110.3 (C-8), 111.0 (C-9a), 116.2 (C-1a), 124.6 (C-4a), 128.3 (C-2′), 131.8 (C-5a), 149.5 (C-8a), 156.4 (C-6′), 159.2 (C-4′), 161.6 (C-5), 164.5 (C-9), 164.6 (C-7), 193.7 (C-4). EI-MS (*m*/*z*): 472 (M^+), 454, 436, 313, 284, 270.

3.1.8. Sophoraflavanone G (8). $[\alpha]_D^{23}$ −31.8° (*c* 0.10, methanol). ^{1}H NMR (400 MHz, δ methanol-d_4 ppm, *J* in Hz): 1.47 (3H, s), 1.55 (3H, s), 1.62 (3H, s), 1.99 (2H, m), 2.49 (1H, m), 2.56 (2H, m), 2.71 (1H, dd, *J* = 17.1, 2.8), 2.97 (1H, dd, *J* = 17.1, 13.3), 4.51 (1H, s), 4.57 (1H, s), 4.96 (1H, m), 5.54 (1H, dd, *J* = 13.3, 2.8), 6.33 (1H, d, *J* = 2.3), 6.34 (1H, dd, *J* = 8.2, 2.3), 7.29 (1H, d, *J* = 8.2). ^{13}C NMR (100 MHz, δ methanol-d_4 ppm): 17.8 (C-7a), 19.1 (C-10a), 25.9 (C-6a), 27.9 (C-1a), 32.3 (C-3a), 43.3 (C-3), 48.4 (C-2a), 75.7 (C-2), 96.2 (C-6), 103.2 (C-5′), 103.3 (C-10), 107.6 (C-3′), 108.6 (C-8), 111.2 (C-9a), 118.3 (C-1a), 124.8 (C-4a), 128.6 (C-2′), 132.0 (C-5a), 149.7 (C-8a), 156.6 (C-6′), 159.5 (C-4′), 162.6 (C-5), 163.1 (C-9), 166.5 (C-7), 199.0 (C-4). EI-MS (*m*/*z*): 424 (M^+), 406, 301, 283, 165.

3.1.9. Kuraridin (9). ^{1}H NMR (400 MHz, δ methanol-d_4 ppm, *J* in Hz): 1.55 (3H, s), 1.64 (3H, s), 1.69 (3H, s), 2.06 (2H, m), 2.55 (1H, m), 2.62 (1H, m), 3.89 (3H, s), 4.56 (2H, m), 5.04 (1H, t, *J* = 7.1), 5.99 (1H, s), 6.33 (1H, d, *J* = 2.3), 6.34 (1H, dd, *J* = 8.2, 2.3), 7.39 (1H, d, *J* = 8.2), 7.92 (1H, d, *J* = 15.7), 7.99 (1H, d, *J* = 15.7). ^{13}C NMR (100 MHz, δ methanol-d_4 ppm): 17.9 (C-7a), 18.7 (C-10a), 25.7 (C-6a), 27.9 (C-1a), 32.1 (C-3a), 48.4 (C-2a), 55.8 (C-2 –OCH_3), 94.4 (C-3), 103.6 (C-3′), 107.4 (C-1), 109.8 (C-5′), 109.9 (C-5), 111.1 (C-9a), 115.5 (C-1′), 125.0 (C-4a), 126.3 (C-α), 131.8 (C-6′), 132.6 (C-5a), 140.6 (C-β), 150.0 (C-8a), 161.1 (C-2′), 162.6 (C-2′), 163.0 (C-4′), 164.0 (C-2), 164.8 (C-4), 167.4 (C-6), 192.1. EI-MS (*m*/*z*): 438 (M^+), 422, 299, 179, 153.

3.2. Biology

3.2.1. Chemicals. Methyl-α-D-glucopyranoside and phlorizin were purchased from Sigma–Aldrich (St. Louis, MO, USA), and methyl (α-D-[U-^{14}C]gluco) pyranoside was purchased from Amersham Biosciences (Piscatway, NJ, USA).

3.2.2. Plasmid construction. To construct the expression vectors, cDNA clones of human SGLT1[24] and SGLT2[25] were amplified by polymerase chain reaction (PCR) using a sense primer homologous to the NH_2-terminal end of the coding sequence and an antisense primer homologous to the COOH-terminal end from human intestine and kidney cDNA libraries. In addition, the coding sequences in each primer were flanked by an *Eco*RI site for hSGLT1 and hSGLT2. The resultant products were cleaved with *Eco*RI to yield fragments of 1995 bp (hSGLT1) or 2038 bp (hSGLT2), and subcloned into *Eco*RI-digested pBluescript SK+ (Stratagene) to yield pSK-hSGLT1 and pSK-hSGLT2, respectively. Following verification of their sequences, the *Eco*RI fragments were excised and subcloned into *Eco*RI-digested pcDNA3.1 (+) (Invitrogen) to yield pCMV-hSGLT-1 and pCMV-hSGLT-2, respectively.

3.2.3. Uptake of methyl-α-D-glucopyranoside in cultured cells expressing SGLT1 or SGLT2. COS-1 cells were cultured at 37 °C in Dulbecco's modified Eagle's/Ham's F-12 medium (1:1) supplemented with 10% fetal calf serum. For the uptake assay, the cells were plated at 1×10^5 cells/24-well plate (Asahi Techno Glass, Tokyo, Japan), and 1 μg of each transporter plasmid was transfected into subconfluent cultures of COS-1 cells using Lipofectamine 2000 (Invitrogen). The cells were used 2–3 days after transfection. They were incubated in a pretreatment buffer [140 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes/Tris (pH 7.5)] at 37 °C for 30 min. An uptake solution containing 80 mM methyl-α-D-glucopyranoside and 4 μCi/ml methyl α-D-[U-^{14}C]glucopyranoside was then added into each well and the mixture was incubated at 37 °C for 30 min. Following incubation, the plates were washed three times with cold stop buffer [140 mM choline chloride, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes/Tris (pH 7.5)] containing 300 μM phlorizin. The cells were then solubilized with 0.1 M NaOH, and their radioactivity was measured with a liquid scintillation counter (3100TR, Perkin-Elmer).

References and notes

1. Lee, W.-S.; Kanai, Y.; Wells, R. G.; Hediger, M. A. *J. Biol. Chem.* **1994**, *269*, 12032.
2. You, G.; Lee, W.-S.; Barros, E. J. G.; Kanai, Y.; Huo, T.-L.; Khawaja, S.; Wells, R. G.; Nigam, S. K.; Heidiger, M. A. *J. Biol. Chem.* **1995**, *270*, 29365.
3. Mackenzie, B.; Panayotova-Heiermann, M.; Loo, D. D. F.; Lever, J. E.; Wright, E. M. *J. Biol. Chem.* **1994**, *269*, 22488.
4. Wright, E. M.; Turk, E. *Pfugers Arch.* **2004**, *447*, 510.
5. Katsuno, K.; Fujimori, Y.; Takemura, Y.; Hitatochi, M.; Itho, F.; Komatsu, Y.; Fujikura, H.; Isaji, M. *J. Pharm. Exp. Ther.* **2007**, 320.
6. Chang, H. M.; But, P. P. H. In *Pharmacology and Applications of Chinese Materia Medica*; World Scientific Publishers: Philadelphia, 1986; Vol. 1, pp 736–737.
7. Ueno, A.; Morinaga, K.; Okuda, S. *Chem. Pharm. Bull.* **1978**, *26*, 1832.
8. Wu, L. J.; Miyase, T.; Ueno, A.; Kuroyanagi, M.; Noro, T.; Fukushima, S. *Chem. Pharm. Bull.* **1985**, *33*, 3231.

9. Kyogoku, K.; Hatayama, K.; Komatsu, M. *Chem. Pharm. Bull.* **1973**, *21*, 2733.
10. Yamamoto, H.; Ichimura, M.; Tanaka, T.; Iinuma, M.; Mizuno, M. *Phytochemistry* **1991**, *30*, 1732.
11. Yamahara, J.; Mochizuki, M.; Fujimura, T.; Takaishi, Y.; Yoshida, M.; Tomimatsu, T.; Tamai, Y. *J. Ethnopharmacol.* **1990**, *29*, 173.
12. Ding, Y.; Tian, R. H.; Kinjo, J.; Nohara, T.; Kitagawa, I. *Chem. Pharm. Bull.* **1992**, *40*, 2990.
13. Woo, E. R.; Kwak, J. H.; Kim, H. J.; Park, H. *J. Nat. Prod.* **1998**, *61*, 1552.
14. Kang, T. H.; Jeong, S. J.; Ko, W. G.; Kim, N. Y.; Lee, B. H.; Inagaki, M.; Miyamoto, T.; Higuchi, R.; Kim, Y. C. *J. Nat. Prod.* **2000**, *63*, 680.
15. Son, J. K.; Park, J. S.; Kim, J. A.; Kim, Y.; Chung, S. R.; Lee, S. H. *Planta Med.* **2003**, *69*, 559.
16. Kim, S. J.; Son, K. H.; Chang, H. W.; Kang, S. S.; Kim, H. P. *Biol. Pharm. Bull.* **2003**, *26*, 1348.
17. Ding, P.; Chen, D.; Bastow, K. F.; Nyarko, A. K.; Wang, X.; Lee, K. H. *HelV. Chim. Acta* **2004**, *87*, 2574.
18. Kim, J. H.; Ryu, Y. B.; Kang, N. S.; Lee, B. W.; Heo, J. S.; Jeong, I.-Y.; Park, K. H. *Biol. Pharm. Bull.* **2006**, *29*, 302.
19. Ryu, S. Y.; Lee, H. Y.; Kim, Y. K.; Kim, S. H. *Arch. Pharm. Res.* **1997**, *20*, 491.
20. Aratanechemuge, Y.; Hibasami, H.; Katsuzaki, H.; Imai, K.; Komiya, T. *Oncol. Rep.* **2004**, *12*, 1183.
21. Kang, T.-H.; Jeong, S.-J.; Ko, W.-G.; Kim, N.-Y.; Lee, B.-H.; Inagaki, M.; Miyamoto, T.; Higuchi, R.; Kim, Y.-C. *J. Nat. Prod.* **2000**, *63*, 680.
22. Jung, M. J.; Kang, S. S.; Jung, H. A.; Kim, G. J.; Choi, J. S. *Arch. Pharm. Res.* **2004**, *27*, 593.
23. Ingham, J. L.; Markham, K. R. *Phytochemistry* **1980**, *19*, 1203.
24. Hediger, M. A.; Turk, E.; Wright, E. M. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5748.
25. Wells, R. G.; Pajor, A. M.; Kanai, Y.; Turk, E.; Wright, E. M.; Hediger, M. A. *Am. J. Physiol.* **1992**, *263*, F459.