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# Design and synthesis of fluorescent SGLT2 inhibitors

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ABSTRACT

The design and synthesis of the first fluorophore-conjugated SGLT2 inhibitors is described. The mode of linking the fluorophore to the SGLT2 pharmacophore was found to be crucial in achieving optimum potency. Superior potency to phlorizin was provided by examples containing TAMRA, BODIPY, Cy3B and NBD fluorophores.



After filtration by the kidney glomerulus, renal glucose is subsequently reabsorbed by two sodium-dependent glucose cotransporters (SGLTs).[1] SGLT1 is a low capacity, high affinity transporter that is expressed in the small intestine and heart in addition to the kidney,[2] whereas SGLT2 is a high capacity, low affinity transporter expressed predominantly in the proximal convoluted tubule of the nephron,[3] where it is responsible for 90% of renal glucose reabsorption. Inhibition of SGLT2 is thus expected to result in elevated urinary glucose excretion, and indeed human SGLT2 gene mutations lead to renal glucosuria in individuals who are otherwise asymptomatic.[4] The consequent lowering of blood glucose levels is anticipated to be achieved without risk of hypoglycemia by virtue of a lack of impact on insulin secretion. In combination with a negative energy balance, such a potential profile is highly attractive for the treatment of diabetes mellitus and obesity. As a result, the development of small-molecule inhibitors of SGLT2 has received widespread attention across the pharmaceutical industry.[5]

Despite the very large number of SGLT2 inhibitors that have been described, all are structurally related to the prototypical inhibitor phlorizin[6] **1** (Fig. 1), sharing the common features of a glucose-based (or glucose-mimicking) ring bearing a lipophilic side-chain at C1. The side chains contain two cyclic systems (generally both aromatic) separated by one to three carbons. In general, known SGLT2 inhibitors are either *O*-glucosides, such as phlorizin **1** and sergliflozin-A[7] **2**, or *C*-aryl glucosides such as dapagliflozin[8] **3**. This intensive focus on such a limited range of chemotypes has led to highly congested chemical space in terms of intellectual property considerations, and limited opportunity for significant differentiation within the existing structural classes. We therefore saw value in seeking an entirely unrelated class of SGLT2 inhibitor.

A major component of our strategy to identify a novel SGLT2-inhibiting chemotype was to perform a high-throughput screen (HTS) of our company compound file. However, the opportunity for undertaking such an HTS was limited by the available assay formats. The most widely used in vitro SGLT2 assays are based on functional inhibition of the uptake of [$^{14}$C]-α-methyl-D-glucopyranoside in various cell lines expressing SGLT2. Although an automated 96-well format of this assay has been described,[9] we considered this still too cumbersome for the purposes of a full-file HTS.

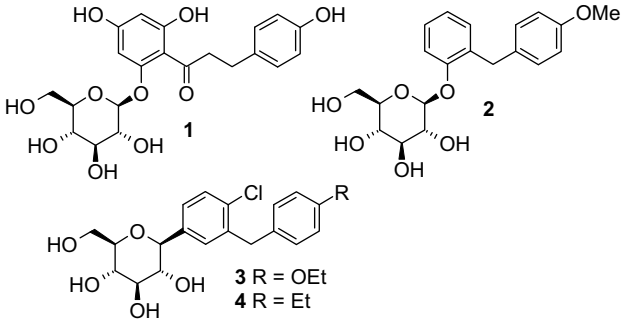


**Figure 1.** Representative SGLT2 inhibitors.

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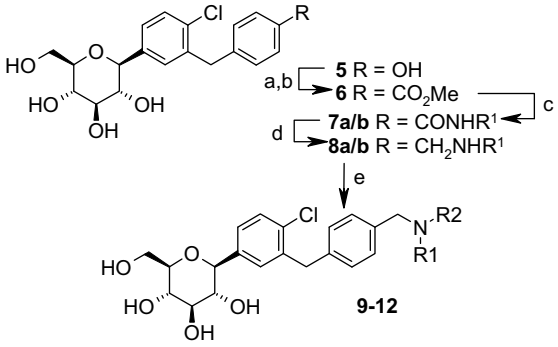
We report here the results of our efforts to design a fluorescent inhibitor of SGLT2 with the aim of enabling development of a fluorescence polarisation high-throughput binding assay. Fluorometric assays offer several advantages over their radiometric counterparts, especially with regard to safety risks, waste disposal, and ease of miniaturisation. Where fluorometric assays tend to present a greater challenge is in the design of an appropriate fluorescent ligand, due to the difficulty in maintaining potency whilst accommodating the steric demands of the fluorophore, particularly when this is conjugated to a small molecule rather than a peptide.[10] Nonetheless, there have been several reports of successful fluorophore-conjugation with small molecule ligands in recent years,[10,11] and in-house experience with a high-throughput fluorometric hERG assay[12] has encouraged pursuit of this approach.

Our first task was to select the location on the typical SGLT-inhibitor framework for conjugation of a fluorophore.[13] Despite the wealth of disclosed SGLT2 inhibitors, the vast majority reside solely within the patent literature, often lacking potency data, and there is a general dearth of well-described structure–activity relationships (SAR) for this transporter. However, we noticed some general flexibility in the nature and size of the substituent on the terminal aromatic ring, and thus chose to target this location, initially employing the nitrogen of amines **8a/b** (Scheme 1) as the conjugation handle. Known phenol **5**[14] was converted to the corresponding triflate,[14] which was then carbonylated to provide novel methyl ester **6**. Reaction of **6** with ammonia or methylamine provided amides **7a** and **7b**, respectively, and these were reduced to benzylic amines **8a/b**.

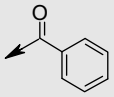
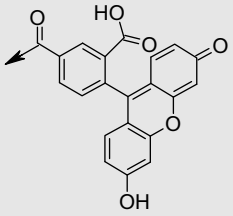
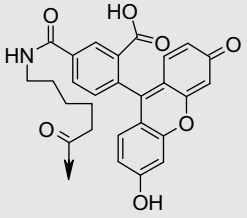
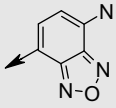
Capping of the amine as a simple benzamide to give **9a/b** (Table 1) suggested that the presence of an amide function close to the lipophilic pharmacophore can be tolerated (albeit with some loss of potency), especially if N-methylated. Due to the high cost of many commercially available fluorophores, we selected the relatively inexpensive fluorescein to begin our survey of fluorophore conjugation.[15] We were encouraged to achieve at least measurable inhibition of SGLT2 with our first compounds (**10a/b**) although the demands of the fluorophore were clearly detrimental to potency, even when separated from the SGLT2 pharmacophore by a C5 tether (**11a/b**). Use of NBD chloride provided an alternative non-amide mode of conjugation (**12a/b**), and demonstrated that sub-micromolar potency was attainable.

Before examining a wider range of fluorophores, we decided to incorporate a linker which would allow greater separation of the pharmacophore from any polarity associated with attachment of the fluorophore. To this end, known tetra-acetylated phenol **13**[14] (an immediate precursor in the preparation of **5**) was alkylated with protected amino-bromoalkanes containing chains ranging from ethyl to octyl (Scheme 2). After global deprotection of **14a–e**, the resulting amines **15a**, **16a**, **17a**, **18** and **19** were either directly conjugated with the fluorophore of choice, or were first alkylated before fluorophore-conjugation, providing targets **20–23**.




**Scheme 1.** Reagents and conditions: (a) $PhN(Tf)_2$, $Et_3N$, DMAP, $CH_2Cl_2$, 63%; (b) $Pd(OAc)_2$, dppp, CO, MeOH, 100 °C, 95%; (c) **7a**: $NH_3$/MeOH, 100 °C, 77% **7b**: $MeNH_2$, EtOH, 100 °C, 90%; (d) $BH_3$-THF, THF, reflux, then MeOH/BuOH quench, **8a** $R^1$ = H 52%, **8b** $R^1$ = Me 68%; (e) benzoic acid succinimidyl ester, or 5-carboxyfluorescein succinimidyl ester (5-FAM, SE), or 6-(fluorescein-5-carboxamido)hexanoic acid succinimidyl ester (5-SFX), or 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD chloride), $Et_3N$, DMF, rt.

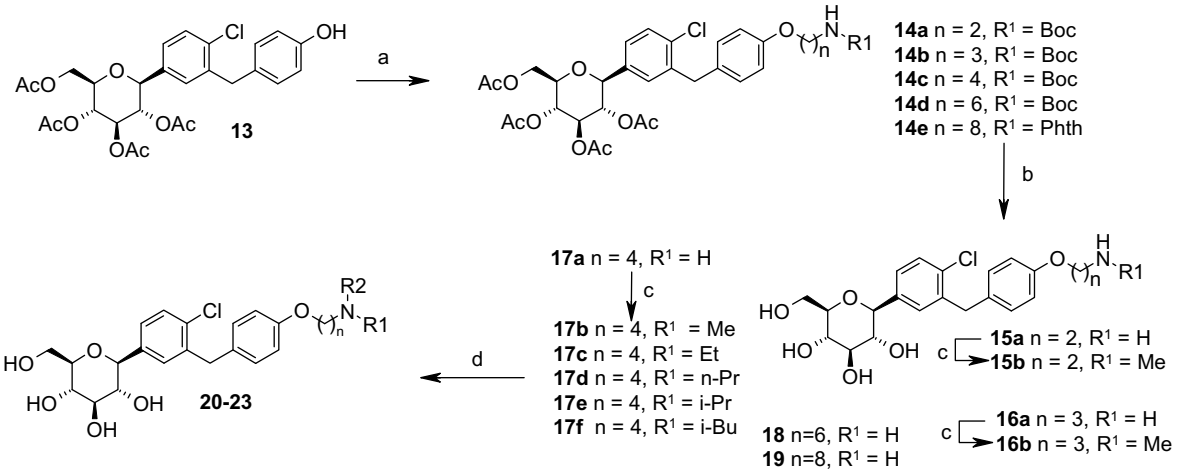
**Table 1**
SGLT2 inhibition data for compounds **1**, **4**,[16] and **5**, and **9–12**

| Compound | $R^1$ | $R^2$ | SGLT2 $IC_{50}$[a] (nM) |
|---|---|---|---|
| **1** | — | — | 752 (545–1040)[b] |
| **4** | — | — | 12 (11–14)[b] |
| **5** | — | — | 33 (28, 39)[c] |
| **9a** | H |  | 495 (480, 510)[c] |
| **9b** | Me | | 45 (36, 53)[c] |
| **10a** | H |  | 11000 (9600, 12400)[c] 3 |
| **10b** | Me | | 350 (3100–3600)[c] |
| **11a** | H |  | 8000 (7500, 8500)[c] |
| **11b** | Me | | 15050 (14600, 15500)[c] |
| **12a** | H |  | 2400 (2300, 2500)[c] |
| **12b** | Me | | 286 (241, 330)[c] |

[a] Inhibition of uptake of [$^{14}$C]-α-methyl-D-glucopyranoside in CHO cells stably transfected with human SGLT2.[17]
[b] **1** $n$ = 13, **4** $n$ = 50 (95% confidence interval in parentheses).
[c] Values are means of $n$ = 2 (individual measurements in parentheses).

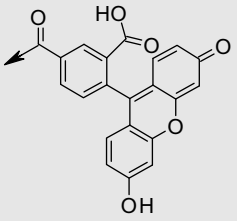
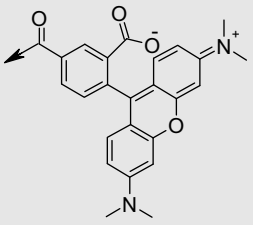
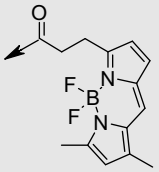
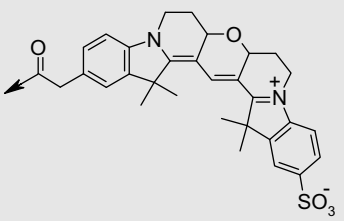
The sub-micromolar potency of **20** (Table 2), the first compound made in this series, immediately suggested that this would be a superior system for attaching the fluorophore compared to utilising the benzylic nitrogen of compound **8a/b**. We therefore expanded the combination of the alkoxy linker with other fluorophores, which we anticipated would have fluorescent properties more suitable for our needs. Potency enhancement was observed on moving from fluorescein-tagged **20**, to TAMRA-tagged **21** to BODIPY-tagged **22e/f**, all containing a 4-carbon linker. At this point we fully explored the impact of linker length and amide substituent $R^1$. The SAR regarding linker length was found to be relatively flat (compare compounds **22a–k**), although there was an indication of a slight preference for increased distance of the fluorophore from the SGLT2 pharmacophore. In contrast, the size of the amide $R^1$ had a much more profound impact on potency, with propyl and butyl substituents (**22g–i**) leading to a marked decrease in potency. We also prepared several examples tagged with Cy3B (**23a–d**) and found this fluorophore to provide marginally improved potencies when compared to BODIPY with the same linker.

Finally, we investigated the impact of introducing a phenyl ring into the linker (Scheme 3). Phenol **13**[14] was reacted under Mitsunobu conditions with benzyl alcohol **24**[18] to generate phenoxy ether **25**, which was then globally deprotected. The resulting amine **26a**

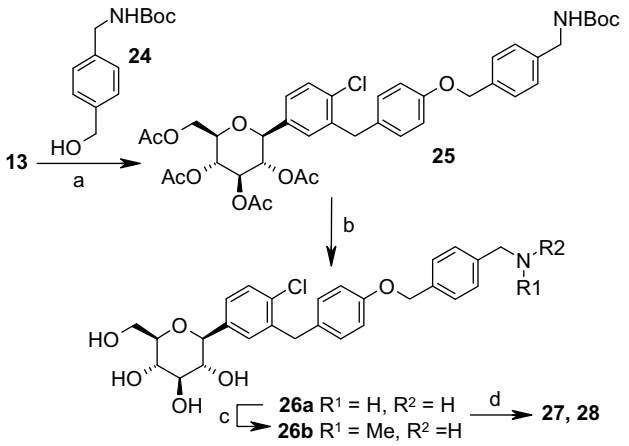



**Scheme 2.** Reagents and conditions: (a) **14a–d**: $Br(CH_2)_nNHBoc$, $Cs_2CO_3$, DMF, **a** 36%, **b** 71%, **c** 75%, **d** 91%; **14e**: *N*-(8-bromooctyl)phthalimide, $Cs_2CO_3$, DMF; (b) **15a**/**16a**/**17a**/**18**/**19**: i—$NH_3$(aq)/MeOH, ii—HCl, MeOH, **15a** 95%, **16a** 100%, **17a** 91%, **18** 60%; **19**: i—$K_2CO_3$, MeOH, 25% (over two steps from **13**), ii—$NH_2NH_2$, MeOH, reflux, 62%; (c) **15b**/**16b**/**17b**: i—ethyl formate, EtOH, reflux, ii—$BH_3$.THF, THF, reflux, **15b** 50%, **16b** 75%, **17b** 43%; **17c**: i—acetyl chloride, NaOAc, THF, $H_2O$, 75%, ii—$BH_3$.THF, THF, reflux, 25%; **17d**: i—propionyl chloride, NaOAc, THF, $H_2O$, 89%, ii—$BH_3$.THF, THF, reflux, 39%; **17e**: i—acetone, MeOH, ii—$NaBH_4$, MeOH, 80%; **17d**: i—isobutyraldehyde, MeOH, ii—$NaBH_4$, MeOH, 84%; (d) 5-carboxyfluorescein succinimidyl ester (5-FAM, SE), or 5-carboxytetramethylrhodamine succinimidyl ester (5-TAMRA, SE), or 4,4-difluoro-5,7-dimethyl-4-bora-3*a*,4*a*-diaza-*s*-indacene-3-propionic acid succinimidyl ester (BODIPY FL, SE), or Cy3B NHS ester, $Et_3N$, DMF, rt.

**Table 2**
SGLT2 inhibition data for compounds **20**–**23**

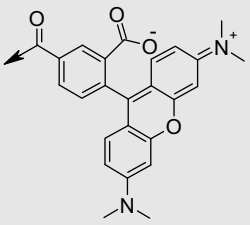
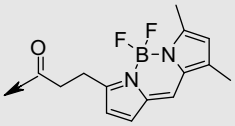
| Compound | Linker | $R^1$ | $R^2$ | SGLT2 $IC_{50}$[a,b] (nM) |
|---|---|---|---|---|
| **20** | *n* = 4 | H |  | 835 (790, 880) |
| **21** | *n* = 4 | H |  | 370 (200, 540) |
| **22a** | *n* = 2 | H |  | 420 (370, 470) |
| **22b** | *n* = 2 | Me | | 315 (310, 320) |
| **22c** | *n* = 3 | H | | 203 (166, 240) |
| **22d** | *n* = 3 | Me | | 430[c] |
| **22e** | *n* = 4 | H2 | | 267 (254, 280) |
| **22f** | *n* = 4 | Me | | 155 (110, 200) |
| **22g** | *n* = 4 | *n*-Pr | | 850[c] |
| **22h** | *n* = 4 | *i*-Pr | | 1570 (1440, 1700) |
| **22i** | *n* = 4 | *i*-Bu | | 2000[c] |
| **22j** | *n* = 6 | H | | 148 (119, 177) |
| **22k** | *n* = 8 | H | | 205 (120, 290) |
| **23a** | *n* = 4 | H |  | 145 (138, 151) |
| **23b** | *n* = 4 | Me | | 122 (79, 164) |
| **23c** | *n* = 4 | Et | | 131 (100, 162) |
| **23d** | *n* = 6 | H | | 102 (100, 104) |

[a] Inhibition of uptake of [$^{14}$C]-α-methyl-D-glucopyranoside in CHO cells stably transfected with human SGLT2.[17]
[b] Values are means of *n* = 2 (individual measurements in parentheses) unless otherwise stated.
[c] *n* = 1.




**Scheme 3.** Reagents and conditions: (a) $PPh_3$, DIAD, THF, 65%; (b) i—$NH_3$/MeOH, ii—HCl/MeOH, 84% (over 2 steps); (c) i—ethylformate, EtOH, reflux, ii—$BH_3$.THF, THF, reflux, 79% (over 2 steps); (d) 5-carboxytetramethylrhodamine succinimidyl ester (5-TAMRA, SE), or 4,4-difluoro-5,7-dimethyl-4-bora-3*a*,4*a*-diaza-*s*-indacene-3-propionic acid succinimidyl ester (BODIPY FL, SE), $Et_3N$, DMF, rt.

**Table 3**
SGLT2 inhibition data for compounds **27-28**

| Compound | $R^1$ | $R^2$ | SGLT2 $IC_{50}$[a,b] (nM) |
|---|---|---|---|
| **27a** | H |  | 55 (54, 55) |
| **27b** | Me | | 154 (107, 200) |
| **28a** | H |  | 123 (120, 126) |
| **28b** | Me | | 95 (91, 98) |

[a] Inhibition of uptake of [$^{14}$C]-α-methyl-D-glucopyranoside in CHO cells stably transfected with human SGLT2.[17]
[b] Values are means of $n = 2$ (individual measurements in parentheses).

was either directly conjugated with a fluorophore, or was first methylated before conjugation. TAMRA-labelled **27a**[19] (Table 3) was found to be considerably more potent than its alkyl-chain-linked analogue **21**, notably over 10-fold more potent than phlorizin **1** and reaching within 5-fold of the potency achieved by **4**, despite the burden of the fluorophore. In contrast, combination of the phenyl-based linker (**28a/b**) with a BODIPY fluorophore provided more modest improvements versus the alkyl chain analogues **22e/f**.

In summary, we have described the first examples of fluorophore-labelled SGLT2 inhibitors. The mode of linking the fluorophore to the SGLT2 pharmacophore was found to be crucial in achieving good potency. A variety of fluorophores are broadly acceptable, which offers the potential for fine-tuning of the fluorescent properties,[20] and sufficient potency has been attained that we anticipate such compounds could find use as pharmacological tools in assay development or other investigations of SGLT2.

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14. Eckhardt, M.; Eickelmann, P.; Himmelsbach, F.; Barsoumian, E. L.; Leo, T. WO2005092877.
15. All fluorophores were purchased from Invitrogen (Molecular Probes), with the exception of Cy3B NHS ester and NBD chloride, which were purchased from GE Healthcare and Sigma–Aldrich, respectively.
16. The structure of dapagliflozin **3** had not been disclosed by the time this work was completed. We had employed close-analogue **4** (Washburn W.; Meng W. US20060063722), alongside phlorizin **1**, to benchmark our SGLT2 assay. In a later, slightly modified, format of the assay, we have since established that **3** and **4** are equipotent.
17. Methodology analogous to that described for SGLT1 by: Lin, J.-T.; Kormanec, J.; Wehner, F.; Wielert-Badt, S.; Kinne, R. K.-H. *Biochim. Biophys. Acta* **1998**, *1373*, 309.
18. Adlington, R. M.; Baldwin, J. E.; Becker, G. W.; Chen, B.; Cheng, L.; Cooper, S. L.; Hermann, R. B.; Howe, T. J.; McCoull, W.; McNulty, A. M.; Neubauer, B. L.; Pritchard, G. J. *J. Med. Chem.* **2001**, *44*, 1491.
19. Preparation of **27a**: 5-TAMRA-SE (5 mg, 0.095 mmol) and amine **26a** (5.7 mg, 0.011 mmol) were dissolved in anhydrous DMF (0.5 mL) and triethylamine (0.019 mL, 0.11 mmol) was then added. The solution was stirred at room temperature for 16 hours in the dark. Solvents were removed in vacuo and the residue was stored under $N_2$ (g) in the freezer until purification, which was performed by preparative HPLC (Column: Waters XBridge C18, 5 μm, 19 × 150 mm, 20 mL/min; Eluent: gradient 20–55% MeCN in water over 15 minutes, then 85% for 5 min, $R_t$ 11.2 min). The product was obtained as a dark purple solid by freeze drying (8.3 mg, 96%). $^1$H NMR ($CD_3OD$, 300 MHz,) rotamers $\delta$ 3.26–3.58 (m, 16H), 3.75 (dd, 2H), 4.02 (d, 2H), 4.08 (d, 1H), 4.63 (s, 2H), 5.04 (s, 2H), 6.87 (d, 2H), 6.90 (s, 2H), 7.00 (d, 2H), 7.11 (d, 2H), 7.22–7.40 (m, 10H), 8.04 (d, 1H), 8.51 (d, 1H). MS (ESI) *m/z* 912.34 $(MH)^+$. Chemical purity by HPLC: Waters XBridge C18, 3 × 150 mm, 5 μm, 0.5 mL/min, gradient 5-95% MeCN in 2% aq. $HCO_2H$ over 15 min, then held for 10 minutes, $R_t$ 10.61 min, 95.93% (223 nm).
20. The excitation-emission profiles of compounds **23d**, **27a**, and **28b** were measured and found to be essentially unchanged from the corresponding commercially available fluorophore precursors.