CHAPTER 1

Recent Developments in DPP-IV Inhibitors as Type 2 Anti-diabetic Agents
1.1 Introduction

Diabetes mellitus is one of the old diseases and it is the most common endocrine disorder currently affecting over 170 million people globally and it is projected that in the year 2030, over 365 million will be affected by this disease. Diabetes mellitus is defined as a condition wherein there is chronic increase in the blood glucose levels. Under normal physiological conditions, glucose homeostasis is maintained by the hormone insulin secreted by the pancreas. It is generally believed that in Diabetes body does not either produce or properly use insulin resulting in glucose imbalance and this in turn adversely affects a number of body organs resulting in irreversible damage. During the last several years considerable research has been expended towards understanding etiology, pathophysiology, genetic and environmental risk factors contributing to the onset and progression of the disease. Currently held view is that diabetes is multifactorial but genetic and environmental factors are major causes for onset of the disease.

There are three main kinds of clinically distinct diabetes namely, Type 1, Type 2 and gestational diabetes. Type 1 diabetes also known as juvenile diabetes or insulin-dependent diabetes wherein, the beta cells of the pancreas no longer make insulin because the body's immune system has attacked and destroyed them. Treatment for type 1 diabetes includes taking insulin, regulating diet and maintaining a physically active life style that would enable patient to lead a normal life. Type 2 diabetes or noninsulin-dependent diabetes is the most common form of diabetes. This form of diabetes usually begins with insulin resistance, a condition in which fat, muscle and liver cells do not use insulin properly. At first, the pancreas keeps up with the added demand by producing more insulin. With the progression of the disease, the pancreas loses the ability to secrete insulin in response to meals. During the past two decades, Type 2 diabetes is on the rise primarily because of inter alia sedentary life style. The third kind of disease is less common but occurs in some women during the later stages of pregnancy but usually goes away after the baby is born. However, they are more likely to develop Type 2 diabetes later in life.

The normal control of glucose metabolism requires not only a normal secretion of insulin but also its normal action in its key target tissues including muscle, liver and fat. Most patients with diabetes pass large volumes of urine, experience an increase in the frequency of urination, undue thirst and hunger and
rapid weight loss. They may also feel tired, irritable, lack of concentration at work, proneness to infection, delay in wound healing, intense itching and need for frequent change of eye glasses. These are some of the clinical symptoms of the disease.

1.2 Therapeutic approaches for Type 2 diabetes

Medical nutrition therapy including increased physical activity and reduced food intake is the traditional first line of treatment for Type 2 diabetes followed by the addition of oral anti-diabetes therapies and ultimately exogenous insulin as required. A lot of attempts have been made to get near normal glycemic control and variety of drugs are now available. However, presently available drugs including sulfonylurea, biguanides, thiazolidinediones, drugs modifying the absorption of glucose, insulin (rapid-acting as well as more long-acting) have disadvantages like hypoglycaemia, weight gain and edema. Therefore there is an imperative need for novel therapeutic approaches for glycemic control that can complement existing therapies and possibly attempt to preserve normal physiological response to meal intake.

There is wealth of information on all aspects of diabetes vide supra. This review highlights only the notable recent progress in the area of Type 2 diabetes therapeutics with specific reference to DPP-IV inhibitors. The review is also intended to provide a brief introduction to the central theme of the present investigation and the results documented in subsequent chapters.

1.3 Incretins

For a given rise in plasma glucose, the increase in plasma insulin is approximately threefold greater when glucose is administered orally as compared to intravenous injection. This enhancement of insulin release is known as the “incretin” effect.

Incretins are a group of gastrointestinal hormones that cause an increase in the amount of insulin released from the beta cells of the islets of Langerhans after eating, even before blood glucose levels become elevated. They also slow the rate of absorption of nutrients into the blood stream by reducing gastric emptying and may directly reduce food intake. As expected, they also inhibit glucagon release from the alpha cells of the Islets of Langerhans.

The most important incretin hormones are: glucagon-like peptide-1 (7-36 amide, GLP-1) and glucose-dependent insulino tropic peptide (GIP). They act as potent insulino tropic hormones and up to two-thirds of the insulin normally secreted
in relation to a meal is thought to be the result of actions of these hormones. Current level of understanding on the physiological role and therapeutic application of GIP is rather modest and research efforts are underway. Therefore a conscious effort is made here to focus more on GLP. Both GIP and GLP-1 are rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-IV). The role of these peptides is depicted in Figure 1.

**Figure 1. Schematic representation of DPP-IV modulated incretin action.**

### 1.3.1 Glucagon-like peptide 1

The incretin hormone GLP-1 [GLP-1 (7-36) amide and GLP-1(7-37), two equipotent bioactive forms] is secreted by intestinal L-cells of the distal small intestine in response to food intake. 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. Meal-stimulated GLP-1 secretion appears decreased in human subjects with Type 2 diabetes. The biological effects of GLP-1 comprise not only an effect on insulin-secreting cells but also on other cells of the islets as well as effects on several extra pancreatic sites (Table 1).
**Table 1. Important physiological functions of GLP-1.**

<table>
<thead>
<tr>
<th>Increases</th>
<th>Decreases</th>
</tr>
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<tbody>
<tr>
<td>• Insulin secretion in the presence of elevated glucose levels</td>
<td>• Glucagon secretion</td>
</tr>
<tr>
<td>• Insulin gene expression and biosynthesis</td>
<td>• Body weight</td>
</tr>
<tr>
<td>• Insulin sensitivity</td>
<td>• Gastric emptying</td>
</tr>
<tr>
<td>• Satiety</td>
<td>• β-cell apoptosis</td>
</tr>
<tr>
<td>• β-cell proliferation</td>
<td></td>
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</tbody>
</table>

### 1.3.2 Strategies for clinical applications of GLP-1

GLP-1 degradation in the body implies that the peptide cannot be immediately employed for clinical treatment of Type 2 diabetes. Therefore, a number of strategies have been explored including development of small molecule agonists for the GLP-1 receptor which have been proven very difficult to produce. Several pharmaceutical companies have undertaken large screening programmes utilizing the cloned GLP-1 receptor, but so far with little success. Another strategy is development of DPP-IV resistant analogues which are produced with far greater ease. As DPP-IV is specific for proline or alanine in the penultimate N-terminal position of the substrate peptides, analogues of GLP-1 substituted at this position (where GLP-1 has alanine) are generally resistant to DPP-IV and retain a prolonged insulinotropic activity compared to native GLP-1. There can be development of inhibitors of DPP-IV which have been taken into consideration due to extreme DPP-IV mediated degradation of GLP-1 in patients with Type 2 diabetes. There is another method to cure Type 2 diabetes in a clinical set up and that is continuous subcutaneous infusion of GLP-1. Some of the DPP-IV resistant GLP-1 analogues currently in usage and/or under different stages of investigation are mentioned below.

**Exendin 4 (Exenatide, Byetta)**

Exendin 4 is isolated from the saliva of lizard *Heloderma suspectum* (Gila monster) and shares 53% sequence homology to GLP-1. Here, amino acid at second position is
substituted by Gly and because of this, exendin 4 is resistant to DPP-IV. US Food and Drug Administration (FDA) has approved Exendin 4 for the treatment of Type 2 diabetes and it is marketed under the brand name Exenatide (Figure 2x).

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln
Leu-Trp-Glu-Ile-Phe-Leu-Arg-Val-Ala-Glu-Glu-Met
Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH₂

Figure 2x. Structure of Exendin 4.

**Liraglutide (NN2211) and CJC-1131**

Liraglutide has been formed by the addition of an acyl chain at amino acid 26 to native GLP-1. This modification leads to improved binding to albumin, thereby reducing access to DPP-IV and at the same time allowing the molecule to escape renal filtration. Because of this dual effect, Liraglutide (Figure 2y) has a half-life of longer than 10 hours thus making it truly a long acting drug. Alternatively, same therapeutic effect has been achieved by modification at the C-terminus of GLP-1 and the molecule under investigation is CJC-1131 (Figure 2z).

H-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser
Glu-OH

Figure 2y. Structure of Liraglutide.

His-D-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Tyr-Leu-Glu

Figure 2z. Structure of CJC-1131.
Albugon

Human Genome Sciences has developed a fusion protein between an analogue of GLP-1 and albumin named Albugon. It has been reported to retain the insulinotropic activities of GLP-1 and to delay gastric emptying.

1.4 Dipeptidyl peptidase-IV inhibitors

Serine proteases are a class of enzymes that cleave peptide bonds in proteins and 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. Members of this family include DPP-IV, DPP-II (also known as quiescent cell proline dipeptidase QPP or DPP-VII), DPP-VIII and DPP-IX. It was the discovery of the role of the enzyme DPP-IV in energy homeostasis which accelerated the design of potential therapeutic agents for the treatment of Type 2 diabetes and led to the first patent application for the use of DPP-IV inhibition in the reduction of blood glucose. Therefore inhibition of DPP-IV has been shown to be a new approach for the treatment of Type 2 diabetes.

1.4.1 Structure of DPP-IV

A number of groups have reported X-ray crystal structures of DPP-IV bound to inhibitors from different chemical scaffolds including a peptide inhibitor that closely resembles the natural substrate. Biochemical evidence and crystal structure have led to delineating detailed molecular architecture of the enzyme per se, in complex with inhibitor; domain interactions, mode and mechanism of substrate/inhibitor binding, catalysis and active site architecture. This information has provided an exciting opportunity for structure based drug design. Herein a brief account of the enzyme structure is mentioned from the medicinal chemistry perspective.

The crystal structure of the extracellular domain (residues 39-766) of DPP-IV has been solved to a resolution of 2.1 Å. The functional unit of the enzyme is a homodimer having active site at the interface as shown in Figure 3a. Each monomer unit has at the N-terminal, a unique eight-bladed beta-propeller domain extending from residues 61-495 and at C-terminal, a α/β hydrolase domain corresponding to residues 39-55 and 497-766. The propeller domain packs against the hydrolase domain and the catalytic triad (S630, D708 and H740) is at the interface of the two domains. The presence of two glutamates (E205 and E206) at the end of an α-helical
segment that protrudes from the β-propeller domain into the active site of the enzyme determines the aminopeptidase function of DPP-IV. The Glu motif functions as a recognition site for the N terminus of peptide substrates and anchors the substrate so that only dipeptides can be cleaved off. The crystal structures of the free form of DPP-IV and complexed with the inhibitor suggest that physiological peptide substrates utilize the side opening, unique to DPP-IV, to access the active site.

Other structural features include well-defined hydrophobic S1 and S2 pockets. The hydrophobic S1 groove is shaped to optimally accommodate and interact with a Pro or Ala residue and explains the strong preference of DPP-IV for peptides with these amino acids in the penultimate position. The S2 subsite preferentially recognizes large hydrophobic and aromatic side chains. It is apparent from the crystal structure that catalytic triad, residues surrounding the scissile peptide bond and non conserved residues along the binding pocket are involved in substrate specificity and catalysis.

Figure 3a. Ribbon diagram showing overall structure of the DPP-IV homodimer, viewed perpendicular to the twofold dyad axis. Secondary structural elements that are involved in dimer formation are represented in red and in blue. The α-helix comprising residues E205 and E206 are indicated in gold.

A schematic diagram showing the binding of peptide derivative with catalytic site of DPP-IV including S1 and S2 pocket of the enzyme and covalent interaction with serine residue is shown in Figure 3b.
1.5 SAR Studies of DPP-IV Inhibitors

Structurally two distinctive classes for example, peptidomimetic and non-peptidomimetic DPP-IV inhibitors have been reported (Table 2).

Table 2. Classification of DPP-IV inhibitors.

<table>
<thead>
<tr>
<th>Structural Type</th>
<th>Peptidomimetic</th>
<th>Non-peptidomimetic</th>
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<tbody>
<tr>
<td>Glycine-based or α-series</td>
<td>Pyrrolidine, thiazolidine piperazine &amp; benzothiazole derivatives</td>
<td>Fused imidazole derivatives</td>
</tr>
<tr>
<td>Triazolopiperazine derivatives</td>
<td>2-Cyano pyrrolidine derivatives</td>
<td>Modifications of P-1 pyrrolidine ring</td>
</tr>
<tr>
<td>Imidazopiperidine/Pyrazolidine derivatives</td>
<td>Modifications of P-2 pyrrolidine ring</td>
<td>Pyrrolidine based inhibitors lacking electrophilic nitrile group</td>
</tr>
<tr>
<td>1,4-Diazepan-2-one derivatives</td>
<td>Fluorinated Pyrrolidine amides</td>
<td>Boronic acid/phosphonate derivatives</td>
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In the following section, studies about the above mentioned derivatives and their SAR will be discussed.

1.5.1 The peptidomimetic series

The peptidomimetic class is further subdivided into (a) glycine-based (α-series) and (b) β-alanine-based (β-series) inhibitors (Figure 4x).
Interactions of α- and β-series with the DPP-IV enzyme (Figure 4x) do not follow the same pattern. For example, a pyrrolidine amide moiety though common to both series, does not occupy the same pocket in the enzyme active site as indicated by SAR studies. Based on the X-ray crystal structure of DPP-IV and computer modeling studies on enzyme bound L-Valine pyrrolidine amide two key interactions have been suggested between the enzyme 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 is observed in case of B but the hydrogen bond to the carbonyl oxygen is not well defined. Furthermore it is observed that compounds of the B series bind to the active site of DPP-IV with the amide moiety in the opposite orientation to that of A. The pyrrolidine ring could afford to have small substituents like nitrile or fluorine in case of A whereas bigger groups are well tolerated either at C-2 or C-3 position in case of B. Moreover, replacement of pyrrolidine ring with thiazolidine is 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-IV inhibitory properties.

1.5.2 Glycine-based inhibitors or α-series

It is noteworthy that pyrrolidine derivatives have been widely explored as DPP-IV inhibitors due to DPP-IV’s specificity for substrate having an amino-terminal proline at C-2. Thus, many DPP-IV inhibitors resemble the cleavage product of P2-P1 dipeptidyl substrate A (Figure 4y) where the P-1 site contains a proline mimic.
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Figure 4y. Design of pyrrolidine derivatives as DPP-IV inhibitors.

Generally, potent inhibitors are 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\[ -P(O)(OPh)\_2 \] or O-acylhydroxamic acid\[ \text{CONHOCOR} \] and (b) boronic acid\[ \text{B(OH)}\_2 \]. A boronic acid moiety provides highly potent inhibitors.

1.5.3 2-Cyano pyrrolidine derivatives

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 led to (i) nanomolar inhibition of DPP-IV and (ii) chemical stability adequate for oral administration. This is exemplified by cyclohexylglycine-(2S)-cyanopyrrolidine\[ 1, \text{Figure 5} \], one of the potent, selective and stable (\( K_i \) of 1.4 nM, >1000-fold selectivity over closely related peptidases except DPP-VIII and \( t_{1/2} \) stability >48 hour in aqueous solution at pH 7.4) inhibitors. Noticing that N-methylglycine was recognized in the substrate P-2 site\[ 20 \], prompted researchers to investigate whether structurally more complicated N-substituted glycines would be tolerated 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 (2S)-cyanopyrrololide in the P-1 site\[ 21,22 \]. One compound from this series NVP DPP728 (2a) (IC\(_{50} \) = 22 nM) was profiled as a potent, selective and short acting DPP-IV inhibitor with excellent oral bioavailability and potent antihyperglycemic activity\[ 23 \] but discontinued later due to its side effects.

Replacing 2-cyanopyrrololide moiety in compound 2a by 5-cyano-4,5-dihydropyrazol provided a potent inhibitor 2b (Figure 5) KR-62436 as a clinical development candidate for Type 2 diabetes. In addition, the compound (10 \( \mu \)M) almost completely inhibited DPP-IV-mediated degradation of GLP-1 \textit{in vitro}. KR-62436 inhibited the enzyme in a competitive manner and exhibited selectivity against several proteases including proline-specific proteases. The \textit{in vivo} efficacy of the
compound was examined by using normal C57BL/6J mice and ob/ob mice. Administration of KR-62436 to C57BL/6J mice either orally or subcutaneously resulted in the suppression of plasma DPP-IV activity, increase in intact GLP-1 and insulin levels in plasma. Furthermore, the plasma glucose concentrations during oral glucose tolerance test (OGTT) were reduced upon oral administration of KR-62436. This study demonstrated that KR-62436 could be a good lead compound for further development as a new anti-diabetic agent.

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-IV activity further SAR study was carried out around this class of compounds. As a result, a follow up compound 3a (IC$_{50}$ = 34 nM) was identified as a clinical candidate.$^{24}$ The pharmacological profile of compound 3a suggest that it is a potent, stable, selective DPP-IV inhibitor possessing excellent oral bioavailability and potent antihyperglycemic activity with potential for once-a-day administration. Replacing the 3-hydroxy group of adamantan moiety by other substituents led to the identification of a new series of potent DPP-IV inhibitor exemplified by a representative compound 3b. Development of novel DPP-IV inhibitors via introducing various C-2 substituents on adamantan ring has also been reported.$^{25}$

Figure 5. 2-Cyano pyrrolidine-based DPP-IV inhibitors.
As evident from the structures of compound 2 and compound 3a that both straight chain and cyclic substituents with polar and lipophilic side chains including bulky adamantyl group are well tolerated at P-2 site. A gem-dimethyl substituent on the side chain of P-2 site is also tolerated as represented by compound 4a, a potent DPP-IV inhibitor (IC$_{50}$ = 15 nM) with high selectivity over DPP-VIII and DPP-II.$^{26}$ Compound 4a suppressed the blood glucose elevation after an oral glucose challenge and also inhibited plasma DPP-IV activity. The results show that compound 4a possesses in vitro and in vivo activities comparable to those of NVP-LAF237 (3a), which is in clinical development. Bridging the carbon and nitrogen at P-2 via making a small ring led to compound 4b, that belongs to tetrahydroisoquinoline-3-carbonylcyanopyrrolidine class which has shown potent inhibition of DPP-IV (IC$_{50}$ = 4.0 nM). Evaluation of a series of 2-cyanopyrrolidines having 4-substituted glutamic acids at the P-2 site against DPP-IV, DPP-VIII and DPP-II indicated that analogues having a bulky substituent at the benzylic position inhibited DPP-IV with 30-fold selectivity over both DPP-VIII and DPP-II. The tert-butyl-substituted analogue 4c showed 53-fold selectivity for DPP-IV over DPP-VIII with a moderate potency (DPP-IV IC$_{50}$ = 251 nM).

1.5.4 Modifications of P-1 pyrrolidine ring: towards enhanced stability

One of the issues encountered with the use of 2-cyano pyrrolidine derivatives is poor 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.$^{24}$ (Scheme 1).

![Scheme 1. Intramolecular cyclization of 2-cyano pyrrolidine derivatives.](image)
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, installation of a cyclopropyl moiety at either the 3,4- or 4,5-position of traditional 2-cyanopyrrolidide proline mimetics led to compounds with potent inhibitory activity against the enzyme. Additionally, cis-4,5 methanoprolinenitriles with β-branching in the N-terminal amino acid provided enhanced chemical stability and high inhibitory potency for compound 5a \((K_i = 4 \text{ nM})\) and compound 5b \((K_i = 71 \text{ nM})\) (Figure 6a) respectively. This class of inhibitors also exhibited the ability to suppress prandial glucose elevations after an oral glucose challenge in male Zucker rats.\(^{27}\)

![Figure 6a. cis-4,5 methanoprolinenitriles.](image)

In the course of further studies exploring SAR around β-quaternary cycloalkylglycine-based inhibitors, potent activity and extended duration of action was observed in ex vivo DPP-IV inhibition studies with compound 5c, which contains a (vinylcyclopentyl)glycine amino acid fragment. However, metabolism and pharmacokinetic studies with 5c revealed uncharacteristically poor oral bioavailability where related small alkyl substituted analogues did not. Efforts to define the role of suspected metabolites resulted in the synthesis of several hydroxymethylcycloalkyl-based analogues that maintained \textit{in vitro} and for some, \textit{in vivo} activity. These hydroxymethyl analogues also displayed favorable pharmacokinetic properties with a tighter correlation of pharmacokinetics to pharmacodynamics. Analogously, hydroxylation of a similarly disposed adamantylglycine-based inhibitor yielded a compound 5d with \textit{in vivo} potency and duration of action superior to other compounds in this series. Consequently, this compound was chosen for development and is currently under clinical investigation for the treatment of Type 2 diabetes. The basis for the enhanced efficacy observed for compound 5d in animal models relative to other agents may be due to contributions from multiple factors, including exquisite
enzyme inhibitory potency and compound distribution to the tissue compartment potentially critical for maximal antihyperglycemic effects.\textsuperscript{28}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6b.png}
\caption{C-4, C-5 modifications of P-1 pyrrolidine ring.}
\end{figure}

Compound 5d also showed robust glucose-lowering effects in a dose-dependent manner in the Zucker fa/\textit{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). Another compound 6 (Figure 6b) obtained via linking the 4-cis substituted L-prolines in the P-2 position with 4,5-methano-pyrrolidine at P-1 site was found to be a potent inhibitor of DPP-IV (\(K_i = 3.6 \text{ nM}\)).\textsuperscript{29} SAR studies in this diprolyl nitrile series demonstrated that the inhibitory activity is greatly enhanced in the five-membered ring cyclic amino acid (proline) relative to either the four membered 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. Most importantly, the 4-position of the P-2 proline can comfortably accommodate cis substituents and aromatic groups in this region significantly enhancing DPP-IV 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-thiovalylpyrrolidine inhibitors of DPP-IV.\textsuperscript{30}
Chapter 1 Recent Developments in DPP-IV Inhibitors

The most promising compound 7a showed good inhibitory properties. It had a longer duration of action in both rat and dog and its pharmacokinetic properties were better as was its stability toward intramolecular cyclization. Addition of the gem-dimethyl group significantly added to the stability of compound 7a toward cyclization as the compound lacking this moiety had a $t_{1/2}$ cyclization of 80 hour. Notably, enhanced DPP-IV inhibitory properties of (4S)-fluoro derivative of 2-cyano pyrrolidine and its higher concentrations in plasma after oral administration to rats have been reported earlier. Compound 7b (Figure 6b) (DPP-IV $IC_{50} = 0.6$ nM) showed good plasma drug concentrations and significant effects on plasma glucose, plasma insulin and plasma DPP-IV activity. 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 compound 7c, a potent and stable DPP-IV inhibitor ($IC_{50} = 4.6$ nM) with a long-term persistent plasma drug concentration and a potent antihyperglycemic activity.31

Modification of the side chain of compound 7c yielded another series of DPP-IV inhibitor as exemplified by compound 7d. A new series of potent DPP-IV inhibitors was identified by introducing an additional fluoro group at the C-4 position of chiral 2-cyano-4-fluoro pyrrolidine moiety32 as represented by compound 7e. Modification of the side chain of compound 7b yielded a series of potent DPP-IV inhibitor and the best compound 7f ($IC_{50} = 22$ nM) progressed into phase 3 clinical trials. Very recently, pharmacological profile of compound 7g with $IC_{50} = 2.6$ nM (mice), 7.3 nM (dog), and 6.2 nM (human) has been reported. Incorporating conformationally restricted N-(aryl or heteroaryl)-3-azabicyclo[3.1.0] hexane moiety in 2-cyanopyrrolidine class provided a new series of DPP-IV inhibitors represented by compound 7h. The most potent compounds displayed off-target activity against DPP-II, DPP-VIII or DPP-IX which precluded further profiling.

1.5.5 Modifications of P-2 pyrrolidine ring: towards enhanced
Potency

The observation that modification at C-4 position of the P-2 pyrrolidine yielded potent inhibitors was exemplified by a new series, that is, 1-(γ-substituted prolyl)-(S)-2-cyanopyrrolidines33 that were designed based on the predicted binding mode of the known DPP-IV inhibitor 2a. SAR study indicated that compounds bearing (S)-stereochemistry at C-4 position of the P-2 pyrrolidine were 20-fold more potent than
the other antipode. Two compounds that showed the highest inhibitory activity are compound 8a with DPP-IV inhibition IC$_{50}$ = 0.13 and 0.17 nM/L in human and rat respectively whereas compound 8b exhibited DPP-IV inhibition IC$_{50}$ = 0.13 and 0.15 nM/L in human and rat respectively (Figure 7). Incorporation of an aryl group at C-4 position of the pyrrolidine ring led to the identification of series of (4-substituted prolyl)prolinenitriles. Among those tested, the 4β-[4-(hydroxyphenyl) prolyl]prolinenitriles showed a potent inhibitory activity with a long duration of action. A representative compound 8c showed DPP-IV inhibition in vitro having IC$_{50}$ = 4.9 nM.

Despite its poor bioavailability compound 8c showed a dose-dependent 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 (Figure 5) a series of substituted pyrrolidine-2,4-dicarboxylic acid amides were designed and synthesized, many of which showed good in vitro DPP-IV inhibition (IC$_{50}$ = 2-250 nM) with selectivity over DPP-II and DPP-VIII enzymes.$^{34}$ Compounds 8d (Figure 7) showed 10-fold improvement in the in vitro DPP-IV inhibition than compound 3a and an in vivo plasma DPP-IV inhibition.
Modification at the C-5 position of the P-2 pyrrolidine also afforded potent inhibitors of DPP-IV. Thus a series of (5-substituted pyrrolidinyl-2-carbonyl)-2-cyanopyrrolidine analogues were evaluated. The C5-substituents make various interactions with the enzyme and affect potency, chemical stability, selectivity and PK properties of the inhibitors. Optimized analogues are extremely potent with subnanomolar $K_i$'s and are chemically stable, show very little potency decrease in the presence of plasma and exhibit more than 1,000-fold selectivity against related peptidases. The best compounds also possess good PK and are efficacious in lowering blood glucose in an oral glucose tolerance test in Zucker diabetic fatty (ZDF) rats. The lead compound 9 ($K_i = 3.1$ nM) exhibited good PK and was efficacious in lowering blood glucose in an OGTT in ZDF rats (30% reduction of glucose excursion at 3.0 mg/kg).

Recently, cis-3-amino-4-(2-cyanopyrrolidide)-pyrrolidines have been shown to be a unique scaffold for the development of potent inhibitors but unexpected plasma instability led to the cessation of work with this series of DPP-IV inhibitors. The lead compound 10 showed excellent DPP-IV inhibitory potency (DPP-IV IC$_{50}$ = 1.3 nM), selectivity, good oral bioavailability in rats and robust maximal DPP-IV inhibition but its unexpected instability in human plasma and blood precluded further development of this molecule.

Further modification led to an extremely novel series of cis-2,5-dicyanopyrrolidine $\alpha$-amino amides. The lead compound 1-({[1-(Hydroxymethyl)cyclopentyl]amino}-acetyl)pyrrolidine-2,5-cis-dicarbonitrile (11a, Figure 8) (DPP-IV IC$_{50}$ = 104 nM) was an achiral, slow-binding inhibitor of DPP-IV that is selective for DPP-IV over other DPP isozymes and proline specific serine proteases, with good oral bioavailability. The mode of binding of the cis-2,5-dicyanopyrrolidine moiety was determined by X-ray crystallography and determined to involve a covalent interaction of S630 with one nitrile group, while the other nitrile forces the Y547 side chain to move and subsequently makes a $\Pi$-stacking interaction and H-bond with Y666. The secondary amine is recognized by E205, E206, N710 and Y662. The covalent nature of the interaction with S630 was indicated by $^{13}$C NMR. Preliminary safety assessments with 11a 5-day toxicity study in male and female Sprague–Dawley rats at 500 mg/kg for 5 days and a single-dose safety study in dog at 20 mg/kg i.v. route indicated no safety liability. The hydrochloride salt of 11a was
subjected to further profiling for development as a potential new treatment for Type 2 diabetes.

Further modification led to an extremely potent clinical candidate, ABT-279, that is orally available, efficacious and well tolerated in preclinical safety pharmacology and toxicology studies. In 4-week studies in rats and dogs, the 'no observed adverse effect level' for compound 11b was greater than 1000 mg/kg/day.\(^{37}\)

### 1.5.6 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 (12) (DPP-IV IC\(_{50} = 420\) nM) and (S)-cyclohexylglycine pyrrolidide\(^{19}\)\(^{13a}\) (DPP-IV IC\(_{50} = 320\) nM) (Figure 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 inhibitor of DPP-IV was advanced into phase 2 clinical trials.\(^{38}\) A further modification of compound 13a led to novel series of potent and selective DPP-IV inhibitors. These are among the most potent compounds reported to date lacking an electrophilic trap. Notable among these is bis-sulfonamide 14b, which is a DPP-IV inhibitor IC\(_{50} = 2.6\) nM with an exceptional selectivity profile.\(^{39}\) This compound however, lacks oral bioavailability in the rat. Sulfonamides 14a and amide 14c both exhibit good pharmacokinetic properties in the rat. Sulfonamide 14a was selected for assessment in the dog where it had an excellent pharmacokinetic profile and oral bioavailability. This compound was evaluated in vivo for glucose lowering potential. Thus, a dose of 3.0 mg/Kg given 1 hour prior to an oral glucose tolerance test (OGTT) in lean
C57BL6/N mice resulted in a 36% reduction in glucose levels when compared to vehicle.

Other inhibitors lacking the electrophilic nitrile group include [(S)-\(\gamma\)-(arylamino)-L-prolyl]thiazolidine derivatives containing a 4\(\beta\)-(amino)-L-prolyl moiety which are the thiazolidine analogs of the previously reported [(S)-\(\gamma\)-(arylamino)prolyl]-(S)-2-cyanopyrrolidine compounds 8a and 8b (Figure 7). One of the representative compound 15a in this series showed a longer duration of plasma DPP-IV inhibition, high oral bioavailability and long half-life in plasma (t\(_{1/2}\) = 5.27 hour). Further optimization to (S)-\(\gamma\)-3,4-dicyanophenylaminosubstituted compound 15b showed the better potency. The SAR of the \(\gamma\)-substituent in the proline moiety of the thiazolidide was similar to that obtained in the (S)-2-cyanopyrrolidide. The \(\gamma\)-substituent in both the (S)-cyanopyrrolidide and the thiazolidide may engage with the S2 binding pocket of DPP-IV and thereby achieve hydrophobic interaction in the
same manner. In case of substituted 2-(pyrrolidine-1-carbonyl)-pyrrolidine-4-carboxylic acid amides the most potent compound 16a showed a maximum plasma DPP-IV inhibition around 30 min and good stability in aqueous media. However, no data is available related to the reduction of plasma glucose by compound 16a during the OGTT in normal rats.

To further explore chemically stable and potent DPP-IV inhibitors derived from 1-(4-substituted prolyl)-(S)-2-cyanopyrrolidine with a long duration of action, a series of 1-[(4β-substituted)-L-prolyl]pyrrolidine analogs lacking the electrophilic nitrile function were evaluated. Structural optimization of a N-(3-phenyl-1,2,4-thiadiazol-5-yl)piperazine analog 16b, which was found by high-speed analog synthesis was carried out to improve the potency and duration of action. Detailed SAR studies of compound 16b DPP-IV IC$_{50}$ = 20 nM, which had 1-N-(sulfur-containing hetero-aryl)piperazin-4-ylcarbonyl as the 4β-substituent of the L-prolyl moiety resulted in the discovery of another structurally new inhibitor 16c lacking an electrophilic nitrile group. The high affinity of compound 16c for DPP-IV was considered to be due to the affinity of the sulfur-containing hetero-aromatic moiety. The compound 16c showed potent, long-lasting ex vivo activity and it demonstrated dose-dependent reduction of the plasma glucose level after the OGTT in normal rats. The compound 17 derived from compound 7c but devoid of nitrile group has been reported as a novel and potent inhibitor of DPP-IV.

1.5.7 Fluorinated pyrrolidine amides

The 3-fluoropyrrolidine analogues obtained by optimization of anti-substituted  $\beta$-polar substituents of biarylphenylalanine derived DPP-IV inhibitors led to the discovery of the potency and selectivity enhancing dimethylamide group at the  $\beta$ -position (Figure 10). Further investigation into pyrrolidine right-hand side replacements and biaryl left-hand side substituents led to the discovery of a series of highly potent and selective phenylalanine derived DPP-IV inhibitors. Since compound 18a exhibits the best balance of potency, selectivity and good oral bioavailability, this compound was profiled further. Compound 18a possesses an excellent pharmacokinetic profile in three species and excellent in vivo efficacy in a lean mouse OGTT. Similarly, other phenylalanine derivatives 18b was identified as potent and selective inhibitor of DPP-IV. A culmination of work with phenylalanine derived DPP-IV inhibitors has led to the discovery of compound 19 obtained by replacing the
4-fluorophenyl group of compound 18 by a heterocyclic moiety. Compound 19 (2S,3S)-3-amino-4-(3,3-difluoropyrrolidin-1-yl)-N,N-dimethyl-4-oxo-2-(4-[1,2,4]triazolo[1,5-a]pyridin-6-ylphenyl)butanamide is a potent DPP-IV inhibitor with good oral bioavailability but it was not selected for further profiling due to some side effects.

![Chemical structures](image)

**Figure 10.** Fluorinated pyrrolidine derivatives.

The phenylalanine series afforded compounds which were potent and selective but exhibited limited oral bioavailability, therefore appropriate modifications provided compound 20 by replacing the phenyl ring of compound 18 by a cyclohexyl that had good intrinsic potency and high selectivity. However, compound 20 showed a lower than anticipated effect in a murine OGTT due mainly to the low oral exposure.

Hybridization of compounds 19 and 20 provided a novel series of 4 arylcyclohexylalanine DPP-IV inhibitors that effectively incorporated the desirable properties of each. One member of this new class, compound 21 was potent (DPP-IV IC\textsubscript{50} = 0.0048 μM) and selective inhibitor with improved PK profile across several preclinical species, had improved selectivity and pharmacokinetic profile. Compound 21 showed good efficacy in a murine OGTT experiment. In an earlier effort to develop 4-amino cyclohexylglycine-based inhibitors of DPP-IV lacking an
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electrophile, 2,4-difluorobenzenesulfonamide\textsuperscript{46} compound 22 (DPP-IV \textit{IC}_{50} = 48 \text{nM}) was found to have good PK properties and produced significant activity. However, further development of compound 22 was halted due to its significant activity against both DPP-VIII and DPP-IX and subsequent SAR study did not improve the selectivity.

Replacing the 4-fluorophenyl group in compound 18b by polar heterocycles such as methylpyridone resulted in identification of compound 23 (DPP-IV \textit{IC}_{50} = 0.034 \mu M), with good PK profile in rats, dogs and monkeys\textsuperscript{47} but \textit{in vitro} and \textit{in vivo} metabolism studies revealed that methylpyridone moiety of 23 was metabolically labile and hence further development of compound 23 was abandoned.

By replacing the central phenyl group of compound 18b with a heterocycle and subsequent SAR work led to the identification of a novel series of oxadiazole-based amides as potent DPP-IV inhibitors.\textsuperscript{48} The optimized compound 24 exhibited excellent selectivity over a variety of DPP-IV homologs. Compound 24 displayed moderate oral bioavailability in rat and excellent oral bioavailability in dogs. It is noteworthy that compound 24 binds in a different manner than the phenylalanine derivatives 18, 19 and 21. The oxadiazole projects into a different binding pocket relative to those of other biaryls described in Figure 10. Unfortunately, compound 24 exhibited a short half-life in both rats (\textit{t}_{1/2} = 1.3 \text{ hour}) and dogs (\textit{t}_{1/2} = 1.75 \text{ hour}) and so further development of compound 24 was not pursued. Highly fluorinated pyrrolidine derivative of cyclohexylglycine amides were explored as new class of DPP-IV inhibitors.\textsuperscript{49} A representative compound tetra-fluoropyrrolidide (compound 25) (DPP-IV \textit{K}_{i} = 81 \text{nM}) showed strong inhibition of DPP-IV and produced significant glucose lowering activity.

\textbf{1.5.8 Boronic acid/phosphonate derivatives}

A boronic acid moiety at the 2-position of the pyrrolidine ring has been shown to be effective for the inhibition of DPP-IV. 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-IV, for example compound 26 (Figure 11).\textsuperscript{18}
While some of them, for example, Val-boro-Pro (26a) inhibited DPP-IV ($K_i = 2.0 \text{ nM}$) 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 were evaluated for DPP-IV inhibitory properties and a representative compound 27a (DPP-IV $IC_{50} = 8.0 \text{ nM}$) was found to be a potent but moderately selective inhibitor of DPP-IV. More recently, several N-acyl-Gly- and N-blocked-boroPro compounds showed selectivity against DPP-IV, for example, compound 27b ($K_i = 68 \text{ nM}$). A similar compound 27c having a pyrrolidinyl group at the side chain has been reported as DPP-IV inhibitor.

1.6 β-Alanine-based inhibitors or β-series

The β-peptide, oligomers of β-amino acids have particular appeal in the understanding of protein structure and stablisation. They are chemically stable and resistant to α-peptidases. β-peptides are also finding increasing utility for the development of novel bioactive peptides including amphiphilic amino acid toxins and antibiotics etc. It may be noted that the β-amino acids and β-peptides are very well tolerated in the human system as evident from the recently released β-amino acids derived blockbuster drug for the treatment of Type 2 diabetes namely Sitagliptin (Januvia,
Figure 12) from Merck co. USA. 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 have been reported of which the key molecules are presented below.

1.6.1 Pyrrolidine, thiazolidine, piperazine and benzothiazole derivatives

Initially, several β-amino acid-based DPP-IV inhibitors were reported and modifications of the β-amino amide backbone for the further optimization of these series were undertaken (Figure 12).
Substitution of alkyl around the β-aminoamide backbone was found to be detrimental to the potency. Functionalization at C-2 on the pyrrolidine ring provided a new series of DPP-IV inhibitor.\textsuperscript{56} Compound 28b was found to be potent (IC\textsubscript{50} = 0.48 nM), selective and safer inhibitor \textit{in vitro}. However, 28b showed poor PK properties 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-IV inhibitors, for example, compound 28c.\textsuperscript{57} Modifications around the amide group in compound 28c provided compound 28d.\textsuperscript{58} Introduction of a fluorine at the 2-position of the phenyl ring proved to be crucial for the potency of these compounds. The 2,5-difluoro and 2,4,5-trifluoro analogues of compound 28a i.e. where R\textsuperscript{1}= 2-F,5-F and R\textsuperscript{1}= 2-F, 4-F ,5-F ; R\textsuperscript{2} and R\textsuperscript{3}=H and X= S were the most potent DPP-IV inhibitors in this series, exhibiting DPP-IV IC\textsubscript{50}=270 and 119nM, respectively. However the alkyl substitution along with other modifications such as lengthening, shortening or tethering were also proven to be ineffective in the corresponding thiazolidine and piperazine series (>10-fold less active). Significantly, truncation of the right-hand side of the piperazine analogs provided low molecular weight compound 29, which maintained good \textit{in vitro} potency (DPP-IV IC\textsubscript{50}=19 nM) and selectivity but showed poor PK properties.

1.6.2 Triazolopiperazine derivatives

To obtain inhibitors having improved metabolic stability and PK properties, piperazine moiety of compound 29 was replaced with metabolically robust heterocycles particularly fused heterocycles (Figure 12). A variety of fused heterocycles was found to be effective in improving metabolic stability and PK properties, in addition to increasing DPP-IV inhibitory potency. A series of β-aminoamides bearing triazolopiperazines were prepared and evaluated as potent, selective and orally active DPP-IV inhibitors. It was demonstrated that β-aminoamides in conjunction with triazolopiperazines with the appropriate substitution provide extremely potent DPP-IV inhibitors showing high selectivity over other related enzymes, good pharmacokinetic profiles and high \textit{in vivo} efficacy in an OGTT in lean mice and ultimately led to the discovery of compound 31 (DPP-IV IC\textsubscript{50} = 18 nM). It showed reduction in blood glucose levels in an OGTT in a dose-dependent manner, good PK profile in a separate OGTT experiment by demonstrating correlation between DPP-IV inhibition, increase in GLP-1 levels and an improvement.
in glucose tolerance and acute lowering of blood glucose. The X-ray crystal structure determination of compound 31 in complex with the DPP-IV 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-IV 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 (α-series) and compound 31 (β-series) bound to the active site of DPP-IV, it was proposed 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 (Figure 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. 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 compound 29, (Figure12) SAR work focused on alkyl substitution around the triazolopiperazine moiety (5-, 6- and 8-positions) in compound 31 was undertaken to provide a series of potent DPP-IV inhibitors beyond sitagliptin. Fine tuning of relative inhibitory properties against DPP-IV and DPP-VIII by variations of substituents (R¹, R², and R³) around the

Figure 13. Interactions of compound 19 (α-series) and compound 31 (β-series) to the active site of DPP-IV and different substituents on compound 31.
triazolopiperazine moiety provided a series of potent DPP-IV inhibitors suitable for further studies.

Among three substituents, \(R^3\) with \((R)\)-stereochemistry at the 8-position was of critical importance for the superior potency and more effective than \(R^1\) at the 5-position. \(R^2\) at the 6-position was least effective. Compound 32a, (Figure 12) (DPP-IV \(IC_{50}= 4.3\) nM), showing a 4-fold increase in DPP-IV activity over compound 31, exhibited pronounced \textit{in vivo} efficacy in the lean mice OGTT. Although compound 32a had an excellent DPP-IV potency, high selectivity, good pharmacokinetic profile and \textit{in vivo} efficacy in hand, unfortunately compound 32a showed unacceptable heart problems which precluded further development of compound 32a. Further refinement of the triazolopiperazines resulted in the discovery of a series of extremely potent compounds with subnanomolar activity against DPP-IV as represented by 4-fluorobenzyl-substituted compound 32b (DPP-IV \(IC_{50} = 0.18\) nM), which is notable for its superior potency but showed poor oral bioavailability in mice.

X-ray crystal structure determination of compounds 32a and 32b in complex with DPP-IV enzyme revealed that \((R)\)-stereochemistry at the 8-position of triazolopiperazines is strongly preferred over \((S)\) with respect to DPP-IV inhibition. Also, the superior DPP-IV potency of compound 32b was thought to be due to the additional water molecule-bridged hydrogen bonding interaction between 4-fluorophenyl and Ser630. Through variation of the hetcroyclic moiety found in sitagliptin by exchanging the 2-position nitrogen with the 3-position substituent, a novel series of potent triazolopiperazine-based DPP-IV inhibitors have been discovered.62 These triazolopiperazine analogs are potent, selective DPP-IV inhibitors and exhibit an excellent oral bioavailability and good overall pharmacokinetic profile. Introduction of alkyl, heteroaryl and benzyl substitution at the \(\alpha\) position resulted in a pronounced boost in DPP-IV potency. Compound 33a, the \((R)\)-diastereomer, exhibited greater DPP-IV potency than its \((S)\) counterpart (DPP-IV \(IC_{50} = 25\) nM) in this series. X-ray studies suggested that the CF3 group and the \((R)\)-p-amino group of compound 33a participated in a similar type of interactions with the DPP-IV enzyme like in the case of compound 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. As in sitagliptin, the fused heterocycle moiety of this series played a positive key role in DPP-IV potency,
selectivity and pharmacokinetics however, further development of compound 33a was discontinued due to unacceptable side effects.

Recently, the novel series of 3-amino-N-(4-aryl-1,1-dioxothian-4-yl)butanamides and 3-amino-N-(4 aryltetrahydropyran-4-yl)butanamides exhibited profound DPP-IV inhibitory effects. The compounds having a 6-substituted-2-benzothiazolyl group were the most potent. Oral administration of the compound 33b (Figure 12) showed potent DPP-IV inhibitory activity (human DPP-IV IC\textsubscript{50} = 3.6 nM) and it also reduced the blood glucose excursion in an OGTT.

1.6.3 Imidazopiperidine/pyrazolidine derivatives

Based on the observation that modification of the piperazine moiety in compound 29 improved the DPP-IV potency by several folds, substituted imidazopiperidine amides have been prepared and evaluated as DPP-IV inhibitors. Substitution at the 1- and 3-positions produced increased selectivity for DPP-IV relative to DPP-VIII and DPP-IX, although improved potency against DPP-IV was not observed. Introduction of a substituent at the 4-position of the imidazopiperidine unit significantly improved DPP-IV inhibition, resulting in compound 34 (Figure 14, DPP-IV IC\textsubscript{50} = 5.8 nM).\textsuperscript{63}

\[\text{Figure 14. Imidazopiperidine-based inhibitor.}\]

Recently, a series of β-aminoacyl-containing cyclic hydrazine derivatives derived from moderately effective pyrazolidine-based DPP-IV inhibitors\textsuperscript{64} have been reported as shown in compound 35 with DPP-IV inhibition (IC\textsubscript{50} = 1.56 μM) when R = C\textsubscript{6}H\textsubscript{4}NO\textsubscript{2}-m. Further modifications in this series led to compound 37, which was found to be competitive inhibitors of DPP-IV and displayed excellent selectivity over related isozymes.\textsuperscript{65}

\[\text{Figure 15. Pyrazolidine and related inhibitors.}\]
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The in vivo efficacy of compound 37 was demonstrated by its inhibition of plasma DPP-IV activity and its suppression of blood glucose elevation. The cyclic hydrazine derivatives evaluated in this effort are members of an interesting chemical class that possess two nitrogens suitable for diverse derivatization. A series of pyrazoline derivatives with β-amino acyl group have been reported as inhibitors of DPP-IV and the compound 36 was found to be the most active inhibitor (IC₅₀ = 0.51 μM).66

1.6.4 1,4-Diazepan-2-one derivatives

In an effort to identify compounds better than sitagliptin, a new series was developed by replacing the triazolopiperazine ring of sitagliptin with various heterocycles. Accordingly, 1, 4-diazepan-2-one derivative67 i.e. compound 38 (Figure 16) was identified as a competitive, highly selective and reversible inhibitor (DPP-IV IC₅₀ = 2.6 nM). In an oral glucose tolerance test (OGTT) in lean mice, this compound significantly reduced blood glucose levels in a dose-dependent manner. In DPP-IV-deficient mice, no inhibition of glucose excursion was observed following its administration indicating that the reduction observed in wild type mice is a mechanism-based effect.

![Figure 16. 1,4-Diazepan-2-one-based inhibitors.](image)

This compound was selected for extensive preclinical development as a potential back-up candidate to sitagliptin. Further optimization studies with different substitution on the seven-membered ring resulted in several highly potent and selective, orally bioavailable and efficacious DPP-IV inhibitors. For example, compound 39 had shown good efficacy after oral dosing in rodents at dose levels correlated well to its intrinsic potency.68

1.7 Non-peptide inhibitors

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-IV active site.

### 1.7.1 Fused imidazole derivatives

A new chemical class of highly potent DPP-IV inhibitors structurally based on the xanthine scaffold has been discovered. A detailed structure activity study on this class of compounds led to the discovery of compound 41 (Figure 17) possessing good inhibitory activity in the low micromolar range (DPP-IV IC\textsubscript{50} = 3900 nM).\textsuperscript{69}

![Figure 17. Fused imidazole-based inhibitors.](image)

Further optimization led to compound 42 bearing a quinazolin-2-ylmethyl at N-1 and the 3-aminopiperidine attached to C-8 which has proved to be a crucial constituent for high inhibitory activity. It is a highly potent, selective (more than 10,000-fold selectivity against DPP-VIII and DPP-IX), long-acting and orally active DPP-IV inhibitor that showed considerable blood glucose lowering in different animal species. Compound 42 showed >70% inhibition of DPP-IV and reduction in plasma glucose excursion in an OGTT. The X-ray crystal structure of 42 in complex with human DPP-IV indicated that the aminopiperidine substituent at C-8 of the xanthine scaffold occupies the S2 subsite (Figure 18). It represents a highly potent, selective and long-acting DPP-IV inhibitor of a novel chemotype that shows promise for once-daily treatment of Type 2 diabetic patients. Compound 42 is currently undergoing phase 3 clinical trials.
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The trifluoroacetate salt of compound 42a (Figure 17), an analogue of 42 inhibited human DPP-IV (IC$_{50}$ = 0.089 μM) with high selectivity and reduced glucose excursion in an OGTT with significant increase in plasma insulin and GLP-1 levels. A further systematic variations of the xanthine scaffold led to 3, 5-dihydro-imidazo[4,5-d]pyridazin-4-ones which provided a series of potent DPP-IV inhibitors. A representative compound 43 (DPP-IV IC$_{50}$ = 1.0 nM) is the most promising inhibitor in this class. A close analogue 43a was also found to be a selective and competitive inhibitor of DPP-IV. Recently, a series of potent benzimidazole-based inhibitors derived from a weak inhibitor of DPP-IV, for example, 2-phenylbenzylamine (DPP-IV IC$_{50}$ = 30 μM) has been reported. The representative compound 44 showed selective inhibition of DPP-IV and excellent oral bioavailability (DPP-IV IC$_{50}$ = 0.062 μM). A xanthine-based analogue 41a related to 41 has been reported as a potent inhibitor DPP-IV (IC$_{50}$ = 18 nM) and is presently undergoing phase 1 clinical trials.

1.7.2 Cyclohexylamine/aminopiperidine derivatives

Molecular modeling was used to design a rigid analog of sitagliptin compound 31. The X-ray crystal structure of sitagliptin bound to DPP-IV suggested that the central β-amino butyl amide moiety could be replaced with a cyclohexylamine group. This was confirmed by structural analysis and the resulting analog 45 was synthesized and found to be a potent DPP-IV inhibitor (DPP-IV IC$_{50}$ = 21 nM) with excellent in vivo activity and pharmacokinetic profile. A further replacement of the central cyclohexylamine in compound 45 with a 3-aminopiperidine provided potent, selective and orally bioavailable DPP-IV inhibitors. A representative DPP-IV inhibitor 46a showed excellent potency and selectivity for DPP-IV inhibition but needed improvement in potassium channel, calcium channel and Cyp2D6 selectivity. Recently, aminotetrahydropyrans represented by 46b (Figure 19) have been reported.
as inhibitors of DPP-IV. Aminopiperidine-based fermentation product 46c isolated from culturebroth of Streptomyces sp. MK251-43F3 showed potent inhibition of DPP-IV.

![Figure 19. Cyclohexylamine/aminopiperidine-based inhibitors.](image)

In another effort, based on the lead compound 47 a cyclohexene-constrained phenethylamine analogue compound 48 (ABT-341) (Figure 20, DPP-IV $K_i = 1.3$ nM) was identified as a candidate for clinical development.\textsuperscript{74}

![Figure 20. Constrained phenethylamine-based inhibitors.](image)

The compound 48 showed good PK, reduction in plasma glucose excursion in an OGTT, increase in GLP-1 and decrease in glucagon levels. It also showed no inhibition of major liver metabolic enzymes. 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 compound 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. Further optimization studies provided several compounds with attractive profiles. Optimized compounds were very potent DPP-IV inhibitors with a low degree of protein binding and high selectivity against DPP-IV homologs. The most promising compound 49a showed good oral bioavailability and reduction in blood glucose excursion in an OGTT.

Expanding the five-membered pyrrolidine ring in this compound to a six membered piperidine led to the identification of a series of piperidinone and piperidine-constrained phenethylamines as novel and potent DPP-IV inhibitors. A representative compound 49b turned out to be potent, selective with 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, compound 50a as a model for optimization, led to a novel series of 4-aminopiperidine DPP-IV inhibitors. An aromatic residue with a small lipophilic group in meta-position yielded the most active inhibitors. The developed SAR and the synthetic procedures could be applied to the 2-aminobenzo[a]quinazoline series, leading to highly active DPP-IV inhibitors, with trans 50b as the best compound (DPP-IV IC<sub>50</sub> = 4.6 nM).

**1.7.3 Quinazolinone/pyrimidinedione/isoquinolone derivatives**

Based on the earlier use of aminopiperidine and cyanobenzyl groups in the development of DPP-IV inhibitors and by using structure-based design, appropriately substituted quinazolinone compound 51a (Figure 21) was designed, prepared and identified as a potent and selective DPP-IV inhibitor (DPP-IV IC<sub>50</sub> = 0.013 μM).
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 and interacted with Arg125. The carbonyl group participated in hydrogen bonding to the backbone NH of Tyr631 and the quinazolin moiety was pie-stacked with Tyr547. Compound 51a suffers from a short metabolic half-life in the rat, making in vivo assessments difficult. To address this problem, fluorinated derivative compound 52 was synthesized. This compound showed a 10-fold improvement in metabolic stability in the rat.\textsuperscript{78} Compound 52 showed improved PK, 50\% inhibition of DPP-IV activity however, compound 52 was found to have some serious side effects which precluded its further development. In an effort to improve upon the activity of this compound, two strategies were adopted. The first relied on modifications to the quinazolinone substituents. The second relied on replacing the quinazolinone with other heterocycles. It was the second strategy that yielded compounds that were chosen for further development. Interestingly, it was found that the phenyl ring of the quinazolinone could be eliminated without loss of DPP-IV inhibition.

Replacing the quinazolinone with a pyrimidinedione resulted in compound 53 (Figure 21) which is a potent inhibitor of DPP-IV ($IC_{50} < 10 \text{ nM}$) and exhibits greater than 10,000 fold selectivity over the closely related serine proteases DPP-VIII and DPP-IX. Compound 53 also produced dose-dependent improvements in glucose tolerance and increased plasma insulin levels. Compound 53 was profiled in a safety
pharmacology screen with very favorable results. Based on the data presented above, this was selected for preclinical evaluation. Following scale-up, GLP toxicology studies in rat and dog demonstrated the compound to be well tolerated. In phase 1 human trials, this compound demonstrated human PK-PD suitable for once daily dosing and it has now progressed to phase III testing. A series of isoquinolone derivatives as represented by compounds 51b and 51c have been reported as potent inhibitors of DPP-IV with IC$_{50}$ = 2.28 nM.

1.7.4 Fluoroolefin derivatives

Novel, potent inhibitors of dipeptidyl peptidase IV containing the fluoroolefin peptide isostere \( \Psi[\text{CF=C}] \), were found to inhibit dipeptidyl peptidase IV competitively.\textsuperscript{79,80} Z-\textsuperscript{+}isomer compound 54 (Figure 22) was evaluated against DPP-IV \( (K_i = 7.69 \text{ and } 6.03 \mu\text{M for diasteromers}) \) and found to be stable at pH 7.6 under buffered conditions.\textsuperscript{79} 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-IV inhibitors has been reported. A representative compound 55 showed potent and selective inhibition of DPP-IV and good PK in rat.

\[
\begin{align*}
\text{NH}_2 & \quad \text{MeO}_2\text{S} \quad \text{N} = \text{O} \\
\text{HCl} & \quad \text{F} \quad \text{C} \quad \text{F} \quad \text{N}=\text{O} \\
\text{NH}_2 & \quad \text{TFA}
\end{align*}
\]

\[ \text{54} \quad \text{55} \]

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fluoroolefins.png}
\caption{Fluoroolefins as DPP-IV inhibitors.}
\end{figure}

Although, X-ray study revealed that fluoroolefin moiety in compound 55 behaved as an effective amide bioisostere both geometrically and electronically it 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 propensity to form reactive metabolites that can irreversibly bind to biomolecules, further evaluation of compound 55 was discontinued.
Table 3. Status summary of selected DPP-IV inhibitors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Company</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Novartis</td>
<td>FDA approval (delayed)</td>
</tr>
<tr>
<td>31&lt;sup&gt;59&lt;/sup&gt;</td>
<td>Merck</td>
<td>Launched</td>
</tr>
<tr>
<td>5d&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Bristol-Myers Squibb/AstraZeneca</td>
<td>Phase 3 (NDA filed in 2008)</td>
</tr>
<tr>
<td>53&lt;sup&gt;77&lt;/sup&gt;</td>
<td>Takeda San Diego Inc./Syrrx</td>
<td>ANDA (2008)</td>
</tr>
<tr>
<td>11b&lt;sup&gt;43&lt;/sup&gt;</td>
<td>Abbot</td>
<td>Phase 3</td>
</tr>
<tr>
<td>42&lt;sup&gt;69&lt;/sup&gt;</td>
<td>Boehringer Ingelheim</td>
<td>Phase 3</td>
</tr>
<tr>
<td>71&lt;sup&gt;82&lt;/sup&gt;</td>
<td>GSK</td>
<td>Phase 3 (study suspended)</td>
</tr>
<tr>
<td>PHX-114&lt;sup&gt;48&lt;/sup&gt; (Dutogliptin)&lt;sup&gt;83&lt;/sup&gt;</td>
<td>Phenomix Corp.</td>
<td>Phase 3</td>
</tr>
<tr>
<td>GRC-8200 (Melogliptin)&lt;sup&gt;84&lt;/sup&gt;</td>
<td>Glenmark Pharma</td>
<td>Phase 2</td>
</tr>
<tr>
<td>PSN-9301&lt;sup&gt;38&lt;/sup&gt;</td>
<td>(OSI) Prosidion</td>
<td>Phase 2</td>
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<tr>
<td>R 1438&lt;sup&gt;85&lt;/sup&gt;</td>
<td>Roche</td>
<td>Phase 2</td>
</tr>
<tr>
<td>TA-6666&lt;sup&gt;85&lt;/sup&gt;</td>
<td>GSK/Tanabe</td>
<td>Phase 1</td>
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<tr>
<td>TS-021&lt;sup&gt;85&lt;/sup&gt;</td>
<td>Taisho/Lilly</td>
<td>Phase 1</td>
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<tr>
<td>SSR-162369&lt;sup&gt;85&lt;/sup&gt;</td>
<td>Sanofi-Aventis</td>
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<td>S-4075&lt;sup&gt;86&lt;/sup&gt;</td>
<td>Servier</td>
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<td>ALS 2-0426&lt;sup&gt;86&lt;/sup&gt;</td>
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<td>ARI-2243&lt;sup&gt;87&lt;/sup&gt;</td>
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1.8 Conclusions

It is apparent from the foregoing discussions that despite many challenges DPP-IV inhibitors have emerged as promising new class of antidiabetics (Table 3) and intense research in this area has resulted in the launch of thirty one new chemical entities that are in different stages of clinical trials. Design of DPP-IV inhibitors offers excellent opportunities for medicinal chemists to explore new chemical scaffolds and/or optimize existing chemical entities with improved therapeutic profile.

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Chapter 1 Recent Developments in DPP-IV Inhibitors


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