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Brief Article

Discovery of Non-Glycoside Sodium-Dependent Glucose Co-Transporter 2 (SGLT2) Inhibitors by Ligand-Based Virtual Screening

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A ligand-based virtual screening strategy (a combination of pharmacophore model generation, shape-based scoring, and structure clustering analysis) was developed to discover novel SGLT2 inhibitors. The best pharmacophore model, generated from eight glycoside inhibitors, was utilized to virtually screen three chemical databases that led to the identification of three non-glycoside SGLT2 inhibitors. This is the first report of the generation of a pharmacophore model from glycosides that has then been used to discover novel non-glycosides hits.

Introduction

The prevalence of diabetes has rapidly increased over the past decades and has become a major public health problem in the developed and developing countries. The number of people worldwide suffering from diabetes mellitus is 246 million in 2007 and could reach 380 million by 2025.[1] Diabetes mellitus is of two types: diabetes mellitus type 1 (T1DM[a]) and diabetes mellitus type 2 (T2DM). T1DM results from absolute deficiency in insulin secretion, and T2DM, comprising 90% of all cases of diabetes, results from insulin deficiency and insulin resistance. T2DM, characterized by the failure to respond to insulin, is a major risk factor for vascular complication such as atherosclerosis, stroke, nephropathy, retinopathy, and neuropathy.[2] Several therapeutic agents are available for monotherapy or for combination therapy with different mechanisms to treat diabetics, such as metformin, rosiglitazone, sitagliptine, acarbose, and glimepiride.[3] However, according to the report by United Kingdom Prevention of Diabetes Study (UKPDS), only 25−50% of T2DM patients are effectively treated by current therapies.[4] Therefore, discovery of new therapeutic agents with novel mechanisms of action is urgently required to treat patients with uncontrolled hyperglycemia or in whom current therapies have been unsuccessful.[3]

Sodium-dependent glucose co-transporters (SGLTs), mediators of reabsorption of glucose in the kidney, have recently emerged as novel drug targets for the treatment of diabetes.[5] They are membrane proteins and function as transporters to actively transfer glucose together with some ions through the proximal convoluted tubule cells of the kidney. Two types of human SGLT proteins, SGLT1 and SGLT2, are encoded by different genes and have been extensively studied in academic institutes and pharmaceutical companies. SGLT2, expressed exclusively in the kidney, is located in the S1 segment of the proximal convoluted tubule of the kidney. It is a low-affinity, high-capacity cotransporter and is responsible for 90% of renal glucose reabsorption.[6] The remaining 10% of glucose is reabsorbed by SGLT1, a low-capacity and high-affinity transporter situated in the S3 segment of the proximal convoluted tubule.[7] In addition to the kidney, SGLT1 is mainly expressed in the small intestine and some in the heart. Inhibition of SGLTs prevents renal glucose reabsorption and leads to glucose excretion in urine, which resulted in reduced the blood glucose level. This mode of action for SGLT inhibitors, which is different from the currently available diabetic therapies that target insulin resistance and insulin deficiency, provides an insulin-independent pathway to control hyperglycemias[3,8] and thus provides a novel option for antidiabetics drugs with minimum adverse effects, such as weight gain and edema.

Several SGLT2 inhibitors have been reported as undergoing clinical trials. Phlorizin,[9] 3-(benzo[*b*]furan-5-yl)-2′,6′-dihydroxy-4′-methylpropiophenone-2′-O-β-D-glucopyranoside (T-1095A),[10] sergliflozin,[11] and remogliflozin[12] are *O*-glycosides and show strong inhibition of SGLT2. They also demonstrate efficacy in vivo when administered orally in rats or mice. They induce a glucosuric response, the result of the blockade of renal glucose reabsorption, and consequently lead to reduction of the blood glucose level and improvement of insulin sensitivity.[13] However, because of the low bioavailability, limited efficacy, poor pharmacokinetic profiles, and severe side effects, such as dehydration and diarrhea, the development of these compounds has been terminated at the preclinical or clinical trials stage.[14] Because of the shortcomings of *O*-glycoside inhibitors, *C*-glycoside SGLT2 inhibitors have drawn much attention in the past few years. Dapagliflozin,[15] currently in phase III clinical trials, is the most advanced compound under development among this SGLT2 inhibitor class. The report of recent clinical trials revealed that dapagliflozin treatment at 2.5, 5.0, and 10.0 mg/day for 24 weeks resulted in significant improvement in glycaemic

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[a] Abbreviations: T1DM, diabetes mellitus type 1; T2DM, diabetes mellitus type 2; UKPDS, United Kingdom Prevention of Diabetes Study; SGLT, sodium-dependent glucose co-transporter; HbA1c, glycosylated hemoglobin; SAR, structure−activity relationship; HA, hydrogen bond acceptor; HD, hydrogen bond donor; AR, aromatic group; HY, hydrophobic; AMG, α-methylglucopyranoside; CPM, counts per minute; DMSO, dimethylsulfoxide; EC_{50}, inhibitor concentration at half-maximal response; KRH, Krebs−Ringer−Henseleit.

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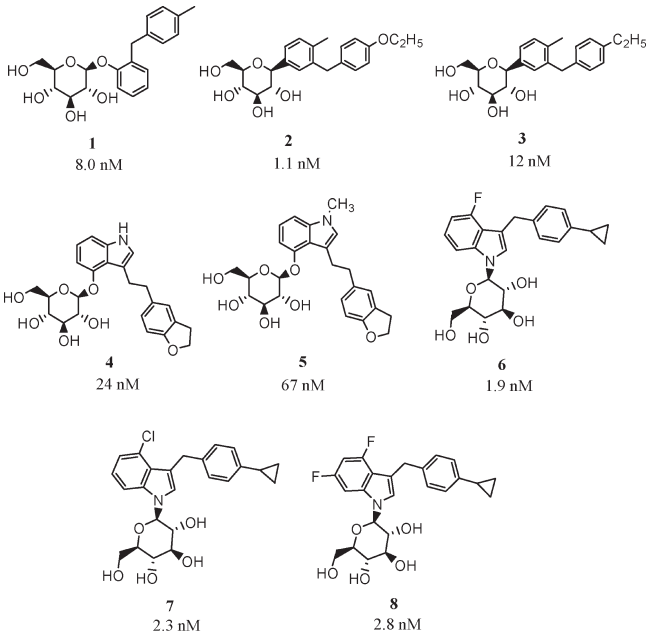


Figure 1. Structure of training set glycosides **1**−**8** and their SGLT2 inhibition EC_{50} values.

control and body weight reduction in patients with poorly controlled T2DM. Incidence of hypoglycemia, one of the major safety issues for many diabetes agents, did not significantly differ between dapagliflozin and placebo groups. Although urinary tract infections and fungal genitourinary infections were higher with the treatment of dapagliflozin, study discontinuations due to adverse events were not significantly different in all of the groups.[16] Along with dapagliflozin, canagliflozin is also in phase III clinical trials. Rosenstock et al. recently presented results from a placebo-controlled, double-blind, dose-ranging study in patients with T2DM on concurrent metformin.[17] Administration of canagliflozin (300 mg/day or 300 mg twice daily) for 12 weeks significantly reduced the HbA1c levels and decreased the body weight. Although the risk of symptomatic genital infections was increased with the treatment of canagliflozin, there was no significant increase in adverse event compared with the placebo group.[17] In addition, ASP1941, a novel *C*-glycoside SGLT2 inhibitor, is currently in phase III trials. In a 12-week double-blind study, administration of 12.5, 25, 50, and 100 mg/day of the compound to 361 Japanese patients with T2DM significantly reduced the HbA1c levels and reached a maximum at the 50 mg dose. Moreover, it reduced the body weight in a dose dependent manner by up to 2 kg in the group receiving 100 mg dose daily. There were no severe side effects observed in patients treated with the compound. Although some cases of urinary tract infections and genital infections were reported, all events were mild and the event rate was similar between the drug treatment group and placebo group.[18]

Although various SGLT2 inhibitors have been developed and are in advanced stages of clinical development, all of them are glycosides. In general, development of glycoside drugs has encountered potential problems such as low tissue permeability, poor stability, and short serum half-time.[19] Moreover, the synthesis of glycoside compounds is much more difficult and expensive than conventional small molecules, which would limit, at least partially, their development into useful therapeutic agents. Therefore, identification of non-glycoside compounds as SGLT2 inhibitors would be advantageous and have better potential for development as antidiabetic therapy.

To date, only few studies have reported the discovery of non-glycoside SGLT2 inhibitors. Sato et al.[20] reported a screening study of traditional Chinese medicines, which led to the discovery of flavonoids isolated from the roots of *Sophora flavescens* as SGLT2 inhibitors. Morita et al.[21] identified cyclic diarylheptanoids, extracted from the barks of *Acer nikoense*, as moderate SGLT2 inhibitors from random screening of traditional medicines. Araj et al[22] reported that picraline-type alkaloids and ajamline-type alkaloid isolated from the leaves of *Alstonia macrophylla* showed inhibition of SGLT2 in the micromolar range. The discovery effort of non-glycoside SGLT2 inhibitors by random screening studies showed only few natural compounds isolated from the plant with the inhibition of SGLT2 in the micromolar range. These results indicate that there is still a need to explore new methods of discovering novel SGLT2 inhibitors more efficiently.

In this work, we report the first application of a computer-aided drug design approach to discover non-glycoside SGLT2 inhibitors. Since the protein structure of human SGLT2 is not available, ligand-based virtual screening was performed to search for novel SGLT2 inhibitors. The pharmacophore models were generated from eight reported SGLT2 glycoside inhibitors which were further validated by their ability to retrieve active compounds from the database. The best model was applied to virtually screen three commercially available chemical databases containing 709 537 non-glycoside small molecules. Shape-based scoring and structural clustering analysis followed by testing of only 80 compounds in a biochemical assay identified three non-glycoside compounds as potential SGLT2 inhibitors. Further analogue searching based on the structure of the most potent hit, **9**, was performed to investigate the structure−activity relationship (SAR) and provide insights on the important pharmacophore features essential for SGLT2 inhibition in this class of compounds.

Results and Discussion

Pharmacophore Model Generation. A training set of eight reported SGLT2 inhibitors with nanomolar inhibition, all containing a sugar moiety, were used to generate the pharmacophore model (Figure 1). The DISCO[23] module implemented in SYBYL was applied to construct the pharmacophore model. By analysis of the chemical nature of the training set compounds, important feature types, such as hydrogen bond acceptor (HA), hydrogen bond donor (HD), aromatic group (AR), and hydrophobic (HY), were selected for each pharmacophore model generation run. Three runs were performed with assignment of specified feature types as listed in Table 1s in the Supporting Information. Each run generated several pharmacophore models. The best models with high pharmacophore score and better structural overlay of the eight training-set compounds were selected. Models 1, 2, and 3 were selected from runs I, II, and III, respectively, and subjected to further studies.

Model 1 consists of two HA, two AR, and one HY features. Model 2 is composed of two HD, two AR, and one HY features, while model 3 contains two HA, one HD, one AR, and one HY features. To further validate these models, their ability to retrieve active compounds from a database was examined. A screening database for validation was prepared, using 106 reported SGLT2 inhibitors with inhibition activity covering over 3 orders.[24] Each model was then used to screen the validation database using the UNITY module of SYBYL. As shown in Table 1s in the Supporting Information, models 1, 2, and 3 retrieved 64, 85, and 71 out of 106 SGLT2 inhibitors,

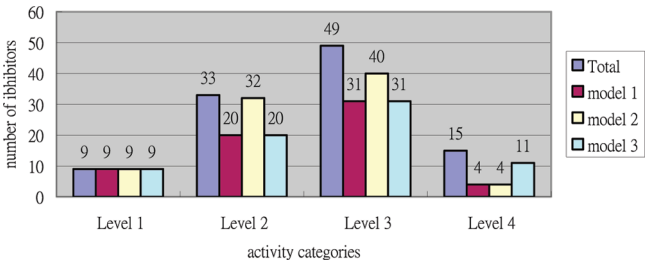


Figure 2. Validation of SGLT2 pharmacophore model screening capability. 106 SGLT2 inhibitors were divided into four activity categories: level 1, activity < 10 nM; level 2, 10 < activity < 100 nM; level 3, 100 < activity < 1000 nM; and level 4, activity > 1000 nM.

respectively. Furthermore, the 106 SGLT2 inhibitors in the database were divided into four activity categories: level 1, activity < 10 nM; level 2, 10 < activity < 100 nM; level 3, 100 < activity < 1000 nM; level 4, activity > 1000 nM. Highly active category level 1 contained nine inhibitors, and the level 2 active category contained 33 inhibitors. The level 3 active category contained 49 inhibitors, and the level 4 active category contained 15 inhibitors. The numbers of compounds retrieved in each activity category by the three pharmacophore models were counted and shown in Figure 2. In the highly active level 1 category (activity < 10 nM), all three models performed equally well to retrieve all nine inhibitors. In the level 2 activity category (10 < activity < 100 nM), model 2 performed better than models 1 and 3 to retrieve 32 out of 33 inhibitors. In the level 3 activity category (100 < activity < 1000 nM), model 2 also performed better than models 1 and 3 to retrieve 40 out of 49 inhibitors. Since the screening capability of model 2 was superior to models 1 and 3, pharmacophore model 2 was selected for further studies.

Virtual Screening with Model 2. The aim of this study is not only to construct a pharmacophore model to elucidate the crucial features important for inhibition but also to discover novel non-glycoside SGLT2 inhibitors by virtual screening chemical databases using the model generated. Three commercially available databases, Maybridge, Chembridge, and Specs in total contain around 700 000 compounds, which were screened with model 2 using the UNITY module in SYBYL. 7989 compounds matching all the features described by model 2 were selected for further analysis. Taking the steric and chemical effects into consideration, shape-based virtual screening was then employed to search for compounds with similar conformation but diverse topology to the reference compounds. Consequently, the 7989 compounds were further screened by the ROCS program, a fast 3D shape comparison program using the minimized conformation of the most potent compounds, **2** and **6**, as reference compound inputs (queries). The ComboScore, the sum of ShapeTanimoto score and ScaledColor score, which represents shape and chemical feature information, was employed to rank the database compounds.[25] The top 1000 compounds with high ComboScore, representing the conformations and chemical features similar to those of **2** and **6**, were selected from each query. Next, these 2000 hits were then filtered to exclude redundant compounds by structural clustering analysis using the program Discovery Studio 2.5. This retrieved 80 compounds with high ComboScore and structurally diverse scaffolds, which were selected to evaluate their inhibition of SGLT2 experimentally. A flowchart representing the various stages of virtual screening, including pharmacophore matching, shape-based screening, and structure clustering analysis, is summarized in Figure 1s in the Supporting Information.

Biological Activity. An SGLT2 inhibition assay was carried out to evaluate the inhibition potency of the 80 selected compounds. Three out of the 80 compounds showed inhibition with $EC_{50} < 10\,\mu M$. In the absence of the test compound as a control experiment (0.5% DMSO), 100% uptake of [^{14}C]α-methylglucopyranoside (AMG), a metabolically stable glucose analogue, was measured. In the presence of **9** (Figure 3), a dose-dependent inhibition of AMG uptake in CHO-K1 (human SGLT2) cells was observed with increasing concentration of **9**. The EC_{50} of **9**, obtained from a sigmoidal dose-response model fit from the plot by the program GraphPad Prism, was 3.85 μM. In similar experiments, **10** (Figure 3) showed inhibition against SGLT2 in a dose-dependent manner with an EC_{50} of 5.92 μM and **11** (Figure 3b) showed inhibition against SGLT2 in a dose-dependent manner with an EC_{50} of 8.98 μM (Figure 3). It is worth noting that **9** and **10** were discovered based on the shape of **6** and that **11** was discovered based on the shape of **2**.

Pharmacophore Feature Mapping. Because of the chemical novelty and activity against SGLT2, the most potent compound, **9**, was subjected to further study. Compound **9** was mapped into the model 2 to reveal the molecular features responsible for its inhibition against SGLT2. Figure 4a presents **9** mapped to model 2. Two AR features were matched onto the benzene and benzimidazole rings to represent the binding feature of the aglycone part. One HY feature was aligned to the *tert*-butyl region to present the hydrophobic interaction. One HD was mapped onto the NH group at the 2-position of the benzimidazole ring, and the other HD feature was mapped onto the OH group adjacent to the *tert*-butyl moiety.

Moreover, since **9** was generated based on the shape of **6**, **6** was also mapped into model 2 for comparison and to provide insight on the binding to SGLT2 (Figure 4b). The benzimidazole scaffold of **9** and the benzene ring of **6** were mapped to the same pharmacophore feature (AR). The HY feature located in the *tert*-butyl group of **9** corresponded to the HY feature mapped into the tetrahydropyranyl ring in the sugar moiety of **6**. The HD features on the OH group and NH group next to the *tert*-butyl group of **9** corresponded to the HD features on the 2- and 3-hydroxyl groups in the sugar moiety of **6**. These results not only indicate that **9** may have similar interactions and functions to **6** but also imply that the sugar moiety could be replaced with the combination of three individual groups (OH, NH, and *tert*-butyl) which may play an important role in the binding to SGLT2.

SAR Study of Compound 9 and Analogues. To further explore the structure−activity relationship (SAR) based on the structure of hit **9**, an analogue search was carried out. Analogues with chemical structures related to hit **9** were selected from the Chemdiv, Specs, and Chembridge databases, and their SGLT2 inhibition was evaluated (Table 1). As shown in Table 1, replacement of the benzene ring with an alkene group or a piperidine group, as in the analogues **12** and **13**, led to loss of SGLT2 inhibition. This result indicates that the aromatic (AR) substitution at this position (R^2) is an important feature for maintaining the inhibition of SGLT2, which is in agreement with the previous discussion in the pharmacophore model section that the AR feature is crucial for the SGLT inhibition. Therefore, we retained the benzyl group and started to find alternative substitution for the *tert*-butyl group in the second round search. When the *tert*-butyl group was replaced with H or a ketone group, as in analogues

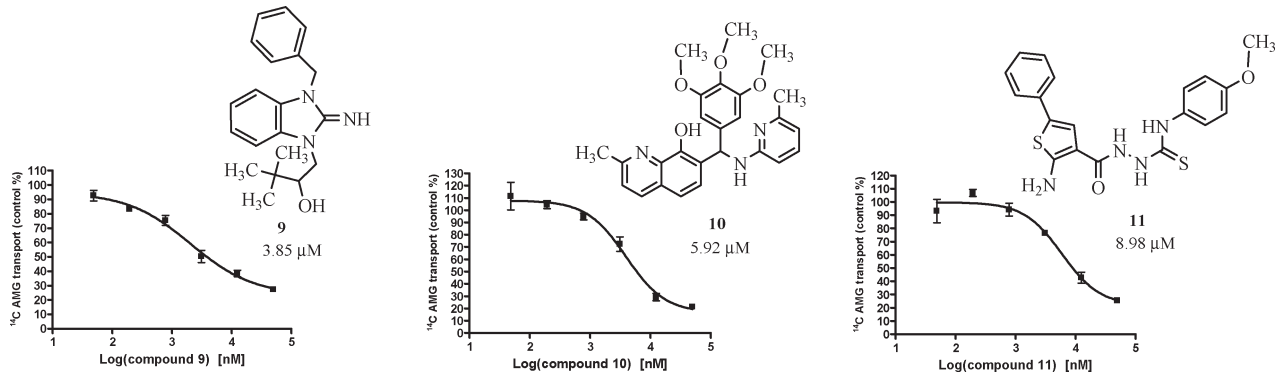


Figure 3. (a) Dose-response curves for SGLT2 inhibition of **9**, **10**, and **11**. (b) Structures of non-glycoside SGLT2 inhibitor hits and their EC_{50} values.

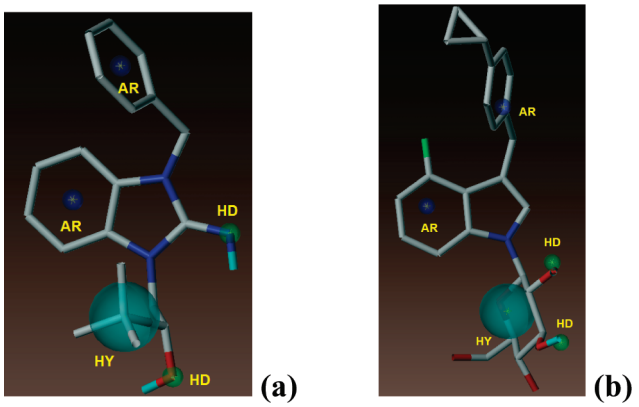


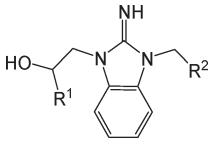
Figure 4. Mapping of **9** (a) and **6** (b) on model 2. The blue spheres represent the AR features. The green spheres represent HD features, and the cyan sphere represents the HY feature.

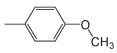
14 and **16**, SGLT2 inhibition was lost. However, the inhibition of SGLT2 was recovered when the *tert*-butyl group was replaced with hydrophobic groups, such as thiophene, furan, or phenyl (**17**, **18**, **20**, **21**). Since the *tert*-butyl group is mapped into the HY feature in the pharmacophore model, this result further validates that the HY feature is essential for inhibition to SGLT2 and further emphasizes that the hydrophobic group in this area is crucial for the activity. Moreover, comparing the inhibition levels of **14** and **15** or **18** and **19** revealed that when the para-substitution of the benzyl ring has an electron withdrawing substituent, the analogues showed an increased SGLT2 inhibition. The knowledge obtained from this preliminary SAR investigation, as discussed above, will provide direction for lead optimization of **9** in the future.

Conclusions

In this study, we have successfully developed a ligand-based virtual screening strategy in which a combination of pharmacophore model generation, shape-based scoring, and structure clustering analysis has led to the discovery of novel SGLT2 inhibitors. Initially, three pharmacophore models were generated based on eight glycoside inhibitors possessing varying levels of inhibition against SGLT2. These three models were subsequently validated by their ability to retrieve active compounds from a database. Since the screening capability of model 2 was superior to those of model 1 and model 3, model 2 was selected for further study. Model 2 is composed of two HD, two AR, and one HY features, which represent the pharmacophore features important for the inhibition of SGLT2. Model 2 was further utilized to screen three

Table 1. SAR of Hit **9** and Analogues



Compound	R^1	R^2	MW	SGLT2 inhibition EC_{50} (μM)[a]
9			323.44	3.85
12			273.37	40.4
13			330.47	>50
14	H		267.33	>50
15	H		301.77	31.1
16	=O		315.75	>50
17			349.45	19.1
18			333.38	34.7
19			367.83	11.0
20			373.45	16.2
21			373.45	4.60

[a] Values are expressed as the mean of at least two independent experiments performed in triplicate and are within ±15%.

chemical databases in silico. 7989 compounds fulfilling the pharmacophore features of model 2 were then subjected to shape-based scoring and structure clustering analysis. Finally, the top scoring, structurally diverse 80 compounds from the above exercise were selected for biological testing, and out of these, three compounds showed inhibition of SGLT2. The EC_{50} of the most potent compound, **9**, is 3.85 μM. As most of the studies so far have been devoted to developing glycoside compounds as SGLT2 inhibitors, this non-glycoside **9** represents a new scaffold for SGLT2 inhibitors and may well serve as a novel hit worthy of lead optimization efforts.

To our knowledge, this strategy is the first successful example to identify non-glycoside SGLT2 inhibitors by computer-aided drug design. Particularly, this is also the first report to generate a pharmacophore model based on glycosides and further to employ the pharmacophore model to discover novel non-glycosides hits. Because of the various drawbacks of glycoside drugs, this ligand-based virtual

screening strategy should provide a useful approach to discover non-glycoside SGLT inhibitors. Furthering addition, an analogue search was carried out based on the structure of the most potent compound, **9**, to explore the SAR. The SAR study of **9** and its analogues confirms the importance of the HY feature contributed by the sugar moiety and the AR feature produced by the aglycone part. The structural optimization of the novel hit **9** is currently in progress.

Experimental Section

SGLT2 Assay. Stably transfected CHO-K1 cells that expressed human SGLT2 were plated at 30 000 cells per well in white-walled 96-well plates (Corning, NY) and incubated for 48 h at 37 °C in a 5% CO_2 atmosphere in growth medium. After 48 h, the culture medium in the 96 wells was taken off and the wells were washed twice with 200 μL of Krebs−Ringer−Henseleit (KRH-Na) solution and incubated in KRH-Na containing 3 μM [^{14}C]AMG in the absence or presence of inhibitors for up to 120 min at 37 °C. The KRH-Na solution contained 120 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2$, 2.2 mM $CaCl_2$, and 10 mM HEPES (pH 7.4). At the end of the uptake period, the transport buffer was removed and the uptake of [^{14}C]AMG was stopped by adding ice-cold KRH-Na containing 0.5 mM phlorizin. The wells were rinsed three times with 100 μL of stop buffer using the microplate washer (TEcan, Männedorf, Switzerland). After the third rinse, the stop buffer was completely removed from the wells and the cells were solubilized by adding 1% sodium dodecyl sulfate (Sigma). After 24 h, the microtiter plate was taken for scintillation counting of radioactive [^{14}C]AMG using a TopCount (Perkin-Elmer). The percent of inhibition of inhibitors was calculated by comparing counts per minute (CPM) in inhibitor-containing wells with CPM in wells containing only DMSO vehicle. Phlorizin was evaluated in parallel in every assay. Each data point was obtained by at least two independent experiments performed in triplicate. A dose-response curve was fitted to a sigmoidal dose-response model using GraphPad software, to determine the inhibitor concentration at half-maximal response (EC_{50}).

Purity of Tested Compounds. The purity of the tested compounds was determined using a Hitachi 2000 series HPLC system using a C-18 column (Agilent ZORBAX Eclipse XDB-C18 5 μm, 4.6 mm ×150 mm), and all compounds were found to be >95% pure except for **10** with the purity of 94.1%.

Supporting Information Available: Parameters for running DISCO to generate the pharmacophore models, Flowchart of virtual screening, Pharmacophore model generation, shape-based scoring, database screening, and structural clustering analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) International Diabetes Federation. *Diabetes Atlas*, 3rd ed; International Diabetes Federation: Brussels, Belgium, 2006.

(2) Porte, D., Jr. Clinical importance of insulin secretion and its interaction with insulin resistance in the treatment of type 2 diabetes mellitus and its complications. *Diabetes Metab. Res. Rev.* **2001**, *17*, 181–188.

(3) Washburn, W. N. Development of the renal glucose reabsorption inhibitors: a new mechanism for the pharmacotherapy of diabetes mellitus type 2. *J. Med. Chem.* **2009**, *52*, 1785–1794.

(4) UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet* **1998**, *352*, 854–865.

(5) Marsenic, O. Glucose control by the kidney: an emerging target in diabetes. *Am. J. Kidney Dis.* **2009**, *53*, 875–883.

(6) Mackenzie, B.; Loo, D. D.; Panayotova-Heiermann, M.; Wright, E. M. Biophysical characteristics of the pig kidney Na^+/glucose cotransporter SGLT2 reveal a common mechanism for SGLT1 and SGLT2. *J. Biol. Chem.* **1996**, *271*, 32678–32683.

(7) Wright, E. M.; Hirayama, B.; Hazama, A.; Loo, D. D.; Supplisson, S.; Turk, E.; Hager, K. M. The sodium/glucose cotransporter (SGLT1). *Soc. Gen. Physiol. Ser.* **1993**, *48*, 229–241.

(8) Chao, E. C.; Henry, R. R. SGLT2 inhibition—a novel strategy for diabetes treatment. *Nat. Rev. Drug Discovery* **2010**, *9*, 551–559.

(9) Toggenburger, G.; Kessler, M.; Semenza, G. Phlorizin as a probe of the small-intestinal Na^+,D-glucose cotransporter. A model. *Biochim. Biophys. Acta* **1982**, *688*, 557–571.

(10) Oku, A.; Ueta, K.; Arakawa, K.; Kano-Ishihara, T.; Matsumoto, M.; Adachi, T.; Yasuda, K.; Tsuda, K.; Saito, A. Antihyperglycemic effect of T-1095 via inhibition of renal Na^+-glucose cotransporters in streptozotocin-induced diabetic rats. *Biol. Pharm. Bull.* **2000**, *23*, 1434–1437.

(11) Fujimori, Y.; Katsuno, K.; Ojima, K.; Nakashima, I.; Nakano, S.; Ishikawa-Takemura, Y.; Kusama, H.; Isaji, M. Sergliflozin etabonate, a selective SGLT2 inhibitor, improves glycemic control in streptozotocin-induced diabetic rats and Zucker fatty rats. *Eur. J. Pharmacol.* **2009**, *609*, 148–154.

(12) Fujimori, Y.; Katsuno, K.; Nakashima, I.; Ishikawa-Takemura, Y.; Fujikura, H.; Isaji, M. Remogliflozin etabonate, in a novel category of selective low-affinity sodium glucose cotransporter (SGLT2) inhibitors, exhibits antidiabetic efficacy in rodent models. *J. Pharmacol. Exp. Ther.* **2008**, *327*, 268–276.

(13) Rossetti, L.; Smith, D.; Shulman, G. I.; Papachristou, D.; DeFronzo, R. A. Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. *J. Clin. Invest.* **1987**, *79*, 1510–1515.

(14) Ehrenkranz, J. R.; Lewis, N. G.; Kahn, C. R.; Roth, J. Phlorizin: a review. *Diabetes Metab. Res. Rev.* **2005**, *21*, 31–38.

(15) Meng, W.; Ellsworth, B. A.; Nirschl, A. A.; McCann, P. J.; Patel, M.; Girotra, R. N.; Wu, G.; Sher, P. M.; Morrison, E. P.; Biller, S. A.; Zahler, R.; Deshpande, P. P.; Pullockaran, A.; Hagan, D. L.; Morgan, N.; Taylor, J. R.; Obermeier, M. T.; Humphreys, W. G.; Khanna, A.; Discenza, L.; Robertson, J. G.; Wang, A.; Han, S.; Wetterau, J. R.; Janovitz, E. B.; Flint, O. P.; Whaley, J. M.; Washburn, W. N. Discovery of dapagliflozin: a potent, selective renal sodium-dependent glucose cotransporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes. *J. Med. Chem.* **2008**, *51*, 1145–1149.

(16) Wilding, J.; Woo, V.; Soler, N.; Pahor, A.; Sugg, J.; Parikh, S. Dapagliflozin in Patients with Type 2 Diabetes Poorly Controlled on Insulin Therapy—Efficacy of a Novel Insulin-Independent Treatment. American Diabetes Association: Alexandria, VA, 2010; Abstract 0078-OR, http://ww2.aievolution.com/ada1001/index.cfm?do=abs.viewAbs&abs=12779.

(17) Rosenstock, J.; Arbit, D.; Usiskn, K.; Capuano, G.; Canovatchel, W. Canagliflozin, an Inhibitor of Sodium Glucose Co-Transporter 2 (SGLT2), Improves Glycemic Control and Lowers Body Weight in Subjects with Type 2 Diabetes (T2D) on Metformin. American Diabetes Association: Alexandria, VA, 2010; Abstract 0077-OR, http://ww2.aievolution.com/ada1001/index.cfm?do=abs.viewAbs&abs=12778.

(18) Kashiwagi, A.; Utsuno, A.; Kazuta, K.; Yoshida, S.; Kageyama, S. ASP1941, a Novel, Selective Sodium-Glucose Co-Transporter (SGLT) 2 Inhibitor, Was Investigated in the Japanese Population. American Diabetes Association: Alexandria, VA, 2010; Abstract 0075-OR, http://ww2.aievolution.com/ada1001/index.cfm?do=abs.viewAbs&abs=12776.

(19) Ernst, B.; Magnani, J. L. From carbohydrate leads to glycomimetic drugs. *Nat. Rev. Drug Discovery* **2009**, *8*, 661–677.

(20) Sato, S.; Takeo, J.; Aoyama, C.; Kawahara, H. Na^+-glucose cotransporter (SGLT) inhibitory flavonoids from the roots of *Sophora flavescens*. *Bioorg. Med. Chem.* **2007**, *15*, 3445–3449.

(21) Morita, H.; Deguchi, J.; Motegi, Y.; Sato, S.; Aoyama, C.; Takeo, J.; Shiro, M.; Hirasawa, Y. Cyclic diarylheptanoids as Na^+-glucose cotransporter (SGLT) inhibitors from *Acer nikoense*. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1070–1074.

(22) Arai, H.; Hirasawa, Y.; Rahman, A.; Kusumawati, I.; Zaini, N. C.; Sato, S.; Aoyama, C.; Takeo, J.; Morita, H. Alstiphyllanines E-H, picraline and ajmaline-type alkaloids from *Alstonia macrophylla* inhibiting sodium glucose cotransporter. *Bioorg. Med. Chem.* **2010**, *18*, 2152–2158.

(23) Martin, Y. C.; Bures, M. G.; Danaher, E. A.; DeLazzer, J.; Lico, I.; Pavlik, P. A. A fast new approach to pharmacophore mapping and its application to dopaminergic and benzodiazepine agonists. *J. Comput.-Aided Mol. Des.* **1993**, *7*, 83–102.

(24) Washburn, W. N. Evolution of sodium glucose co-transporter 2 inhibitors as anti-diabetic agents. *Expert. Opin. Ther. Pat.* **2009**, *19*, 1485–1499.

(25) Kirchmair, J.; Distinto, S.; Markt, P.; Schuster, D.; Spitzer, G. M.; Liedl, K. R.; Wolber, G. How to optimize shape-based virtual screening: choosing the right query and including chemical information. *J. Chem. Inf. Model.* **2009**, *49*, 678–692.