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Pyrazole-*O*-Glucosides as Novel Na^+-Glucose Cotransporter (SGLT) Inhibitors

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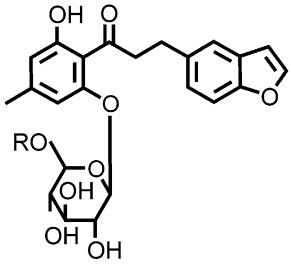
Abstract—*O*-glucuronides and *O*-glucosides of a series of pyrazoles analogues were synthesized and evaluated for their SGLT inhibitory activity in brush border membrane vehicles (BBMVs) of rat kidney. *O*-glucosides of certain pyrazole analogues inhibited the transport of [^{14}C]-glucose in BBMVs, and induced glucosuria in Wistar rats by intravenous injection.

Introduction

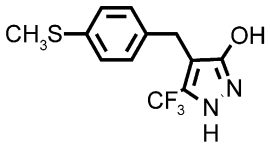
Na^+-glucose cotransporter (SGLT) is a membrane protein that plays an important role in the reabsorption of glucose in the kidney. It has been reported that there are at least three isoforms of SGLTs (SGLT1, SGLT2 and SGLT3).[1–3] SGLT2 mainly exists in renal uriniferous tubule and SGLT1 exists in the kidney and the intetestine. Glucose that is filtered through glomeruli is reabsorbed at the renal uriniferous tubules via SGLT1 and SGLT2. It is expected that the inhibition of SGLT at the kidney could decrease glucose reabsorption and this could result in the increase of urinary sugar excretion and decrease of blood glucose level. Thus, SGLT inhibitors are thought to be promising candidates for diabetes treatment.

Tsujihara et al. reported a SGLT inhibitor T-1095A (**1**) and its prodrug T-1095 (**2**) as anti-diabetic agents (Fig. 1).[4–6] T-1095A lowers blood glucose level by inhibiting SGLT2 in the kidney, leading to increase urinary glucose excretion.

We were interested in this mechanism of action and started searching for new anti-diabetic agents that have



R=H T-1095A (1)
R=$COOCH_3$ T-1095 (2)



WAY-123783 (3)

Figure 1.

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SGLT2 inhibitory activity. By database searching, we found WAY-123783 (**3**), which was reported to increases glucosuria and lower blood glucose levels in db/db mice. (Fig. 1).[7,8] The authors suggested that WAY-123783 was a SGLT2 inhibitor in their report.

To clarify the profile of WAY123783 (**3**) and related compounds, we synthesized **3** and WAY-2-12 (**7**) and tested their activity in several assays. Oral administration of **3** increased urinary glucose excretion in mice but did not induce the same effect in rats. In addition, compound **3** did not inhibit the transport of [^{14}C]-glucose in BBMVs of rat kidney at all.[5]

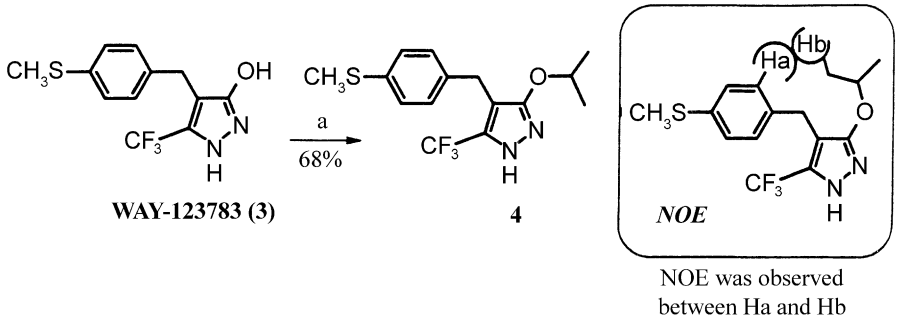
From these results, we hypothesized that WAY-123783 was converted to a metabolite after oral administration in mice and this metabolite induced glucosuria in vivo. Several polar metabolites were observed in mice plasma after oral administration of WAY-123783 by HPLC analysis. From mass spectrum, one of the metabolites showed the molecular weight of a glucuronide.

It has been shown that phenolic compounds can be metabolized to give the corresponding glucuronides.[9] Furthermore, metabolic glucosidation of phenolic compounds has also been reported.[10] Since SGLT2 recognizes glucose as a substrate, we thought it is possible that sugar analogues such as the glucuronide and glucoside of WAY-123783 could inhibit SGLT2. Therefore, we synthesized these compounds and tested their SGLT inhibitory activity in vitro and in vivo.

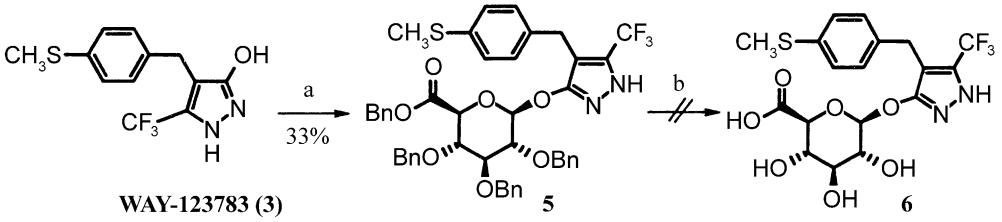
Synthesis

We attempted to synthesize the glucuronide and glucoside of WAY-123783 by the Mitsunobu procedure.[11] Phenolic OH and a nitrogen atom in the pyrazole ring of WAY-123783 were thought to be the reaction site. First, we examined the regioselectivity of the Mitsunobu reaction. Treatment of a THF solution of WAY-123783 and isopropanol with ethyl azodicarboxylate and triphenylphosphine provided single product **4** in 68%. To elucidate the position of isopropyl group, we used NOE method. Cross peak was obtained between the proton on the benzene ring (Ha) and the proton on the isopropyl group (Hb). Compound **4** obtained was confirmed as an *O*-isopropyl analogue (Scheme 1).

Synthesis of a glucuronide of WAY-123783 is shown in Scheme 2. By using the Mitsunobu procedure, glucuronide **5** was obtained in 33%. Debenzylation of **5** with

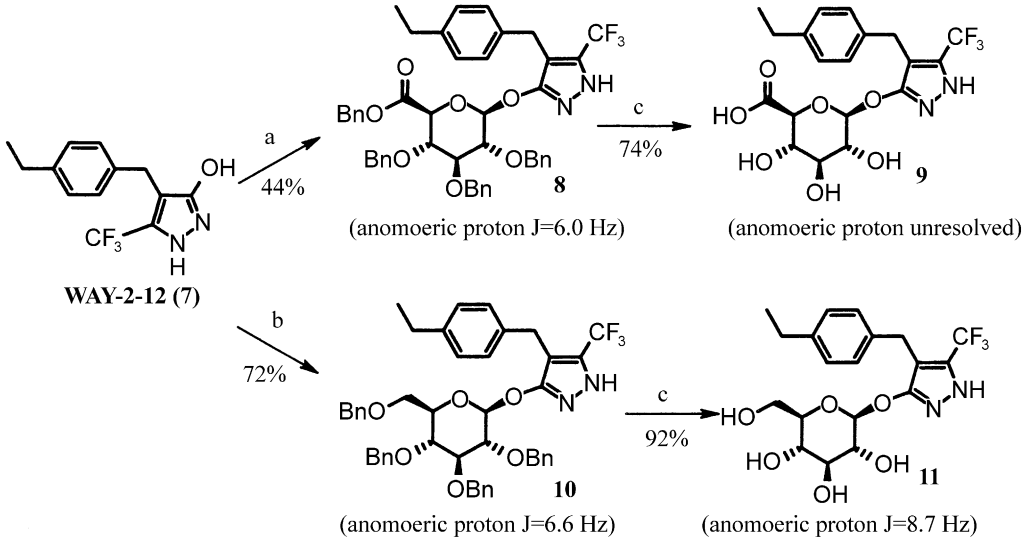


Scheme 1. Reaction of WAY-123783 and isopropanol by Mistunobu reaction. (a) Isopropanol, 40% DEAD/toluene, PPh_3.



Scheme 2. Synthesis of *O*-glucuronide of WAY-123783. (a) 2,3,4-*O*-tribenzyl-D-glucopyranosideuronic acid benzylester, PPh_3, 40% DEAD/toluene THF; (b) 20% Pd $(OH)_2$, MeOH-EtOAc.

Figure 2.



Scheme 3. Synthesis of *O*-glucuronide and *O*-glycoside of WAY-2-12. (a) 2,3,4-*O*-tribenzyl-D-glucopyranosideuronic acid benzylester, 40% DEAD/toluene, PPh_3, THF; (b) 2,3,4,6-tetrabenzyl-D-glucopyranose, 40% DEAD/toluene, PPh_3, THF; (c) 20% Pd $(OH)_2$ MeOH–EtOAc.

Table 1. Effect of pyrazole analogues on rat kidney SGLT inhibitory activity

Compd	Inhibition rate (%)
9	30% (100 μM)
11	84% (10 μM)
T-1095A **(1)**	90% (10 μM)
WAY-123783 **(3)**	Not active (100 μM)

Inhibition of [^{14}C]-glucose uptake in BBMV's of rat kidney.
Each value represents the mean of three experiments.

Table 2. Effect of pyrazole analogues on urinary glucose excretion in rat

Compd	Glucosuria (mg/24 h)
	iv (3 mg/kg)
9	2.6 mg
11	63 mg
T-1095A **(1)**	300 mg
WAY-123783 **(3)**	0 mg

Each value represents the mean of three experiments.

20%Pd $(OH)_2$ was sluggish, and did not give the desired product **6**. The thiomethyl group on the benzene ring was thought to diminish the activity of the palladium catalyst, so we used WAY-2-12 (**7**) instead of WAY-123783 (**3**). WAY-2-12 has a 4-ethyl group in place of the thiomethyl group of WAY-123783. In Wyeth-Ayerst's report, WAY-2-12 showed as potent an anti-diabetic effect as WAY-123783 (Fig. 2).[8]

WAY-2-12 was glucuronidated by the Mitsunobu procedure to give benzyl-protected glucuronide derivative **8** in 44%. The stereochemistry of **8** at the anomeric center was assigned as beta by NMR studies. ($J_{\text{anomeric H}} = 6.0$ Hz). Glucuronide **8** obtained was successfully debenzylated with 20%Pd$(OH)_2$ under a hydrogen atmosphere to give compound **9** in 74%.[12]

In a similar fashion, WAY-2-12 was converted to benzyl-protected glucoside derivative **10** in 72%. The stereochemistry of **10** at the anomeric center was assigned as beta by NMR studies. ($J_{\text{anomeric H}} = 6.6$ Hz). This glucoside **10** was debenzylated with 20%Pd $(OH)_2$ to give the desired glucoside **11** in 92% (Scheme 3).[13]

Biological Result

The SGLT inhibitory activity of *O*-glucuronide **9** and *O*-glucoside **11** against [^{14}C]-glucose transport in BBMVs of rat kidney was evaluated.[4,14] *O*-glucuronide **9** showed weak inhibitory activity against rat kidney BBMVs (30% inhibition at 100 μM). On the other hand, *O*-glucoside **11** showed potent inhibitory activity. (84% inhibition at 10 μM). T-1095A, an active form of T-1095 showed potent inhibitory activity (90% inhibition at 10 μM) (Table 1).

Next, the effect on urinary glucose excretion activity of *O*-glucuronide **9** and *O*-glucoside **11** were evaluated in Wistar rats by intravenous injection.[6,15] 3 mg/kg of *O*-glucuronide **9** induced only marginal urinary sugar excretion (2.6 mg). On the other hand, 3 mg/kg of *O*-glucoside **11** induced 63 mg of urinary sugar excretion. At the same dose, T-1095A induced 300 mg of glucosuria in Wistar rats (Table 2).

Conclusion

We have synthesized *O*-glucoside and *O*-glucuronide of WAY-123783 analogue based on the hypothesis that WAY-123783 shows SGLT2 inhibitory activity after metabolism in mice. We found that glucoside **11** was a potent inhibitor of SGLT in rat kidney BBMVs. Intravenous injection of glucoside analogue **11** increased urinary sugar excretion in Wistar rats. This glucoside is thought to be a promising candidate as an anti-diabetic agent. Although, glucoside **11** showed inhibitory activity as potent as T-1095A in rat kidney BBMVs, it showed only one-fifth the effect of T-1095A on urinary glucose

excretion in rats. This discrepancy was thought to be due to the difference in stability between glucoside **11** and T-1095A in rats. Optimization of *O*-glucoside **11** is now underway to improve stability and selectivity against SGLTs.

During the course of this work, compound **11** was claimed for SGLT inhibitors (WO 0116147). To date, details of the method of discovery have not been published.

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12. **4-(4-Ethylphenyl)methyl-5-trifluoromethyl-1H-pyrazol-3-yl-β-D-glucopyranoside uronic acid 9** A mixture of 2,3,4-tri-*O*-benzyl-D-glucopyranoside uronic acid benzyl ester (199 mg, 0.36 mmol),1,2-dihydro-4-(ethylphenyl)methyl-5-trifluoromethyl-3H-pyrazol-3-one **7** (99 mg, 0.37 mmol), triphenylphosphine (109 mg, 0.42 mmol) and anhydrous THF (0.5 mL) were stirred in ice-water bath. To this solution, 40% ethyl azodicarboxylate/toluene (0.18 mL, 0.40 mmol) was added dropwise. After 1.5 h, the reaction mixture was purified by silica-gel chromatography (20% EtOAc–hexane) to give benzyl 4′-(4′-ethylphenyl) methyl-5′-trifluoromethyl-1H-pyrazol-3′-yl-2,3,4-*O*-tribenzyl-β-D-glucopyranouronate (127mg, 44%) **8** as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22–7.36 (16H, m), 7.08–7.18 (4H, m), 7.07 (2H, d, $J=8.7$), 7.00 (2H, d, $J=8.7$), 5.24 (1H, anomeric d, $J=6.0$), 5.15 (1H, d, $J=12.0$), 5.10 (1H, d, $J=12.0$), 4.76 (1H, d, $J=11.4$), 4.70 (1H, d, $J=11.4$), 4.69 (1H, d, $J=11.1$), 4.54 (1H, d, $J=11.1$), 4.49 (1H, d, $J=11.1$), 4.46 (1H, d, $J=11.1$), 4.12 (1H, d, $J=9.6$), 3.98 (1H, t, $J=8.4$), 3.80 (2H, d, $J=3.0$), 3.76-3.84 (2H, brd), 3.71 (1H, t, $J=7.5$), 3.67 (1H, t, $J=6.9$), 2.49 (2H, q, $J=7.5$), 1.12 (3H, t, $J=7.5$). Compound **8** (122 mg, 0.15 mmol) was hydrogenated over 20% $Pd(OH)_2$ (200 mg) in EtOAc (4 mL)-methanol (4 mL) for 8 h. The catalyst was removed by filtration,. The filtrate was purified by SepPack column (10% MeOH/H_2O to 100% MeOH) to give desired product **9** as white solid (22 mg, 33%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.09 (2H, d, $J=8.4$ Hz), 7.06 (2H, d, $J=8.4$ Hz), 5.00–5.20 (1H, br), 3.70–3.90 (3H, m), 3.52–3.65 (1H, m), 3.35–3.51 (2H, m), 2.58 (2H, q, $J=7.5$ Hz), 1.19 (3H, t, $J=7.5$ Hz), ESI-MS(*m*/*z*) 445[(M-H)$^-$], 447[(M + H)$^+$].
13. **4-(4-Ethylphenyl)methyl-5-trifluoromethyl-1H-pyrazol-3-yl-*O*-β-D-glucopyranoside 11**. A mixture of 2,3,4,6-*O*-tetrabenzyl-D-glucopyranose (107 mg, 0.39 mmol) 1,2-dihydro-4-(ethylphenyl)methyl-5-trifluoromethyl-3H-pyrazol-3-one **7** (214 mg, 0.39 mmol) triphenylphosphine (102 mg, 0.39 mmol) and anhydrous THF (5 mL) were stirred in ice-water bath. To this solution, 40% Ethyl azodicarboxylate/toluene (0.175 mL, 0.39 mmol) was added dropwise. After stirring for 24 h, the reaction mixture was purified by silica-gel chromatography (25% EtOAc–hexane) to give 4′-(4′-ethylphenyl)methyl-5′-trifluoromethyl-1H-pyrazol-3′-yl-*O*-2,3,4,6-*O*-tetrabenzyl-β-D-glucopyranoside **10** (223 mg, 72%) as oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98–7.34 (24H, m), 5.09 (1H, anomeric, d, $J=6.6$), 4.46–4.88 (8H, m), 3.81 (2H, s), 3.56–3.74 (6H, m), 2.53 (2H, q, $J=7.5$), 1.14 (3H, t, $J=7.5$). ESI-MS (*m*/*z*) 791 [(M–H)$^-$], 793[(M + H)$^+$].

Compound **10** (220 mg, 0.28 mmol) was hydrogenated with 20% $Pd(OH)_2$ (100 mg) in EtOAc (4 mL)-methanol (4 mL) for 1.5 h. The catalyst was removed by filtration, and the filtrate was evaporated. The residue was purified by silica gel chromatography (10% MeOH–CH_2Cl_2) to give desired product **11** as white solid (110 mg, 92%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.08 (4H, s), 5.11(1H, brs), 5.02(1H, brs), 4.89 (1H, anomeric d, $J=8.7$), 3.74 (2H, s), 3.66 (1H, d, $J=12.0$), 3.48 (1H, dd, $J=5.1$, 12.0), 3.10-3.24 (4H, br), 2.53 (2H, q, $J=7.5$), 1.13 (3H, t, $J=7.5$). ESI-MS (*m*/*z*) 431 [(M–H)$^-$], 863 [(2M–H)$^-$].
14. Rat kidney BBMVs assay method

Brush border membrane vesicles (BBMVs) were prepared by the Ca^{2+} precipitation method from kidney of male Wistar rats (8–10 weeks old). The test compounds, D-glucose (0.2 mM), [^{14}C]-D-glucose (0.1 μCi) and NaCl (100 mM) were added and incubated with BBMV's for one min. The glucose uptake reaction was terminated by addition of 1mM phlorizin in 40 mM Hepes–Tris (pH 7.4). The vehicles were immediately centrifuged and filtered, then the radioactivity on the membrane was measured by a liquid scintillation counter.
15. Urinary glucose excretion in rats

Test compounds were dissolved in DMSO and diluted by saline. The compounds were intravenously administered to male Wistar rats (4 week old, Charles River Japan, Tokyo, Japan) housed in metabolic cages. Twenty-four hours after the administration, urine was collected and urine volume was measured. Glucose concentration in urine was measured using an auto-analyzer (FUJI DRI-CHEM, Fuji Photo Film Co., Ltd, Tokyo, Japan).