1. Introduction

Type 2 diabetes mellitus (T2DM) is a major metabolic, multi-causal and heterogeneous disorder which causes significant morbidity and mortality with considerable burden to healthcare resources. The number of deaths due to T2DM highlights the insufficiency of the currently available drugs for controlling the disease and its complications and more needs to be done.

Areas covered: This paper reviews the updated pathobiology of T2DM that should be targeted in drug discovery. Further, the article provides discussion on the mechanism of action, side effects and structure of the currently available synthetic drugs. The authors specifically evaluate two newer classes of anti-diabetic agents: dipeptidyl peptidase IV (DPP-4) and sodium-glucose transporter-2 (SGLT2). They also present information on newer synthetic compounds. The article also highlights the key interactions between synthetic compounds and DPP-4 active site residues for rational drug design.

Expert opinion: Numerous anti-hyperglycaemic drugs are currently available but many are limited by their adverse effects. The identification of the 3D structure of DPP-4 has opened new avenues for design, thus aiming to produce drugs that directly exploit the structural characteristics of this binding site. Further, structural- and ligand-based screening techniques have been developed for designing novel DPP-4 and SGLT2 inhibitors. There has also been progress with the design and development of novel T2DM therapeutics including: PPARα/dual agonists, Sirtuin 1 activators, glycogen phosphorylase inhibitors and protein tyrosine phosphatase 1B inhibitors. Finding new targets and synthesis strategies is still essential but it is becoming accepted that no diabetic therapy is ‘best suited’ with each patient responding differently.

Keywords: anti-diabetic agents, dipeptidyl peptidase IV inhibitors, sodium-glucose transporter-2 inhibitors, synthetic drugs, type 2 diabetes
The incidence of T2DM continues to grow rapidly worldwide and is associated with multiple comorbidities. Peripheral insulin resistance and β-cell failure represent the core pathophysiological defects in T2DM. Currently available synthetic drug therapies include metformin, sulphonylurea and other insulin secretagogues, thiazolidinediones, α-glucosidase inhibitors, GLP1 agonists, dopamine-2 agonists, bile acid sequestrants, DPP-4 and SGLT2 inhibitors. Two latter classes (DPP-4 and SGLT2 inhibitors) are novel and promising drugs that target novel mechanisms. Finding new drug targets and new faster and cheaper synthesis methods are the main goals of T2DM drug discovery.

This box summarises key points contained in the article.

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2. Pathophysiology of T2DM

 Unlike simple characterisation of type 1 diabetes, the pathogenesis of T2DM is more complex and still remains a matter of argument. The most problem is that the specific aetiologies have not yet been clearly elucidated. For instance, unlike type 1 diabetes, autoimmune destruction of cells does not occur and ketoacidosis seldom occurs [33]. As mentioned earlier, peripheral insulin resistance and β-cell dysfunction are rather involved in T2DM [34]. Insulin resistance and the related consequences called insulin resistance syndrome (IRS) or the metabolic syndrome are common in T2DM, deemed mainly linking to patients’ genetic background [35].

Insulin resistance is also linked to obesity [36], dyslipidemia, hypertension and increased cardiovascular risk [37]. However, risk alleles in some loci seem having a primary impact on insulin sensitivity. Peroxisome proliferator-activated receptor-γ (PPAR-γ), glucokinase regulator and insulin-like growth factor 1 have been proved as insulin resistance locus from T2DM susceptibility loci [38]. Obesity and lifestyle are independent risk factor for T2DM [1,39]. According to short-term studies, numerous benefits of weight loss can be achieved in overweight or obese patients with T2DM. The finding of recent study indicated that the intensive lifestyle intervention do not reduce the rate of cardiovascular morbidity and mortality in overweight or obese patients with T2DM [40]. Gastric bypass induces substantial and sustained weight loss and is a highly effective treatment for obesity-related diabetes. Laboratory data suggest that gastric bypass exhibits reprogramming of intestinal glucose metabolism which renders the intestine a major tissue for glucose disposal, contributing to the improvement in glycaemic control after surgery [41].

Several studies have indicated that obesity correlates with a low-grade inflammation of the white adipose tissue. This results from activation of some pro-inflammatory signalling pathways which end up with insulin resistance, impaired glucose tolerance and diabetes [39,42]. Recent data indicate that white adipose tissue in obese patients is infiltrated by macrophages [43]. Several pro-inflammatory factors, such as TNF-α and IL-6, are derived not only from adipocytes but also from infiltrated macrophages [39]. The role of cytokines and insulin signalling pathways should not be missed as this interaction results in change of insulin action [44,45]. Normally, insulin acts through binding to and activation of its cell-surface receptors. Such receptors are made up of two α subunits and two β subunits, which are disulfide linked into α2β2 heterotetrameric complex. The actions of insulin are initiated when the receptor is bound to the extracellular α subunits, causing phosphorylation of intracellular tyrosine kinase domain of the β subunits and inducing a conformational change [46-48]. Phosphorylation of the insulin receptor can activate insulin receptor substrate complexes (IRS-1 – IRS-4). The activated IRS binds to the p85 regulatory subunit of phosphatidylinositol (PI) 3-kinase that results in activation of p110 catalytic subunit (47,49,50). PI(3,4,5) P3 which is produced by enzymatic activity of PI3-kinase is a key lipid second messenger in various metabolic effects of insulin [51]. PI(3,4,5)P3 mediates the signal transduction to downstream molecules including Akt and atypical protein

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**Article highlights.**

- The incidence of T2DM continues to grow rapidly worldwide and is associated with multiple comorbidities.
- Peripheral insulin resistance and β-cell failure represent the core pathophysiological defects in T2DM.
- Currently available synthetic drug therapies include metformin, sulphonylurea and other insulin secretagogues, thiazolidinediones, α-glucosidase inhibitors, GLP1 agonists, dopamine-2 agonists, bile acid sequestrants, DPP-4 and SGLT2 inhibitors.
- Two latter classes (DPP-4 and SGLT2 inhibitors) are novel and promising drugs that target novel mechanisms.
- Finding new drug targets and new faster and cheaper synthesis methods are the main goals of T2DM drug discovery.

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such as coronary artery disease, stroke, hypertension, nephropathy, peripheral vascular disease, neuropathy and retinopathy [9-13]. It has been suggested that higher glucose levels may be a risk factor for dementia [14]. Although T2DM was traditionally seen in individuals over the age of 40, recent data from several countries confirm that T2DM occurs in younger people even in childhood [15,16]. Cases of T2DM that occurs before 30 years of age usually develop diabetic nephropathy, renal failure, blindness and atherosclerotic vascular disease in their 30s [17].

Although there are several therapeutic options available for the management of diabetes, most of them respond only in the short-to-medium term. Further, most of current therapies are associated with an increased risk of adverse effects such as weight gain (sulphonylureas, thiazolidinediones and insulin), hypoglycaemia (sulphonylureas and insulin), gastrointestinal intolerance (metformin) and myocardial infarction (rosiglitazone) [18,19]. Researchers are trying to find therapies better than the three oldest classes, for example, insulin, sulphonylureas and biguanides but the newer ones have not shown more potency and were often less effective in lowering glycaemia [19].

Alternative to these synthetic agents, new insulin analogues, inhaled insulin and many medicinal plants are being investigated for possible benefits in diabetes [20-30]. Moreover, insulin–mimetic complexes have been synthesized in the thought zone) [18,19]. Researchers are trying to find therapies better than the three oldest classes, for example, insulin, sulphonylureas and biguanides but the newer ones have not shown more potency and were often less effective in lowering glycaemia [19].

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kinase C. These are the key signalling molecules in the activation of glucose uptake, inhibition of apoptosis and synthesis of proteins [49,52-54].

It is thought that in adipose and retinal tissues, phosphorylation on specific serine sites (Ser307) on IRS-1 reduces the tyrosine phosphorylation of the insulin receptor [48,55]. The cytokine TNF-α plays a major role through phosphorylation of the IRS-1 protein on Ser307 site. TNF-α-induced inhibition of IRS-1 signalling can reduce Akt phosphorylation and stop the insulin signalling pathway [39,48]. Also, it has been shown that TNF-α causes marked downregulation of the adipocytes and muscle cells insulin-regulable glucose transporter [56].

According to recent studies, the circulating IL-6 correlates with insulin sensitivity in obese humans [57]. This means that both TNF-α and IL-6 and other adipocyte-specific signalling elements such as resistin and leptin can alter insulin sensitivity by provoking diverse key steps in the process of insulin action [36,58]. Any defect in insulin action or the IRS triggers β-cells in a way to cause T2DM [59]. The interplay between insulin resistance and β-cell dysfunction remains highly complex [60]. Studies of insulin-resistant animals illustrate an important role for expansion of the β-cell mass and enhanced β-cell function as the compensatory mechanisms [61]. In most obese and insulin-resistant individuals, β-cell compensation reduces due to hyperglycaemia and lipid toxicity [62,63]. Continuous decline of β-cell function usually ends up in β-cell exhaustion and finally β-cell failure and diabetes [60].

Numerous studies have demonstrated that enhanced pro-inflammatory cytokines, free radicals and oxidative stress are central events to the development of diabetic complications [13,64-66]. Dominant cause of oxidative stress in diabetes is glucose autoxidation that leads to production of free radicals [67]. Oxidative stress causes β-cell death via induction of mitochondrial stress during development of diabetes. In pancreatic β cells, important targets for an oxidant insult are ATP-dependent potassium (K_{ATP}) channels and cell metabolism [68]. The efficacies of different approaches in the reduction of diabetes-induced oxidative stress have been studied and usage of antioxidants as the supplement to drug regimen of T2DM patients has been recommended [69-79]. Briefly, the pathobiology of T2DM which are considered as targets for classic and current synthetic drugs are summarised in Figure 1.

3. Overview of current synthetic drugs in the treatment of T2DM

3.1 Biguanides

The class of biguanides includes the metformin and two withdrawn agents phenformin and buformin. The reason for removing phenformin and buformin from the market was the occurrence of fatal lactic acidosis [80,81]. Introduced in the market in 1950, metformin is a well-accepted first-line
choice for the treatment of T2DM due to its good efficacy, low price and low rate of adverse effects especially in long-term use [82-85]. Metformin reduces fasting plasma glucose concentrations by reducing rates of hepatic glucose production through a reduction in gluconeogenesis and glycogenolysis [86-88]. Metformin also affects peripherally and improves skeletal myocyte glucose uptake, reduces the overall plasma free fatty acid (FFA) concentration and induces mild weight loss through reduction of caloric intake [89,90].

Activation of adenosine monophosphate-activated protein kinase (AMPK) is required for metformin’s inhibitory effects on glucose production by hepatocytes and acetyl-CoA carboxylase activity and its inducing effects on glucose uptake by skeletal muscles and fatty acid oxidation. It should be noticed that AMPK is a major cellular regulator of lipid and glucose metabolism [91,92]. Metformin, by improving insulin sensitivity, also improves a variety of other factors related to increased cardiovascular risk. It reduces the rate of myocardial infarction and all-cause mortality [93]. However, gastrointestinal intolerance, such as nausea, abdominal pain and diarrhea, happens as a side effect in about 30% of the users that limits the compliance of patients to consume top effective doses [94,95]. Although rare, fatal lactic acidosis might be observed in some users [96,97] and thus it should not be used in patients with liver disease [95], renal dysfunction [97] and in those with a slight degree of creatinine elevation [98]. The structure of biguanide (red highlight in metformin structure in Table 1) is conventionally represented in a wrong tautomeric form which was corrected in 2005 [99]. They are moderately strong bases and form HCl salts quite readily. This aspect is being exploited in the preparation of oral formulations of metformin with desired properties [99,100].

### 3.2 Sulphonylureas

Sulphonylureas as the fast insulin secretagogues are the oldest available class of oral glucose-lowering agents which were introduced in the market in the 1940s and approved for use in 1994 for T2DM before metformin [85,95,101].

These drugs specially stimulate insulin secretion in a glucose-independent manner by binding to a regulatory protein called sulphonylureas receptor on the pancreatic β-cells. After binding to the receptors, they trigger closure to K\textsubscript{ATP} channels, membrane depolarisation and influx of calcium through voltage-dependent channels, which subsequently ends up in insulin secretion [102-104]. In addition to their pancreatic effects, sulphonylureas can enhance insulin-stimulated peripheral glucose utilisation through triggering insulin action on adipose tissue glucose transport and lipogenesis and skeletal muscle glycogen synthase [105,106].

This class of oral drugs include the second-generation agents glipizide, gliclazide, glibornuride, glibidone, glimepiride, glyclopyramide and glibenclamide as well as the first-generation agents acetohexamide, chlorpropamide, tolbutamide, tolazamide and tolbutamide [107]. The third-generation sulphonylurea glimepiride is as effective as second-generation sulphonylureas and appears to have several clinical advantages over conventional sulphonylureas [108]. In clinical studies, glimepiride provides more stable blood glucose control, lowers risk of hypoglycaemia and induces less weight gain in comparison to other sulphonylureas [108,109]. Owing to glimepiride pancreatic tissue specificity, its use may be safer in patients with cardiovascular disease. Cardiovascular side effects of these compounds result from their effect on K\textsubscript{ATP} channels present in extra-pancreatic tissues of the cardiac and vascular smooth muscle [110,111].

All sulphonylureas contain a central S-phenyl sulphonylurea structure (red highlight in the glimepiride structure in the Table 1) [107]. The pharmacokinetic and pharmacological differences among the available sulphonylureas are a consequence of substitutions at the para-position on the benzene ring and the other at a nitrogen residue in the urea moiety [112]. First-generation sulphonylureas (e.g., tolbutamide, acetohexamide, tolazamide and chlorpropamide) have relatively small, polar, hydrophilic substitutions. Second-generation sulphonylureas have large, non-polar, lipophilic substitutions that penetrate cell membranes more easily, giving them greater potency [107,112].

#### 3.3 N-urea insulin secretagogues

Anti-diabetic and insulino-tropic properties of the non-sulphonylurea moiety of glibenclamide, subsequently named meglitinide, have been discovered > 30 years ago. Repaglinide, nateglinide and mitiglinide are proposed as non-sulphonylurea insulinotropic agents exhibiting structural analogy with meglitinide [113,114]. Repaglinide and nateglinide have been approved for treatment of T2DM and have been released into market in 1998 and 2001, respectively [115]. Repaglinide and nateglinide have a mechanism of action that is similar to that of sulphonylureas [104]. They are short-acting insulin secretagogues with high affinity and rapid association–dissociation kinetic activity to the K\textsubscript{ATP} at the outer membrane of β-cells. This property of these compounds results in restoration of early phase insulin secretion [107,113]. The most common adverse events of repaglinide and nateglinide are upper respiratory tract infection, sinusitis, constipation, arthralgia, headache and vomiting [116].

Nateglinide is D-phenylalanine derivative (phenylalanine is highlighted as red in the Table 1) which blocks K\textsubscript{ATP} channels in the pancreatic β-cells to stimulate insulin release [117,118]. In therapeutic concentrations, nateglinide with no sulphonylurea or benzamido moiety induces high selectivity for the pancreatic K\textsubscript{ATP} subtype over the cardiovascular subtype [119]. Nateglinide sensitizes pancreatic β-cells to limited amount of glucose and reduces the glucose concentration needed to stimulate insulin secretion [118]. In contrast to other insulin secretagogues, nateglinide has a low propensity to cause hypoglycaemia because of its selective, early phase insulin secretion and transient effect [120].
<table>
<thead>
<tr>
<th>Drug class</th>
<th>Drug structure</th>
<th>Mechanism of action</th>
<th>Side effects</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biguanide</td>
<td></td>
<td>Reduces hepatic glucose production, increases peripheral glucose utilisation and insulin sensitivity</td>
<td>Gastrointestinal complaints and lactic acidosis</td>
<td>Bharatam et al. 2005, Lipska et al. 2011 [99,236]</td>
</tr>
<tr>
<td>Sulphonylureas</td>
<td></td>
<td>Stimulates insulin release from pancreas and reduces post-absorptive rates of endogenous glucose production</td>
<td>Very rare adverse effects compared to other sulphonylureas side effects such as hypoglycaemia and weight gain</td>
<td>Korytkowski 2004, Becic et al. 2003 [107,237]</td>
</tr>
<tr>
<td>Non-sulphonylureas</td>
<td></td>
<td>Increases insulin secretion in the pancreas</td>
<td>Mild gastrointestinal complaints and weight gain</td>
<td>Chachin et al. 2003, Campbell 2005 [119,120]</td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td></td>
<td>Lowers insulin resistance in peripheral tissue by activating PPAR-γ</td>
<td>Weight gain, fluid retention and heart failure</td>
<td>Yki-Jarvinen 2004, Defronzo 2009 [125,128]</td>
</tr>
<tr>
<td>Disaccharidase inhibitors</td>
<td></td>
<td>Inhibits intestinal α-glucosidase enzymes</td>
<td>Gastrointestinal disturbance such as flatulence, abdominal distension, stomach rumble and diarrhoea</td>
<td>Balfour et al. 1993, Chssold and Edwards 1988 [141,238]</td>
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</table>
Table 1. Current anti-diabetic synthetic drug classes (continued).

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Drug structure</th>
<th>Mechanism of action</th>
<th>Side effects</th>
<th>Study</th>
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<tbody>
<tr>
<td>GLP1 agonists</td>
<td>![GLP1 agonist structure]</td>
<td>Stimulates insulin biosynthesis and secretion, inhibits glucagon secretion and slows gastric emptying</td>
<td>Nausea and vomiting</td>
<td>Wajcberg and Amarah 2010, Madsbad 2009 [159,239]</td>
</tr>
<tr>
<td>Liraglutide</td>
<td>![Liraglutide structure]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPP-4 inhibitors</td>
<td>![DPP-4 inhibitor structure]</td>
<td>Inhibits DPP-4 that improves glycaemic control</td>
<td>Few gastrointestinal disturbance</td>
<td>Gupta et al. 2009, Panina 2007 [168,240]</td>
</tr>
<tr>
<td>Vildagliptin</td>
<td>![Vildagliptin structure]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGL2 inhibitors</td>
<td>![SGL2 inhibitor structure]</td>
<td>Inhibits SGLT2 in the kidneys</td>
<td>Low incidence of hypoglycaemia and genital infections in females</td>
<td>Nomura et al. 2010, Rosenstock et al. 2012 [179,241]</td>
</tr>
<tr>
<td>Invokana (canagliflozin)</td>
<td>![Invokana structure]</td>
<td>Resets abnormally elevated hypothalamic drive for increased plasma glucose, triglyceride and FFA levels</td>
<td>Fatigue, nausea, vomiting, dizziness and headache</td>
<td>Keche 2010, Kumar et al. 2012 [181,182]</td>
</tr>
<tr>
<td>Dopamine-2 agonists</td>
<td>![Dopamine-2 agonist structure]</td>
<td></td>
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<tr>
<td>Bromocriptine</td>
<td>![Bromocriptine structure]</td>
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3.4 Thiazolidinediones
Troglitazone was the first thiazolidinedione approved as a glucose-lowering therapy for patients with T2DM in 1997 [98,121]. Troglitazone was subsequently withdrawn from use, in March 2000, because of causing severe hepatic toxicity [122]. Two currently available PPAR-γ agonists, rosiglitazone and pioglitazone, were approved in 1999. This class of agents also known as glitazones like the biguanide metformin do not increase insulin secretion but rather increase insulin sensitivity in muscle and adipose tissue and in the liver by activating PPAR-γ and affecting gene regulation in the target cells [123-125].

PPAR-γ is essential for normal metabolism of lipids such as adipocyte differentiation and proliferation as well as fatty acid uptake and storage [126,127]. Thiazolidinediones exert their insulin-sensitising actions by promoting genesis of small adipocytes and redirect fat from non-adipose tissues, such as liver fat, to (subcutaneous) adipose depots [125,128]. Although thiazolidinediones may enhance insulin sensitivity by keeping fat where it belongs and sparing other tissues such as the liver, skeletal muscle and possibly β-cells from lipotoxicity, indirect effects may also be involved via alteration of gene transcription such as adiponectin. Adiponectin, an adipocytokine, produced exclusively by adipose tissue increases insulin sensitivity [129-131].

The potential of thiazolidinediones to lower glucose production by the liver or improve glucose transport into muscle and adipose tissue have been shown [14,132]. The main adverse events associated with the thiazolidinediones class are weight gain and fluid retention that may be severe enough to exacerbate or precipitate heart failure [133,134]. These drugs also cause slight decrease in the haemoglobin level and haematocrit, probably without clinical consequence [125]. Pioglitazone and rosiglitazone are licensed for use as the second-line therapy to metformin and glyburide, agents that have demonstrated efficacy in decreasing the microvascular and macrovascular complications associated with T2DM [14].

Thiazolidine-2,4-dione structure (red highlight in the rosiglitazone structure in the Table 1) is common to all thiazolidinediones [135]. Thiazolidinedione ring has been investigated as a potent polar head group which is a critical binding motif in the active site of PPAR ligand-binding domain. However, these polar head groups are prone to racemisation under physiological conditions [136].

3.5 Disaccharidase inhibitors (α-glucosidase inhibitors)
The α-glucosidase inhibitors acarbose and miglitol are two of these agents which were released in the market in 1996 [95]. Initial investigations of voglibose as another disaccharidase inhibitor have not been proceeded further [137,138]. Absorption of carbohydrates requires eventual breakdown of disaccharides into monosaccharides by the α-glucosidase enzyme in the brush border of the small intestine. Disaccharidase inhibitors, such as acarbose and miglitol, inhibit digestion of carbohydrates by affecting the breakdown of disaccharides to monosaccharides in the intestinal epithelium. As a consequence, delayed and decreased absorption of the sugars happen. The α-glucosidase inhibitors decrease both postprandial blood glucose and postprandial insulin levels and in that way these improve sensitivity to insulin and release the stress on β-cells [139].

The safety of α-glucosidase inhibitors may be a definite help in elderly patients with T2DM [140]. The efficacy of acarbose and miglitol is limited by the adverse reactions caused by a large amount of non-absorbed disaccharides in the intestinal tract with the attendant symptoms of abdominal bloating, diarrhoea and flatulence. These compounds do not induce hypoglycaemia or weight gain [139,141].

According to some studies, acarbose does not directly alter insulin resistance but may lower postprandial plasma insulin levels, fasting blood glucose, glycosylated haemoglobin, plasma triglycerides and/or cholesterol concentrations [141,142]. Acarbose is only minimally absorbed from the gut and thereby considered as a non-absorbable inhibitor that makes this drug with no systemic adverse effects even after long-term administration. The symptoms, which is due to undigested carbohydrates, occur in ~ 30 – 60% of patients and tend to decrease with time and seem to be dose-dependent [142,143]. Acarbose is composed of an acarviosin moiety with a maltose at the reducing terminus. Acarviosin is a sugar composed of cyclohexitol unit linked to a 4-amino-4,6-dideoxy-D-glucopyranose unit, which is part of the acarbose and its derivatives (highlighted as red in the Table 1) [144].

3.6 Glucagon-like peptide 1 agonists
In April 2005, the first glucagon-like peptide 1 (GLP-1) agonist, exenatide, was approved for the treatment of T2DM [145]. In 2010, other analogue liraglutide was approved for use as an adjunct to diet and exercise to improve glycaemic control in adults with T2DM [146]. Several additional GLP-1 agonists, including exenatide long-acting release [15,147] albiglutide and taspoglutide, are under development and in various stages of clinical trials [148].

GLP-1 is a gut-derived incretin hormone, which is secreted by the more distally located intestinal L cells. Incretin hormones are a group of gastrointestinal hormones released in response to nutrient ingestion, which cause an increase in the amount of insulin released even before elevation of blood glucose [149]. Incretin hormone, GLP-1 stimulates insulin biosynthesis and secretion in response to meal ingestion, inhibits glucagon secretion, slows gastric emptying, reduces appetite and promotes the regeneration and proliferation of pancreatic β-cells [149-151].

Since GLP-1, gastric inhibitory polypeptide (GIP) and glucagon are all pivotal in glucose homeostasis, the G-protein-coupled receptors represent important drug targets in T2DM. One novel diabetes treatment strategy is activation of GLP-1 receptors and inhibition of the glucagon signal [152]. The structure of the human glucagon class B G-protein-coupled receptor has been reported more recently. The distinct structural
features and larger binding pocket of this receptor provide new insights into the molecular details of peptide ligand binding and a more reliable structural template for the design of specific and potent small molecules for the treatment of T2DM [153].

The short half-life (t_{1/2} ~ 1 – 1.5 min) of GLP-1, because of their rapid inactivation by dipeptidyl peptidase IV (DPP-4) in the circulation, is a major difficulty for its use [149,154]. Exenatide is a synthetic form of exendin-4 that occurs naturally in the saliva of the Gila monster (a large venomous lizard native to southwestern United States) and its amino acid sequence shows 53% homology to human GLP-1 [155]. Exendin-4 and GLP-1 exhibit similar insulinoergic and glucose-dependent secretion of insulin and decreased blood glucose levels in rodents and humans [156]. Exenatide and liraglutide exhibit increased resistance to DPP-4 degradation and thus provide pharmacological levels of GLP-1. Exenatide as short-acting GLP-1 receptor agonists (t_{1/2} ~ 2.4 h) is recommended for twice daily dosing [154]. Nausea, vomiting and hypoglycaemia were the most frequently reported side effects and were indifferent to dose while headache and nasopharyngitis were seen more at lower dose [147]. Liraglutide, but not short-acting exenatide, was associated with thyroid C-cell hyperplasia and tumours in rodents, probably due to continuous high-dose GLP-1 agonist exposure [157]. Rare cases of acute pancreatitis in association with use of incretin-based classes have been reported but most of the ensuing reports showed inconsistent results [85,158]. Liraglutide is a long-acting GLP-1 analogue (t_{1/2} ~ 24 h), with 96% structural identity to human GLP-1. The addition of a C16 fatty acid side chain using a γ-glutamic acid spacer at the γ-amino group of lysine-26 (fatty acid palmitate is highlighted as red in the Table 1), which allows non-covalent binding to albumin, increases the stability through formation of heptamer mediated by the fatty acid side chain [159].

3.7 DPP-4 inhibitors

DPP-4 inhibitors are promising new class of anti-diabetics that are extremely studied [10]. In October 2006, the first DPP-4 inhibitor, sitagliptin, was introduced in the market. Indeed, several other drugs such as vildagliptin, saxagliptin, alogliptin, linagliptin and anagliptin have been approved in certain countries for the treatment of T2DM. Other candidates have been demonstrated to be in an advanced stage of clinical trials for T2DM [160-163].

Inhibition of DPP-4, a serine protease, enhances endogenous GLP-1 activity by decreasing the rate of GLP-1 degradation, which represents a promising approach to the treatment of T2DM [164]. DPP-4 inhibitors increase circulating GLP-1 and GIP levels in humans, which leads to increased glucose-dependent secretion of insulin and decreased blood glucose, haemoglobin A1C and glucagon levels [165,166]. DPP-4 is a 766 residue N-terminal dipeptidyl exopeptidase that specifically cleaves an amino acid sequence having proline or alanine at the N-terminal penultimate (P1) position but may also cleave substrate with non-preferred amino acids at this position [167].

DPP-4 inhibitors include diverse structural types. Many DPP-4 inhibitors have five-membered heterocyclic rings such as pyrrolidine, cyanopyrrolidine, thiazolidine and cyanothiazolidine as a proline mimetic in the P1 part. Vildagliptin structure as a cyanopyrrolidine derivatives are presented in the Table 1 (cyanopyrrolidine ring is highlighted in red) [18]. Despite the positive results of vildagliptin in clinical trials, one of the issues encountered with the use of 2-cyano pyrrolidine derivatives is their stability in solution due to the participation of cyano group in an intramolecular cyclisation process leading to inactive products [168].

However, at this time, the tolerance and safety profile of DPP-4 inhibitors are considered as excellent. Possible increased risk of acute pancreatitis in the short term and occurrence of chronic pancreatitis in the longer term are among concerns attributed to increase in GLP-1 levels. Non-consistent results regarding a possible effect of GLP-1 and DPP-4 inhibitors on the exocrine pancreas were reported in various animal models [169]. In view of likely toxic side effects associated with the inhibition of other members of DPP family (inhibition of which was linked to toxicity in animal studies), it seems necessary to design selective inhibitors targeting DPP-4 over DPP-8 and DPP-9 [170]. Achieving desired selectivity toward the inhibition of DPP-4 over other related peptidases such as DPP-8 and DPP-9 and long-acting potential for maximal efficacy are the main challenges.

3.8 Sodium-glucose transporter-2 inhibitors

Canagliflozin from the new class of medications called sodium-glucose transporter-2 (SGLT2) inhibitors was approved by FDA in March 2013 [171]. Although there are several candidates, SGLT2 inhibitors such as dapagliflozin and BI10773 are now in various stages of clinical development, and the phlorizin, sergliblozin and remogliflozin have been discarded [172].

Kidney plays a key role in glucose homeostasis, primarily by the reabsorption of filtered glucose especially by the SGLT2 located in the proximal convoluted tubule. SGLT1, the other SGLT isoform, is the key transporter for glucose absorption in the gastrointestinal tract and plays only a minor role in the kidney [173,174]. The expression of SGLT2 and other renal glucose transporters were elevated in diabetic patients [175]. SGLT2 inhibitors, with a greater selectivity for SGLT2 versus SGLT1, offer a considerable advantage as potential anti-diabetic medications, because of their ability to inhibit renal glucose reabsorption and subsequent plasma glucose-lowering effect without inducing excessive insulin secretion [172,176].

Canagliflozin, an oral selective SGLT2 inhibitor improves glycaemic control in T2DM by reducing renal threshold for glucose reabsorption and increasing the urinary glucose excretion ending up in weight loss [177]. Various SGLT2 inhibitors have been proposed based on the glucose...
structure (glucoside ring is highlighted in red in canagliflozin structure in the Table 1). Since, the O-linkage of the structure of SGLT2 inhibitors is a metabolic target for β-glucosidase enzymes that can restrict the activity of SGLT2 inhibitors in vivo, newer candidate with a C-glucoside linkage such as canagliflozin have been synthesized [178]. Canagliflozin as a C-glucoside bearing a heteroaromatic ring has improved metabolic stability in comparison to O-glucoside [179].

3.9 Bile acid sequestrants/dopamine-2 agonists
From this category, two classes of drugs were already approved for other diseases. Colesevelam hydrochloride is a bile acid sequestrant that had been originally approved for the treatment of hypercholesterolaemia in the 2000s and approved in January 2008 to improve glycaemic control in T2DM adults. The exact mechanism influencing glucose metabolism remains unexplained [180].

The bromocriptine mesylate, a quick release formulation by trade name Cycloset, as an adjunct to diet and exercise has been approved to improve glycaemic control in adults with T2DM on 2009 [181]. Bromocriptine mesylate is a sympatholytic D2 dopamine agonist which can reverse many of the metabolic alterations associated with insulin resistance and obesity via resetting dopaminergic and sympathetic tone within the central nervous system [182].

Clinically, bromocriptine is used in treating Parkinson’s disease through activity at dopamine receptors and in treating hyperprolactinaemia and acromegaly. Bromocriptine was used for 30 years for other indications and based on its activity has been chemically designated as 2-bromoergocryptine monomethanesulfonate (salt) [182]. More information is presented in the Table 1.

4. New synthetic compounds as DPP-4 inhibitors
The DPP-4 is responsible for the degradation of the incretin hormones GLP-1 and GIP and has therefore a strong influence on insulin secretion and glucose homeostasis [184]. In contrast to therapy with GLP-1 analogues, because of limitations due to its swift inactivation, DPP-4 inhibitors increase effective incretin levels into a more physiological range [185].

4.1 Peptidomimetic inhibitors
The β-methylphenylalanine-derived amides have been shown to be potent DPP-4 inhibitors, exhibiting suboptimal selectivity and pharmacokinetics [187,188]. A series of phenylalanine and cyclohexylalanine derivatives also known as fluorinated pyrrolidine amides [10] were designed and assayed for their inhibitory potency against the DPP-4 enzyme as well as their selectivity over the related proline-specific enzymes quiescent cell proline dipeptidase (QPP) (DPP-II), DPP-8 and DPP-9. The phenylalanine series afforded compounds such as compound (1) that were potent and selective. Among cyclohexylalanine derivatives, the acetamide β-methyl substitute (2) was the most potent with better oral bioavailability [189].

The effect of substitution on the imidazopiperidine-based β-amino acid derivatives inhibitory activity against DPP-4, DPP-8 and DPP-9 was evaluated in another study. Introduction of a substituent at the 4-position of the imidazopiperidine unit (3) produces compounds having DPP-4 IC50 values < 10 nM [190].

A series of novel azobicyclo[3,3,0] octane derivatives substituted with pyrrolidine-2-carbonitrile were synthesized and evaluated against DPP-4, DPP-8 and DPP-9. Among them, compound (4) exhibited good DPP-4 activity, high selectivity, moderate pharmacokinetic profiles and excellent in vivo efficacy in an oral glucose tolerance test (oGTT) in lean mice [191].

Anagliptin is a potent and highly selective DPP-4 inhibitor which has been approved in Japan for the treatment of T2DM in 2012 [162,192]. A series of pyrazolo[1,5-α]pyrimidines were found to be novel DPP-4 inhibitors. Compounds were evaluated in vitro for inhibition of human recombinant DPP-4 and also screened for selectivity over dipeptidyl peptidase 8 and 9. Structure–activity relationships (SARs) for compounds showed N-[2-(amino)-2-methylpropyl]-2-methylpyrazolo[1,5-alpyrimidine-6-carboxamide hydrochloride (5) (anagliptin hydrochloride salt) as a potent and selective DPP-4 inhibitor with unique pharmacological profile [193].

It is noteworthy that many known DPP-4 inhibitors have the P-1-P-2 fragment, where the P-1 site contains a proline mimic [10,194]. Pyrrolidine derivatives (vildagliptin and saxagliptin) have been widely explored as DPP-4 inhibitors due to DPP-4’s specificity for substrate having an amino-terminal proline at C-2 [10]. Their cyanopyrrolidine moiety bind to the S1 sub-site and form a covalent bond between the nitrile group and hydroxyl of Ser630 in the catalytic triad. Further, their hydroxy adamantyl groups bind to the S2 subsite [195]. Vildagliptin was chemically unstable due to intramolecular cyclisation between the nitrile group and the P-2 basic amine moiety. Saxagliptin has an improved chemical stability by introduction of a cyclopropyl ring (cis-4,5-methanobridge) to the prolinenitrile which minimises cyclisation [160,196]. The DPP-4 inhibitors possessing the electrophilic trap such as a nitrile group have low selectivity against other related prolyl
Figure 2. Schematic representation of chemical structure of some examples of the peptidomimetic DPP-4.
peptidases, DPP-8 and DPP-9 [197]. The key interactions of the vildagliptin and saxagliptin structures with DPP-4 active site residues are presented in Table 2.

Yoshida et al. designed novel pyrrolidine derivatives without electrophilic nitrile moiety focused on the substituent at the \( \gamma \)-position of proline moiety of prolylthiazolidine core structure towards increasing the affinity to the S2 sub-site of DPP-4[194,198,199]. According to previous reports, introducing an electron-deficient 4-arylpiperazine results in highly potent and long-lasting inhibitors [200]. Fused bicyclic heteroarylpyrrolidine substituted at the \( \gamma \)-position of the proline structure in the investigation of L-prolylthiazolidines lacking the electrophilic nitrile was previously explored. Compound (6) (2-trifluoroquinolyl) was the most potent, long-lasting and selective DPP-4 inhibitor. X-ray crystal structure determination of compound (6) indicates that compound (6) made many interactions with the active site of DPP-4 (Table 2). The SAR study of fused bicyclic heteroarylpyrrolidine parts

![Chemical structures of DPP-4 inhibitors](image)
Table 2. Key interactions between inhibitor structures and DPP-4 active site residues.

<table>
<thead>
<tr>
<th>DPP-4 inhibitor</th>
<th>Involved inhibitor structure</th>
<th>Interaction type</th>
<th>DPP-4 residue</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linagliptin</td>
<td>Amino group on the piperidine</td>
<td>Hydrogen bonding interactions</td>
<td>Glu205, Glu206 and Tyr662</td>
<td>Eckhardt et al. 2007 [202]</td>
</tr>
<tr>
<td></td>
<td>C-6 carbonyl of the xanthine Quinazoline group Uracl group</td>
<td>Hydrogen bonding interactions</td>
<td>Glu205, Glu206 and Tyr662</td>
<td>Metzler et al. 2008 [242]</td>
</tr>
<tr>
<td>Saxagliptin</td>
<td>Amino group (primary)</td>
<td>Hydrogen bonding interactions</td>
<td>Glu205, Glu206 and Tyr662</td>
<td>Metzler et al. 2008 [242]</td>
</tr>
<tr>
<td></td>
<td>Carbonyl group Hydroxyl group on the adamantyl moiety Imidate nitrogen 4,5-Methanopyrrolidine ring</td>
<td>Hydrogen bonding interactions</td>
<td>Glu205, Glu206 and Tyr662</td>
<td>Metzler et al. 2008 [242]</td>
</tr>
<tr>
<td>Saxagliptin</td>
<td></td>
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</tr>
<tr>
<td>Sitagliptin</td>
<td>Nitrile of the cyanoxyopyridinβ-Amino group</td>
<td>Covalent bond</td>
<td>Glu205, Glu206 and Tyr662</td>
<td>Zhu et al. 2013, Kim et al. 2005 [163,243]</td>
</tr>
<tr>
<td></td>
<td>Carbonyl group Triazolopiperazine Trifluoromethyl group on the triazolopiperazine Trifluorobenzyl group</td>
<td>Hydrogen bonding interactions</td>
<td>Glu205, Glu206 and Tyr662</td>
<td>Metzler et al. 2008 [242]</td>
</tr>
<tr>
<td>Sitagliptin</td>
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<tr>
<td>Vildagliptin</td>
<td>Amino group</td>
<td>Salt bridge interactions</td>
<td>Glu205, Glu206</td>
<td>Nabeno et al. 2013 [195]</td>
</tr>
<tr>
<td></td>
<td>Carbonyl group Hydroxyl group on the adamantyl moiety</td>
<td>Hydrogen bonding interactions</td>
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<td>Hydrogen bonding interactions</td>
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<tr>
<td>Vildagliptin</td>
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<tr>
<td>6</td>
<td>Nitrile of the cyanoxyopyridine</td>
<td>Covalent bond</td>
<td>Glu205, Glu206 and Tyr662</td>
<td>Yoshiida et al. 2012 [194]</td>
</tr>
<tr>
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<td>6-Amino group of the proline Carbonyl group Quinolyl ring</td>
<td>Hydrogen bonding interactions</td>
<td>Glu205, Glu206 and Tyr662</td>
<td>Yoshiida et al. 2012 [194]</td>
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<tr>
<td>7 (teneligliptin)</td>
<td>6-Amino group of the proline Carbonyl group Phenyl on the pyrazolyl ring Piperazinyl ring Pyrazolyl ring</td>
<td>Hydrogen bonding interactions</td>
<td>Glu205, Glu206 and Tyr662</td>
<td>Yoshiida et al. 2012 [197]</td>
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<tr>
<td>8</td>
<td>Trifluoromethyl group on the quinolyl ring Amino group</td>
<td>Hydrogen bonding interactions</td>
<td>Glu205, Glu206 and Tyr662</td>
<td>Yoshiida et al. 2012 [197]</td>
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<td>Hydrogen bonding interactions</td>
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<td>14</td>
<td>Amino group on the quinolyl ring 2-Isobutyl group 2-Oxo group on the piperazin-2,5-dione 5-Oxo group on the piperazin-2,5-dione Piperazin-2,5-dione</td>
<td>Hydrogen bonding interactions</td>
<td>Glu205, Glu206 and Tyr662</td>
<td>Maezaiki et al. 2011 [207]</td>
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<tr>
<td></td>
<td></td>
<td>Hydrogen bonding interactions</td>
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<tr>
<td>15</td>
<td>Amino group at the piperidine moiety (primary) Carbonyl group on the quinolyl ring Carboxyl group on the benzene ring 3H-imidazo[4,5-c]quinolin-4(5H)-one moiety</td>
<td>Hydrogen bonding interactions</td>
<td>Glu205, Glu206 and Tyr662</td>
<td>Ikuma et al. 2012 [208]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydrogen bonding interactions</td>
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<tr>
<td>6</td>
<td>Nitrile of the cyanoxyopyridine</td>
<td>Covalent bond</td>
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<td>7 (teneligliptin)</td>
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<tr>
<td>8</td>
<td>Trifluoromethyl group on the quinolyl ring Amino group</td>
<td>Hydrogen bonding interactions</td>
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<td>Hydrogen bonding interactions</td>
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<td>14</td>
<td>Amino group on the quinolyl ring 2-Isobutyl group 2-Oxo group on the piperazin-2,5-dione 5-Oxo group on the piperazin-2,5-dione Piperazin-2,5-dione</td>
<td>Hydrogen bonding interactions</td>
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of previous study revealed that the non-linear (L-shaped) structure was more suitable than the linear (I-shaped) one in DPP-4 inhibitory activity. Moreover, the X-ray crystal structure determination of compound (6) in complex with human DPP-4 indicated that interaction between the quinolyl ring and the S2 extensive sub-site plays an important role for DPP-4 inhibition [194]. Hence, the introduction of another non-linear structure, a linked bicyclic heteroaryl group on the piperazine or piperidine moiety, instead of a fused bicyclic heteroaryl group, was addressed in the latter study which led to discovery of 3-[(2S,4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)piperazin-1-yl]pyrrolidin-2-ylcarbonyl]thiazolidine (7), as highly potent, selective, long-lasting and orally active DPP-4 inhibitor. The X-ray co-crystal structure of compound (7) in DPP-4 demonstrated that the characteristic five rings of compound (7) fit into the active site of DPP-4 and the key interaction between the phenyl substituent on the pyrazolyl ring and the S2 extensive sub-site of DPP-4 not only raised potency, but also increased selectivity (Table 2). Compound (7) significantly inhibited the increase of plasma glucose levels after an oral glucose load in Zucker fatty rats. Compound (7) (teneliglitin) has been approved for the treatment of T2DM in Japan on June 2012 [197].

The crystal structures of DPP-4 in complex with bound sitagliptin and alogliptin have shown that a sub-pocket formed by the catalytic residue Ser630 and nearby residues is usually occupied by a hydrophobic ring group such as the trifluorobenzyl group of sitagliptin or the benzonitrile of alogliptin. Two acidic residues – Glu205 and Glu206 – in the S2 sub-site of DPP-4 forms interactions with amine group of sitagliptin (Table 2). These interactions between sitagliptin and DPP-4 give a large unoccupied space around the right terminal portion (the fused heterocyclic ring) which could be modified to generate a new series of DPP-4 inhibitors. With this rationale, well-established click chemistry was used to quickly explore the SAR of the linker and the terminal portion of sitagliptin. A series of 4-[(2,4,5-trifluorophenyl)butane-1,3-diamines were designed and elaborated as DPP-4 inhibitors. The results showed that compound (8) had desirable efficacy, selectivity and pharmacokinetics properties. It is noteworthy that crystal structures of DPP-4 in complex with bound compound (8) revealed that the trifluoromethyl heterocycle is rotated ~180° and directed towards a sub-pocket different from the sub-site bound by sitagliptin, providing clues for the design of new DPP-4 inhibitors. The key interaction between compound (8) and DPP-4 active site are presented in Table 2 [163].

4.2 Non-peptidomimetic inhibitors

Monocyclic, bicyclic, bicyclic lactams and triazolopyridazines-substituted 3-aminopiperidines were synthesized and evaluated for in vitro inhibition of human DPP-4, QPP, DPP-8 and DPP-9. Bicyclic lactams series were potent (DPP-4 IC₅₀ 1.2 – 8 nM) and in general had good-to-excellent selectivity. Compound (9) was found to be the most potent DPP-4 inhibitor with excellent selectivity but poor pharmacokinetic properties [201].

The X-ray crystal structure of linagliptin complexed with DPP-4 indicated the manner in which these residues provided a good fit (Table 2) [202]. A class of 3,5-dihydro-imidazo[4,5-d]pyridazin-4-ones with different substituent provided. In addition to in vitro DPP-4 inhibitory activity, the effects of compounds on the muscarinic receptor M1 and inhibition of DPP-4 in rats was tested. It should be noticed that significant M1 receptor inhibition was a characteristic of some early xanthines. Compound (10) seems to be a potential candidate showing very potent DPP-4 inhibitor activity in vitro as well as in vivo and only poor affinity to M1 receptors. The long-lasting strong DPP-4 inhibition (> 70% 24 h post-administration) of compound (10) is particularly noteworthy. Therefore, further works on this series, and compound (10) in particular, will be helpful in the development of better drugs for T2DM [203].

Pyrazolopyrimidines are a class of compounds under investigation. A series of pyrazolopyrimidines with different substituents on the pyrazole ring which include alkyl, aryl, substituted carboxyl and carboxyl groups were tested in vitro against purified human DPP-4. Favourable DPP-4 activity, selectivity over DPP-8 and DPP-9 and ample opportunity for further optimisation around the pyrazole ring were the three desirable characteristics of this class of compounds. A 5-aminomethyl imidazopyrimidines derivative compound (11) was the most potent against DPP-4 with no significant DPP-8/DPP-9 inhibition. The oGTT in ob/ob mice by compound (11) showed dose-dependent increase in plasma insulin levels [204].

While substituted 4-amino cyclohexylglycine analogues in the α-amino acid series showed good activity against DPP-4 [205], the substituted cyclohexane and piperidine derivatives were prepared and assessed for DPP-4 inhibitory activity and selectivity profiles against other proline-specific peptidases. In the cyclohexyl with sulphonamide substituent series, N-methyl-(2,5-dimethyloxazol-yl)sulphonamide (12) showed the most promising potency against DPP-4 and excellent selectivity against the off-target enzymes but poor pharmacokinetic. Among the N-substitution piperidine derivatives, 3-(5-amino-carbonyl)pyridyl piperidine) (13) displayed excellent DPP-4 activity with good selectivity versus other proline enzymes [206].

As mentioned in Table 2, many of the reported DPP-4 inhibitors including vildagliptin and saxagliptin make a covalent interaction with the Ser630 residue in the S1 pocket. Sitagliptin and linagliptin form non-covalent interactions with the S1, S2 or S2 extensive sub-sites in addition to the S1 and S2 sub-sites. It appears that excess interactions may increase DPP-4 inhibition beyond the level yielded by the fundamental interactions with the S1 and S2 sub-sites and are more effective than forming a covalent bond with Ser630 in the S1 subunit [195,207]. Design of novel non-covalent inhibitors of DPP-4 using structural information
derived from DPP-4 structures co-crystallised with small molecules originating from different chemical classes is now a vital investigation in medicinal chemistry.

Based on the X-ray co-crystal structure of some compounds, the hydrogen bonding interaction with Lys554 may be applicable in the novel design of DPP-4 inhibitors. Hence, Maezaki et al. developed a novel series of non-covalent quinolone-based inhibitors to target the side chain of Lys554. Synthesis and evaluation of designed compounds against DPP-4, QPP, DPP-8 and DPP-9 enzyme and determination of absorption, distribution, metabolism and excretion and pharmacokinetic profiles revealed 1-[3-(aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl) quinolin-6-yl]piperazine-2,5-dione compound (14) - a potent, selective and orally active DPP-4 inhibitor with long-lasting ex vivo activity in dogs and potent glucose-lowering effects in rats. A docking study of compound (14) suggested that an efficient fit into the binding site and a hydrogen-bonding interaction with the side chain of Lys554 are important in enhancing the inhibitory activity. Also, in comparison with other members of this series, it seems that compound (14) achieved excellent activity by taking advantage of the desirable piperazin-2,5-dione substituent and forming hydrophobic and two-hydrogen bonding interactions, including the one with Lys554 (Table 2) [207].

Ikuma et al. found a potent DPP-4 inhibitor by high-throughput screening of laboratory chemical library and proper optimisation based on results of published DPP-4 enzyme-inhibitor docking study. Further docking studies were carried out to design a strategy for optimisation of lead compound with 3H-imidazo[4,5-c]quinolin-4(5H)-one as skeleton. Compound (15) with 2-chloro-5-fluorobenzylsubstituent on the imidazoquinoline group was found as potent and highly selective DPP-4 inhibitor [208]. The results of docking study are shown in Table 2.

5. New synthetic compounds as SGLT2 inhibitors

Inhibition of glucose reabsorption and increasing urinary glucose excretion lead to a negative energy balance, making these series of compounds a unique promising therapeutic glucose excretion lead to a negative energy balance, making these compounds a unique promising therapeutic approach in the novel design of SGLT2 inhibitors. Hence, the first-generation SGLT2 inhibitors with O-glycosidic bond were discontinued due to their short half-lives in vivo resulting from glycosidase cleavage [211,212]. Second-generation SGLT2 inhibitors adopted other forms of glycosidase linkage resistant to glycosidase activity such as C-glucoside structure [213].

5.1 C-glucoside analogues of SGLT2 inhibitors

Many C-aryl glucoside analogues containing conjugated aryl moieties with glucose moiety by C–C bond instead of stable C–O–C bond have been designed and synthesized [179,214]. Among these analogues, dapagliflozin has been used as a lead compound as many synthetic derivatives have been further made on its SAR data. A series of fluorinated analogues of dapagliflozin were designed and synthesized by incorporation of a gem-difluoromethylene group at C-4 position. The in vitro inhibitory activities of these series against human SGLT2 showed that some of the analogues (compound 16) with CF2 at C-4 are better SGLT2 inhibitors compared with dapagliflozin (Figure 4) [215].

The structural similarity of D-xylose and D-glucose and the positive results of O-xylosides and N-β-D-xylosides [216] led to the design of a new series of C-linked indolylxylosides. SAR studies indicated that a p-cyclopropylphenyl group in the distal position and substituents at the 7-position of the indole moiety were necessary for optimum inhibitory activity. The pharmacokinetic and animal studies demonstrated that the most potent compound 7-substituted indolylxylosides bearing a distal p-cyclopropylphenyl group (17) is metabolically stable with significant efficacy on lowering blood glucose levels of streptozotocin-induced diabetic rats [217].

Ikegai et al. investigation led to the discovery of other C-glucoside SGLT2 inhibitors with azulene motifs [218]. Although azulene is not a common motif in medicinal chemistry of anti-diabetic drug discovery, a search of the literature indicates that certain azulene derivatives possess significant pharmacological and therapeutic activity [219-221]. Hence, a series of C-glucosides with azulene rings in the aglycone moiety was synthesized; SAR of azulene-derived C-glucoside and the inhibitory activities towards hSGLT1 and hSGLT2 were explored. Incorporation of a phenolic hydroxyl group at the central benzene ring afforded a more potent and selective SGLT2 inhibitor (18), which exerted a strong and sustained anti-hyperglycaemic effect in rodent diabetic models. A mono choline salt of compound (18) (YM543) was selected as a clinical candidate for use in treating T2DM [218].

A new class of potent and selective SGLT2 inhibitors, by modifying the glycone side chain incorporating a structurally novel dioxabicyclo-[3.2.1]octane ring system, was prepared [222]. At first, a series of C-5-spirocyclic C-glucoside were synthesized with relatively good potency and selectivity for human SGLT2 but suboptimal pharmacokinetics [223]. The medicinal chemistry strategy by innovative chemistry
that allowed difficult, yet very desirable, targets to be synthesized in an analogue-friendly fashion and the development of a pharmacokinetics/pharmacodynamics (PKPD) model brought more hope. These efforts led to deprioritisation of the C-5-spirocyclic C-glycoside SGLT2 inhibitors and focusing on the dioxabicyclo[3.2.1]octane class. It is believed that the bridged ketal system would confer rigidity with potentially positive impact on potency and selectivity. Moreover, introduction of a hydroxymethylene H-bond donor group at C-5 (in place of the spirocycle) brought more anticipation to reduce the rate of human Phase II metabolism and further improvement of the potency. These efforts supported the advancement of compound (19) (PF-04971729) into clinical development that is being evaluated for the treatment of T2DM [222].

Vyas et al. performed a ligand-based 3D quantitative SARs (QSARs) study on C-aryl glucoside SGLT2 inhibitors (180 analogues), which belong to various diversified

<table>
<thead>
<tr>
<th>Chemical Structure</th>
<th>SGLT2 IC₅₀</th>
<th>SGLT1/SGLT2</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phlorizin [215]</td>
<td>46.03 nM</td>
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<tr>
<td>Dapagliflozin [214]</td>
<td>1.1 nM</td>
<td>1200 fold</td>
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<tr>
<td>Sergliflozin-A [249]</td>
<td>2.39 nM</td>
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<tr>
<td>16 [215]</td>
<td>0.35 nM</td>
<td></td>
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<tr>
<td>17 [217]</td>
<td>47 nM</td>
<td>6 fold</td>
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<tr>
<td>18 [218]</td>
<td>8.9 nM</td>
<td>280 fold</td>
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</tr>
<tr>
<td>19 [222]</td>
<td>0.887 nM</td>
<td>&gt; 2234 fold</td>
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<tr>
<td>20 [225]</td>
<td>3.85 μM</td>
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<tr>
<td>21 (pseudo-sergliflozin) [227]</td>
<td>2.45 nM</td>
<td>&gt; 200000 fold</td>
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<tr>
<td>22 [228]</td>
<td></td>
<td></td>
<td>Inhibition rate of blood glucose levels in oGTT: 74.85%</td>
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<tr>
<td>23 [216]</td>
<td>161 nM</td>
<td>1.3 fold</td>
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</table>

Figure 4. Schematic representation of chemical structures of some SGLT2 inhibitors together with their experimental inhibitory and selectivity activities.
structures such as benzothiazole, indolizine-β-D-glucopyranoside, thiazolylmethylphenyl, pyridazine, thiazole and pyrimidinylmethylphenyl glucoside analogues. Comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) were used to further explore the structural requirements of C-aryl glucoside analogues for SGLT2 inhibition. CoMFA and CoMSIA contour map analysis offered enough information to understand the importance of the substituent at particular positions with respect to steric, electrostatic, hydrophobic, hydrogen-bond donor and acceptor properties for better activity of C-aryl glucoside SGLT2 inhibitors. For example analysis of CoMFA and CoMSIA contour plots revealed the importance of hydrophobic and hydrogen-bond donor and acceptor properties.

5.2 SGLT2 inhibitors with other structures

The application of ligand-based virtual screening strategy comprising the combination of pharmacophore model with shape-based scoring and structure clustering analysis led to the discovery of non-glycoside SGLT2 inhibitors. A total of 7989 compounds matching all the features described by pharmacophoreCE50 < 10 µM. The non-glycoside compound with EC50 = 3.85 µM was the most potent compound.

Most SGLT2 inhibitors in clinical trials are taken from two distinct series: C-aryl glycosides (e.g., dapagliflozin) and O-aryl glycosides (e.g., sergliflozin and remogliflozin) [223]. The abovementioned compounds were C-glucoside derivatives but showed higher cancer risk [226]. It is thought that the metabolic instability of the O-glucoside SGLT2 inhibitors can be solved by modifying the sugar core of sergliflozin-A. Several small-molecule carbohydrate mimics such as pseudo-sergliflozin were synthesized by a region- and stereo-selective allylic substitution reaction. Then the SGLT2/SGLT1 inhibitory activities of these newly synthesized compounds were studied. The endocyclic oxygen atom of sergliflozin-A was replaced by a methylene unit to render the molecule free from glycosidase degradation. In this way, compound (21) was found as a potent and selective inhibitor of SGLT2 that considered a lead compound for further development [227].

A series of thiadiazole-based S-glucosides were synthesized from D-glucose, D-galactose and a variety of phenylacetic acids and then evaluated in vivo with an oGTT test. Of these, 5-benzyl-1,3,4-thiadiazol-2-yl 1-thio-β-D-glucopyranoside (22) was the most efficacious to suppress the blood glucose excursion during oGTT with the inhibition rates of blood glucose levels equal to 74.85% [228].

Since, N-linked glucosides were expected to have greater metabolic stability compared to O-glucosides, a novel series of N-linked β-D-xylidoses were synthesized and evaluated for inhibitory activity against SGLT2 in a cell-based assay. Among them, the 4-chloroindolyl-N-xyloside with the 4-cyclopropylbenzyl at the distal position (23) was found the most potent inhibitor with no selectivity for SGLT2 over SGLT1. According to pharmacokinetic data, compound (23) was metabolically stable with a low clearance and good oral bioavailability in Sprague Dawley rats [216].

7. Conclusion

More recently, the need to develop new drugs for T2DM as a multi-causal and complicated disease has been emphasized, as the worldwide incidence of this disease is increasing dramatically. Various drug options are now available for the management of T2DM, but almost all are associated with restrictions and most do not address all aspects of the defects in diabetic patients. The recently introduced DPP-4 and SGLT2 inhibitors effectively lower the blood glucose without weight gain and risk of hypoglycaemia.

The co-crystal structures of human DPP-4 with some approved inhibitors are available. So, in recent years, new synthetic DPP-4 inhibitors with diverse chemical structures have been found, according to the X-ray crystal structure of DPP-4 and computer-modelling studies. Remarkable creativity and thoughts have been demonstrated by medicinal chemists in designing novel and potent series of DPP-4 inhibitors. Various SGLT2 inhibitors based on the glucoside structure of chlorizin, sergliflozin, dapagliflozin, canagliflozin and other inhibitors in clinical trial have since been proposed. Alternative candidate SGLT2 inhibitors that have also been considered include modified sugar rings, N-glucosides, S-glucosides, non-glycoside and, more recently, bridged ketal ring, azulene motifs. The other promising agents targeting novel molecular mechanisms are under development. The hope is to discover more selective and effective anti-diabetic agents in the near future.

8. Expert opinion

Despite a rapid increase in the number of drugs available to treat hyperglycaemia, diabetes still remains a major global concern. Although three oldest classes comprising insulin, sulphonylureas and the biguanides are effective in improving outcome, they are unable to sustain adequate glycaemic control over time in the diabetic patients. A variety of anti-hyperglycaemic drugs with different and complementary mechanisms of action are currently available. Moreover, the use of current medications is often limited by their side effects, mostly sudden hypoglycaemia, weight gain, gastrointestinal effects or oedema. These factors necessitate the ongoing quest for novel agents that would address fundamental defects of T2DM and have minimal adverse effects.

Among the main anti-diabetic drug classes, DPP-4 and SGLT2 inhibitors are novel and promising therapy for T2DM. The details of synthesis backgrounds of many new DPP-4 inhibitors, in the recent years, are described in this
review. In this review, the structures of some compounds with biological properties are also presented. Phenylalanine and cyclohexylalanine derivatives and imidazopiperidine-based β-amino acid derivatives were designed based on the observation that modification of the phenylalanine, cyclohexylalanine or piperazine moiety improved the DPP-4 potency by several folds. Also the 3-fluoropyrrolidine analogues obtained by replacing the pyrrolidine ring compounds (1) and (2) showed more activity compared to parent compound [189,190]. Since bicyclo[3.3.0]octane derivative with pyrrolidine-2-carbonitrile was previously reported to be a potent DPP-4 inhibitor [191], azobicyclo[3.3.0]octane compounds were synthesized. The discovery of anagliptin was according to studies showed that pyrazolo[1,5-a]pyrimidine functions as a bioisostere conveying much metabolic stability and safety [193].

A costly component of drug discovery approaches is structure-based screening (docking), which is a design strategy for new chemical entities, or optimisation of lead compounds identified by other methods, using the 3D structure of the DPP-4. The 3D structure of DPP-4 could be obtained by X-ray or nuclear magnetic resonance studies or from homology models. The protein data bank is a repository for the 3D structural data of proteins. The crystal structures of DPP-4 have been previously disclosed and the discovery of teneligliptin performed by aid of mentioned in silico methods which comprise computationally assessed ligand-binding interactions with DPP-4. Non-peptidomimetic inhibitors of DPP-4 such as 3-aminopiperidines with bicyclic lactams substituents were originated from sitagliptin—a peptidomimetic DPP-4 inhibitor, X-ray crystallographic structure along with molecular modelling [201]. Also non-covalent quinoline-based inhibitors which target the side chain of Lys554 were designed based on the X-ray co-crystal data of some compounds containing hydrogen bonding interaction with Lys554 [207]. Numerous DPP-4 inhibitors that were discovered and/or optimised using in silico methods have reached the level of clinical studies or have gained approval of use in some countries.

Stability and selectivity of SGLT2 inhibitors is an important issue in drug design. The SARs studies of phlorizin and other candidates led to discovery of another selective and potent SGLT2 inhibitor with excellent pharmacokinetic properties. Discovery of dioxabicyclo[3.2.1]octane was supported by analogue-friendly fashion and PKPD model [222].

The X-ray crystal structure of SGLT2 is yet not available; thus, to explore the structural requirements for SGLT2 inhibition, the ligand-directed drug model was used to screen active compounds as templates. Ligand-based screening techniques mainly focus on comparing molecular similarity analyses of compounds with known and unknown moieties. The 3D QSAR methods of CoMFA and CoMSIA models have been developed as ligand-based approaches. As mentioned, CoMFA and CoMSIA models were successfully used in providing useful information for designing new SGLT2 inhibitors [224].

Most classic anti-diabetic drugs target the major pathophysiological defects in T2DM, namely insulin resistance and impaired insulin secretion, while the new developing drugs focus on other options such as SGLT2 inhibitors that affect the kidney through insulin-independent mechanisms. Recently, PPARα/δ agonists [230], SIRT1 activators [231,232], glycogen phosphorylase inhibitors [233] and protein tyrosine phosphatase 1B inhibitors [233-235] were luckily designed for the treatment of T2DM.

There is no doubt that T2DM and its related complications are associated with oxidative stress that plays a role in the pathogenesis of T2DM. Antioxidant (natural or synthetic) therapy can protect β-cells from apoptosis and might possibly work in control of hyperglycaemia by activating the production and release of insulin [13,66,74].

Finding new targets and new faster and cheaper synthesis methods are the main goal in T2DM drug discovery. No single diabetes treatment is best for everyone as each person responds in a different way to medication. Together, the available and rising drug candidates should provide physicians with a broad range of pharmacological choices to successfully individualise patient treatment.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.
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Importance of synthetic drugs for T2DM drug discovery

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