Electronic Supplementary Information

Development of a novel non-radioactive cell-based method for the screening of SGLT1 and SGLT2 inhibitors using 1-NBDG

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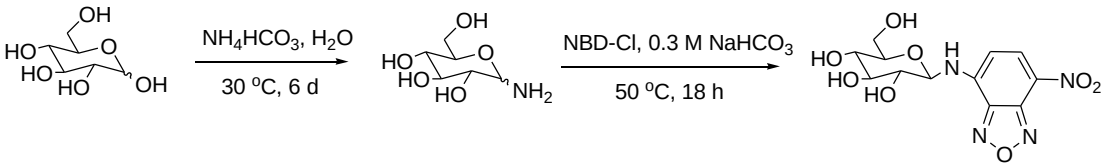
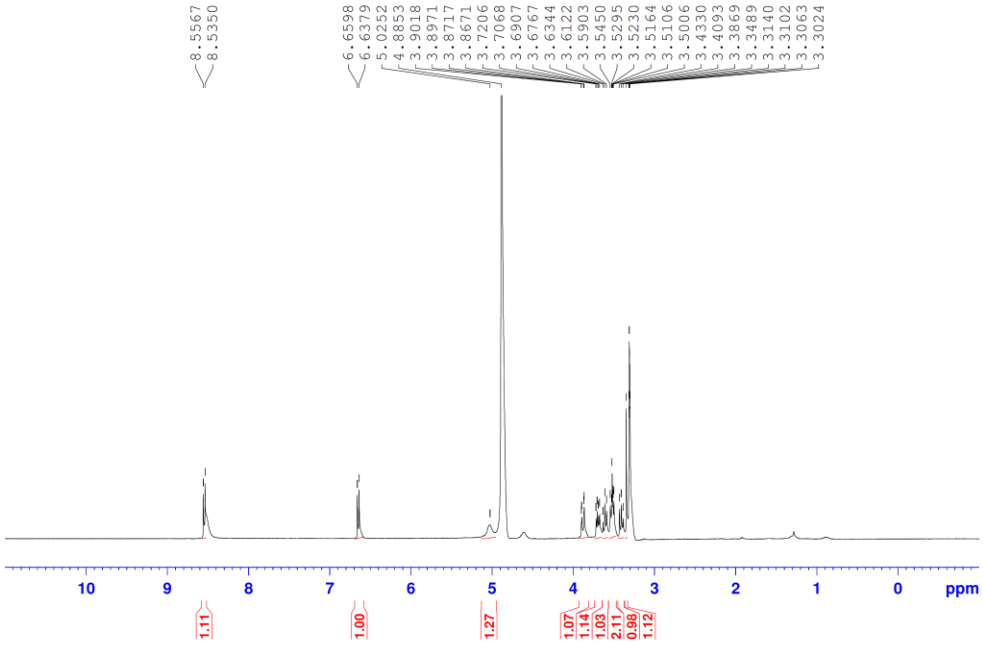
A

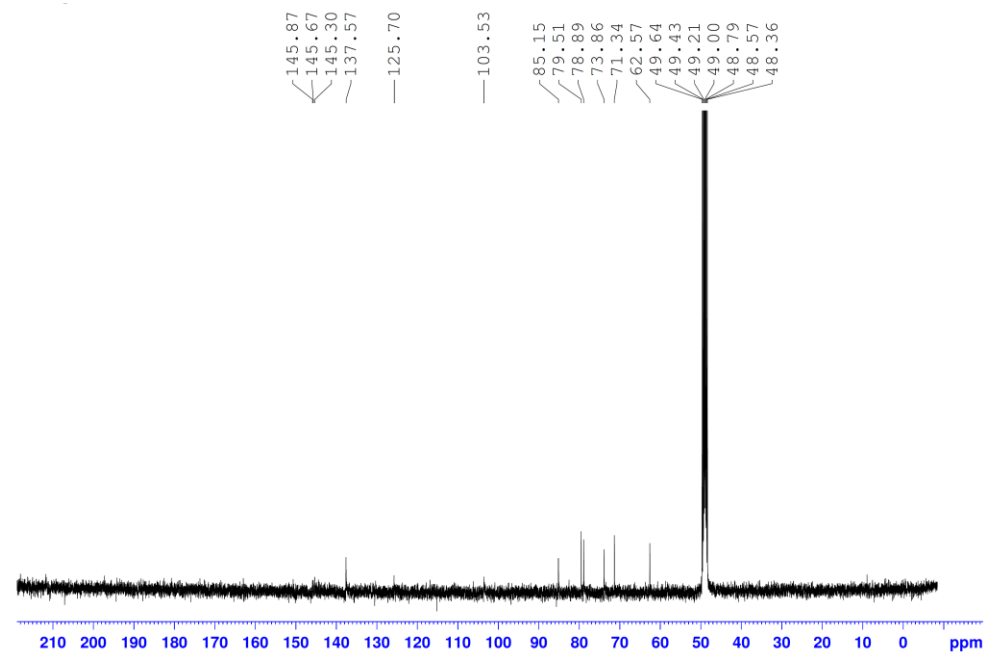


B

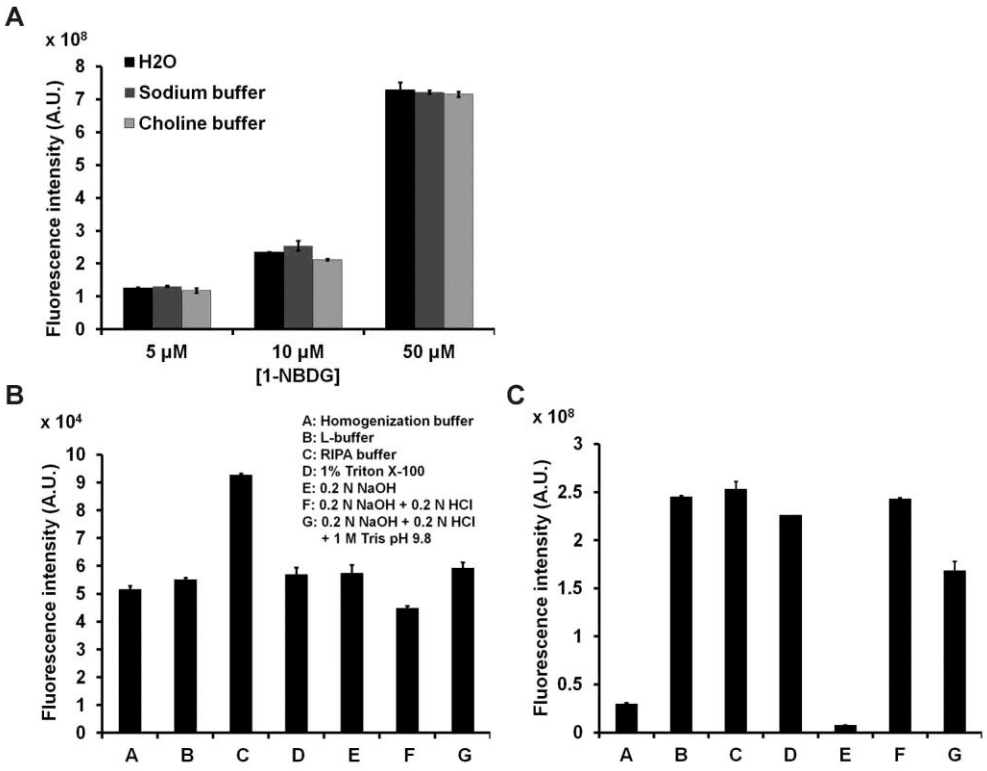
^{1}H-NMR Spectrum



^{13}C-NMR Spectrum

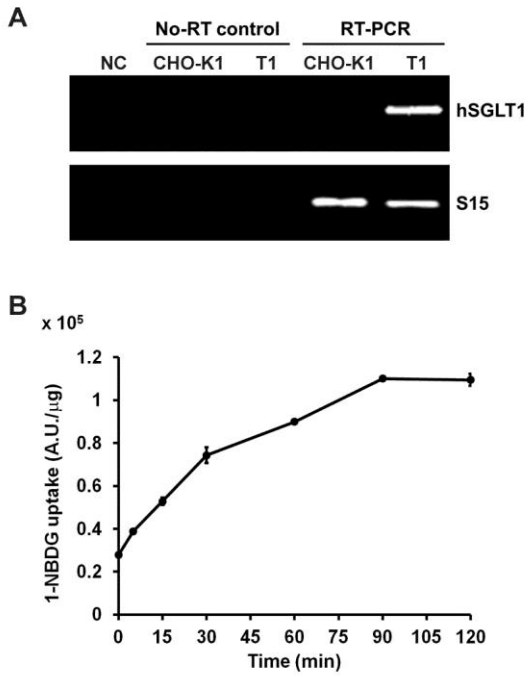


Supplementary Figure 1 1-NBDG synthesis (A) and NMR spectra (B).



Supplementary Figure 2 Optimization of uptake buffers and lysis solutions. (A) The effect of uptake buffers on 1-NBDG fluorescence. 1-NBDG was dissolved in deionized water, sodium buffer or choline buffer. Sodium or choline buffer did not affect 1-NBDG fluorescence. (B) Background fluorescence of cell lysis solutions. All the solutions tested showed relatively low background fluorescence. Among them, solution C (RIPA buffer) had the highest and solution F (0.2 N NaOH + 0.2 N HCl) had the lowest background fluorescence. (C) Fluorescence of 1-NBDG at 10 μM in cell lysis solutions. 1-NBDG fluorescence intensities were relatively high in solutions B, C, D, and F; but dramatically quenched in solutions A and E. Intermediate fluorescence intensity of 1-NBDG was detected in solution G. Solution F (0.2 N NaOH + 0.2 N HCl) was selected as the lysis solution for 1-NBDG uptake assay based on low background fluorescence and high 1-NBDG fluorescence. Solutions A and E were excluded because of a quenching effect. Solutions B, C and D were also excluded because the presence of detergents may interfere with protein assay. Experiments were performed in triplicate and data are presented as mean ± S.E. Cell lysis solutions used in (B) and (C):

A. homogenization buffer: 7.5 mM Na_2HPO_4, 1 mM EDTA, pH 7.4; B. L-buffer: PBS with 0.1% NP-40 and 0.1% Triton X-100; C. RIPA buffer: 50 mM Tris, pH 8.0, 150 mM NaCl, 0.1% SDS, 0.1% sodium deoxycholate, 1% NP-40; D. 1% Triton X-100 in deionized water; E. 0.2 N NaOH; F. a mixture of equal volumes of 0.2 N NaOH and 0.2 N HCl; G. a mixture of equal volumes of 0.2 N NaOH and 0.2 N HCl, adjusted to alkaline pH with 1/10 volume of 1 M Tris, pH 9.8.



Supplementary Figure 3 Expression of hSGLT1 in a CHO-K1 cell clone and the time course of 1-NBDG uptake in this cell clone. (A) Expression of hSGLT1 detected by RT-PCR. CHO-K1: the parental CHO-K1 cell line. NC: negative control for PCR. S15: a housekeeping gene rig/S15 used as a positive control for RT-PCR. (B) Time course of 1-NBDG uptake. 1-NBDG uptake was performed in sodium buffer. Fluorescence intensity was normalized with protein content (μg). Experiments were performed in triplicate and data are presented as mean ± S.E. The results showed that the initial rate was within 30 min.

Supplementary Table 1 IC_{50} values of phlorizin for hSGLT1.

IC_{50} (μM)	Reference
0.11	Chang et al., using ^{14}C-AMG
0.11	Chang et al., using 1-NBDG
0.33	Meng et al. (2008)
0.17	Pajor et al. (2008)
0.2	Sato et al. (2007)
0.21	Tahara et al. (2012)

Summary of published phlorizin IC_{50} values for hSGLT1. The IC_{50} values acquired from our non-radioactive cell-based assay system are very close to the published results obtained using ^{14}C-AMG.

Supplementary Table 2 IC_{50} values of the selective SGLT2 inhibitor dapagliflozin for hSGLT2 and hSGLT1.

IC_{50} (nM)		Selectivity	Reference
hSGLT2	hSGLT1		
1.86	880	473	Chang et al., using 1-NBDG
4	370	92.5	Li et al. (2011)
6	400-800	67-133	Hummel et al. (2012)
6.7	885	132	Xu et al. (2009)
1.1	1390	1200	Meng et al. (2008)

Summary of published IC_{50} values of dapagliflozin for hSGLT2 and hSGLT1. The results acquired from our non-radioactive cell-based system are within the range of the published data obtained using ^{14}C-AMG.

Supplemental References

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