Exploring Newer Target Sodium Glucose Transporter 2 for the Treatment of Diabetes Mellitus

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Abstract: Diabetes mellitus is an independent risk factor for the development of coronary artery disease, myocardial infarction, hypertension, and dyslipidemia. The treatment of diabetes has been mainly focused on maintaining normal blood glucose concentrations. Insulin and hypoglycemic agents have been used as standard therapeutic strategies. However, these are characterized by limited efficacy and adverse side effects, making the development of new therapeutic alternatives mandatory. Inhibition of glucose reabsorption in the kidney, mediated by Sodium Glucose Transporters (SGLT's), represents a promising therapeutic approach. The high-affinity sodium glucose cotransporter (SGLT1) is expressed to some extent in the kidney and contributes to glucose reabsorption, However Genetic mutations in the SGLT1 gene leading to a functional defect are responsible for glucose/galactose malabsorption. The low-affinity sodium glucose cotransporter (SGLT2), which is expressed specifically in the kidney, plays a major role in renal glucose reabsorption in the proximal tubule. We focused on SGLT2 as a molecular target for this review because it plays a major role in renal glucose reabsorption, and its tissue distribution is limited in the kidney to reduce the likelihood for mechanism-based side effects. Phlorizin, a natural phenolic O-glucoside has been known to induce glucosuria for more than 100 years. As it is not a specific SGLT inhibitor, later on o-glucoside is replaced by c-glycoside as it is resistant to hydrolysis by β-glucosidases. The present review summarizes the concept of SGLT2 selective target based therapy for diabetes mellitus and the current clinical and preclinical development of SGLT2 inhibitors.

Keywords: Sodium glucose transporters, diabetes mellitus, dyslipidemia, glucotoxicity, glucose facilitated transporters, β-glucosidases, hypoglycemia, dyslipidemia.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a disorder characterized by elevated serum glucose. Hyperglycemia is well established as a major risk factor for microvascular and potentially macrovascular complications of diabetes. In addition, there is strong evidence to suggest that hyperglycemia *per se* has a deleterious effect on insulin secretion and reduces insulin sensitivity an effect referred to as glucotoxicity and this, in turn, contributes to the progression of diabetes [1, 2]. Diabetes mellitus has a high incidence worldwide. The International Diabetes Federation estimated to be 246 million, and this number is projected to reach at least 380 million people in the world to have diabetes with in 20 years [3]. Approximately 90-95% of people who are diagnosed with diabetes have type 2 diabetes. Diabetes both type 1 and type 2, poses two to six fold higher risk for progressive cardiovascular disease; emerging evidence suggests aggressive glycemic control may have some benefits in terms of modifying this risk [4-6]. T2DM is associated with serious complications and comorbidity and is quickly becoming one of the leading causes of death and disability in the world [7-9]. Complications of diabetes arise from chronic hyperglycemia, which can cause damage to large and small blood vessels and peripheral nerves, potentially leading to heart attack, stroke, blindness, the need for limb amputation, and kidney failure [10, 11]. Current therapies act to improve metabolism by increasing insulin secretion, improving insulin sensitivity, or replacing insulin altogether [12]. Most of these agents lose their glycemic efficacy over time [7, 13]. They are mainly classified as: α-Glucosidase inhibitors, Sulfonylureas, Meglitinides, Biguanides, Thiazolidinediones (TZDs), Glucagon-Like Peptide-1 (GLP-1) and DPP-IV inhibitors Fig. (**1**). Several other new approaches are being taken in the search for the treatment of diabetes and the list includes: the *hepatic-derived fibroblast growth factor 21* (FGF21), the *renal sodium-glucose transporter-2* (SGLT2), and the *NAD^+-dependent deacetylase SIRT1 or sirtuin 1*. Several types of oral hypoglycemic drugs are available for the clinical control of blood glucose [14]. Among these, sulfonylureas are insulin secretagogues with efficacies that depend on insulin secretion [15]. Frequently, inappropriate, excessive insulin secretion is induced by sulfonylureas, and this causes hypoglycemia, a major clinical side effect [16]. β-Glycosidase inhibitors are effective at preventing post-prandial hyperglycemia by delaying carbohydrate digestion [17], and thus gastrointestinal symptoms such as soft feces or diarrhea are their main side effects [18]. Thiazolidinediones promote adipocyte differentiation and enhance insulin sensitivity [19], but body weight control may be compromised because glucose is accumulated as fat [20]. Biguanides exerts hypoglycemic effects by acting on energy production [21]. Thus there is an urgent need to develop newer therapeutic strategy to treat diabetes, which does not have such side effects. This review summarizes recent pharmacological approaches based on

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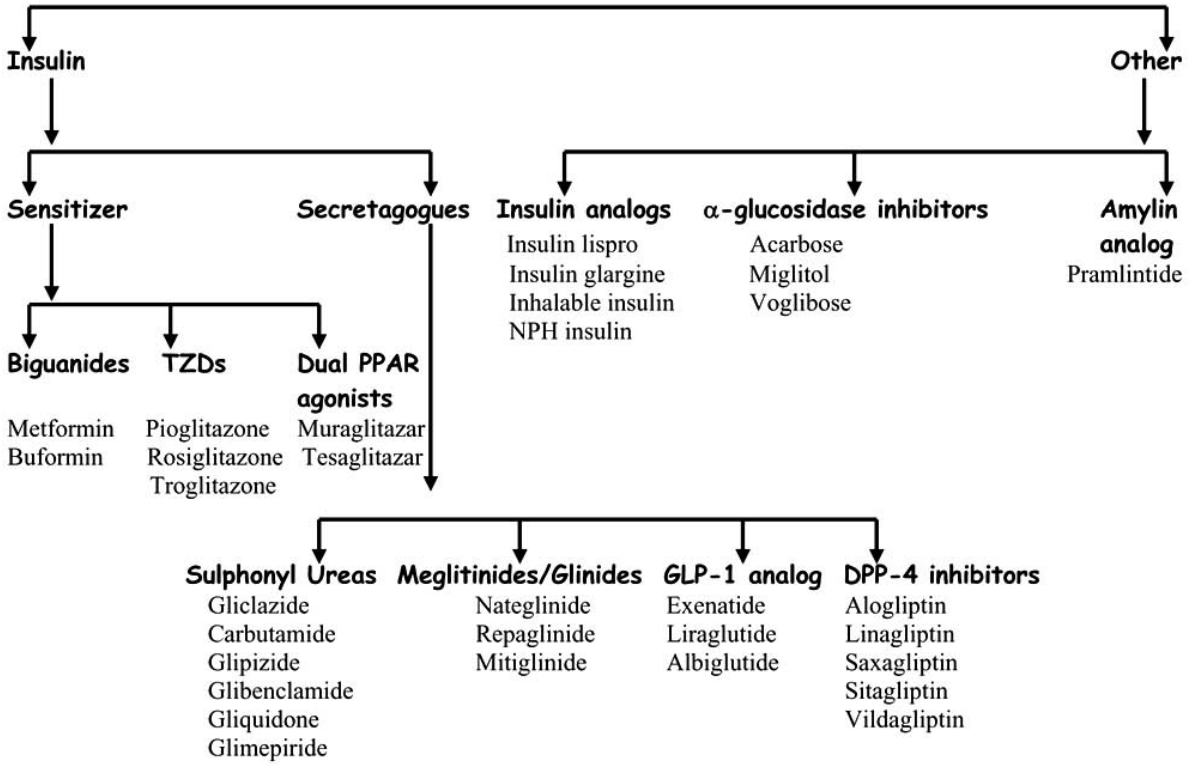

Fig. (1). Classification of the anti diabetic drugs based on the mechanism of action.

renal sodium-glucose transporter-2, a new class of orally administered compounds that targets renal glucose transport and inducers of glucosuria and are currently being tested for efficacy in type 2 diabetes treatment. The main section includes mechanism of action and its clinical implications; several important structural classes of the non specific SGLT and specific SGLT2 are described and compared based on literature data. The last section of this review is providing a brief overview of some efficacy data from recent clinical studies with SGLT2.

Diabetes results from insulin resistance combined with relative insulin deficiency [22]. Both insulin resistance and deficiency leads to hyperglycemia due to altered glucose transport into the cells. Cellular glucose uptake requires transport proteins because it does not freely permeate the plasma membrane [23]. Glucose transport proteins are divided in two groups: glucose facilitated transporters (GLUT) and sodium dependent D-glucose co-transporters (SGLT). GLUT allows transport of glucose down its concentration gradient, while SGLT transports glucose against its concentration gradient. The causes of type 2 diabetes are numerous and complex, but physical inactivity is an important factor. Exercise, the major physiological activator of muscle glucose transport, regulates the expression of GLUT4 in skeletal muscle [24, 25], and induces its translocation from the intracellular pool to the plasma membrane [26, 27]. However, sustained insulin deficiency leads to a decreased number of GLUT4 transporters, resulting in impaired responsiveness of glucose transport to both insulin and exercise [25, 28]. People with type 2 diabetes have been shown to have defective insulin-dependent glucose transport in skeletal muscle [29]. This is of concern given that skeletal muscle plays an important role in glucose homeostasis, primarily due to its effect on postprandial glucose uptake [30]. The SGLT transports glucose (and galactose), with different affinities, *via* a secondary active transport mechanism. The Na^+-electrochemical gradient provided by the Na^+–K^+ ATPase pump is utilized to transport glucose into cells against its concentration gradient. This form of glucose transport takes place across the lumenal membrane of cells lining the small intestine and the proximal tubules of the kidneys.

Historically, phlorizin was the first natural sodium glucose transporter inhibitor. It is a natural o-glycoside largely available from the root and bark of the apple, pear and cherry [31]. However, several of its properties are disadvantageous for this purpose. Phlorizin is a nonselective SGLT inhibitor and is thus not suitable for distinguishing between SGLT1 and SGLT2 [32]. Phloretin, the aglycon of phlorizin, is produced as a metabolite and may inhibit facilitative glucose transporter (GLUT1), which is responsible for glucose uptake in various tissues [31, 33]. Several researchers have focused on the renal glucose reabsorption system as a way of improving hyperglycemia in type 2 diabetes, and SGLT inhibitors have been developed [32, 34-38].

RATIONALE FOR SGLT2 INHIBITION

The inhibition of renal glucose reabsorption is a novel approach for the treatment of diabetes. In normal individuals, glucose present in the plasma is filtered by the kidneys, but virtually all of it is reabsorbed, such that <1% of glucose is excreted in urine and 99% of glucose reabsorbed through early S1 segment of the proximal convoluted tubule [39-41]. Inhibition of this reabsorption process is predicted to reduce the renal threshold for glucose, allowing the excretion of

excess glucose in the urine and thus lowering plasma glucose levels. Because this mechanism of action does not require insulin secretion or insulin action to affect glucose lowering, it could be efficacious in a wide variety of diabetic patients. Furthermore, since hyperglycemia per se has been shown to reduce insulin sensitivity and impair β-cell function in animal models, the correction of hyperglycemia is predicted to improve these important physiological defects in type 2 diabetes [42-49]. The level of specificity for SGLT2 inhibition is an important consideration, since inhibition of SGLT1 is associated with potentially serious side effects. Although SGLT1 plays a minor role in renal glucose reabsorption, this high-affinity, low-capacity co-transporter facilitates absorption of dietary glucose in the intestine. SGLT1 is also expressed in a number of other tissues as well, although its function is less understood [50]. Evidence for the deleterious effects of SGLT1 inhibition is derived from individuals with mutations in the SGLT1 gene, these patients experience glucose-galactose malabsorption syndrome, which results in severe, sometimes fatal diarrhea [51]. In contrast, genetic alterations in SGLT2, which are exclusively expressed in renal proximal tubule cells, lead to increased renal glucose excretion with no apparent adverse effects on carbohydrate metabolism [31, 52]. Evidence suggests SGLT2 to be responsible for the majority of renal reabsorption thus it has become potential target of therapeutic interest.

SODIUM GLUCOSE TRANSPORTERS

The SGLTs are expressed mainly in the brush-border membrane of the small intestines and kidney proximal tubules [53]. There are three to six, SGLTs (SGLT1–SGLT6; gene name SLC5A, 11 genes) identified till date [54-56]. *In vitro* perfusion studies of rabbit proximal tubules provided evidence for the existence of a low affinity Na^+-coupled glucose transporter in S1 segments and a high affinity Na^+-coupled glucose transporter located in S3 segments [57]. The first of this type of glucose transport protein to be cloned was the high-affinity transporter from rabbit intestine, SGLT1 [58]. The human analogue soon followed by homology cloning [59]. SGLT1 has a limited tissue expression and is found essentially on the apical membranes of small-intestinal absorptive cells (enterocytes) and renal proximal straight tubules (S3 cells).

A second Na^+–glucose transporter, SGLT2, is of low affinity and is predominantly expressed on the apical membrane of renal proximal convoluted tubules (S1 and S2 cells) [60, 61]. It is currently accepted that in the kidney, SGLT2 (low affinity, high capacity) transports the bulk of plasma glucose from the glomerular filtrate. Any remaining glucose is recovered by SGLT1 (high affinity, low capacity) thus preventing glucose loss in the urine. However, controversy exists as to whether SGLT2 is the major renal glucose transporter [54, 62]. Later on it was reported that the renal reabsorption of glucose occurs mainly through SGLT2 because a homozygous nonsense mutation and compound heterozygous mutations in the SGLT2 gene were recently found in patients suffering from renal glucosuria [63, 64]. The function of SGLT3, which is a human ortholog of porcine SGLT2 and formerly called SAAT1 [65, 66], was recently reevaluated [67]. The SGLT3 genes encode proteins containing 659–672 residues, with a predicted mass of 73 kDa. The amino acid sequence alignments show that SGLT3 and SGLT2 have 70 and 59% similarities with SGLT1 respectively. SGLT2 or SGLT3 has been predicted to have the same secondary structure profile as SGLT1, even though there have been virtually no experimental studies. The affinity of SGLT1 for D-glucose is 10 times higher than SGLT2 or 3. The Na^+/glucose coupling ratio is two for SGLT1 and SGLT3, and is believed to be one for SGLT2. Therefore, the major differences in function between SGLT1 and SGLT2/SGLT3 relate to differences in the selectivity for glucose transport. Besides glucose reabsorption, a wide variety of physiological roles have been found for the SGLT family: human SGLT1 functions as a water channel and human SGLT3 functions as a glucose sensitive sodium channel. This suggests that SGLT3 is not a glucose transporter but rather a glucose sensor in the plasma membrane of cholinergic neurons, skeletal muscle and other tissues. Additional members, SGLT4–6, have been assigned but their complete functional and structural characterization has not been reported.

REGULATORY MECHANISMS OF SGLTs IN PTCs

The model for glucose transport across the tubule is similar to that first reported for the small intestine, that is, glucose is first accumulated within the epithelium by SGLTs in the brush-border membrane and is then transported out of the cell across the basolateral membrane by GLUTs Fig. (**2**). Both active and facilitative glucose transporters have distinct distribution profiles along the proximal tubule related to their particular kinetic characteristics. This provides a proximal tubule environment through which 90% of the filtered glucose is reabsorbed by the low-affinity / high-capacity SGLT2 and GLUT2 located in the early S1 segments of the proximal tubule. On the other hand, the high-affinity/low-capacity transporters, SGLT1 and GLUT1, scavenge the residual glucose that is presented to the later portions (S3 segments) of the proximal tubule [68].

ADVANTAGES AND DISADVANTAGES

Very first argument is that SGLT2 inhibition is not targeting the underlying pathophysiology of diabetes; this is also true for many current treatments, including commonly used drugs, such as metformin and sulfonylureas. The advantages of this approach include the loss of glucose (and therefore energy) in the urine, which may help weight loss or weight maintenance, a key target for any type 2 diabetes treatment. Secondly, the energy deficit, which, depending on the dose used, is likely to be about 100-300 kcal per day and in the same range as that seen with the intestinal lipase inhibitor orlistat [69]. SGLT2 inhibitors do not stimulate insulin secretion; therefore, they would be expected to have a low hypoglycemia risk, unless used in combination with insulin secretagogues or insulin itself. Preclinical studies have suggested that the therapeutic effect of SGLT2 inhibitors is likely to lead to reductions in hepatic glucose production and amelioration of glucotoxicity, both of which are important therapeutic goals in diabetes [44]. Another potential benefit would be small increases in sodium excretion; this would be analogous to the mild diuretic effect seen with thiazide diuretics, and might theoretically result in modest reductions in arterial blood pressure. This is supported by a

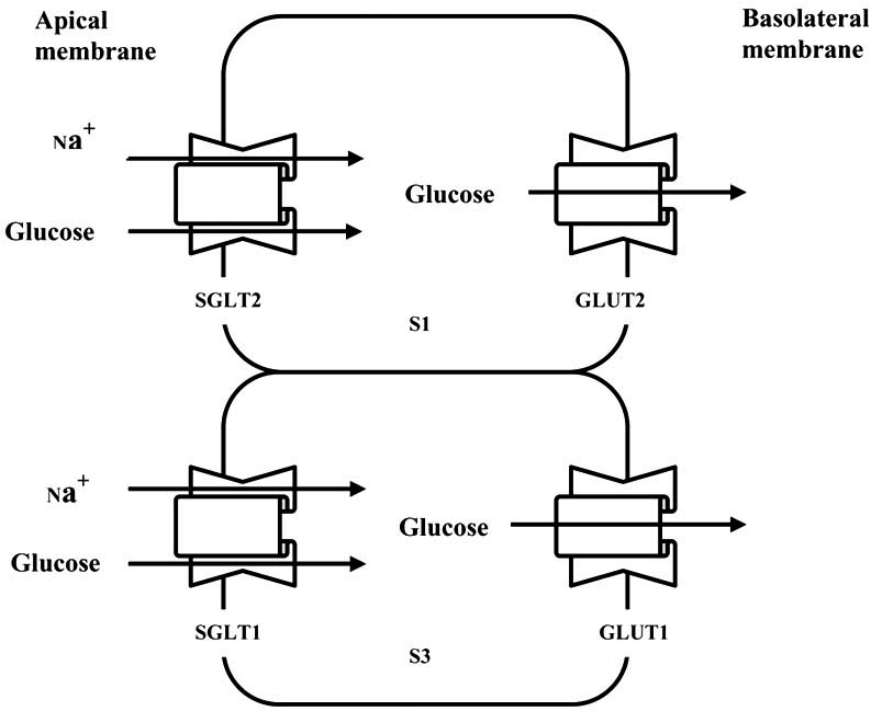

Fig. (2). Renal glucose transporters and their action.

recently published study which showed that tubular SLGT2 expression was up-regulated in spontaneously hypertensive rats, probably mediated *via* increased angiotensin 2 *via* the angiotensin II type 1 (AT1) receptor which may be associated with the increase in sodium reabsorbtion [67]. If this is the case, then SGLT2 inhibition may be helpful to the diabetic patients having hypertension but still it needs to be studied in clinical stage.

The main drawback with glucosuric agents is the damage to kidney, but till date it is not observed in any study. Secondly, the effects of improved glycemia should reduce glomerular hyperfiltration and have long-term beneficial effects in terms of reduction of diabetic nephropathy. Increases in urinary glucose excretion may lead to polyuria and increased thirst, but this has not been reported as a major problem in the trials till date, or in familial renal glucosuria. Another theoretical problem in relation to the genitourinary tract is increased risk for either bacterial or fungal infection. However, there is little direct evidence that diabetes increases the risk for bacterial urinary tract infections, and although fungal infections, such as vaginal candidiasis in women and balanitis in men, are associated with hyperglycemia, [68] it is not known whether this is a result of systemic hyperglycemia or the associated glucosuria.

Due to the mechanism of action of SGLT2 inhibitors, some patients would experience salt-wasting, as has been described in one individual with an SGLT2 mutation [51]. The small trials reported to date have not suggested a major problem in this respect, but it remains a possibility that will have to be considered in ongoing and future studies. It is also important to consider the likelihood of other unpredicted adverse effects, but there is a lack of clinical data to comment further on this possibility for this class of drug.

CURRENT STATUS OF SGLT2's

Phlorizin, a natural phenolic *O*-glucoside present in many plants including apple trees, has been known to induce glucosuria for more than 100 years [31]. More recently, studies showed that phlorizin administration reduced plasma glucose levels and improved insulin sensitivity in diabetic animal models [42, 45, 48]. However, this *O*-glucoside is a nonspecific SGLT inhibitor impacting both SGLT1 and SGLT2 activity, which limits its usefulness as a treatment for T2DM patients. Moreover, metabolic instability of phlorizin due to *β*-glucosidase cleavage in the intestinal tract precludes oral administration. To improve the selectivity and stability of phlorizin its structure-activity relationship (SAR) has been developed and analysis of dihydrochalcone analogues of phlorizin revealed that the addition of para lipophilic groups to the distal ring enhanced both SGLT2 inhibitory activity and greater selectivity for SGLT2 than SGLT1 [32, 70].

The *O*-glycoside T-1095A exhibited sufficiently promising potency, selectivity, and *in vivo* efficacy when administered orally, if protected from intestinal glucosidases, that it entered clinical trials as the methyl carbonate prodrug T-1095 [71-73]. The report from Wyeth that administration of 4-benzylpyrazolones derivative WAY-123783 (Fig. **3**) could induce a glucosuric response in mice prompted investigations at Kissei and Bristol-Myers Squibb that independently led to the realization that *in vivo* glucosylation of 4-benzylpyrazolones must occur to generate a series of potent SGLT2 inhibitors [74]. Subsequent changes in this lead molecule results in *O*-glycosides of *o*-benzylphenols as a second potent series of SGLT2 inhibitors [36, 75-80]. The *o*-benzylphenolic *O*-glucoside Sergliflozin-A, KGT 1075 and the benzylpyrazolone *O*-glucoside (remogliflozin) emerged from this effort at Kissei [81, 82]. Subsequent exploration of

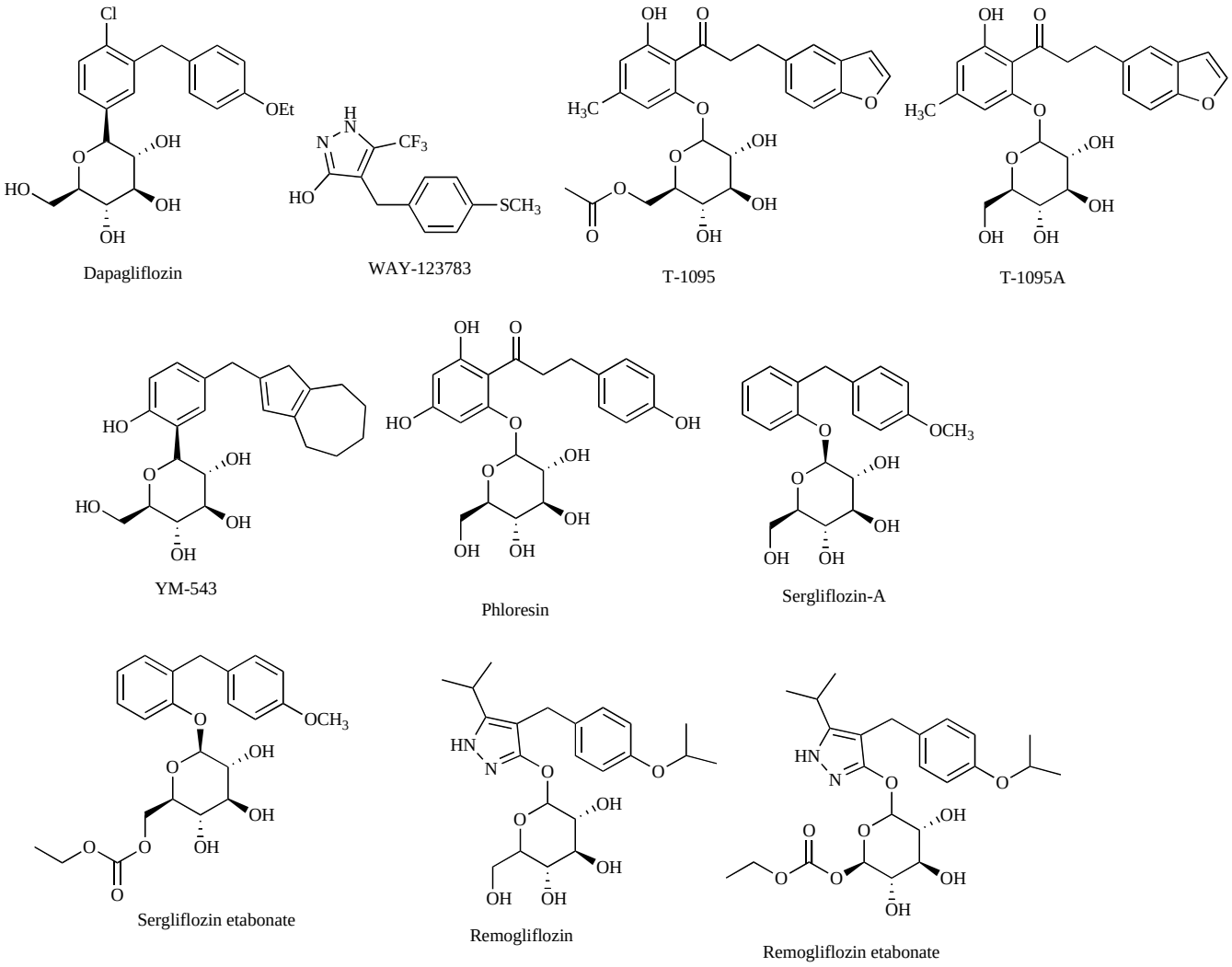

Fig. (3). Chemical structure of the known SGLT 2 inhibitors.

the SAR space for *O*-glucoside based SGLT2 inhibitors by a number of other groups produced disclosures of a variety of novel *O*-and *N*-glycosides only partially exemplified [83-85] a more complete representation may be found in prior reviews [86, 87].

Now a day's O-glycosides are replaced by C-glycosides as they are resistant to hydrolysis by β-glucosidases. This effort culminated in the discovery of dapagliflozin (BMS-512148), which subsequently has exhibited sufficient promise to warrant initiation of phase III clinical studies in 2007. It is noted that SGLT2 inhibition is not limited to low molecular weight glycosides. Two other approaches targeting inhibition of renal glucose reabsorption are in preclinical development. One uses antisense molecules exemplified by ISIS 388626 to reduce expression of SGLT2; the other uses SGLT specific peptide antagonists [88-90].

In preclinical trials, the abilities of O-glucoside-based SGLT2 inhibitors to blunt postprandial glucose (PPG) excursions, promote glucosuria, and reduce hyperglycemic levels in normal and diabetic rats are well established [32, 81, 82, 70-73]. The *in vivo* profile of dapagliflozin suggests that C-glucoside based inhibitors are no different, when administered at 0.1-1.0 mg/kg to normal and diabetic rats, dapagliflozin dose dependently increased glucosuria, resulting in urinary glucose excretion of up to 1.0 g/day without causing hypoglycemia. An oral glucose tolerance test (OGTT) test revealed that doses of 1 and 10 mg/kg reduced the glucose AUC of normal rats by 30% and 50%, respectively, thereby demonstrating the ability to blunt PPG excursion [91].

In clinical phase trial dapagliflozin, recently reported results from a 12 week phase II B study comprising 389 T2DM patients provide further confirmation of both the efficacy and safety of dapagliflozin in T2DM patients [92, 93]. The most common adverse events were urinary tract infection, nausea, dizziness, headache, fatigue, back pain, and nasopharyngitis. The incidence of reported but unverified hypoglycemic events was higher than placebo but similar to that of metformin, a hyperglycemic agent known to present little risk of hypoglycemia. No clinically meaningful changes in serum sodium, potassium, or calcium levels were detected, but serum magnesium was elevated and phosphate was reduced. Currently phase III studies with dapagliflozin are in progress.

Table 1. SGLT2 Inhibitors Currently Under Development for the Treatment of Diabetes

Drug	Source	Phase
Dapagliflozin	Bristol-Myers Squibb/Astrazenica	III
ASP-1941	Astellas Pharma/ Kotobuki	II
AVE-2268	Sanofi-aventis	II
JNJ-23431754	Johnson and Johnson	II
Remogliflozin etabonate	Glaxo SmithKlin/ Kissei	II
TA-7284	Mitsubishi Tanabepharma/ Johnson and Johnson	II
YM-543	Astellas Pharma/ Kotobuki	II
R-7201	Roche/Chugai Pharmaceuticals	II
SAR-7226	Sanifi-aventis	I
ISIS-388626	Isis Pharmaceuticals	I

Several other SGLT2 inhibitors are also being actively evaluated in the clinic (Table **1**). GSK/Kissei had completed several phase II studies with sergliflozin as an antidiabetic agent before apparently replacing it with remogliflozin etabonate [94, 95]. Sanofi-Aventis completed a phase IIB study with AVE 2268 in 300 patients. However, subsequent progression has been discontinued [95, 96]. Similarly, Taisho, after conducting a phase II study in Japan with TS-033 halted clinical progression of TS-033 in favor of an alternative undisclosed candidate [97]. Astellas has completed a 3-month phase IIB study with the C-glucoside YM-543 [98]. Boehinger-Ingelheim has evaluated two SGLT2 inhibitors, BI 10773 and BI 44847, in a 4-week phase I/IIA study in diabetics [95, 99]. Despite the extensive number of studies listed in the clinical trials database, only GSK and Sanofi-Aventis have disclosed any clinical results. Sanofi-Aventis disclosed the urinary glucose excreted over 24 h following administration of a single 1.2 and 2 g dose of AVE 2268 to healthy volunteers. GSK reported that in two early phase 1 single dose studies, sergliflozin was well tolerated when administered at doses of 5-500 mg to healthy males and diabetics. Dose dependent urinary glucose excretion was observed to plateau at the higher doses in both studies.

Comparison of findings from and doses utilized in seasonal affective disorder (SAD) studies of AVE-2268 and sergliflozin with that of dapagliflozin reveals dapagliflozin to be a much more potent glucosuric agent over 24 h. Just as was observed in rodents, all indications are that O-glucoside containing SGLT2 inhibitors will require much higher doses to compensate for either the presumed more rapid clearance mediated in part by glucosidases or lower bioavailability in order to achieve clinical efficacy comparable to that of C-glycosides such as dapagliflozin.

CONCLUSIONS

Selective inhibition of the SGLT2 transporters in the kidney offers promising weight-neutral approach for treatment of T2DM. Low nano-molar inhibitors contain a glucose residue for targeting which is covalently bound by either a *C*- or *O*-glycoside linkage to a large properly oriented hydrophobic planar moiety to increase affinity. The spatial orientation conducive to high affinity differs for *O*- and *C*-glycosides. C-glycosides appear to be more potent and specific to the target. It also possesses relatively good metabolic stability against glycosidase hydrolysis. Combined with the observation of C-glycosides did not show any gastrointestinal side effects or hypoglycemia. These agents induce glucosuria, reduce fasting and postprandial hyperglycemia, improve insulin sensitivity, and reduce the need for insulin production. Moreover, the caloric loss induced by SGLT2 inhibition, not surprisingly, was found to produce significant weight loss in animal models, especially if compensatory food consumption was restrained.

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