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Review

Medicinal chemistry approaches to the inhibition of dipeptidyl peptidase-4 for the treatment of type 2 diabetes

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ABSTRACT

Emerging as an epidemic of the 21st century type 2 diabetes has become a major health problem throughout the globe. The number of deaths attributable to diabetes reflects the insufficient glycemic control achieved with the treatments used in recent past. DPP-4 inhibitors have been investigated as a new therapy with novel mechanisms of action and improved tolerability. DPP-4, a protease that specifically cleaves dipeptides from proteins and oligopeptides after a penultimate N-terminal proline or alanine, is involved in the degradation of a number of neuropeptides, peptide hormones and cytokines, including the incretins GLP-1 and GIP. As soon as released from the gut in response to food intake, GLP-1 and GIP exert a potent glucose-dependent insulinotropic action, thereby playing a key role in the maintenance of post-meal glycemic control. Consequently, inhibiting DPP-4 prolongs the action of GLP-1 and GIP, which in turn improves glucose homeostasis with a low risk of hypoglycemia and potential for disease modification. Indeed, clinical trials involving diabetic patients have shown improved glucose control by administering DPP-4 inhibitors, thus demonstrating the benefit of this promising new class of antidiabetics. Intense research activities in this area have resulted in the launch of sitagliptin and vildagliptin (in Europe only) and the advancement of a few others into preregistration/phase 3, for example, saxagliptin, alogliptin and ABT-279. Achieving desired selectivity for DPP-4 over other related peptidases such as DPP-8 and DPP-9 (inhibition of which was linked to toxicity in animal studies) and long-acting potential for maximal efficacy (particularly in more severe diabetic patients) were the major challenges. Whether these goals are achieved with the present series of inhibitors in the advanced stages of clinical development is yet to be confirmed. Nevertheless, treatment of this metabolic disorder especially in the early stages of the disease via DPP-4 inhibition has been recognized as a validated principle and a large number of inhibitors are presently in various stage of pre-clinical/clinical development. Sitagliptin is a new weapon in the arsenal of oral antihyperglycemic agents. This review will focus on the journey of drug discovery of DPP-4 inhibitors for oral delivery covering a brief scientific background and medicinal chemistry approaches along with the status of advanced clinical candidates.

Contents

Abbreviations: IUBMB, international union of biochemistry and molecular biology; DPP, dipeptidyl peptidase; T2D, type 2 diabetes; MOA, mechanism of action; PPAR-γ, perxisome proliferator-activated nuclear receptor-γ; Postprandial, occurring after intake of a meal; Antidiabetogenic, preventing or treating diabetes; HbA1c, glycosylated hemoglobin A1c—a marker of long-term (two to three months) control of diabetes; FAP, fibroblast activation protein-α; SAR, structure activity relationship; OGTT, oral glucose tolerance test; K_i, inhibition constant; hERG, human ether-a-go-go related gene; *F*, oral bioavailability; PK, pharmacokinetic; PD, pharmacodynamics; QPP, quiescent cell proline dipeptidase; PD, pharmacodynamic; ZDF rats, Zucker diabetic fatty rats; POP, prolyl oligopeptidases; FAP_α, fibroblast activation protein α (also called seprase); CYPs, cytochrom P450 enzyme; HTS, high-throughput screening; SD rats, Sprague–Dawley rats; DIO, diet-induced obese.

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1. Introduction: diabetes—an emerging epidemic of the 21st century

Diabetes mellitus (often referred as diabetes) is a group of metabolic diseases characterized by abnormally high levels of plasma glucose or hyperglycemia in the fasting state or after administration of glucose during an oral glucose tolerance test. The World Health Organization recognizes mainly two distinct clinical forms of diabetes, for example, type 1 and 2 (Chart 1). Type 1 diabetes, also known as insulin-dependent or juvenile onset diabetes is usually diagnosed in children and young adults, and is caused by the destruction of the insulin-producing beta cells of the Islets of Langerhans in the pancreas, leading to a deficiency of insulin. Insulin, a peptide hormone composed of 51 amino acid residues with a molecular weight of 5808 Da, is produced in the Islets of Langerhans in the pancreas and causes glucose uptake into liver, muscle and fat and storage as glycogen in the liver and muscle. Type 2 or non-insulin-dependent diabetes mellitus (NIDDM) is the most common form of diabetes and is primarily characterized by insulin resistance or reduced insulin sensitivity, combined with reduced insulin secretion and hyperglycemia. Thus the important contributing factors for type 2 diabetes (T2D) include (i) body cell resistance to insulin, (ii) increased hepatic glucose production (e.g., from glycogen degradation), (iii) decreased insulin-mediated glucose transport into muscle and adipose tissues and (iv) impaired beta-cell function leading to loss of early phase of insulin release in response to hyperglycemic stimuli. Patients having T2D are at high risk of macro and micro vascular complications such as coronary artery disease, stroke, hypertension, nephropathy, peripheral vascular disease, neuropathy and retinopathy (Chart 2). This metabolic disease is a growing public health problem, affecting approximately 194 million people worldwide according to the World Health Organization, and this number is projected to be 366 million by 2030.[1] A recent study demonstrated that for every percentage reduction in HbA1c [glycosylated hemoglobin A1c—a marker of long-term (two to three months) control of diabetes], there was a 35% reduction in the risk of complications in patients with T2D. While current T2D therapies that increase insulin secretion have proven therapeutically beneficial effects, these often suffer from undesirable side effects such as hypoglycemia and weight gain. Thus, there is a significant unmet medical need for effective drugs to treat T2D. Recently, inhibition of dipeptidyl peptidase-4 (DPP-4) has emerged as a new treatment option for T2D. This review highlights the design and structure activity relationships of the DPP-4 inhibitors reported over the last 10 years, along with the status of advanced clinical candidates. An attempt was also made to review DPP-4 inhibitors reported in various scientific journals along with selected patent literature.

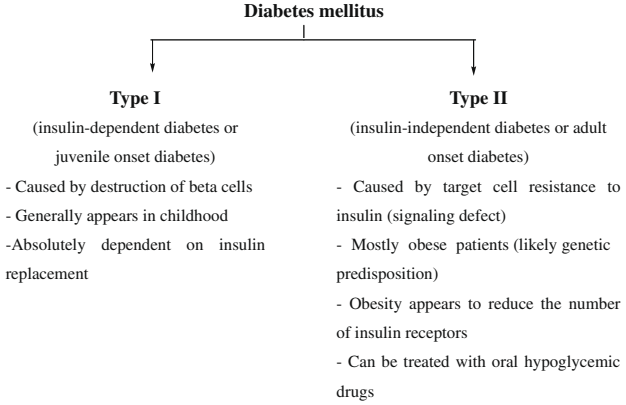


Chart 1. Classification of diabetes mellitus.

2. Current treatments of T2D: insufficient for glycemic control?

Enhancement of insulin secretion by pancreatic islet β-cells has been a major goal for the treatment of T2D. The current therapeutic agents,[2a] although effective in increasing insulin secretion, are associated with undesirable side effects, including hypoglycemia, abnormalities in cardiovascular responses and β-cell apoptosis. A

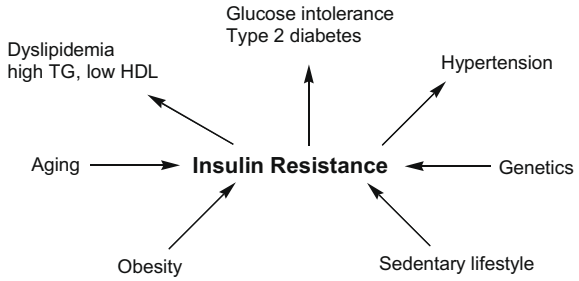


Chart 2. Cause and consequences of insulin resistance.

list of these agents along with their molecular target, mechanism of action (MOA) and adverse events related to their use are summarized in Table 1. Due to their adverse side effects most of these treatments are considered to be unsatisfactory in terms of prevention of complications and preservation of quality of life.[2a] α-Glucosidase inhibitors such as acarbose and miglitol, although effective in decreasing the absorption of glucose by interfering the action of α-glucosidases present in the small intestinal brush border, are often associated with abdominal bloating, diarrhea and flatulence. Conventional insulin secretagogues such as sulfonylureas (SUs) and the newer class of glinides [e.g., *S*(+)2-ethoxy-4(2((3-methyl-1-(2-(1-piperidinyl) phenyl)-butyl) amino)-2-oxoethyl) benzoic acid or Repaglinide] both suffer from induction of hypoglycemia. While metformin is the only therapeutic agent that has been demonstrated to reduce macrovascular events in T2D, its use is not recommended in conditions in which a patient has decreased renal or hepatic function. Agonists of peroxisome proliferator-activated nuclear receptor (PPAR), thiazolidinediones are able to reduce insulin resistance but are under intense scrutiny because of safety issues.[2b] Notably, insulin which is used to treat type 1 diabetes patients (for whom the hormone is no longer produced internally) is also used occasionally for patients having T2D when other medications fail to control blood glucose levels adequately. However, hypoglycemia and weight gain are common side effects. Thus, new approaches are needed to treat T2D. One of the desirable approaches to achieve this goal would be to identify agents that promote/enhance glucose (nutrient)-dependent insulin secretion.

3. Dipeptidyl peptidase—4: a new therapeutic target in type 2 diabetes

Inhibition of DPP-4 (DPP-IV, DP-4, CD26, IUBMB Enzyme Nomenclature EC 3.4.14.5), a serine protease and also known as serine exopeptidase or serine aminodipeptidase, has been shown to be a new approach for the treatment of T2D.[3] Serine proteases are a class of enzymes that cleave peptide bonds in proteins and as implicated by the name, one of the critical amino acids in the active site of the enzyme that cleaves peptide bonds is serine. The dipeptidyl peptidases (DPPs) are a subclass of the serine protease family.[3e] Members of this family include DPP I–IV [DPP II is also known as quiescent cell proline dipeptidase (QPP) or DPP VII (DPP7)], fibroblast activation protein-α (FAP), DPP-8 and DPP-9. Except DPP-4 all these enzymes remain poorly characterized and their natural substrates have not yet been identified. These peptidases are often referred to as DPP-4 activity- and/or structure-homologues (DASH) proteins. DPP-4 is a non-classical serine protease in that the catalytic triad of Ser, Asp, His, found in the C-terminal region of the enzyme, is in reverse order to that found in classical serine proteases. DPP-4 or CD26 is a membrane-associated peptidase of 766 amino acids, that is, widely distributed in numerous tissues. It is constitutively expressed on epithelial and endothelial cells of a variety of different tissues, for example, intestine, liver, lung, kidney and placenta. DPP-4 is also expressed on circulating T-lymphocytes and has been shown to be synonymous with cell-surface antigen, CD-26. DPP-4 also exists as a soluble circulating form in plasma and significant DPP-4 like activity is detectable in plasma. While the soluble form has structure and function identical to the membrane-bound form of the enzyme, it however lacks the hydrophobic transmembrane domain. The principal role of DPP-4 is its enzymatic function. It has many physiological relevant substrates such as chemokines, RANTES (regulated on activation normal T cell expressed and secreted), eotaxin, macrophage-derived chemokine, neuropeptides such as neuropeptide Y (NPY) and substance P, vasoactive peptides. It is responsible for the metabolic cleavage of certain endogenous peptides (Table 2). DPP-4 prefers substrates with an amino-terminal proline or alanine at position 2, but may also cleave substrate with non-preferred amino acids at position 2. Nevertheless, DPP-4 cleaves dipeptides (Xaa-Pro- or Xaa-Ala-) from the N terminus of biologically active peptides, transforming them into inactive or even antagonistic species. Researchers have found that the activity of two potent stimulators of insulin secretion, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (gastric inhibitory polypeptide or GIP), is rapidly cleaved by DPP-4. The structure of GIP, GLP-1 and GLP-2 reveals a highly conserved alanine at position 2, rendering these peptides ideal substrates for the DPP-4.[6]

Table 1
Current therapeutic agents for T2D other than DPP-4 inhibitors

Drug class	Molecular target	Mechanism/ actions	Adverse events
Insulin	Insulin receptor	Correct insulin deficiency	Hypoglycemia, weight gain
Sulphonylureas	SU receptor/ ATP-potassium channel	Stimulate insulin secretion	Hypoglycemia, weight gain
Metformin (Biguanides)	Unknown	Inhibition of hepatic glucose output	Gastrointestinal disturbances, lactic acidosis
Acarbose	α-Glucosidase	Retard carbohydrate absorption	Gastrointestinal disturbances
Thiazolidinediones (Pio and Rosiglitazone)	PPAR-γ	Increase insulin sensitivity	Weight gain, edema, anemia
GLP-1 analogues (Byetta)	GLP-1 receptor	Stimulate insulin secretion	Gastrointestinal disturbances, nausea, abdominal pain, weight loss

4. Glucagon: playing the opposite role of insulin in glucose regulation

Glucagon, a linear peptide of 29 amino acids is synthesized as proglucagon in the intestinal L cell and proteolytically processed to yield glucagon within alpha cells of the pancreatic islets. However, proglucagon expressed within the intestinal tract is processed into a family of glucagon-like peptides (enteroglucagon) instead of glucagon. Glucagon plays a major role in maintaining normal glucose levels in blood via increasing it when blood glucose levels begin to fall below the normal range (Fig. 1). Thus, glucagon is often described as having the opposite effect of insulin. Glucagon is secreted in response to hypoglycemia or low blood concentrations of glucose and its secretion is inhibited by high levels of blood glucose. Glucagon secretion is also inhibited by another hormone called somatostatin. Glucagon maintains the normal blood glucose

Table 2
Peptides that are inactivated by DPP-4[a]

Peptides with an N-terminal proline at position 2	Peptides with an N-terminal alanine at position 2
Substance P	GLP-1
NPY	GLP-2
GRP	GIP
Insulin-like growth factor I (IGF-I)	Growth-hormone-releasing hormone (GRH)
Peptide YY (PYY)	Peptide histidine methionin (PHM)
Enterostatin	

[a] Only those peptides are listed that are related to glucose homeostasis and body weight control.

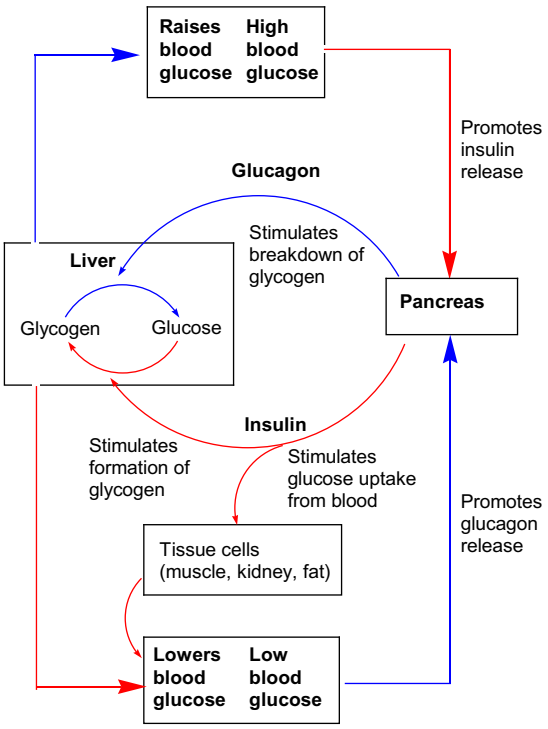


Figure 1. Regulation of blood glucose.

level with the help of two pivotal metabolic pathways within the liver: (a) as soon as secreted, it binds to glucagon receptors on hepatocytes to activate the enzymes that depolymerize glycogen stored in the liver through a process called glycogenolysis and (b) as the glycogen storage in the liver is depleted, glucagon then encourages the liver to synthesize additional glucose from non-hexose substrates such as amino acids via gluconeogenesis. The glucose generated is released into the bloodstream thus preventing the development of hypoglycemia.

5. GLP-1: the most important incretin hormone and substrate of DPP-4

Incretin hormones are defined as intestinal hormones released in response to nutrient ingestion, which potentiate the glucose-induced insulin response (the incretin effect). GLP-1 is an incretin hormone (Fig. 2) secreted by intestinal L-cells of the distal small intestine in response to food intake, that is, oral nutrients.[4] While oral nutrients such as glucose and fat are potent physiological regulators of GLP-1 secretion, neuromodulators acetylcholine and gastrin releasing peptide (GRP) have also been identified as non-nutrient stimulators of GLP-1. The active form of GLP-1 is a 30-amino acid peptide, which via binding to the GLP-1 receptor on pancreatic β-cells stimulates insulin gene expression, insulin biosynthesis and glucose-dependent insulin release (Chart 3).[5] It also inhibits glucagon release, promotes satiety, slows gastric emptying and promotes growth of pancreatic β-cells (β-cell dysfunction is an early pathophysiological defect in T2D mellitus). Each of these effects is beneficial in the control of glucose homeostasis in patients with T2D. Indeed, T2D patients infused with GLP-1 have demonstrated efficacy in normalizing both fasted and prandial glycemia. The combined beneficial effects of GLP-1 have made this peptide a promising new treatment for type 2 diabetes. While a GLP-1 analogue is currently available though in an injectable form only, the pharmacological side effect of GLP-1 is nausea and vomiting when administered in high doses. Moreover, the active form of GLP-1 (7-36) is rapidly inactivated by the plasma DPP-4, which cleaves a dipeptide from the N-terminus[6,7] (Fig. 2) via converting it into GLP-1 (9-36). The short half-life ($t_{1/2} \sim 1$–1.5 min) of GLP-1 in the circulation is a major obstacle for its use as a therapeutic agent. Continuous administration of GLP-1 or development of DPP-4 resistant GLP-1 agonists, such as exenatide[8] circumvents this problem. Exenatide is a synthetic version of a metabolic hormone called exendin-4 that occurs naturally in the saliva of the Gila monster (a large venomous lizard native to several southwestern American states) and displays properties similar to human GLP-1. Alternatively, inhibition of DPP-4 is expected to extend the half-life of endogenously secreted GLP-1 (7-36) (Fig. 3). Preclinical studies have demonstrated that this approach is effective in enhancing endogenous levels of GLP-1, which in turn enhances insulin secretion and improves the glucose tolerance in glucose-intolerant and diabetic animal models. Moreover, DPP-4 inhibitors have proved to be efficacious in patients with T2D both as monotherapy and in combination with metformin for the duration of 3–12 months. These inhibitors reduced fasting and postprandial glucose concentrations leading to decrease in glycosylated haemoglobin levels, while preserving β-cell function in animal models.

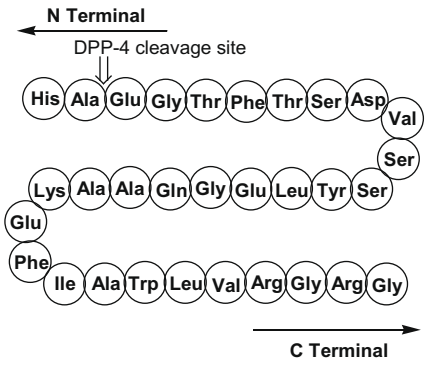


Figure 2. The incretin hormone GLP-1.

6. GIP: the other incretin hormone and substrate of DPP-4

The first and the other most important incretin hormone GIP was purified and characterized in 1970s. Until that the identity of the putative incretin factor(s) remained elusive. Initially GIP was believed to neutralize stomach acid to protect the small intestine

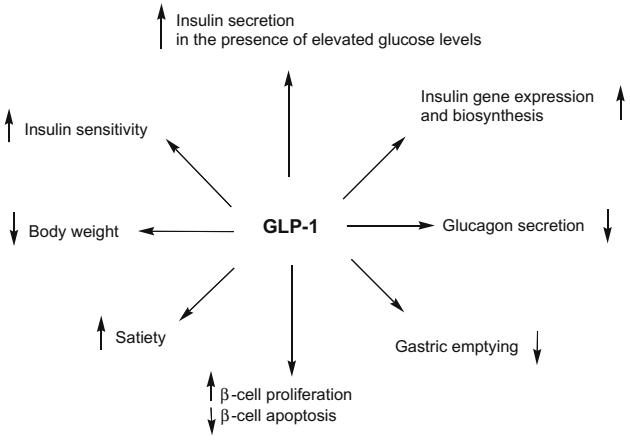


Chart 3. Important physiological effects of GLP-1.[5]

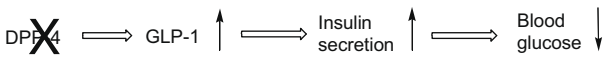


Figure 3. Effect of DPP-4 inhibition.

from acid damage, reduce the rate at which food is transferred through the stomach, and inhibit the GI motility and secretion of acid. However, later it was established that like GLP-1, GIP also plays a key role in glucose homeostasis.[9] GIP, a 42 amino acid peptide is secreted by K cells from the upper small intestine within minutes of nutrient ingestion, facilitates the rapid disposal of ingested nutrients. After the discovery of its insulin secretion properties GIP has been evaluated as a potential antidiabetic agent.[10] GIP mediates its physiological actions through a GPCR belonging to the glucagon receptor super family, which includes receptors for other structurally related gut-derived peptides, including GLP-1, GLP-2, glucagon, secretin and growth hormone–releasing hormone. GIP receptor activation in the β-cell has been shown to increase adenylyl cyclase activity leading to the increases in cAMP as well as in intracellular calcium ion levels. This pathway leads to downstream activation of Protein Kinase A (PKA) and enhances hormone release, most notably that of insulin from the β-cell. GIP also promotes energy storage via direct actions on adipose tissue, and enhances bone formation via stimulation of osteoblast proliferation and inhibition of apoptosis. However, human subjects with T2D exhibit relative resistance to the actions of GIP[9] and therefore GIP is not an effective blood glucose lowering agent in T2D subjects.[11] Like GLP-1 it is also inactivated rapidly in vivo through the action of DPP-4. Thus, full length GIP(1–42) is rapidly converted to bioinactive GIP(3–42) within minutes of its secretion from the gut K cell. Thus, one of the objectives of DPP-4 inhibition is to stabilize GIP[12] in addition to prolong the beneficial effects of endogenous GLP-1.

7. DPP-4 inhibitors: a major breakthrough in metabolic research

Various clinically feasible approaches for the use of GLP-1 to treat T2D have been investigated. These include development of (a) DPP-4 resistant GLP-1 analogues (peptide based) such as exenatide LAR and liraglutide, (b) small molecules as agonist for the GLP-1 receptor (non-peptide based) and (c) small molecules as DPP-4 inhibitors. DPP-4 inhibitors,[13] a new class of oral hypoglycemic agents that block DPP-4, are of considerable interest to the pharmaceutical industry. Their mechanism of action is thought to result from increased incretin levels (GLP-1 and GIP), which, inhibit glucagon release thereby decreasing the blood glucose and more importantly increase insulin secretion and decrease gastric emptying. In 1996 for the first time it was demonstrated in rats that application of a specific DPP-4 inhibitor isoleucyl thiazolidine completely blocked the degradation of GLP-1 in vivo resulting in improved insulin response accompanied by accelerated peripheral glucose disposal. Several research groups confirmed these results. Thus improvement of glucose tolerance in mammal via application of DPP-4 inhibitors marks a major breakthrough in metabolic research bearing the potential of a new revolutionary diabetes therapy. DPP-4 inhibitors offer several potential advantages over existing therapies including decreased risk of hypoglycemia, potential for weight loss, and the potential for regeneration and differentiation of pancreatic β-cells.[3a] More importantly, DPP-4 inhibitors can be administered orally. While certain concerns are raised against the use of DPP-4 inhibitors (Table 3), at present treatment of metabolic disorder via DPP-4 inhibition is a validated principle and several inhibitors are in various stages of clinical development.[14] Apart from T2D, DPP-4 inhibitors are also believed to be useful for several other related disease conditions such as diabetic dyslipidemia, conditions of impaired glucose tolerance (IGT), conditions of impaired fasting plasma glucose (IFG), metabolic acidosis, ketosis, appetite regulation and obesity. Extensive research efforts from both the academia and the pharmaceutical industry have resulted in a number of potent DPP-4 inhibitors.

Table 3
Main advantages and concerns of DPP-4 inhibitors

Advantages	Concerns
(a) Orally available (b) Well tolerated (c) Weight neutral (d) GLP-1/GIP receptor-dependent MOA	(a) Side effects due to other functions[a] in addition to degrading regulatory peptides, or because of the effect on multiple substrates of DPP-4
(e) Enhancement of endogenous incretin action	(b) Selectivity against other closely related enzymes DPP-2 or QPP, DPP-8, DPP-9 and FAP is an important issue

[a] Other functions of DPP-4 include a role in the immune system, influencing T-cell activity.

Remarkable creativity and imagination have been demonstrated by medicinal chemists in designing novel and potent series of inhibitors some of which are presented in the following sections.

8. Development of selective DPP-4 inhibitors: a challenging task

Development of small molecules as selective inhibitors of DPP-4 is a major challenge. Although the in vivo function of other members of DPP family, that is, DPP-2, DPP-8, DPP-9 etc. are largely unknown, the physiological effects of their inhibition has been documented in the literature.[15a] For example, inhibition of DPP-2 has been shown to result in the apoptosis of quiescent T cells. DPP-8 is a cytoplasmic protease with a 51% homology with DPP-4 at the amino acid level. Selective inhibition of DPP-8/DPP-9 in animals resulted in severe toxic reactions, including alopecia, thrombocytopenia, anemia, enlarged spleen, multiple histological pathologies and increased mortality.[15b] Notably, it has been shown very recently that inhibition of DPP-8 and DPP-9 did not lead to organ toxicities and mortality in rodents and thus, a mechanism other than DPP-8/DPP-9 inhibition has been suggested to be responsible for the observed toxicities associated with the inhibitors of DPP-8/DPP-9.[15c] Nevertheless, in view of likely toxic side effects associated with the inhibition of other members of DPP family it was become necessary to design selective inhibitors targeting DPP-4. Experimental observation indicated that the S2 pocket (see Section 9.1) of DPP-4 might be similar to that of DPP-8 and consequently inhibitors designed for DPP-4 might show an inhibitory effect on DPP-8 as well. However, extensive SAR work has proved that desired selectivity for DPP-4 inhibition can be achieved via introducing appropriate substituent or group as discussed in the following sections. Nevertheless, once synthesized, it has therefore become mandatory to determine the DPP-4 selectivity of an inhibitor over closely related other DPPs. Recently, another study has demonstrated that high levels of GLP-1 should be maintained for 24 h for optimal glycemic control.[15d] Thus, in addition to focusing on potency and selectivity, development of long-acting inhibitors is also desirable that could potentially provide maximal efficacy, particularly in patients suffering from severe diabetes (e.g., HbA1c >9%).

Structurally, two distinctive classes, for example, peptidomimetic and non-peptidomimetic DPP-4 inhibitors have been reported. The peptidomimetic class can be subdivided into (a) glycine-based (α-series **A**) and (b) β-alanine-based (β-series **B**) inhibitors (Fig. 4). Interactions of α- and β-series with the DPP-4 enzyme (Fig. 4) do not follow the same pattern. For example, a pyrrolidine amide moiety though common to both these series, did not occupy the same pocket in the enzyme active site as indicated by SAR studies. Based on the X-ray crystal structure of DPP-4 and computer modeling studies on enzyme bound L-valine pyrrolidine amide two key interactions has been suggested between the en-

zyme and **A**, for example, (i) a salt bridge between the free amino group and Glu205 and/or Glu206 and (ii) a hydrogen bond between the carbonyl oxygen and Arg125. While a similar salt bridge was observed in case of **B** but the hydrogen bond to the carbonyl oxygen was not well defined. Notably, the X-ray crystal structure determination showed that **B** binds to the active site of DPP-4 with the amide moiety in the opposite orientation to that of **A** and was in agreement with the reported SAR. The pyrrolidine ring could afford to have small substituents like nitrile or fluorine in case of **A** whereas bigger groups were well tolerated either at C-2 or C-3 position in case of **B**. Moreover, replacement of pyrrolidine ring with thiazolidine was well tolerated in case of **A** though a similar ring containing other hetero atom, for example, oxazolidine or larger ring, for example, piperidine, homopiperidine decreased the activity. Replacing the pyrrolidine ring by other groups like piperazine or other cyclic amines in case of **B** did not alter the DPP-4 inhibitory properties.

9. The peptidomimetic series

9.1. Glycine-based inhibitors or α-series

It is noteworthy that pyrrolidine derivatives have been widely explored as DPP-4 inhibitors due to DPP-4's specificity for substrate having an amino-terminal proline at C-2. Thus, many DPP-4 inhibitors resemble the cleavage product of P2–P1 dipeptidyl substrate **A** (Fig. 4x) where the P-1 site contains a proline mimic. A schematic diagram showing the binding of **A** with catalytic site of DPP-4 including S1 and S2 pocket of the enzyme and covalent interaction with serine residue is shown in Figure 4y. According to the X-ray data, the DPP-4 active site can be defined by a catalytic triad (S630, H740, D708), an oxyanion hole (Y547 OH, Y631 N), and specificity residues (E205, E206, Y662, Y666, N710) including the S1 pocket (Y662, Y666, V656, V711, Y631, W659). Generally, potent inhibitors were obtained by replacing the amide moiety of the cleaved P-1 substrate by an electrophile which forms an adduct with the active site serine 630. For example, (a) diphenylphosphonate ester[16] [–P(O)(OPh)$_2$] or *O*-acylhydroxamic acid[17] (CONHOCOR′) and (b) boronic acid[18] [B(OH)$_2$], nitrile (CN) or hydrogen at the C-2- of the pyrrolidine ring (**B**, Fig. 4x) provided irreversible and reversible inhibitors, respectively.

9.1.1. 2-Cyano pyrrolidine derivatives: the most extensively studied inhibitors

2-Cyano pyrrolidine-based inhibitors have been studied most extensively because apart from behaving as a proline mimic, the presence of the nitrile on the five-membered ring provided (i) nanomolar inhibition of DPP-4 and (ii) chemical stability adequate for oral administration. This was exemplified by cyclohexylglycine-(2*S*)-cyanopyrrolidine[19] (**1**, Fig. 5), one of the potent, selective and stable (K_i of 1.4 nM, >1000-fold selectivity over closely related peptidases except DPP-8 and $t_{1/2}$ stability >48 h at pH 7.4) inhibitors. While a basic amine at P-2 was necessary for inhibition (Fig. 4y), the presence of protonable primary amine in compound **1** and

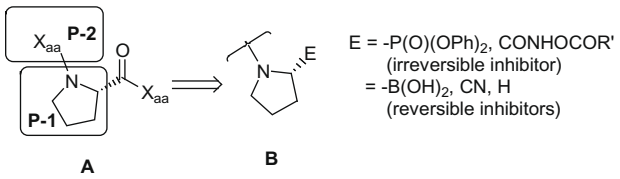


Figure 4x. Design of pyrrolidine derivatives as DPP-4 inhibitors.

the cleavage of substrates containing *N*-methylglycine at P-2 site[20] prompted researchers to study a range of structurally diverse N-substituted glycines for better toleration at the P-2 site. Accordingly, a number of diverse P-2 site N-substituted glycines were prepared and found to provide potent inhibition when combined with a (2*S*)-cyanopyrrolidide in the P-1 site.[21–24] One compound from this series (**2**, Fig. 5) (IC_{50} = 22 nM) was found to be potent, selective and short-acting DPP-4 inhibitor.[21a] Replacing 2-cyanopyrrolidine moiety in **2** by 5-cyano-4,5-dihydropyrazol provided a potent inhibitor KR-62436 [DPP-4 IC_{50} = 0.78 (rat plasma), 0.49 (porcine kidney) and 0.14 μM (human)][21b] that improved glucose tolerance in the *ob/ob* mouse model of T2D. Compound **2**, because of its excellent oral bioavailability and potent antihyperglycemic activity, was progressed into clinical trials[25] but discontinued later due to its side effects. Since the 2-cyanopyrrolidide moiety was responsible for a slow-binding mechanism characterized by high potency, competitive behaviour, and rapid reversibility in inhibiting both human and rodent DPP-4 activity further SAR study was carried out around this class of compounds. As a result, a follow up compound **3a** (Fig. 5) (IC_{50} = 34 nM) was identified as a clinical candidate.[26] Pharmacological studies suggested that **3a** was a potent, selective, and orally active inhibitor of DPP-4 (*F* > 90% in normal cynomolgus monkeys) that improved insulin secretion and glucose homeostasis with a profile wholly consistent with increased action of GLP-1. Replacing the 3-hydroxy group of adamantan moiety by other substituents led to the identification of a new series of potent DPP-4 inhibitor exemplified by a representative compound **3b**.[27a] Development of novel DPP-4 inhibitors via introducing various C-2 substituents on adamantan ring has also been reported.[27b] As evident from the structures of **2** and **3a** that

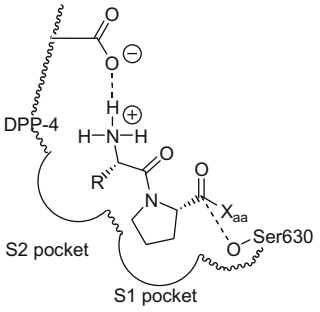


Figure 4y. Interaction of **A** with DPP-4.

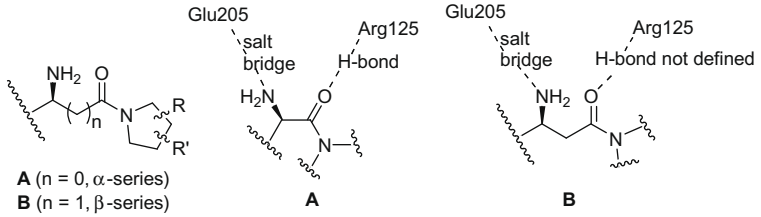


Figure 4. Classification of DPP-4 inhibitors and their key interactions with the DPP-4 enzyme.

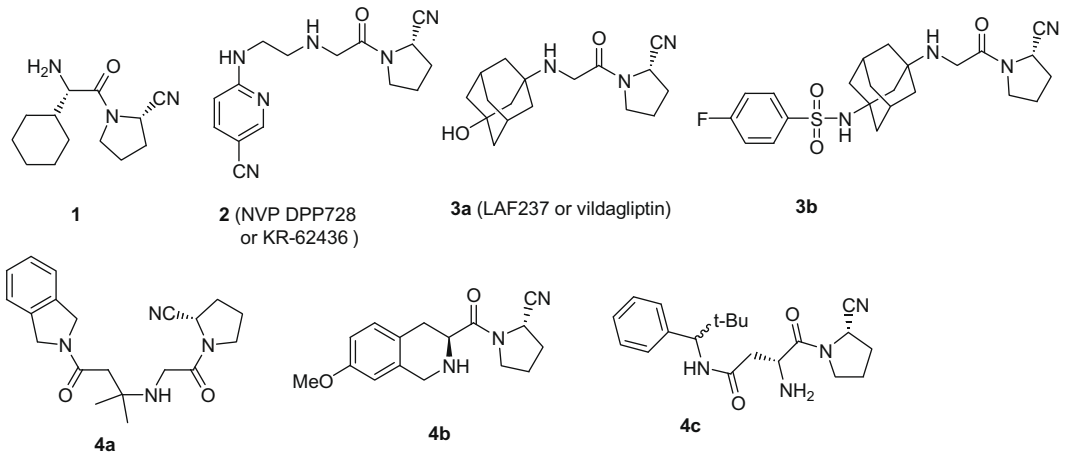


Figure 5. 2-Cyano pyrrolidine-based DPP-4 inhibitors.

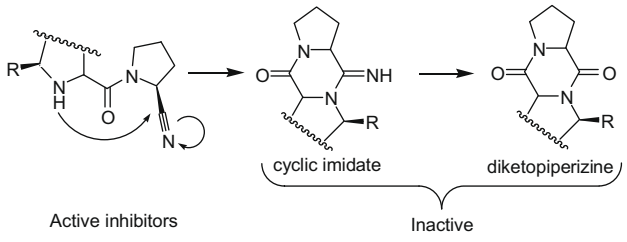
both straight chain and cyclic substituents with polar and lipophilic side chains including bulky adamantly group were well tolerated at P-2 site. A *gem*-dimethyl substituent on the side chain of P-2 site was also tolerated as represented by compound **4a** (Fig. 5), a potent DPP-4 inhibitor (IC_{50} = 15 nM) with high selectivity over DPP8 (IC_{50} > 100 μM) and DPP-II (IC_{50} > 100 μM).[28a]

Bridging the carbon and nitrogen at P-2 via making a small ring was tolerated. For example, **4b** (Fig. 5), that belongs to tetrahydroisoquinoline-3-carbonylcyanopyrrolidine class has shown potent inhibition of DPP-4 (IC_{50} = 4.0 nM).[28b] Evaluation of a series of 2-cyanopyrrolidines which bear 4-substituted glutamic acids at the P2-site against DPP-4, DPP-8 and DPP-2 indicated that analogues having a bulky substituent at the benzylic position inhibited DPP-4 with 30-fold selectivity over both DPP-8 and DPP-2.[28c] The *tert*-butyl-substituted analogue **4c** (Fig. 5) showed 53-fold selectivity for DPP-4 over DPP-8, with a moderate potency (DPP-4 IC_{50} = 251 nM).

9.1.2. Modifications of P-1 pyrrolidine ring: towards enhanced stability

One of the issues encountered with the use of 2-cyano pyrrolidine derivatives was its stability in solution that hampered the formulation efforts. The cyano group and the P-2 basic amine moiety underwent an intramolecular cyclization to form inactive cyclic imidates and/or their diketopiperazine hydrolysis products[26] (Scheme 1).

However, as observed in case of compound **3a**, this process could be slowed down by creating an appropriate steric crowding on the P-2 fragment. Alternatively, a cyclopropyl ring fused on the P-1 pyrrolidine moiety minimized this cyclization.[29] Thus a series of methanoprolinenitrile-containing dipeptide mimetics were evaluated as DPP-4 inhibitors where *cis*-4,5-methanoprolinenitriles with β-branching in the N-terminal amino acid provided enhanced chemical stability and high inhibitory potency.[30] This



Scheme 1. Intramolecular cyclization of 2-cyano pyrrolidine derivatives.

class of compounds exhibited the ability to suppress prandial glucose elevations after an oral glucose challenge in male Zucker rats. Efforts to further elucidate SAR within this series led to the investigation of vinyl substitution at the β-position of α-cycloalkyl-substituted glycines. Despite poor systemic exposure, vinyl-substituted compounds showed extended duration of action in an ex vivo plasma DPP-4 inhibition model in normal rats. In order to define the role of suspected metabolites, oxygenated metabolites (i.e., hydroxymethylcycloalkyl-based analogues) were prepared that maintained the potency and extended duration of action of their precursors in efficacy models measuring glucose clearance in Zucker$^{fa/fa}$ rats. They also displayed favourable PK properties with a tighter correlation of PK to PD. Extension of this approach to adamantylglycine-derived inhibitors led to the discovery of highly potent inhibitors, including hydroxyadamantyl compound **5** (Fig. 6).[30a] A highly efficacious (human DPP-4 K_i = 0.6 nM; 87% ex vivo plasma DPP-4 inhibition @ 4 μmol/kg in normal rats at 30 min and 4 h), stable, and long-acting DPP-4 inhibitor **5** (Fig. 6) showed a slow rat liver microsomal turnover rate, no CYP3A4 inhibition up to 100 μM, and good oral exposure (F = 75%, $t_{1/2}$ = 2.1 h). Compound **5** also showed robust glucose-lowering effects in a dose-dependent manner in the Zucker$^{fa/fa}$ rat OGTT model and efficacy in reducing postprandial glucose AUC in *ob*/*ob* mice. This compound was effective in elevating insulin levels after an OGTT in *ob*/*ob* mice and is in phase 3 clinical trials (NDA filed in 2008).[30b]

Another compound **6** (Fig. 6) obtained via linking the 4-cis-substituted L-prolines in the P2 position with 4,5-methano-pyrrolidine at P1 site was found to be a potent inhibitor of DPP-4 (K_i = 3.6 nM).[31] SAR studies in this diprolyl nitrile series revealed that the five-membered cyclic amino acid (proline) provided enhanced inhibitory activity than four- or six-membered ring analogues, and that the L-cyclic amino acid is the preferred stereoisomer to bind in the S2 pocket of the enzyme. Moreover, the C-4 position of P2 proline could accommodate cis substituents, and aromatic groups to enhance the DPP-4 inhibitory potency.

In another effort, SAR work centered on the P-2 fragment and blocking groups was carried out to decrease the cyclization process. Incorporation of a fluoro substituent at C-4 position of the pyrrolidine ring provided a series of 2-cyano-4-fluoro-1-thiovalyl-pyrrolidine inhibitors of DPP-4.[32a] The most promising compound **7a** showed good inhibitory properties (K_i = 53 nM, DPP-4 inhibition at 6 h @ 1 mpk in rat = 84%), selectivity (>422 over DPP-II and >442 over seprase), and PK profile (F = 32% in rat and 80% in dog). Additionally, compound **7a** showed a long duration of action in both rat and dog and good stability towards cyclization ($t_{1/2}$ at 37 °C at pH 7.2 = 1733 h) due to the presence of *gem*-di methyl group.

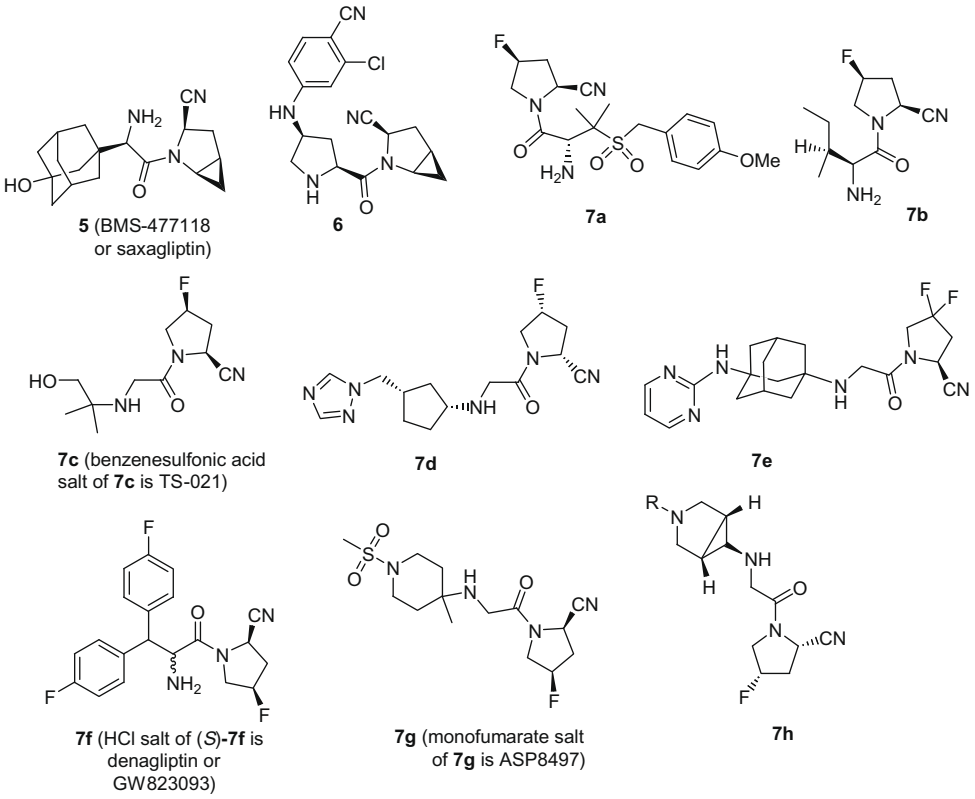


Figure 6. C-4, C-5 modifications of P-1 pyrrolidine ring.

Notably, enhanced DPP-4 inhibitory properties of (4*S*)-fluoro derivative of 2-cyano pyrrolidine and its higher concentrations in plasma after oral administration to rats have been reported earlier.[32b] Compound **7b** (Fig. 6) (DPP-4 IC_{50} = 0.6 nM) showed good plasma drug concentrations (C_{max} = 372 ng/mL at 10 min @ 1.0 mg/kg in Wistar rats) and significant effects on plasma glucose (reduction in increase), plasma insulin (enhancement of secretion) and plasma DPP-4 activity (complete inhibition) during OGTT in Zucker fatty rats (1 or 4 mg/kg po). However, as compound **7b** failed in chemical stability and persistence effect, further SAR work was carried out on 2-cyano-4-fluoropyrrolidine with N-substituted glycine at the 1-position. This work has led to the identification of **7c** (Fig. 6), a potent and stable DPP-4 inhibitor (IC_{50} = 4.6 nM) with a long-term persistent plasma drug concentration and a potent antihyperglycemic activity.[32c] An X-ray crystallographic structure of DPP-4 indicated that a 2-hydroxy-1,1-dimethylethyl side chain could interact with the pocket created by Phe357, Arg358, Ser201, His126 and Arg358, thereby accounting the high affinity of **7c**. Modification of the side chain of **7c** yielded another series of DPP-4 inhibitor as exemplified by **7d** (Fig. 6).[32d] A new series of potent DPP-4 inhibitors was identified by introducing an additional fluoro group at the C-4 position of chiral 2-cyano-4-fluoro pyrrolidine moiety[32e] and the representative compound **7e** is shown in Figure 6. Modification of the side chain of **7b** yielded a series of potent DPP-4 inhibitor and the best compound[32f] **7f** (*S*-isomer, Fig. 6) (IC_{50} = 22 nM) progressed into phase 3 clinical trials. Very recently, pharmacological profile of **7g** (Fig. 6), a potent [plasma DPP-4 IC_{50} = 2.6 nM (mice), 7.3 nM (dog), and 6.2 nM (human)] and selective [IC_{50} = 1700 nM (DPP8) and 100 nM (DPP9)] inhibitor, has been reported.[32g] Incorporating conformationally restricted *N*-(aryl or heteroaryl)-3-azabicyclo[3.1.0]hexane moiety in 2-cyanopyrrolidine class provided a new series of DPP-4 inhibitors represented by **7h** (Fig. 6).[32h]

9.1.3. Modifications of P2 pyrrolidine ring: towards enhanced potency

The observation that modification at C-4 position of the P2 pyrrolidine yielded potent inhibitors was exemplified by a new series, that is, 1-(γ-substituted prolyl)-(*S*)-2-cyanopyrrolidines[33a] that were designed based on the predicted binding mode of the known DPP-4 inhibitor **2**. SAR study indicated that compounds bearing (*S*)-stereochemistry at C-4 position of the P2 pyrrolidine were 20-fold more potent than the other antipode. Two compounds that showed the highest inhibitory activity are **8a** (DPP-4 inhibition; IC_{50} = 0.13 and 0.17 nM/L in human and rat) and **8b** (DPP-4 inhibition; IC_{50} = 0.13 and 0.15 nM/L in human and rat) (Fig. 7). Incorporation of an aryl group at C-4 position of the pyrrolidine ring led to the identification of [4-(hydroxyphenyl)prolyl]prolinenitrile dipeptides that were stable [due to the 2,6-disubstitution of the 4β-(hydroxyphenyl) residues] and highly effective long-acting inhibitors of DPP-4.[33b] A representative compound **8c** (Fig. 7) showed DPP-4 inhibitory properties in vitro (human DPP-4 IC_{50} = 4.9 nM) and ex vivo (95% and 83% plasma DPP-4 inhibition @ 1 mg/kg po in normal rats at 6 and 10 h, respectively). Despite its poor bioavailability (3% in rat) **8c** showed a dose-dependent (0.01, 0.03 and 0.1 mg/kg po) suppression of plasma glucose after an OGTT in normal rats indicating the appearance of an active metabolite (glucuronate) after oral dosing.

By introducing ring-constraint in compound **3a** (Fig. 5) a series of substituted pyrrolidine-2,4-dicarboxylic acid amides were designed and synthesized, many of which showed good in vitro DPP-4 inhibition (IC_{50} = 2–250 nM) with selectivity over DPP-2, DPP-8 and FAP enzymes.[33c] Compounds **8d** (Fig. 7) [IC_{50} = 0.0017 μM (DPP-4); 2.727 μM (DPP-8); >20 μM (DPP-II); 0.175 μM (FAP)] showed 10-fold improvement in the in vitro DPP-4 inhibition than **3a** and an in vivo plasma DPP-4 inhibition after oral administration in Wistar rats (10 mpk po).

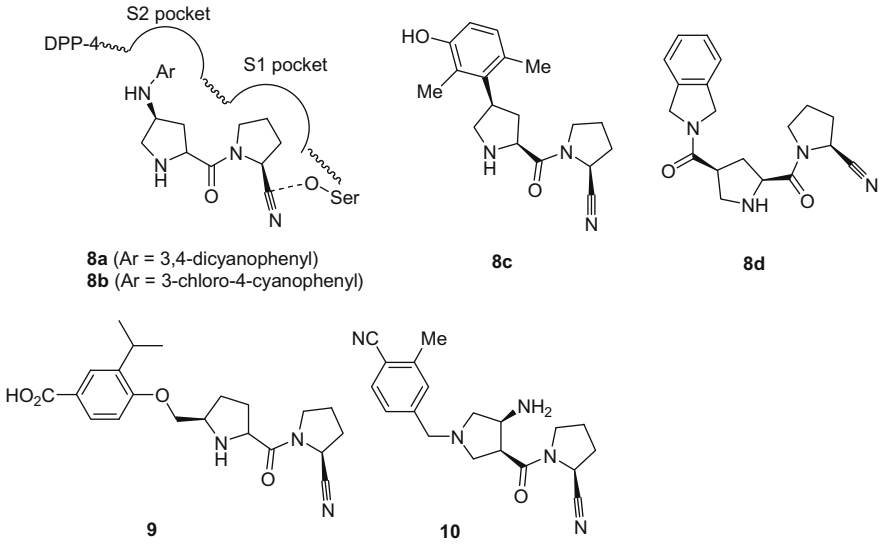


Figure 7. C-4, C-5 and other modifications of the P-2 pyrrolidine ring.

Modification at the C-5 position of the P2 pyrrolidine also afforded potent inhibitors of DPP-4. Thus a series of (5-substituted pyrrolidinyl-2-carbonyl)-2-cyanopyrrolidine analogues was evaluated.[34] The optimized analogues were found to be potent (with subnanomolar K_i's) and chemically stable. These compounds showed very little potency decrease in the presence of plasma, and exhibited more than 1000-fold selectivity against related peptidases. X-ray crystallography data showed that these cyanopyrrolidine inhibitors bind to the active site through the nitrile group, forming a covalent bond with the hydroxyl group of the active site serine, Ser630.[35] Consistent with the known binding of the P2 amino group of peptide inhibitors to two glutamates, the amino group of the P2 pyrrolidine was observed to be in close proximity to the side chains of Glu205 and Glu206. The C5-substituents made various interactions with the enzyme and affected potency, chemical stability, selectivity and PK properties of the inhibitors. The lead compound **9** (Fig. 7) (K_i = 3.1 nM) exhibited good PK (F = 34% in rat) and was efficacious in lowering blood glucose in an OGTT in Zucker diabetic fatty (ZDF) rats (30% reduction of glucose excursion @ 3 mg/kg).

Recently, *cis*-3-amino-4-(2-cyanopyrrolidide)-pyrrolidines have been shown to be a unique scaffold for the development of potent DPP-4 inhibitors.[36] The lead compound **10** (Fig. 7) showed excellent DPP-4 inhibitory potency (human DPP-4 IC_{50} = 1.3 nM), selectivity (human DPP-4 IC_{50} = 460 nM), good oral bioavailability in rats (81% @ 5 mg/kg po) and robust maximal DPP-4 inhibition in rats (5 mg/kg po dose) inhibiting rat DPP-4 > 90% beyond 8 h post-dose. While compound **10** tested negative in a human lymphocyte aberration (HLA) assay implying less potential for cytotoxicity its unexpected instability in human plasma and blood precluded further development of this molecule.

While molecular modeling studies based on known DPP-4 enzyme-inhibitor crystal structure did not encourage the use of achiral *cis*-2,5-dicyanopyrrolidine template for the design of new DPP-4 inhibitors, a series of *cis*-2,5-dicyanopyrrolidine α-amino amides however, were evaluated for DPP-4 inhibition.[37a] The choice of this template was prompted by the fact that binding site residues of DPP-4 can move to accommodate a ligand. Thus, **11a** (Fig. 8) (DPP-4 IC_{50} = 104 nM) was identified as an achiral inhibitor of DPP-4 that showed selectivity over DPP-2, DPP-3, DPP-8, DPP-9, APP and FAP. It exhibited oral bioavailability in the rat (77%), dog (93%) and monkey (42%) and displayed in vivo efficacy in a mouse OGTT model (67% reduction of glucose excursion @ 10 mg/kg). The X-ray crystallography data suggested that *cis*-2,5-dicyanopyrrolidine moiety was involved in a covalent interaction with S630 through one nitrile group, while the other nitrile forced the Y547 side chain to move and subsequently made a π-stacking interaction and H-bond with Y666. The secondary amine was recognized by E205, E206, N710 and Y662.[37a] Preliminary safety assessments with **11a** (5-day toxicity study in male and female Sprague–Dawley rats @ 500 mg/kg for 5 days and a single-dose safety study in dog @ 20 mg/kg iv) indicated no safety liability. Based on better solubility profile the hydrochloride salt of **11a** was selected for clinical development.

Interestingly, an alkyne-substituted 2-cyanopyrrolidine provided superior selectivity profiles as exemplified by **11b** (Fig. 8), a clinical compound presently in further development.[37b] Compound **11b** showed excellent potency and selectivity for DPP-4 inhibition [K_i = 1.0 nM (DPP-4), >30 μM (DPP-8), >30 μM (DPP-9), >30 μM (DPP-7), >30 μM (POP), >30 μM (FAP-α)]. It was orally available (F = 28% (rat), 11% (monkey) and 35% (dog)], efficacious, and well tolerated in preclinical safety pharmacology (IC_{50} > 30 μM for CYP3A4, CYP2D6, and CYP2C9 inhibition; K_i > 45.1 μM for binding to the hERG channel) and toxicology studies. In 4-week studies in rats and dogs, the 'no observed adverse effect level' for **11b** was greater than 1000 mg/kg/day.

9.1.4. Pyrrolidine-based inhibitors lacking electrophilic nitrile group

Removal of nitrile moiety from 2-cyano pyrrolidine derivative is an obvious solution to the intramolecular cyclization related problem of this class of compounds. Indeed inhibitors lacking nitrile moiety, for example, (*S*)-isoleucine thiazolidide[38a] **12** (DPP-4 IC_{50} = 420 nM) and (*S*)-cyclohexylglycine pyrrolidide[19] **13a** (DPP-4 IC_{50} = 320 nM) (Fig. 9) were found to be stable though their inhibitory potency was moderate. Compound **12** showed good effects in limited clinical trials despite moderate inhibitory properties. Indeed, glutaminyl thiazolidine (PSN-9301), a short acting

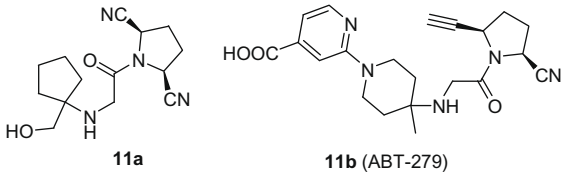


Figure 8. 5-Substituted 2-cyanopyrrolidine derivatives.

inhibitor of DPP-4 was advanced into phase 2 clinical trials.[38b] A further modification of compound **13a** led to the identification of sulfonamide[39] **13b** (Fig. 9) [IC_{50} = 88 nM (DPP-4) and 8800 nM (QPP)] that showed an 100-fold selectivity over QPP, good PK properties in rat and dog (F = 100%) and found to be efficacious in an OGTT in lean C57BL6/N mice (36% reduction in glucose excursion @ 3 mpk po). The thiazolidide derivatives represented by compound **14** was found to be potent, selective and orally bioavailable inhibitors of DPP-4.[39]

Other inhibitors lacking the electrophilic nitrile group include [(*S*)-γ-(arylamino)-L-prolyl]thiazolindine derivatives containing a 4β-(amino)-L-prolyl moiety.[40] One of the representative compounds in this series **15a** (Fig. 9) [DPP-4 IC_{50} = 25.4 nM (human) and 30.4 nM (rat)] showed a longer duration of plasma DPP-4 inhibition, high oral bioavailability (F = 83.9%) and long half-life in plasma ($t_{1/2}$ = 5.27 h) when dosed to SD rats @ 10 mg po. The γ-substituent in the proline moiety, that is, 5-cyano-2-pyridyl group was thought to interact with the S2 binding pocket of DPP-4 maintaining a hydrophobic interaction. Its close analogues, for example, **15b** and **15c** (Fig. 9) have also been reported as DPP-4 inhibitors.

In case of substituted 2-(pyrrolidine-1-carbonyl)-pyrrolidine-4-carboxylic acid amides[33c] the most potent compound **16a** (Fig. 9) [IC_{50} = 0.050 μM (DPP-4); >20 μM (DPP-8); >20 μM (DPP-II); 7.951 μM (FAP)] showed a weak FAP inhibition, maximum plasma DPP-4 inhibition around 30 min (after oral dosing @ 10 mpk in Wistar rats) and good stability in aqueous media (analyzed by LC–MS). However, no data is available related to the reduction of plasma glucose by compound **16a** during the OGTT in normal rats.

A series of (4β-substituted)-L-prolylpyrrolidine analogs lacking the electrophilic nitrile function has been reported as stable DPP-4 inhibitors[41a] that showed long-lasting and potent ex vivo DPP-4 inhibition in normal rats after oral dosing. A representative compound **16b** (Fig. 9) [DPP-4 IC_{50} = 14 nM (human enzyme), 41 nM (human plasma) and 60 nM (rat plasma)] showed good ex vivo inhibitory activity (95% and 72% plasma DPP-4 inhibition @ 3 mg/kg po, normal rats at 30 min and 9 h) and effectively reduced the plasma glucose level after the OGTT in normal rats. The high affinity of **16b** for DPP-4 was thought to be due to the affinity of the sulfur-containing hetero-aromatic moiety of *N*-(3-methyl-1,2,4-thiadiazol-5-yl)piperazine residue which might have reversed the reduction of inhibitory activity caused by removal of the reactive nitrile group. The compound **17a** (Fig. 9) derived from **7c** but devoid of nitrile group has been reported as a novel and potent inhibitor of DPP-4.[41b] Recently, β-branched natural and unnatural amino acids **17b** (Fig. 9), particularly adamantylglycines, linked to a (2*S*,3*R*)-2,3-methanopyrrolidine-based scaffold have been described as potent DPP-4 inhibitors.[41c] Another compound, that is, *N*-(cyanomethyl)-*N*-ethyl-L-prolinamide, was identified as a potent inhibitor of DPP-4 (human DPP-4 IC_{50} = 24 nM, plasma DPP-4 inhibition @ 0.3 mg/kg po in normal rats after 6 h = 73%) and was evaluated to determine its effect on the plasma glucose level.[41d]

9.1.5. Fluorinated pyrrolidine amides as DPP-4 inhibitors

The 3-fluoropyrrolidine analogues obtained by replacing the pyrrolidine ring in **13a** by 3-fluoropyrrolidine moiety were shown to have activity equal or superior to that of the parent compounds. Thus, phenylalanine derivatives **18** (Fig. 10) were identified as potent and selective inhibitors of DPP-4 [for **18a**, IC_{50} = 0.012 (DPP-4), 24 (QPP), >100 (DPP-8) and 69 μM (DPP-9), F = 67% in rat; for **18b**, IC_{50} = 0.064 (DPP-4), 2.7 (QPP), 88 (DPP-8) and 86 μM (DPP-9), F = 85% in rat].[42,43] However, due to their hERG ion channel binding properties (IC_{50} = 4.6 and 1.1 μM for **18a** and **18b**) and demonstrated high serum potency shift (>10-fold increase in apparent IC_{50}) thought to be caused by the highly lipophilic biaryl moiety a further SAR study was carried out. Replacing the 4-fluorophenyl group of **18** by a heterocyclic moiety yielded the compound **19** (Fig. 10) [IC_{50} = 4.3 nM (DPP-4), >100,000 nM (QPP), >100,000 nM (DPP-8) and >100,000 nM (DPP-9), F = 43% in rat and 94% in dog] which had an improved hERG profile (IC_{50} = 86 μM).[44] However, due to the potential for metabolic activation though moderate in both rat and human, **19** was not selected for further profiling. Replacing the phenyl ring of **18** by a cyclohexyl ring followed by appropriate modifications provided compound **20** (Fig. 10) [IC_{50} = 0.016 μM (DPP-4), 25 μM (DPP-8) and >100 μM (DPP-9), F = 56% (rat) and 83% (dog)] that had good intrinsic potency, high selectivity against DPP-8 and DPP-9, and low ion channel binding (hERG IC_{50} > 90 μM).[45] However, compound **20** showed a lower than anticipated effect in a murine OGTT due mainly to the low oral exposure (as evidenced by AUC_n) in rodents. A combination of the structural features present in **19** and **20** provided a novel series of 4-arylcyclohexylalanine DPP-4 inhibitors of which compound **21** (Fig. 10) was identified as a potent [DPP-4 IC_{50} = 0.0048 μM (0% human serium), 0.13 μM (50% human seri-

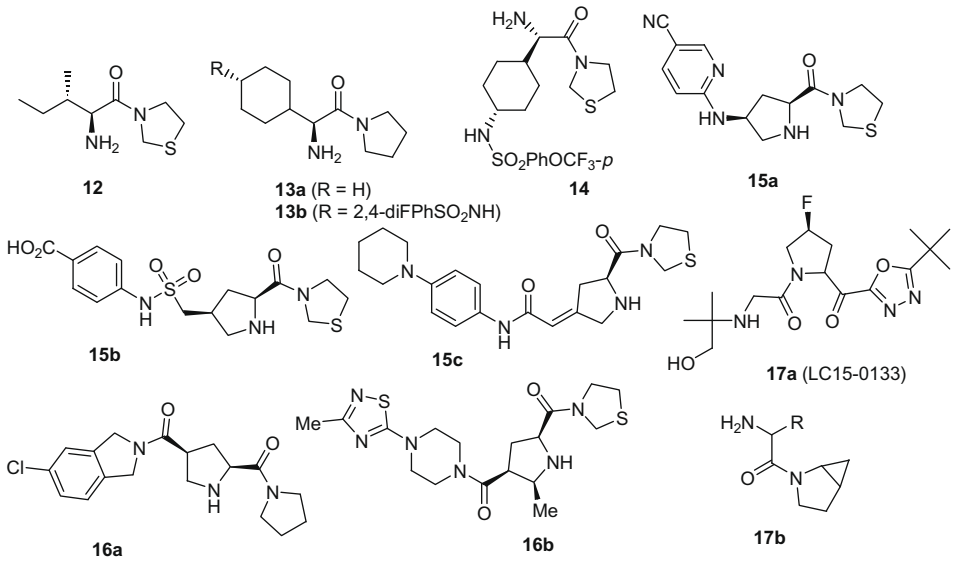


Figure 9. Inhibitors lacking electrophilic nitrile group.

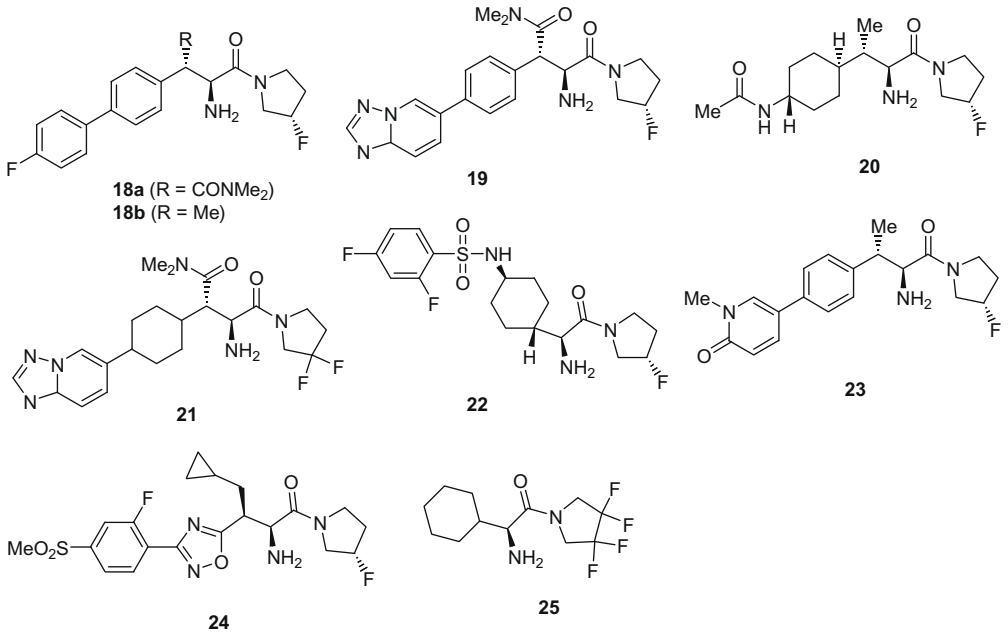


Figure 10. Fluorinated pyrrolidine derivatives.

um); DPP-8 IC_{50} = 30 μM; DPP-9 IC_{50} = 38 μM] and selective inhibitor with improved PK profile across several preclinical species [F = 63% (rat), 100% (dog) and 97% (rhesus)]. It was inactive against the hERG ion channel (IC_{50} > 90 μM).[46] Evaluation of **21** in an OGTT demonstrated that this compound effectively reduced glucose excursion in lean mice (23% reduction in blood glucose @ 0.03 mpk po). X-ray crystal structures of **21** bound to the active site of DPP-4 suggested a hydrogen bonding interaction between the heterocyclic moiety and Arg358.

In an earlier effort to develop 4-amino cyclohexylglycine-based inhibitors of DPP-4 lacking an electrophile, 2,4-difluorobenzenesulfonamide[47] **22** (Fig. 10) (DPP-4 IC_{50} = 48 nM, hERG K_i = 49,000 nM) was found to have good PK properties (F = 55% in rat and 75% in dog) and produced significant activity in an OGTT in lean C57BL6/N mice (42% reduction in the glucose excursion @ 3 mg/kg po). However, further development of **22** was halted due to its significant activity against both DPP-8 (IC_{50} = 993 nM) and DPP-9 (IC_{50} = 2720 nM) and subsequent SAR study did not improve the selectivity.

Replacing the 4-fluorophenyl group in **18b** by polar heterocycles such as methylpyridone resulted in identification of a potent DPP-4 inhibitor **23** (Fig. 10) [IC_{50} = 0.034 μM (DPP-4), 8.0 μM (QPP), >100 μM (DPP-8), >100 μM (DPP-9); F = 34% (rat), 95% (dog) and 50% (rhesus); hERG IC_{50} > 100 μM] with reduced serum shift and a good PK profile in rats, dogs and monkeys.[48] However, the methylpyridone moiety of **23** being metabolically labile, formed the free pyridone ring upon metabolism. Since the corresponding metabolite was also found to be a potent DPP-4 inhibitor [IC_{50} = 0.025 μM (DPP-4), 5.0 μM (QPP), >100 μM (DPP-8), >100 μM (DPP-9); F = 10% (rat); hERG IC_{50} = 8.3 μM] and was active at various ion channels, hence further development of **23** was abandoned.

By replacing the central phenyl group of **18b** with a heterocycle and subsequent SAR work led to the identification of a novel series of oxadiazole-based amides as potent DPP-4 inhibitors.[49] The optimized compound **24** (Fig. 10) [IC_{50} = 0.019 μM (DPP-4), 17.6 μM (QPP), >100 μM (DPP-8), >100 μM (DPP-9)] showed selectivity over a variety of DPP-4 homologs, good PK (F = 36% in rat and 95% in dog) and hERG profile (IC_{50} = 58.5 μM). Notably, as revealed by the X-ray crystal structure, compound **24** had a *syn* relationship between the β-methyl and the primary amine. It is mention worthy that compound **24** bind in a different manner than the phenylalanine derivatives **18**, **19** and **21**. The oxadiazole projects into a different DPP-4 binding pocket relative to those other biaryls described in Figure 10. Further development of compound **24** was not pursued due to the short half-life observed upon oral administration in both rats ($t_{1/2}$ = 1.3 h) and dogs ($t_{1/2}$ = 1.75 h).

Highly fluorinated pyrrolidine derivative of cyclohexylglycine amides were explored as new class of DPP-4 inhibitors.[50] A representative compound tetra-fluoropyrrolidide **25** (Fig. 10) (DPP-4 K_i = 81 nM) showed strong inhibition of DPP-4 and produced significant activity in an OGTT in KK mice (59% reduction in the glucose excursion @ 10 mg/kg po).

9.1.6. Boronic acid/phosphonate derivatives as inhibitors of DPP-4

A boronic acid moiety at the 2-position of the pyrrolidine ring has shown to be effective for the inhibition of DPP-4. A number of boronic acid inhibitors of the type Xaa-boroPro (-boroPro refers to proline in which the C-terminal carboxylate has been replaced by a boronyl group and Xaa represents any unblocked amino acid) has been reported as potent inhibitors of DPP-4, for example, **26** (Fig. 11).[18] While, some of them, for example, Val-boro-Pro (**26a**) inhibited DPP-4 (K_i = 2.0 nM) and QPP (K_i = 125 nM) and many of them underwent a reversible, pH-dependent intramolecular cyclization between the N-terminal amine and the C-terminal boron (the cyclic structure, favoured at high pH, was devoid of inhibitory activity) compound **26b** however, progressed into phase 3 clinical trials.

Another series, that is, *N*-Alkyl Gly-boro-Pro derivatives[51a] were evaluated for DPP inhibitory properties and a representative compound **27a** (Fig. 11) [IC_{50} = 8.0 nM (DPP-4), 46 nM (FAP) and 420 nM (DPP-7)] was found to be a potent but moderately selective inhibitor of DPP-4. More recently, several *N*-acyl-Gly- and *N*-blocked-boroPro compounds showed low nanomolar inhibitory activity against FAP and prolyl oligopeptidase (POP) along with selectivity against DPP-4, for example, **27b** (Fig. 11) [K_i = 68 nM (DPP-4), 146 nM (FAP) and 101 nM (POP)].[51b] A similar compound

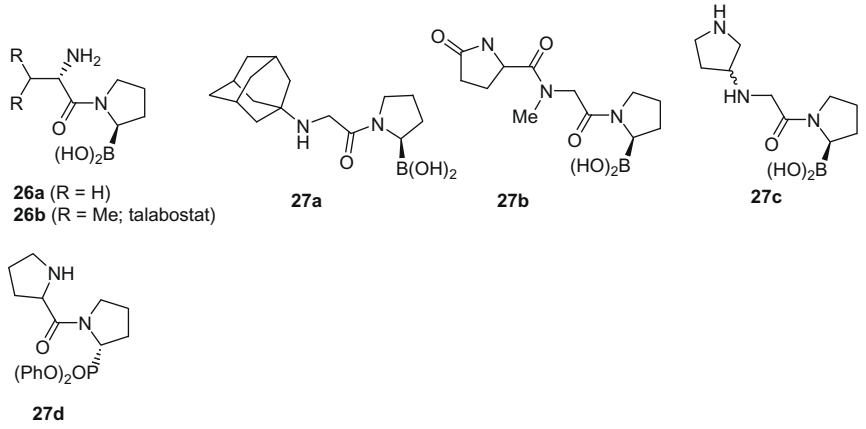


Figure 11. Boronic acid/phosphonate derivatives.

27c (Fig. 11) having a pyrrolidinyl group at the side chain has been reported as DPP-4 inhibitor.[51c] When tested in a number of immunological models, an irreversible inhibitor, that is, Pro-Pro-diphenyl phosphonate **27d** (Fig. 11) along with **26a** showed inhibition of hind paw swelling in collagen- and alkyldiamine-induced models of arthritis in rats.[51d]

9.2. β-Alanine-based inhibitors or β-series

The α-series was mainly developed by using available information and data generated for compounds that were previously described in the literature. In contrast, the β-series as observed in many cases was developed from an initial lead obtained via high-throughput screening (HTS). A number of inhibitors based on β-amino amide backbone has been reported of which the key molecules are presented below.

9.2.1. Pyrrolidine, thiazolidine piperazine and benzothiazole derivatives

Initially, several β-amino acid-based DPP-4 inhibitors were reported (**28a**, Fig. 12) and modifications of the β-aminoamide backbone for the further optimization of these series were undertaken. Substitution of alkyl around the β-aminoamide backbone was found to be detrimental to the potency. Alkyl substitution along with other modifications such as lengthening, shortening, or tethering were also proven to be ineffective in the corresponding thiazolidine[52a] and the piperazine series[52b] (**28a** and **29**; >10-fold less active). Moreover, inhibitors such as **29a** (Fig. 12) (DPP-4 IC_{50} = 139 nM) was reported with high DPP-4 inhibitory potency but poor PK properties, resulting from an extensive metabolism of the heterocycle moiety.[52b] Functionalization at C-2 on the pyrrolidine ring provided a new series of DPP-4 inhibitor.[52c] Compound **28b** (Fig. 12) was found to be potent (IC_{50} = 0.48 nM),

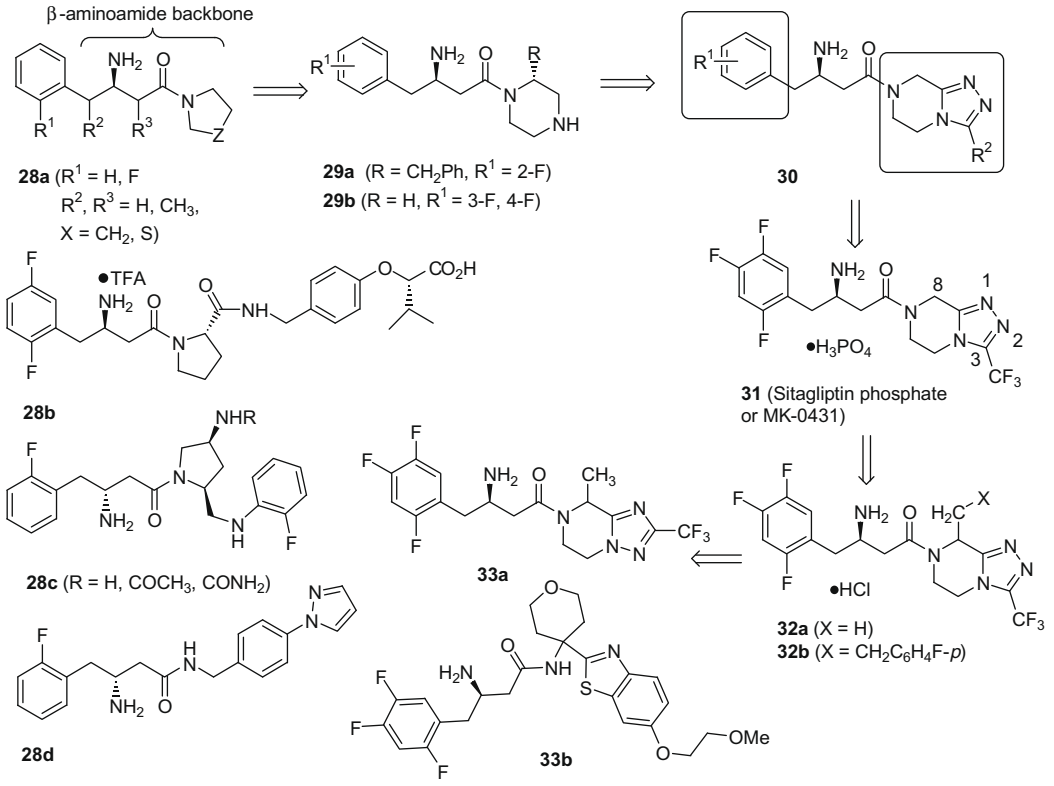


Figure 12. Pyrrolidine, thiazolidine, (triazolo)piperazine and benzothiazole derivatives.

selective [IC_{50} > 100 μM (QPP), >100 μM (DPP-8), >86 μM (DPP-9), 21 μM (FAP)] and safer inhibitor (hERG IC_{50} = 76 μM) in vitro. However, **28b** showed poor PK properties (F = 1.2%) in rat due to the poor absorption thought to be caused by the carboxylic acid moiety. Additional substitution at C-4 in addition to C-2 on the pyrrolidine ring provided another series of DPP-4 inhibitors, for example, **28c** (Fig. 12).[52d] Modifications around the amide group in **28c** provided **28d** (Fig. 12).[52e]

9.2.2. Triazolopiperazine derivatives

To obtain inhibitors having improved metabolic stability and PK properties piperazine moieties of **29** were replaced with metabolically robust heterocycles particularly fused heterocycles. A variety of fused heterocycles was found to be effective in improving metabolic stability and PK properties, in addition to increasing DPP-4 inhibitory potency. Accordingly, a series of β-aminoamides bearing triazolopiperazines was identified as potent, selective, orally active DPP-4 inhibitors (**30**, Fig. 12). Efforts to optimize this β-aminoamide series by using a variety of substituents R^1 and R^2 on the left phenyl and the right triazolopiperazine, respectively, ultimately led to the discovery of **31**,[53] (Fig. 12) [IC_{50} = 18 nM (DPP-4), >100,000 nM (QPP), 48,000 nM (DPP-8) and >100,000 nM (DPP-9), F = 76% in rat, 100% in dog and 68% in monkey]. Compound **31** showed reduction in blood glucose excursion in an OGTT in a dose-dependent manner (23% and 55% reduction @ 0.1 and 3.0 mg/kg in lean mice), good PK (PD) profile in a separate OGTT experiment (by demonstrating correlation between DPP-4 inhibition, increase in GLP-1 levels, and an improvement in glucose tolerance) and acute lowering of blood glucose in diet-induced obese (DIO) mice (near normalization of glucose excursion observed @ 3 mg/kg po). The X-ray crystal structure determination of **31** in complex with the DPP-4 enzyme demonstrated that the amide moiety of compound **31** attained the opposite orientation of that of α-series **A** and the S1 hydrophobic pocket in the DPP-4 enzyme was fully occupied by the 2,4,5-trifluorophenyl moiety. Also, the (*R*)-β-amino group interacted with glutamate residues Glu205 and Glu206 through four hydrogen bonding interactions. Recently, based on the overlay of X-ray structures of compound **19**[44] (α-series) and **31** (β-series) bound to the active site of DPP-4, it was proposed[54] that the fluoropyrrolidine amide moiety of the α-series and the fluorobenzyl group of the β-series occupied the same pocket in the enzyme active site (Fig. 13). In this new orientation, the carbonyl oxygen of **31** formed a hydrogen bond to Tyr547 through a bridging water molecule in the active site. In the complex with compound **19**, the water molecule was displaced by the dimethyl amide moiety which forms a hydrogen bond directly with Tyr547, which was re-oriented to have a better alignment for hydrogen bonding.[44] Compound **31** has been approved by the US Food and Drug Administration (FDA) for the treatment of type 2 diabetes.

Based on significant increase in potency (>20-fold) previously observed with the incorporation of a benzyl substituent into the piperazine moiety [**29a** vs **29b** (DPP-4 IC_{50} = 3700 nM), Fig. 12] SAR work focused on alkyl substitution around the triazolopiperazine moiety (5-, 6- and 8-positions) in **31** was undertaken to provide a series of potent DPP-4 inhibitors beyond sitagliptin.[55] Thus compound **32a** [(*R*)-methyl isomer, Fig. 12] was identified with excellent in vitro potency [IC_{50} = 4.3 nM (DPP-4), >100,000 nM (QPP), 17,000 nM (DPP-8) and >100,000 nM (DPP-9), F = 57% in rat, 91% in dog and 85% in monkey] against DPP-4, high selectivity over other enzymes, and good PK profiles. It showed reduction in blood glucose excursion in an OGTT (34% and 55% reduction @ 0.1 and 1.0 mg/kg in lean mice), improved PD profile (in a separate OGTT experiment) than **31** [in agreement with the improved DPP-4 activity of **32a** compared to **31** in mice (IC_{50} = 11 nM vs 69 nM] and acute lowering of blood glucose in DIO mice (near normalization of glucose excursion observed @ 3 mg/kg po). However, in studies in anesthetized dogs to assess cardiovascular activity, compound **32a** showed unacceptable dose-dependent prolongation of QRS and QTc intervals in the ECG, which precluded its further development. A further modification of the methyl group of **32a** resulted in a series of compounds with subnanomolar activity against DPP-4.[55] The 4-fluorobenzyl-substituted compound **32b** (Fig. 12) was notable for its superior potency [IC_{50} = 0.18 nM (DPP-4), >33,000 nM (QPP), 332 nM (DPP-8) and 20,000 nM (DPP-9), F = 76% in rat, 100% in Zucker$^{fa/fa}$ rats and 6% in mice] but showed poor oral bioavailability in mice. In general, the (*R*)-stereochemistry of the substituent at the 8-position of triazolopiperazines was preferred over (*S*) with respect to DPP-4 inhibition as indicated by the X-ray crystal structure determination of compounds **32a** and **32b** in complex with DPP-4 enzyme. Also, the superior DPP-4 potency of compound **32b** was thought to be due to the additional water molecule-bridged hydrogen bonding interaction between 4-fluorophenyl and Ser630.

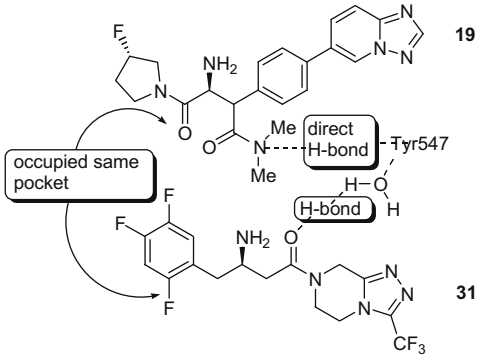


Figure 13. Interactions of compound **19** (α-series) and **31** (β-series) to the active site of DPP-4.

Moving the nitrogen from the 2- to the 3-position in the triazolopiperazine ring of **31** yielded a new family of compounds and subsequent modification at the 2-position as well as the 8-position of the resulting bicyclic ring provided potent, selective and orally active DPP-4 inhibitors.[56a] The compound **33a** [(*R*)-methyl isomer, Fig. 12] showed best overall profile [IC_{50} = 25 nM (DPP-4), 25,000 nM (QPP), >100,000 nM (DPP-8) and >100,000 nM (DPP-9), F = 74% in rat, 67 in dog and 65% in monkey] in this series and reduction in blood glucose excursion in an OGTT (14% and 44% reduction @ 0.1 and 3.0 mg/kg in lean mice). X-ray studies suggested that the CF_3 group and the (*R*)-β-amino group of **33a** participated in a similar type of interactions with the DPP-4 enzyme like **31**. Several water-mediated interactions were present between the nitrogen atoms of the triazolopiperazine and protein atoms. The triazolopiperazine was stacked against the side chain of Phe357. Recently, derivatives incorporating the 6-substituted benzothiazole group have been reported as new series of DPP-4 inhibitors. Compound **33b** (Fig. 12) showed potent DPP-4 inhibitory activity (human DPP-4 IC_{50} = 3.6 nM) and reduced blood glucose excursion in an OGTT.[56b]

9.2.3. Imidazopiperidine/pyrazolidine derivatives

Based on the observation that modification of the piperazine moiety of **29** improved the DPP-4 potency by several folds, an imidazopiperidine analog **34** (Fig. 14) was prepared that showed modest potency (IC_{50} = 0.97 μM).[57] Subsequent SAR work in this series indicated that substitution at the 1- and 3-positions produced increased selectivity for DPP-4 relative to DPP-8 and DPP-9 as exemplified by compound **35** [(*R*)-methyl isomer, Fig. 14], a potent and selective DPP-4 inhibitor [IC_{50} = 0.045 μM (DPP-4), 39 μM (QPP), 58 μM (DPP-8) and >100 μM (DPP-9), F = 40% in rat].[57]

Recently, a series of β-aminoacyl-containing cyclic hydrazine derivatives derived from moderately effective pyrazolidine-based DPP-4 inhibitors[58] **36a** [Fig. 15, DPP-4 IC_{50} = 1.56 μM (rat plasma), ED_{50} = 80 mg/kg (po, 1 h) when R = $C_6H_4NO_2$-*m*] have been described as potent inhibitors of DPP-4.[59a] One member of this series, **37** (Fig. 15) showed potent in vitro activity, good selectivity and in vivo efficacy in mouse models [IC_{50} = 50 nM (DPP-4), 18,190 nM (DPP-2), 89,120 nM (DPP-8) and 48,660 nM (DPP-9)]. Compound **37** blocked 85% and 46% DPP-4-mediated degradation of GLP-1 (analyzed by MALDI-TOF mass spectrometry) over a period of 24 h when used in concentrations of 10 and 1 μM, respectively. Also compound **37** showed 90% inhibition of plasma DPP-4 activity (after 1 h in normal C57BL/6 J mice @ 50 mg/kg po) and reduction in blood glucose excursion in an OGTT (52% and 77% reduction @ 10 and 50 mg/kg in TallyHo mice). The binding mode of compound **37**, as determined by X-ray crystallography indicated that the β-aminoacyl group had a conformation similar to that of **31**. However, the cyclic hydrazine moiety had different binding modes with the carbonyl oxygen of its benzoyl moiety forming water-bridged hydrogen bonding interactions with the side chains of His 126 and Ser 209 and the carbonyl group of Glu205. A series of pyrazoline derivatives with β-amino acyl group have been reported as inhibitors of DPP-4 and the compound **36b** (Fig. 15) was found to be the most active inhibitor (IC_{50} = 0.51 μM).[59b]

9.2.4. 1,4-Diazepan-2-one derivatives

To identify a follow-up compound for **31**, a new series was developed by replacing the triazolopiperazine ring of **31** with various heterocycles. Accordingly, 1,4-diazepan-2-one derivative[60] **38** (Fig. 16) was identified as a potent (DPP-4 IC_{50} = 2.6 nM) and selective inhibitor of DPP-4 [IC_{50} = 2.6 nM (DPP-4), 59,000 nM (QPP) and 28,000 nM (DPP-8), *F* = 36% in rat and 95% in dog] and was efficacious in an OGTT in mice. Compound **38** showed reduction in blood glucose excursion in an OGTT (16% and 46% reduction @ 0.1 and 3.0 mg/kg in lean mice) and no inhibition of glucose excursion in DPP-4-deficient mice (10 mg/kg po) indicating that the reduction observed in wild type mice is a mechanism-based effect. X-ray structure of compound **38** bound to active site of DPP-4 indicated that the basic amine group formed hydrogen bonds with the side chains of Glu205 and Glu206. The C-2 fluorine of aryl group was within hydrogen bonding distance from the side chain of Asn710 and the amide carbonyl interacted with the side-chain hydroxyl of Tyr547 through a water molecule. The seven-membered ring group filled the hydrophobic area above the side chain of Phe357. Compound **38** was selected for extensive preclinical development as a potential back-up candidate to **31**.

A further optimization of **38** with different substitution on the seven-membered ring resulted in several highly potent and selective, orally bioavailable, and efficacious DPP-4 inhibitors. For example, compound **39** (Fig. 16) [IC_{50} = 9.7 nM (DPP-4), 38,000 nM (QPP) and 21,000 nM (DPP-8), *F* = 49% in rat, 77% in dog and 71% in monkey][61] showed reduction in blood glucose excursion in an OGTT (27%, 46% and 49% reduction @ 0.03, 0.1 and 0.3 mg/kg in lean mice) and good correlations among plasma concentration, DPP-4 inhibition, GLP-1 elevation and glucose AUC reduction.

Based on the observation that C-2 fluorine of the benzyl group in **31** occupied the same space as the pyrrolidine amide oxygen in **19** and formed a hydrogen bond to Asn710 (Fig. 13), valerolactam-based DPP-4 inhibitor[54] **40** was designed and synthesized. Compound **40** showed potent and selective inhibition of DPP-4 [IC_{50} = 20 nM (DPP-4) and 41,000 nM (DPP-8)] but poor oral bioavailability in rat.

10. Non-peptidomimetic inhibitors: structural deviation from the traditional series

A number of non-peptidomimetic inhibitors that are structurally different from the traditional α- or β-series have been reported. In most of the cases X-ray crystal studies have shown that in spite of their distinct structural features these inhibitors interacted well with the DPP-4 active site.

10.1. Fused imidazole derivatives

Fused imidazole-based inhibitors were found to be a new chemical class of potent DPP-4 inhibitors for the potential treatment of T2D. The initial lead was derived from the xanthine scaffold. A HTS on xanthine class of compounds led to the discovery of compound **41** (Fig. 17) possessing good inhibitory activity in the low micromolar range (DPP-4 IC_{50} = 3900 nM).[62a] A systematic structural modification on the xanthine scaffold provided compound **42** (*R*-isomer, Fig. 17) [IC_{50} = 1.0 nM (DPP-4) and 295 nM (muscarinic receptor M_1), did not show hERG channel inhibition @ 1.0 μM, *F* = 51% in rat and 50% in cynomolgus monkey], a highly potent, selective (more than 10,000-fold selectivity against DPP-8 and DPP-9), long-acting, and orally active DPP-4 inhibitor that showed considerable blood glucose lowering in different animal spe-

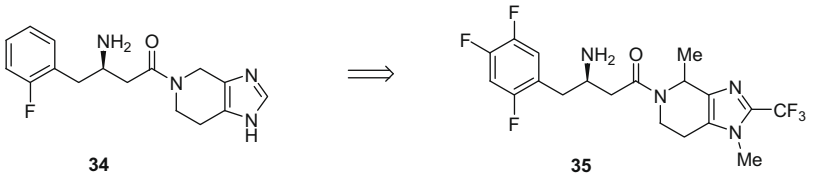


Figure 14. Imidazopiperidine-based inhibitors.

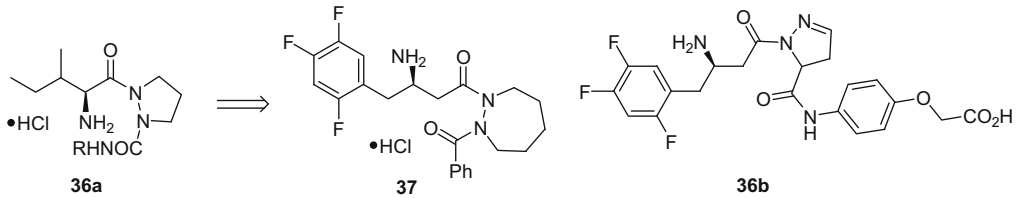


Figure 15. Pyrazolidine and related inhibitors.

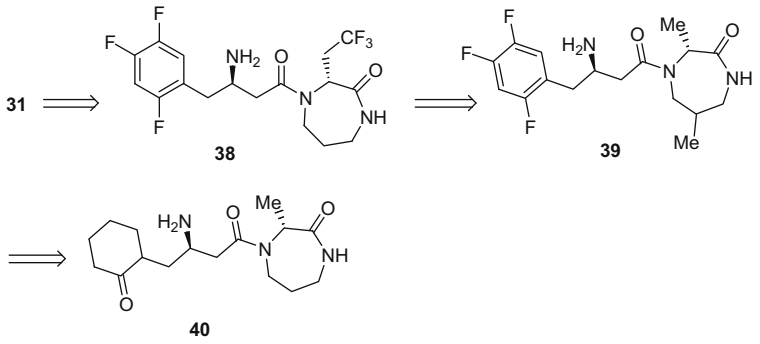


Figure 16. 1,4-Diazepan-2-one-based inhibitors.

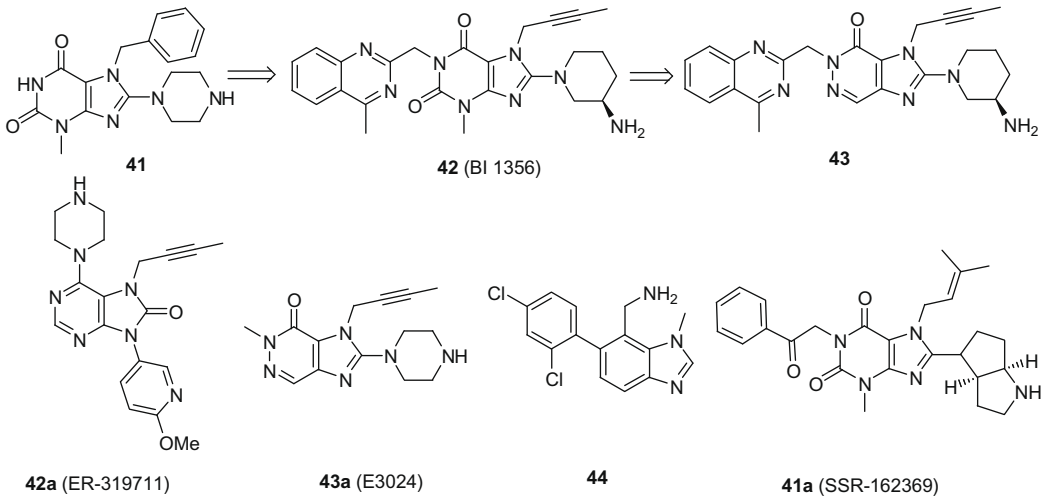


Figure 17. Fused imidazole-based inhibitors.

cies.[62,63] Compound **42** showed >70% inhibition of DPP-4 for >7 h in three species (e.g., male Wistar rats, Beagle dogs and Rhesus monkeys @1 mg/kg po) and reduction in plasma glucose excursion in an OGTT (15% and 66% reduction @ 0.1 and 1.0 mg/kg in db/db mice). It did not show interaction with CYP-450 enzymes up to 50 μM. The X-ray crystal structure of **42** in complex with human DPP-4 indicated that the aminopiperidine substituent at C-8 of the xanthine scaffold occupies the S2 subsite (Fig. 18). Its primary amine formed a network of charge-reinforced hydrogen bonds to Glu205, Glu206 and Tyr662 (amino acid residues that constituted the recognition site for the amino terminus of peptide substrates of DPP-4) and the butynyl substituent at N-7 occupied the hydrophobic S1 pocket of the enzyme. The xanthine moiety was positioned such that its uracil moiety lies on top of Tyr547, forming aromatic π-stacking interactions with the phenol of Tyr547. Thus, the side chain of Tyr547 was pushed from its relaxed position in the uncomplexed enzyme. The C-6 carbonyl function of the xanthine scaffold formed a hydrogen bond to the backbone NH of Tyr631. The quinazoline substituent at N-1 was placed on a hydrophobic surface patch of the protein and interacts with Trp629 by π-stacking its phenyl ring with the pyrrol ring of the amino acid side chain. Compound **42** is currently undergoing phase 3 clinical trials and holds the potential for once-daily treatment of T2D. The trifluoroacetate salt of **42a** (Fig. 17), an analogue of **42** inhibited human DPP-4 (IC_{50} = 0.089 μM) with high selectivity (IC_{50} > 100 μM for human DPP8 and DPP9) and reduced glucose excursion in an OGTT (@ 1 mg/kg in Zucker$^{fa/fa}$ rats), with significant increases in plasma insulin and GLP-1 levels.

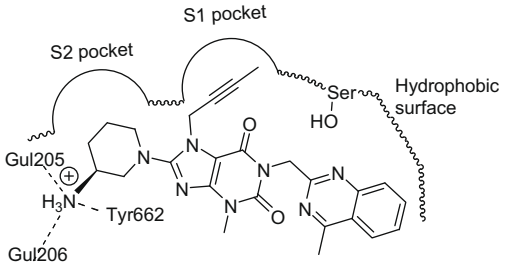


Figure 18. Interactions of compound **43** with the active site of DPP-4.

A further systematic variations of the xanthine scaffold led to the class of 3,5-dihydro-imidazo[4,5-*d*]pyridazin-4-ones which provided a series of potent DPP-4 inhibitors. A representative compound[64] **43** (*R*-isomer, Fig. 17) [IC_{50} = 1.0 nM (DPP-4) and 1190 nM (muscarinic receptor M_1), low affinity to hERG channel inhibition @ 1.0 μM, *F* = 15% in rat], the most promising inhibitor in this class showed 71% DPP-4 inhibition in rats, ex vivo after 24 h (10 mg po) and no interaction with CYP-450 enzymes up to >50 μM. A close analogue **43a** (Fig. 17) was also found to be a selective and competitive inhibitor of DPP-4.

Recently, a series of potent benzimidazole-based inhibitors derived from a weak inhibitor of DPP-4, for example, 2-phenylbenzylamine (DPP-4 IC_{50} = 30 μM) has been reported. The representative compound **44** (Fig. 17) showed selective inhibition of DPP-4 and excellent oral bioavailability[65a] [IC_{50} = 0.062 μM (DPP-4) and

25 μM (DPP-8), F = 100% in rat]. A xanthine-based analogue **41a** (Fig. 17), related to **41** has been reported as a potent inhibitor of DPP-4 (IC_{50} = 18 nM) and is presently in phase 1 trial.[65b]

10.2. Cyclohexylamine/aminopiperidine derivatives

Based on molecular modeling studies and the X-ray crystal structure of **31** bound to DPP-4 the central β-amino butyl amide moiety of **31** was replaced with a cyclohexylamine group to provide a rigid analog **45** (Fig. 19).[66] Compound **45** showed potent inhibition of DPP-4, excellent in vivo activity and PK profile [IC_{50} = 21 nM (DPP-4), >100 nM (QPP), >100 nM (DPP-8) and 21,000 nM (DPP-9), F = 100% in rat, 95% in dog and 94% in monkey]. It showed reduction in blood glucose excursion in an OGTT (26%, 48%, 53%, 60% and 54% reduction @ 0.03, 0.1, 0.3, 1.0 and 3.0 mg/kg in lean male mice). In a separate OGTT, compound **45** (@ 0.1 mg/kg) showed 94% inhibition of plasma DPP-4 activity resulting in a 3.5-fold increase in active GLP-1 levels and full efficacy in glucose reduction.

A further replacement of the central cyclohexylamine in **45** with a 3-aminopiperidine provided potent, selective and orally bioavailable DPP-4 inhibitors.[67a] A representative DPP-4 inhibitor **46a** (Fig. 19) was evaluated for hERG potassium channel, calcium channel, Cyp2D6 and PK properties [IC_{50} = 0.006 μM (DPP-4), 74 μM (QPP), >100 μM (DPP-8), >100 μM (DPP-9), 20.3 μM (hERG), 2.0 μM (Ca), 8.8 (Cyp2D6); F = 100% in rat]. While **46a** showed excellent potency and selectivity for DPP-4 inhibition but needed improvement in potassium channel, calcium channel and Cyp2D6 selectivity. Recently, aminotetrahydropyrans represented by **46b** (Fig. 19) has been reported as inhibitors of DPP-4.[67b] Aminopiperidine-based natural product **46c** (Fig. 19), isolated from culture broth of Streptomyces sp. MK251-43F3 showed potent inhibition of DPP-4.[67c]

In another effort, starting with the HTS hit **47** (Fig. 20) (DPP-4 IC_{50} = 0.8 μM), cyclohexene-constrained phenethylamine **48** (ABT-341) [K_i = 1.3 nM (DPP-4), >30,000 nM (DPP-8) and 4000 nM (DPP-9)] was identified as a candidate for clinical development.[68] The compound showed good PK (F = 67% in rat, 104% in dog and 54% in monkey), reduction in plasma glucose excursion in an OGTT (23%, 37% and 51% reduction @ 0.3, 1.0 and 3.0 mg/kg in female ZDF rats), increase in GLP-1 (151%, 163% and 291% @ 0.3, 1.0 and 3.0 mpk) and decrease in glucagon levels (36%, 46% and 61% @ 0.3, 1.0 and 3.0 mpk). It also showed no inhibition of major liver metabolic enzymes such as CYP3A4, CYP2D6 and CYP2C9 (IC_{50} > 30 μM). It was negative in both mini-Ames and clastogenicity tests (up to 2000 μg/well). Compound **48** exhibited no hERG binding (dofetilide K_i > 50 μM, IC_{50} > 300 μM in patch clamp using HEK 293 cells). In a 5-day study in rats compound **48** caused no toxicological effects when dosed up to 1000 mg/kg/day. The X-ray study indicated that the trifluorophenyl group of **48** occupied the hydrophobic S1 pocket and the amino group on the cyclohexene ring was in close proximity to the side chains of Glu205 and Glu206 for an electrostatic interaction. The carbonyl oxygen of **48** was oriented towards a water molecule positioned for a bridging hydrogen-bonding interaction with the side chain of Arg669. A favourable hydrophobic interaction of the heterocycle with the side chain of Phe357 was observed.

A further SAR work on the same HTS lead (**47**) afforded a series of pyrrolidine-constrained phenethylamines as novel and potent DPP4 inhibitors.[69a] The most promising compound **49a** (Fig. 20) [IC_{50} = 2.1 nM (DPP-4), 4350 nM (DPP-8), 11,100 nM (DPP-9), >30,000 nM (POP), >30,000 nM (FAP_α)] showed good oral bioavailability (F = 74% in rat, 42% in monkey and 100% in dog], insignificant CYP inhibition (IC_{50} > 10 μM for CYP2D6, CYP2C9 and CYP3A4) and reduction in blood glucose excursion in an OGTT (32%, 39% and 37% reduction @ 0.3, 1.0 and 3.0 mg/kg in female ZDF rats).

Expanding the five-membered pyrrolidine ring in **49a** to a six-membered one led to the identification of a series of piperidinone- and piperidine-constrained phenethylamines as novel and potent DPP4 inhibitors.[69b] A representative compound **49b** (Fig. 20) [IC_{50} = 4.0 nM (DPP-4), >30,000 nM (DPP-8), >30,000 nM (DPP-9); F = 90% in rat] was potent, selective and showed excellent PK properties. X-ray crystallographic data showed that halogenated phenyl ring occupied the S1 pocket. The middle piperidine ring acted to orient the exocyclic primary amino group and the appendages off the endocyclic nitrogen atom in the correct directions.

Using 1,3-disubstituted 4-aminopiperidine, for example, **50a** (Fig. 20) (DPP-4 IC_{50} = 600 nM) as a model compound for optimization, a new series of DPP-4 inhibitor based on 2-aminobenzo[*a*]quinolizine was developed.[70] For example, compound **50b** (Fig. 20) was identified as a potent inhibitor of DPP-4 (IC_{50} = 4.6 nM) with insignificant CYP inhibition [IC_{50} = 15 (2C9), 11 (2D6) and > 50 μM (3A4)]. It reduced glucose levels in the OGTT (in *fa/fa* rats) by 41% @ 0.3 mg/kg po.

10.3. Quinazolinone/pyrimidinedione/isoquinolone derivatives

Based on the earlier use of aminopiperidine and cyanobenzyl groups in the development of DPP-4 inhibitors,[71] and by using structure-based design appropriately substituted quinazolinone[72a] **51a** (Fig. 21) was designed, prepared and identified as a potent and selective DPP-4 inhibitor [IC_{50} = 0.013 μM (DPP-4) and >100 μM (DPP-8)]. X-ray studies indicated that the aminopiperidine moiety provided a salt bridge to Glu205/Glu206 while a cyanobenzyl group at *N*-3 occupied the S1 pocket (formed by V656, Y631, Y662, W659, Y666 and V711) and interacted with Arg125. The car-

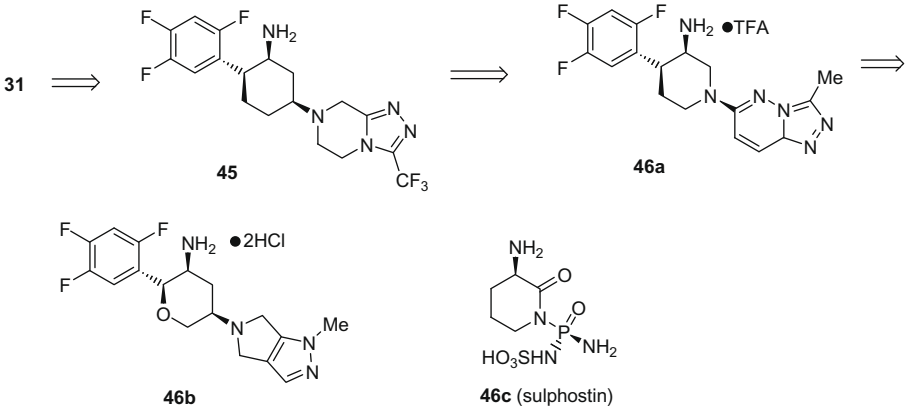


Figure 19. Cyclohexylamine/aminopiperidine-based inhibitors.

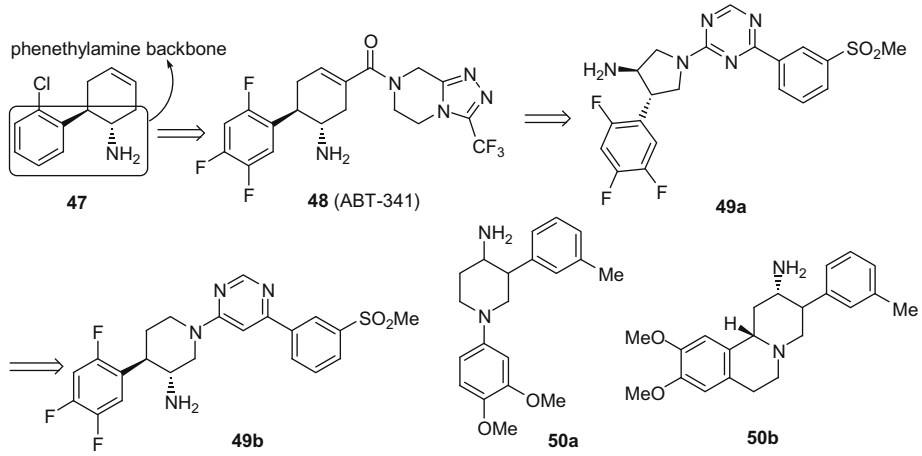


Figure 20. Constrained phenethylamine-based inhibitors.

bonyl group participated in hydrogen bonding to the backbone NH of Tyr631, and the quinazolin moiety was π-stacked with Tyr547. However, due to the short metabolic half-life of **51a** in rat, caused by the metabolism via oxidation at C-5 or C-6 of the fused benzene ring, fluorinated derivative[73] **52** (Fig. 21) was synthesized. Compound **52** showed improved PK (F = 83% in rat), 50% inhibition of DPP-4 activity (after 12 h @ 10 mpk) and reduction in glucose excursion in an OGTT in mice. While favourable safety data were obtained for **52** in an Ames test and a 4-day rat toxicology study, inhibition of CYP450 3A4 (IC_{50} = 2.5 μM) and blocking of the hERG channel at micromolar concentrations precluded its further development. Replacing the quinazolinone with a pyrimidinedione resulted in **53** (Fig. 21), a potent inhibitor of DPP-4 (IC_{50} < 10 nM) that exhibited greater than 10,000-fold selectivity over the closely related serine proteases DPP-8 and DPP-9. In addition to good PK [F = 68% (HCl salt in dog) and 87% (benzoate salt in monkey)] compound **53** also showed dose-dependent improvements in glucose tolerance and increased plasma insulin levels in female Wistar fatty rats. Compound **53** did not inhibit CYP-450 enzymes nor block the hERG channel at concentrations up to 30 μM and was well tolerated in toxicology studies in rat and dog. Presently, **53** is undergoing phase 3 clinical trials. A series of isoquinolone derivatives[72b] as represented by **51b** (Fig. 21) and related compound such as **51c**[72c] (Fig. 21) (DPP-4 IC_{50} = 2.28 nM) have been reported as potent inhibitors of DPP-4.

10.4. Fluoroolefin derivatives

The fluoroolefin derivatives have been explored as DPP-4 inhibitors earlier.[74,75] For example, *Z*-isomer **54** (Fig. 22) was evaluated against DPP-4 (K_i = 7.69 and 6.03 μM for diasteromers) and found to be stable at pH 7.6 under buffered conditions.[74] Based on these observation and replacing the central amide bond in compound **24** by a fluoroolefin moiety a new series of potent and selective DPP-4 inhibitors has been reported.[76] A representative compound **55** (Fig. 22) showed potent and selective inhibition of DPP-4 [IC_{50} = 0.0075 μM (DPP-4), 0.33 μM (QPP) and 19 μM (hERG)] and good PK (F = 62%) in rat. X-ray study revealed that fluoroolefin moiety in **55** behaved as an effective amide bioisostere both geometrically and electronically. However, **55** was found to be less stable in rat liver microsome due to the oxidative metabolism at the cyclopentanyl fluoroolefin moiety. Moreover, due to its high pro-

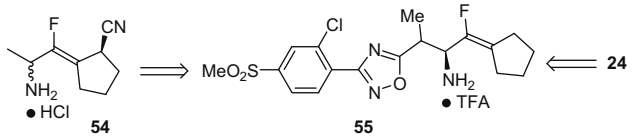


Figure 22. Fluoroolefins as DPP-4 inhibitors.

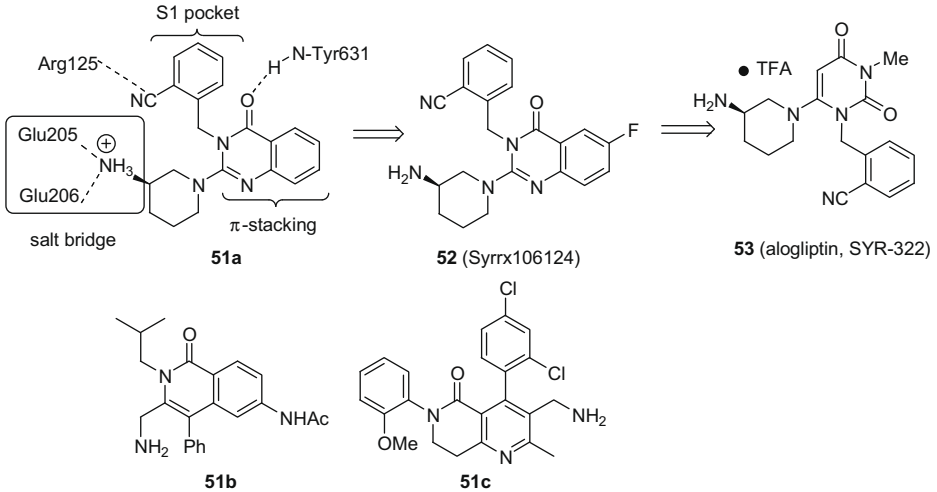


Figure 21. Quinazolinone/pyrimidinedione/isoquinolone-based inhibitors.

pensity to form reactive metabolites that can irreversibly bind to biomolecules, further evaluation of **55** was discontinued.

11. Conclusions: DPP-IV inhibitors are new weapons in the arsenal of oral antihyperglycemic agents

There are nine classes of antihyperglycemic agents including DPP-4 inhibitors are now available for the treatment of T2D. Compound **31** being the first in a new class has been in patient's use since early 2008 either as a monotherapy or in combination with metformin or thiazolidinediones. Combination therapy, as indicated by clinical trails data, would be a preferred option for patients not adequately controlled on monotherapy.[77] Accordingly, pairing **31** with metformin is beneficial because the later drug is used as initial therapy due to the absence of weight gain and hypoglycemia. As a monotherapy, DPP-4 inhibitors with disease-modifying potential offer the advantage of no weight gain and can be an alternative treatment option for patients suffering from metformin (or thiazolidinediones) related side effects such as decreased renal function or intolerable gastrointestinal disturbances.

While the maximum beneficial effects of DPP-4 inhibitors have been demonstrated in patients in the early stage of T2D, several questions however need to be answered before their long-term usage. Data showed that more than 50% of patients would require a second medication to control diabetes after being on monotherapy for three years.[78] Thus, instead of simply delaying the insulin therapy if DPP-4 inhibitors show better control of HbA1c for a longer period of time then their use would be preferred over the currently available drugs. It is still unknown if DPP-4 inhibitors can prevent weight gain in long term. It is also unclear if DPP-4 inhibitors can alter the course of T2D in humans via elevating the GLP-1 level though the long-term effects of GLP-1 in animal studies demonstrated increases in β-cell mass and β-cell efficiency.[79] While improvement in the markers of α- and β-cell function (e.g., glucagon suppression, indices of insulin secretion etc.) have been demonstrated in some of the clinical studies but long-term studies are needed to confirm these findings and delay in disease progression. The effectiveness of DPP-4 inhibitors in patients having diabetes for a longer period (e.g., 10–15 years) with low or no β-cell function need to be assessed via long-term studies and post-marketing evaluation. This is essential because the use of **3a** and **31**, as indicated by a recent study, might lead to the increased risk of infection (e.g., nasopharyngitis and urinary tract infection) and headache due to the potential involvement of DPP-4 in immune functions.

From the viewpoint of drug design, selectivity towards the inhibition of DPP-4 over DPP-8 and -9 was mostly attained through empirical iterative optimization. While homology models of DPP-8 and DPP-9 have been proposed, the molecular basis of the origin of the selectivity remained unclear. Thus, X-ray crystal structures of DPP-8 and -9 would help in the rational design of selective DPP-4 inhibitors with improved safety profiles. Dual inhibition of DPP-4 and neutral endopeptidase 24.11 (NEP) could be another way forward as implicated by the observation that combination of a DPP-4 and NEP inhibitor increased GLP-1 levels. However, beneficial effects of dual inhibition over DPP-4 inhibition alone along with the potential side effects need to be assessed.

With the long journey of drug discovery of DPP-4 inhibitors that have been full of twists and turns, excitements and setbacks, a remarkable progress have been made towards understanding the biology of DPPs and incretin pathways. This has been reflected in the creativity and imagination demonstrated by the researchers in designing recent inhibitors. Until recently, the known inhibitors had all been dipeptidomimetic in nature bearing structural resemblance to the N-terminal dipeptide of the enzyme substrates. However, the use of HTS in combination with rational drug design has resulted in a major shift from the structural features of traditional series and a diverse class of compounds has been generated as potent and selective inhibitors of DPP-4.

Table 4
Status summary of selected DPP-4 inhibitors

Compound	Company	Status
3a[26]	Novartis	FDA approval (delayed)
31[53]	Merck	Launched
5[30]	Bristol-Myers Squibb/ AstraZeneca	Phase 3 (NDA filed in 2008)
53[72]	Takeda San Diego Inc./Syrrx	ANDA (2008)
11b[37b]	Abbot	Phase 3
42[62a]	Boehringer Ingelheim	Phase 3
7f[32f]	GSK	Phase 3 (study suspended)
26b[18]	Point therapeutics	Phase 3 (study on hold)
PHX-1149 (Dutogliptin)[81]	Phenomix Corp.	Phase 3
GRC-8200 (Melogliptin)[82]	Glenmark Pharma	Phase 2
PSN-9301[38b]	(OSI) Prosidion	Phase 2
R 1438[83]	Roche	Phase 2
TA-6666[83]	GSK/Tanabe	Phase 1
TS-021[83]	Taisho/Lilly	Phase 1
SSR-162369[83]	Sanofi-Aventis	Phase 1
S-40755[84]	Servier	Phase 1
SYR-619[83]	Takeda	Phase 1
ALS 2-0426[84]	Alantos Pharma	Phase 1
ARI-2243[85]	Arisaph Pharma	IND

Overall, DPP-4 inhibitors are promising new class of antidiabetics and intense research in this area has resulted in the launch of **31** (a new weapon in the arsenal of oral antihyperglycemic agents) and FDA approval of **3a** (Table 4). The findings from clinical study revealed that patients with T2D on **3a** were able to take lower dosages of insulin and had fewer incidences of hypoglycemia.[80] The side effects and the rate of hypoglycemia in patients taking of **3a** was similar to **31** and placebo respectively and no anticipated dosage adjustment was required for renal insufficiency.[80] The other inhibitors that have advanced into preregistration/phase 3 include **5**, **53**, **11b** and **42** (Table 4). While these inhibitors do not lower the glucose level to a greater extent than existing therapies, they offer many potential advantages, including the ability to achieve sustainable reductions in HbAlc with a well-tolerated agent that has a low risk of hypoglycemia and weight gain. Also, these agents can be administered as a once-daily oral dose. Thus, DPP-4 inhibitors could compete with traditional oral antidiabetic agents, although because of cost, lack of familiarity and no endpoint data, likely they will be used mainly in combination treatment in early years after launch. DPP-4 inhibitors also have potential in the treatment of diseases beyond diabetes such as obesity, intestinal injury and other related diseases.

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