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Discovery of non-glucoside SGLT2 inhibitors

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ABSTRACT

A series of benzothiazinone and benzooxazinone derivatives were discovered as SGLT2 inhibitors. The optimization led to the discovery of compounds **31** and **32**, which exhibited similar potency and better SGLT1 selectivity compared to dapagliflozin. These compounds may provide novel promising scaffolds, which are different from phlorizin-based SGLT2 inhibitors.

Sodium glucose co-transporter 2 (SGLT2) is primarily expressed in the S1 segment of renal proximal tubules and accounts for 90% of renal glucose reabsorption.[1] Inhibition of SGLT2 prevents renal glucose reuptake from the glomerular filtrate and subsequently lowers the blood glucose level. The efficacy and safety of this insulin independent treatment has been validated by human genetic evidence and late stage clinical trials with SGLT2 inhibitors. Human with genetic defects of SGLT2 excrete as much as 140 g/day of glucose in urine (180 g/day filtered in the kidney), but live a normal lives without adverse effect on carbohydrate metabolism.[2] Phase II and III clinical trials of dapagliflozin, the leading SGLT2 inhibitor in development, showed dose-dependent increases in urinary glucose excretion, reduction in fasting and postprandial blood glucose levels and moderate weigh loss.[3] All the evidence has demonstrated SGLT2 to be the promising target for treatment of type 2 diabetes mellitus.

Another well-characterized subtype in the renal glucose reuptake is SGLT1, which is responsible for the other 10% of renal glucose reabsorption. In addition to its expression in the kidney, SGLT1 is also highly expressed in small intestine and responsible for absorption of dietary glucose and galactose. Inhibition of SGLT1 is likely to cause severe side effect, as demonstrated by mutations of SGLT1, which have been associated with glucose-galactose malabsorption syndrome in humans. The syndrome is characterized by severe diarrhea and dehydration on a glucose or galactose-containing diet.[4] Therefore achieving SGLT1 selectivity for SGLT2 is important.

With few exceptions,[5–7] most of the reported SGLT2 inhibitors are glucoside derivatives and can be traced back to a naturally-occurring *O*-aryl glucoside phlorizin (**1**). It is a fairly potent inhibitor of SGLT2 (IC_{50} = 36 nM), with some selectivity against SGLT1 (IC_{50} = 330 nM).[8] It is well documented that administration of **1** caused glucosuria in humans and animals.[9] However, the lack of selectivity and metabolic instability prevent its further application. Currently, dapagliflozin (**2**),[3] BI10773,[10] and canagliflozin[11] as *C*-aryl glucosides are undergoing phase III clinical trials. Several other SGLT2 inhibitors, including ASP194,[12] TA-7284,[13] CSG452[14] and LX4211[15,16] are in phase II clinical trials.

It is somewhat puzzling to see the overwhelming majority of the reported SGLT2 inhibitors are phlorizin related glucosides. They may be unique for SGLT2 inhibition, but structurally diverse SGLT2 inhibitors may offer unforeseen advantages. In this Letter, we describe our efforts to discover a series of novel non-glucoside SGLT2 inhibitors with high potency and SGLT1 selectivity. To our knowledge, this is the first description of an industry effort from HTS to optimization for non-glucoside SGLT2 inhibitors in a peer reviewed Letter (see Fig. 1).

High-throughput screening identified compound **3** as a SGLT2 inhibitor (Table 1). Compound **3** inhibited transportation of [^{14}C]-AMG[17] into SGLT2 expressed CHO cell with IC_{50} of 1.4 μM for human, 0.4 μM for rat and 1.2 μM for mouse. The selectivity of SGLT2 versus SGLT1 is less than 25-fold across different species as shown in Table 1. Herein, we describe our lead optimization efforts to improve both activity and selectivity of compound **3**.

A series of compound **3** analogues was prepared as illustrated in Scheme 1. Compound **5** was synthesized by heating hydrazinecarbothioamide **4** and 2-hydroxyacetate in the presence of base. Alkylation of **5** led to key intermediate **6**, which was then

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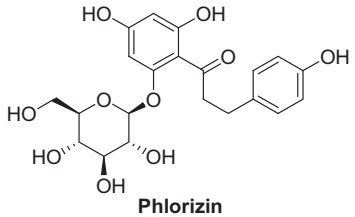
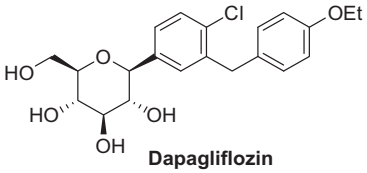


Phlorizin

1

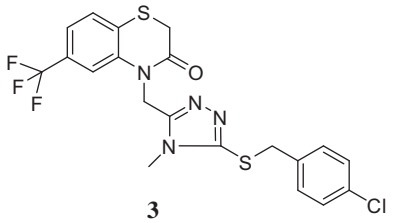


Dapagliflozin

2

Figure 1.

Table 1
Activity of the HTS hit (**3**) across species



3

Species	hSGLT2 inhibition IC_{50}[a] (μM)	hSGLT1 inhibition IC_{50}[a] (μM)
Human	1.40	7.00
Rat	0.40	9.00
Mouse	1.20	3.00

[a] Inhibition of [^{14}C]AMG transportation into the CHO cells that express hSGLT2 or hSGLT1. Assay conducted in a buffer containing 0.1% BSA. See Ref.[17] for assay protocol. Values are means of three experiments, standard deviation is ±30%.

reacted with benzothiazinone or benzooxazinnone to provide compounds **3**, **7**–**10**, **15**–**22**.

In addition, the synthesis of another series of analogues with different substituents on the right-hand side is shown in Scheme 2. Acid **11** was coupled with amines to provide various amides, which were then converted to intermediates **12** by reacting with Lawesson's reagent. Cyclization with formic acid hydrazide yielded **13**. The installation of hydroxymethyl group was achieved by heating paraformaldehyde with **13**. Final compounds **23**–**32** were obtained using previously described Mitsunobu conditions (step c of Scheme 1).

Initially, N-substitutions of the triazole were explored. Replacement of methyl by ethyl resulted in 3-fold of increased activity of SGLT2 and slightly improved selectivity versus SGLT1. Larger groups, such as phenyl, cyclopropyl and isopropyl were also tolerated (Table 2, **8**–**10**).

Compound **3** showed high metabolic instability with less than 2% remaining after incubation in human or rat microsomes for 30 min. In order to increase the microsomal stability, the replacement of the sulfur atoms was studied. We were pleased to discover that benzothiazinone can be replaced by benzooxazinone without a loss in activity (compare **7** and **15** in Table 3). Therefore, benzooxazinones were used for subsequent SAR studies.

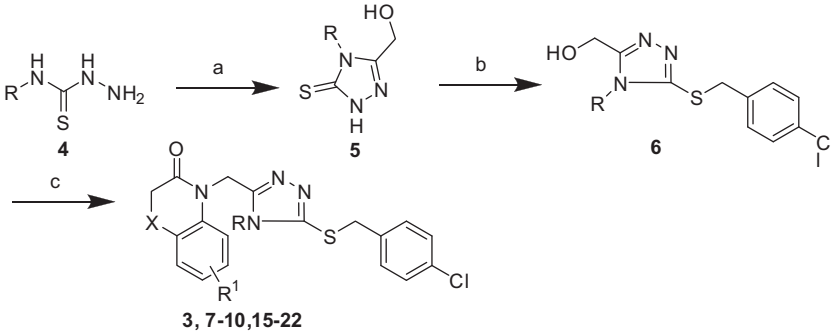
Evaluation of phenyl substitutions of benzooxazinone demonstrated that a variety of lipophilic groups can be tolerated on 6, 7 or 8 position (Table 3). 6-Chloro, bromo, methyl and 8-chloro analogues (**17**, **18**, **19** and **22**) displayed an approximately 2- to 3-fold increased activity compared to 6-trifluoromethyl counterpart (**15**). However, replacement of 6-trifluoromethyl by a 6-nitro group resulted in 10-fold loss of potency (**16**). Interestingly, variation of these substitutions (at the 6 or 8 position) has no impact in SGLT1 activity. Introduction of halogen at the 7-position (Cl or Br) slightly decreased the SGLT2 activity (**20** and **21**).

Replacement of sulfur next to triazole by a carbon as in compound **15** provided compound **23**, which is only 2-fold less potent than **15** for SGLT2 (Table 4).

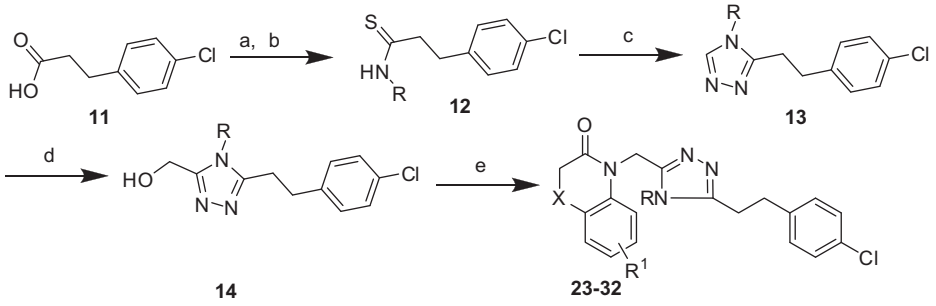
With the two sulfur atoms replaced, we decided to revisit N-substitutions of the triazole as shown in Table 4. *n*-Propyl (**25**) was as optimal as other bigger substituents (**26**, **27**, **29**). α-Branched substituent resulted in significant loss of activity (**30**).

Since both 6-chloro and 8- chloro were beneficial for SGLT2 activity (Table 3, compounds **18** and **22**), 6, 8-dichlorosubstituented compounds **31** and **32** (Table 5) were synthesized and indeed exhibited much better potency in hSGLT2 glucose uptake assay, comparable to that of dapagliflozin (**2**). They also displayed more than 1000-fold selectivity toward SGLT2 versus SGLT1 and SGLT6, which were better than Dapagliflozin (**2**). In addition, compounds **31** and **32** displaced tritium labeled dapagliflozin with IC_{50} of 16 nM and 20 nM respectively.[18] However, these two compounds had poor microsomal stability with 4% remaining after incubation in human or rat microsomes for 30 min.

In summary, we discovered and optimized a series of non-glucoside SGLT2 inhibitors. Very potent and highly selective SGLT2 inhibitors were obtained. N-substituents of triazole and phenyl substituents of benzooxazinone play a key role in activity and selectivity. Further optimization to improve solubility and pharmacokinetic properties will be reported in due course.

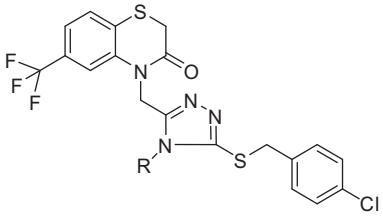


Scheme 1. Reagents and conditions: (a) methyl 2-hydroxyacetate, NaOH, EtOH, reflux, 95%; (b) 1-(bromomethyl)-4-chlorobenzene, potassium carbonate, DMF, rt, 1 h, 90%; (c) DEAD, Ph_3P, THF, rt, 50%–65%.



Scheme 2. (a) EDC, HOBt, RNH_2, DMF, rt, 100%; (b) Lawesson's reagent, toluene, 85 °C, 3 h, 99%; (c) formic acid hydrazide, mercuric acetate, dioxane, reflux, 60%–70%; (d) paraformaldehyde, xylene, 125 °C, 2 h, 80%–90%; (e) DEAD, Ph_3P, THF, rt, 65%.

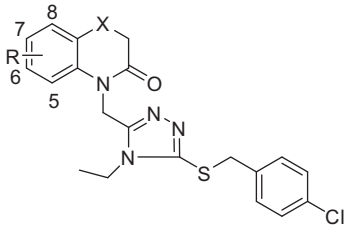
Table 2
N-substituents of triazole



Compound	R	hSGLT2 inhibition IC_{50}[a] (μM)	hSGLT1 inhibition IC_{50}[a] (μM)
3	Me	1.40	7.00
7	Et	0.37	4.75
8	$CH(CH_3)_2$	1.20	2.00
9	Cyclopropyl	1.10	3.75
10	Ph	2.11	3.47

[a] Inhibition of [^{14}C]AMG transportation into the CHO cells that express hSGLT2 or hSGLT1. Assay run in buffer containing 0.1% BSA. See Ref.[17] for assay protocol. Values are means of three experiments, standard deviation is ±30%.

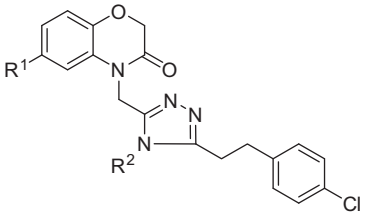
Table 3
Phenyl substitutions of benzooxazinone and benzothiazinone



Compound	R	X	hSGLT2 inhibition IC_{50}[a] (μM)	hSGLT1 inhibition IC_{50}[a] (μM)
7	6-CF_3	S	0.37	4.75
15	6-CF_3	O	0.25	6.64
16	6-NO_2	O	2.10	5.61
17	6-Br	O	0.09	5.32
18	6-Cl	O	0.15	7.34
19	6-Me	O	0.08	7.13
20	7-Cl	O	0.43	3.29
21	7-Br	O	0.52	2.53
22	8-Cl	O	0.15	7.34

[a] Inhibition of [^{14}C]AMG transportation into the CHO cells that express hSGLT2 or hSGLT1. Assay run in buffer containing 0.1% BSA. See Ref. 17 for assay protocol. Values are means of three experiments, standard deviation is ±30%.

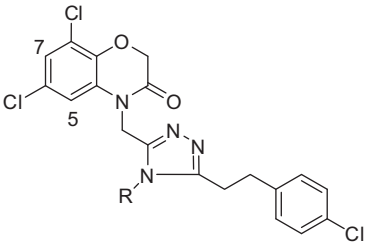
Table 4
N-substituents of triazole



Compound	R^1	R^2	hSGLT2 IC_{50}[a] (μM)	hSGLT1 IC_{50}[a] (μM)
23	CF3	CH_2CH_3	0.64	46.00
24	CF3	$(CH_2)_2CH_3$	0.18	9.88
25	Cl	$(CH_2)_2CH_3$	0.04	13.60
26	Cl	$(CH_2)_3CH_3$	0.03	11.80
27	Cl	$CH_2CH(CH_2)_2$	0.03	7.28
28	Cl	$CH_2CH(CH_3)_2$	0.16	6.90
29	Cl	$CH_2CH_2CF_3$	0.07	13.80
30	Cl	$CH(CH_2)_2$	1.58	25

[a] Inhibition of [^{14}C]AMG transportation into the CHO cells that express hSGLT2 or hSGLT1. Assay run in buffer containing 0.1% BSA. See Ref.[17] for assay protocol. Values are means of three experiments, standard deviation is ±30%.

Table 5
Activity and selectivity of top two compounds



Compound	R	hSGLT2 IC_{50}[a] (μM)	hSGLT1 IC_{50}[a] (μM)	hSGLT6 IC_{50}[a] (μM)	hSGLT2 plasma IC_{50}[b] (μM)
31	$(CH2)_2CH_3$	0.009	9.14	14.40	0.04
32	$(CH_2)_2CF_3$	0.010	6.29	9.20	0.019
2		0.004	0.37	0.38	0.022

[a] Inhibition of [^{14}C]AMG transportation into the CHO cells that express hSGLT2 or hSGLT1. Assay run in buffer containing 0.1% BSA. See Ref.[17] for assay protocol. Values are means of three experiments, standard deviation is ±30%.
[b] Assay conducted in the presence of 100% plasma.

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17. *[^{14}C] Glucose transporter assay*: Chinese hamster ovary (CHO) cells stably expressing human SGLT1 and 2 cDNA were seeded in 96-well Cytostar scintillant coated plates (PerkinElmer Biosciences). Cells were rinsed briefly with assay buffer (10 mM HEPES, 5 mM Tris, 140 mM NaCl, 2.5 mM KCl, 1.25 mM $CaCl_2$, pH 7.4). Ligands were serially diluted via BioMek 3000 (Beckman Coulter, Inc.) in the assay buffer supplemented with 0.1% BSA. After addition of ligands to the assay plate, [^{14}C] α-methyl glucopyranoside ([^{14}C]AMG, 10 uCi/ml, Moravek) was added and incubated at 37 °C incubator for 60 min. Assay plate was briefly washed with the buffer before air drying, and counted in Microbeta plate counter (PerkinElmer Biosciences).
18. *Radioligand binding affinity assay:* For competition binding studies, compounds were diluted serially in the assay buffer (10 mM HEPES, 5 mM Tris, 140 mM NaCl, 2.5 mM KCl, 1.25 mM $CaCl_2$, 1.25 mM $MgCl_2$, pH7.4) via BioMek 3000. To each well of assay plate, diluted compounds, 8 nM of radioligand ([^{3}H]dapagliflozin, 39.7 Ci/mmol; Moravek, Irvine, CA) and CHO cell membrane protein stably expressing human SGLT2 (10–20 μg/well) were added and incubated at 37 °C for 60 min. Plates were harvested in GF/B filtermat with three washes in cold wash buffer (10 mM HEPES). 50 μl scintillant was added to each well of plate and read on TopCount (Perkin Elmer). IC_{50} and K_i values were calculated using GRAPHPAD PRISM 5 software (GraphPad, San Diego, CA).