# Chapter 1  

## Background Overview

**Table of Contents**

BACKGROUND OVERVIEW ........................................................................................................ 2

1.1 Introduction to diabetes ................................................................................................. 2

1.1.1 Current therapeutic agents ...................................................................................... 5

1.1.2 Lacking of available therapies ............................................................................... 7

1.1.3 Incretin concept ..................................................................................................... 8

1.1.4 DPP-4 and structure homologues ......................................................................... 12

1.1.5 DPP-4 as novel target for T2DM .......................................................................... 15

1.2 Drug design and molecular modeling ......................................................................... 18

1.2.1 Types of drug designing ....................................................................................... 19

1.2.2 Drug design Techniques ......................................................................................... 21

1.2.3 Drug design protocol for DPP-4 inhibitors .......................................................... 25

1.3 Review of Literature ................................................................................................... 26

1.3.1 Classification of DPP-4 inhibitors ........................................................................ 26

1.3.2. Other DPP inhibitors ......................................................................................... 47

1.3.3. Clinical progress ................................................................................................. 52

1.4. Critical issue .............................................................................................................. 57

1.5 References .................................................................................................................... 58
BACKGROUND OVERVIEW

1.1 Introduction to diabetes

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 [1]. The World Health Organization recognizes mainly two distinct clinical forms of diabetes, for example, type 1 and 2 [2]. 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. 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 [3,4]. Out of this, another type of diabetes is Gestational diabetes, which appears during pregnancy, can lead to serious health risks to the mother and her infant and increase the risk for developing type 2 diabetes later in life [5].

Type 1 diabetes is caused by an autoimmune reaction, where the body’s defense system attacks the insulin-producing beta cells in the pancreas. As a result, the body can no longer produce the insulin it needs. Why this occurs is not fully understood. The disease can affect people of any age, but usually occurs in children or young adults. People with this form of diabetes need insulin every day in order to control the levels of glucose in their blood. Without insulin, a person with type 1 diabetes will die [6, 7].

T2DM presents a major challenge to health care systems around the world which is characterized by both insulin resistance and Islet dysfunction, resulting in a decline in insulin secretion and increased blood glucose levels [8]. Endogenous insulin replacement is eventually required to avoid the complications associated with poor glycemic control. Islet dysfunction is characterized by reduced glucose sensitivity in both α and β cells; causing and imbalance in insulin and glucagon secretion, respectively. Glucose insensitivity in the β cell causes a reduction in meal-induced insulin secretion and in the first phase insulin response to a glucose challenge, by an elevated proinsulin: insulin ratio, and by an abnormal secretion of islet amyloid polypeptide [9].
Thus the important contributing factors for type 2 diabetes (T2D) include [10]

(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.

Although the reasons for developing type 2 diabetes are still not known, there are several important risk factors [11]. These include:

1. Obesity
2. Poor diet
3. Physical inactivity
4. Advancing age
5. Family history of diabetes
6. Ethnicity
7. High blood glucose during pregnancy affecting the unborn child

People with diabetes have an increased risk of developing a number of serious health problems. Consistently high blood glucose levels can lead to serious diseases affecting the heart and blood vessels, eyes, kidneys, nerves and teeth. In addition, people with diabetes also have a higher risk of developing infections. In almost all high-income countries, diabetes is a leading cause of cardiovascular disease, blindness, kidney failure, and lower limb amputation [12].

Global facts

T2DM is a global epidemic with an estimated worldwide prevalence of 12% (415 million people) in 2015, and forecast to rise to ~642 million diabetics by 2040 beside this 46.5% of adults with diabetes are undiagnosed. A further 318 million adults are estimated to have impaired glucose tolerance, which puts them at high risk of developing the disease. There are 320.5 million people of working age (20-64 years) with diabetes and 94.2 million people aged 65-79 with diabetes. In high-income countries, approximately 87% to 91% of all people with diabetes are estimated to have type 2 diabetes, 7% to 12% are estimated to have type 1 diabetes and 1% to
Chapter 1

Background Overview

3% to have other types of diabetes. About 75% live in low- and middle income countries. If these trends continue, by 2040 some 642 million people, or one adult in ten, will have diabetes. The largest increases will take place in the regions where economies are moving from low-income to middle-income levels (Table 1.1).

There is little gender difference in the global number of people with diabetes for 2015 or 2040. There are about 15.6 million more men than women with diabetes (215.2 million men vs 199.5 million women). This difference is expected to decrease to about 15.1 million more men than women (328.4 million men vs 313.3 million women) by 2040. Currently there are more people with diabetes in urban (269.7 million) than in rural (145.1 million) areas. In low- and middle-income countries, the number of people with diabetes in urban areas is 186.2 million while 126.7 million live in rural areas. By 2040, globally the difference is expected to widen, with 477.9 million people living in urban areas and 163.9 million in rural areas. The total global health expenditure on diabetes, though these three countries are home to only 35.1% of people with diabetes. India is home to the second largest number of adults living with diabetes worldwide, after China. People with diabetes in India, Bangladesh, and Sri Lanka make up 99.0% of the region’s total adult diabetes population [13].

Table 1.1 Top countries/Territory for number of people with diabetes (20-79 years), 2015 and 2040

<table>
<thead>
<tr>
<th>Country/Territory</th>
<th>2015 (millions)</th>
<th>2040 (millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>109.6</td>
<td>150.7</td>
</tr>
<tr>
<td>India</td>
<td>69.2</td>
<td>123.5</td>
</tr>
<tr>
<td>United states of America</td>
<td>29.3</td>
<td>35.1</td>
</tr>
<tr>
<td>Brazil</td>
<td>14.3</td>
<td>23.3</td>
</tr>
<tr>
<td>Russian Federation</td>
<td>12.1</td>
<td>12.4</td>
</tr>
<tr>
<td>Mexico</td>
<td>11.5</td>
<td>20.6</td>
</tr>
<tr>
<td>Indonesia</td>
<td>10.0</td>
<td>16.2</td>
</tr>
<tr>
<td>Egypt</td>
<td>7.8</td>
<td>15.1</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>7.1</td>
<td>13.6</td>
</tr>
</tbody>
</table>
1.1.1 Current therapeutic agents

Oral hypoglycaemic drugs (OHD) are considered only after a regimen of dietary treatment combined with exercise has failed to achieve the therapy targets set. Anti-diabetic medications treat diabetes mellitus by lowering glucose levels in the blood. With the exceptions of insulin, exenatide, and pramlintide, all are administered orally and are thus also called oral hypoglycemic agents or oral antihyperglycemic agents. There are different classes of anti-diabetic drugs, and their selection depends on the nature of the diabetes, age and situation of the person, as well as other factors (Table 1.2) [14].

Diabetes mellitus type 1 is a disease caused by the lack of insulin. Insulin must be used in Type I, which must be injected. Diabetes mellitus type 2 is a disease of insulin resistance by cells. Treatments include

- Agents which increase the amount of insulin secreted by the pancreas,
- Agents which increase the sensitivity of target organs to insulin, and
- Agents which decrease the rate at which glucose is absorbed from the gastrointestinal tract.

Although much effort has been made to delay the natural progression of T2DM, it remains inadequately controlled in most parts of the world. To achieve glucose control and prevent diabetic complications, currently receive therapeutic agents such as glucosidase inhibitors, sulfonylureas, metformin, thiazolidinediones (TZDs) GLP-1 mimetics, DPP-4 inhibitors and insulin injections [15]. Several groups of drugs, mostly given by mouth, are effective in Type II, often in combination.

Sulfonylureas were the first widely used oral anti-hyperglycaemic medications. They are insulin secretagogues, triggering insulin release by inhibiting the KATP channel of the pancreatic beta cells. Sulfonylureas bind strongly to plasma proteins. Sulfonylureas are only useful in Type II diabetes, as they work by stimulating endogenous release of insulin. Biguanides reduce hepatic glucose output and increase uptake of glucose by the periphery, including skeletal muscle. Although it must be used with caution in patients with impaired liver or kidney function, metformin, a biguanide, has become the most commonly used agent for type 2 diabetes in children and teenagers. α-glucosidase inhibitors are "diabetes pills" but not technically hypoglycemic agents because they do not have a direct effect on insulin secretion or
sensitivity. These agents slow the digestion of starch in the small intestine, so that glucose from the starch of a meal enters the bloodstream more slowly, and can be matched more effectively by an impaired insulin response or sensitivity. Thiazolidinediones (TZDs), also known as "glitazones," bind to PPARγ, a type of nuclear regulatory protein involved in transcription of genes regulating glucose and fat metabolism. These PPARs act on peroxysome proliferator responsive elements (PPRE). The PPREs influence insulin sensitive genes, which enhance production of mRNAs of insulin-dependent enzymes. The final result is better use of glucose by the cells [16].

Insulin is most commonly used when adequate glycaemic control can no longer be achieved with oral agents alone. As type 2 diabetes is a progressive disorder, with loss of beta cell function occurring over time, insulin is often needed to achieve good glycaemic control, and should be considered for all patients on maximum oral therapy whose HbA1c is > 6.5% [17].

Table 1.2: Available Therapeutic agent for clinical diabetes management released since 1990

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Drug class</th>
<th>Drugs</th>
<th>Brand name</th>
<th>Discovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sulfonylureas/Secretagogues</td>
<td>Glipizide</td>
<td>Glucotrol XL</td>
<td>1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glimepiride</td>
<td>Amaryl</td>
<td>1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Repaglinide</td>
<td>Prandin</td>
<td>1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nateglinde</td>
<td>Starlix</td>
<td>2000</td>
</tr>
<tr>
<td>2.</td>
<td>Bigunides</td>
<td>Metformin</td>
<td>Glcophage</td>
<td>1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metformin/glyburide</td>
<td>Glucovarance</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rosiglitazone/metformin</td>
<td>Avandamet</td>
<td>2002</td>
</tr>
<tr>
<td>3.</td>
<td>Alpha-glucosidase inhibitors</td>
<td>Acarbose</td>
<td>Precose</td>
<td>1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Miglitol</td>
<td>Glyset</td>
<td>1999</td>
</tr>
<tr>
<td>4.</td>
<td>Thiazolidinediones</td>
<td>Rosiglitazone</td>
<td>Avandia</td>
<td>1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pioglitazone</td>
<td>Actos</td>
<td>1999</td>
</tr>
<tr>
<td>5</td>
<td>Insulin</td>
<td>Lispro</td>
<td>Humalog</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lispro/Protamine</td>
<td>Mixtard 7525</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspart</td>
<td>Novolog</td>
<td>2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspart/NPH</td>
<td>(75/25)</td>
<td>2002</td>
</tr>
</tbody>
</table>
1.1.2 Lacking of available therapies

Most of these treatments have limited efficacy and are associated with side-effects, including weight gain, hypoglycemia, gastrointestinal disturbances, lactic acidosis, edema and anemia. Sulphonylureas increase insulin secretion by binding to the SUR1 receptors on the pancreatic β cells resulting in the closure of the ATP sensitive K-channels and release of insulin in a glucose-independent manner. Their main side effects are weight gain and hypoglycaemia. There are no absolute contraindications to their use, but they need to be used with caution in patients at increased risk of hypoglycaemia including those with renal failure and the elderly [18]. Gastrointestinal intolerance is the most common side effect of metformin and Renal, liver and heart failure are the main contraindications because of the fear of lactic acidosis[19]. 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 [20]. Thiazolidinediones and Peroxisome proliferator- activated nuclear receptor (PPAR) agonist are able to reduce insulin resistance but are under intense scrutiny because of safety issues. (Table 1.3)[21].

Table 1.3 Available therapeutic agents and their side effect [84]

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Molecular target</th>
<th>Mechanism /action</th>
<th>Adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Insulin receptor</td>
<td>Correct insulin deficiency</td>
<td>Hypoglycemia, weight gain</td>
</tr>
<tr>
<td>Sulphonylureas</td>
<td>SU receptor/ATP K⁺ Channel</td>
<td>Stimulate insulin secretion</td>
<td>Hypogycemia, weight gain</td>
</tr>
<tr>
<td>Metformin (Biguanides)</td>
<td>Unknown</td>
<td>Inhibition of hepatic glucose output</td>
<td>Gastrointestinal disturbance, lactic acidosis</td>
</tr>
<tr>
<td>Acarbose</td>
<td>A-Glucosidase</td>
<td>Retard carbohydrate absorption</td>
<td>Gastrointestinal disturbance</td>
</tr>
<tr>
<td>Thiazolidinediones (Pio and Rosiglitazones)</td>
<td>PPAR-γ</td>
<td>Increase insulin sensitivity</td>
<td>weight gain, edema, anemia</td>
</tr>
<tr>
<td>GLP-1 analoges (Byetta)</td>
<td>GLP-1 receptor</td>
<td>Stimulate insulin secretion</td>
<td>Gastrointestinal disturbance, nausea, abdominal pain, weight loss</td>
</tr>
</tbody>
</table>
Chapter 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. Thus, there is an imperative need for novel therapeutic approaches for glycemic control that can complement existing therapies and possibly attempt to preserve the normal physiological response to meal intake. One of the desirable approaches to achieve this goal would be to identify agents that enhance glucose (nutrient)-dependent insulin secretion like incretin hormone. [22].

1.1.3 Incretin concept

A new perspective is the use of incretin hormones and incretin enhancers. Incretins are defined as being responsible for the higher insulin release after an oral glucose load compared to an intravenous glucose load. The incretin effect is believed to be mediated by mainly two incretin hormones: glucose dependent insulinotropic polypeptide (GIP, originally referred to as gastric inhibitory peptide) and GLP-1(glucagon-like peptide-1) [23].

The incretin hormones glucagon like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) play an important role in glucose homeostasis with effects on the pancreas, gastrointestinal tract, muscle tissue, and brain [24]. Various clinically feasible approaches for the use of GLP-1 and GIP to treat T2D have been investigated (Fig 1.1).

Glucagon like peptide-1 (GLP-1)

GLP-1 is an incretin hormone secreted by intestinal L-cells of the distal small intestine in response to food intake, that is, oral nutrients 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 nonnutrient stimulators of GLP-1 [25]. 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. GLP-1 enhances glucose-stimulated insulin secretion from the β-cells of the pancreas, promotes insulin biosynthesis, and inhibits postprandial glucagon secretion. Administration of GLP-1 reduces the rate of gastric emptying, suppresses appetite and, importantly, promotes β-cell mass [26].
Glucose-dependent insulinotropic polypeptide (GIP)

The first incretin to be identified, glucose-dependent insulinotropic polypeptide (GIP), was purified from porcine intestinal extracts; it has weak effects on gastric acid secretion but potent insulinotropic actions. The GIP gene is expressed mainly in K cells, enterochromaffin cells of the proximal small intestine (enteroendocrine duodenal and jejunal mucosa). GIP, a 42-amino acid hormone, is stimulated by enteral glucose, lipids and products of meal digestion in a concentration dependent manner. Elevated GIP plasma levels augment glucose stimulated insulin secretion. Consistent with the incretin concept, GIP acts as a feed-forward mechanism to signal the endocrine pancreas of impending substrate fluxes from the gut [27].

Fig. 1.1 Role of incretins in glucose homeostasis and the action of DPP-4 enzyme [84]

Some other antidiabetic actions of the incretin hormones are as follows:

- Inhibition of food intake and weight gain,
- Retardation of gastric emptying which can attenuate the meal-associated increases in blood glucose,
- Promotion of insulin-stimulated incorporation of fatty acids into triglycerides
1.1.3.1 Incretin based therapeutic approaches

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 via converting it into GLP-1 (9-36). The short half-life ($t_{1/2}$ 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 circumvents this problem. Alternatively, inhibition of DPP-4 is expected to extend the half-life of endogenously secreted GLP-1 (7-36). Like GLP-1, GIP 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 in addition to prolong the beneficial effects of endogenous GLP-1 [28]. So, under physiological conditions, the incretin effect is brief because of the short half-life of the incretin hormones due to the action of DPP-4 enzyme [18]. DPP-4 is a serine protease that cleaves a dipeptide from the N-terminus of the active form of GLP-1, GIP, neuropeptides, and chemokines, and renders them inactive [29].

Various clinically feasible approaches for the use of GLP-1 to treat T2D have been investigated. These include development of

(a) Peptide based GLP-1 analogues: Exenatide LAR and liraglutide,
(b) Non-peptide based small molecules as agonist for the GLP-1 receptor
(c) Small molecules as DPP-4 inhibitors

Exendin-4 (36 amino acids) was discovered in a lizard (salivary gland venom of the Gila monster) and found to be more stable and less rapidly degraded than GLP-1. Although Exenatide, synthetic version of exendin-4 is transcribed from a distinct gene, it has a 53% overlap of the amino acid sequence with mammalian GLP-1. Exendin-4 has an in vivo potency up to 5-10 times higher than GLP-1 itself. 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 [30].

Liraglutide (NN 2211) is a long-acting GLP-1 analogue with the following chemical modification (Arg34Lys substitution, and a glutamic acid and 16-C free-fatty-acid addition to
Lys26). The fatty-acyl-GLP-1 structure binds to interstitial albumin at the injection site and the GLP-1 resembling structure is released slowly from the albumin complex to be absorbed into the circulation. Liraglutide inhibits appetite. The counter regulation by glucagon induced by a hypoglycaemia is not impaired by liraglutide. Liraglutide inhibits apoptosis and increases β-cell mass. As expected from exenatide, GI symptoms (e.g., nausea, vomiting) are prominent adverse effects of liraglutide and led to discontinuation in 3% of patients [31].

GLP-1 as an endogenous peptide is characterized by a very short half-life of 2-3 min due to the hydrolysis by DPP-4. Thus DPP-4 came into the focus as a relevant drug target. This discovery has led to the development of DPP-4 inhibitors to increase the half-life of circulating incretin hormones and normalize glucose homeostasis.

### 1.1.3.2 Reasons in favour of using incretin-based medications

Both DPP-4 inhibitors and incretin mimetics have shown efficacy in terms of reducing fasting and postprandial glucose concentrations and glycated haemoglobin of particular relevance are the studies comparing glucose control on a background of oral anti-diabetic agents (preferably metformin) with other accepted anti-diabetic medications [32].

**No weight gain**

Reasons to recommend the use of incretin mimetics or DPP-4 inhibitors in combination with metformin include no apparent weight gain (DPP-4 inhibitors) and even the potential to lower body weight (incretin mimetics).

**Low risk of hypoglycaemia**

The low risk of provoking hypoglycaemic episodes that has been observed in clinical studies is compatible with our understanding of the mode of action of glucagon-like peptide-1 (GLP-1), which stimulates insulin secretion in a strictly glucose-dependent manner, and only above a permissive level of glycaemia. Therefore, even high concentrations of GLP-1 are unable to cause hypoglycaemia.

**Absence of threatening adverse events**
The main adverse events reported in studies with incretin mimetics have been ‘gastrointestinal’ in nature, and typically include mild-to-moderate nausea, and sometimes vomiting, diarrhoea, abdominal fullness or pain. Therefore, both incretin mimetics and DPP-4 inhibitors are not known to cause any potentially severe or even life-threatening diseases or events. Potential cardiovascular benefits Beneficial effects of GLP-1 have been shown in both animal and human studies of coronary ischaemia and in left-ventricular failure due to cardiomyopathy. In patients with acute myocardial infarction treated by angioplasty, both type 2 diabetics and nondiabetics, an intravenous infusion of GLP-1 led to less wall-motion abnormality and better overall left-ventricular function.

**Effects on β-cell health**

In line with GLP-1 effects on β-cell proliferation and differentiation, insulin content, apoptosis, functional status including recruitment of individual β-cells to the insulin secretory process, treatment with incretin mimetics and DPP-4 inhibitors has improved β-cell numbers and function in animal models of type 2 diabetes.

**Reasons for hesitating to recommend incretin-based anti-diabetic drugs**

- Lack of long-term studies describing hard clinical end points
- Lack of databases to conclude long-term safety
- Treatment costs
- Recommendations of official bodies representing health-care systems

**1.1.4 DPP-4 and structure homologues**

Membrane-bound proteases are broadly spread out among various cell systems. Their expression in a particular cell type is finely regulated, specific functional cell implications and involved in defining physiological pathways. Protein turnover, ontogeny, inflammation, tissue remodeling, cell migration and tumor invasion are among the many physiological and pathological events in which membrane proteases play a crucial role, both as effectors as well as regulatory molecules. The proline specific dipeptidyl peptidases (DPPs) like DPP-4, fibroblast activation protein (FAP), DPPII, DPP8 and DPP9, cleaves after X-Pro, are believed to be involved in many of
these processes [33]. These DPPs are issued as an important protease family and their Inhibitors have an interesting therapeutic potential, mainly in diabetes, oncology and hematology.

1.1.4.1 Dipeptidyl peptidases (DPP-4)

DPP-4/CD26 is a cell-surface protease belonging to the prolyloligopeptidase family. DPP-4 was first reported in 1966 as glycyl-prolyl-β-naphthylamidase and later named dipeptidyl peptidase-4, as recommended by the Enzyme Commission [34]. In the 1970s, the enzyme served initially as a model protein for the study of the catalytic mechanism of serine peptidases and for the investigation of the specifics of proline peptide bonds. In the 1980s, the potential of the enzyme to convert bioactive peptides was discovered, which intensified the search for its function. In the middle of the 1990s, the involvement of DPP-4 in metabolism and the regulation of the cytokines, chemokines and different peptide hormones triggered programs for the development of DPP-4 inhibitors [35]. It was the discovery of the role of the enzyme in energy homeostasis which accelerated the design of potential pharmaceutical agents for the treatment of that most important of metabolic diseases, Type 2 diabetes, and led to the first patent application for the use of DPP-4 inhibition in the reduction of blood glucose [36]. Physico-chemical, X-ray crystallographic and cryotransmission electron microscopic studies have provided considerable information on the structure and mode of action of DPP-4. This knowledge is critical for rational design of optimal inhibitors. Cross-linking and analytical ultracentrifugation studies have shown that human DPP-4 exists as a dimer and [37]. It has been proposed that homodimerization of DPP-4 is essential for its serine protease activity [38]. The catalytic triad is situated in a large cavity formed by α/β hydrolase fold and an eight-bladed propeller domain that constitutes part of the dimerization interface. A short helix, with a Glu205–Glu206 sequence motif constitutes a binding region for the N-termini of peptide substrates. DPP-4 is only capable of hydrolyzing small peptides physiologically, because of restricted entry to the active site [39]. Dipeptidyl peptidase-4 exhibits a strong preference for peptides with proline (Pro) or alanine (Ala) as the penultimate (P1) amino acid, but it is now established that it can also act efficiently on peptides with N-termini consisting of Xaa-Serine (Ser) [40] and with less efficient cleavage of hydroxyproline, dehydroxyproline, glycine, valine, threonine or leucine at P1 [40-44].
1.1.4.2 Fibroblast Activation Protein (Seprase)

Fibroblast activation protein is a serine protease bearing probably dual DPP-4-like and gelatinase enzymatic activity. Only a single active site was found to mediate its enzymatic activity [45, 46]. Fibroblast activation protein is expressed in tissue remodeling sites, reactive stromal fibroblasts of over 90% of human malignant tumors, granulation tissue of healing wounds, and some fetal mesenchymal tissues but not in normal adult human tissues [47].

1.1.4.3 Dipeptidyl Peptidase II (DPP-II)

Dipeptidyl peptidase II is a serine protease acting preferentially in the acidic pH. DPPII was first reported in 1966 as dipeptidylarylaminidase II [48]. Based on the enzyme's ability to only cleave dipeptides from β-naphtylamide derivatives with a un-substituted NH2-terminus, the enzyme was renamed dipeptidylaminopeptidase II [49, 50]. Along the time, DPPII was also referred to as brain dipeptidylaminopeptidase A, DPP V, carboxytripeptidase, DPP-7 and quiescent cell proline dipeptidase (QPP) [51-54]. Inhibitors of post-proline cleaving dipeptidyl peptidases such as Val-boro-Pro caused apoptosis in quiescent lymphocytes in a process independent of DPP-4. This effect was attributed to inhibition of a novel dipeptidyl peptidase named QPP. Later QPP and DPPII were proven to be identical [55]. The possible physiological role of DPPII is its localization and limited substrate selectivity (tripeptides), it's presumed that DPPII has a role in the final steps of peptide degradation as of the autophagic process or the degradation of endocytosed protein. There is still no clarity on the possible role of DPPII in the prevention of cell death in leukocytes. The widespread distribution of DPPII suggests a general role for DPPII as one of the housekeeping proteins. Even in the absence of specialized functions, DPPII as the only lysosomal DPP able to cleave post-proline peptide bonds probably play an important role in cellular protein economy [56].

1.1.4.4 DPP-8 and DPP-9

Dipeptidyl peptidase 8 is a ubiquitous soluble nonglycosylated serine protease localized in the cytoplasmic (non-lysosomal) compartment and acting preferably at neutral pH. Dipeptidyl peptidase 8 (DPP-8) consists of 882 amino acids and has a molecular weight of 100 kDa. DPP-8 is distributed ubiquitously with its highest expression in testis and brain. Based on the structural similarity with DPP-4, DPP-8 was proposed to be involved in the T-cell activation [57].
However, functional studies dealing with DPP-8 have not been published. Dipeptidyl peptidase 9 (DPP-9) has previously been reported to be active as a cytosolic monomer comprised of 971 amino acids with a molecular weight of approximately 100 kDa [58]. DPP9 is ubiquitously distributed, with its highest expression in liver, heart and skeletal muscle [59,60]. Its physiological function is not known so far. Due to their shortest gene size, lowest number of exons and the active site being located in one exon in comparison to DPP-4 and FAP, DPP-8 and DPP-9 have been suggested to be the most ancient DPP-4 like enzymes. It should be mentioned that side effects obtained during the course of toxicological studies of a nonselective inhibitor were due the inhibition of DPP-8 and/or DPP-9 [61a]. In 2008, Burkey et al reported association between inhibition of dipeptidyl peptidase (DPP)-8 and/or DPP-9 organ toxicities and mortality in rodents. They found that at high dose, the toxicities of a selective DPP-8/DPP-9 inhibitor that were reported previously (100% mortality in mice, alopecia, thrombocytopenia, reticulocytopenia, enlarged lymph nodes, splenomegaly and 20% mortality in rats) were not observed. Thus, Inhibition of DPP-8 and DPP-9 does not lead to organ toxicities and mortality in rodents [61b,84]

1.1.5 DPP-4 as novel target for T2DM

DPP-4 inhibitors have now become a currently, both saxagliptin (OnglyzaTM), sitagliptin (Januvia®) and vildagliptin (Galvus®) are DPP-4 inhibitors approved as adjuncts to diet and exercise to improve glycemic control in adults with T2DM; vildagliptin is also commercially available but only outside the United States [62]. Sitagliptin was approved by the FDA and EMEA for the treatment of T2DM patients who fail to achieve hyperglycemic control with diet and exercise, alone or in combination with another drug such as metformin or a glitazone [63]. The FDA has approved linagliptin as a monotherapy or in combination with other commonly prescribed medications for type 2 diabetes such as metformin, sulphonylurea and pioglitazone to reduce haemoglobin A1c (HbA1c or A1c) levels by a mean of up to -0.7 percent (Table 1.4) [64]. More than 100 patents have been issued for DPP-4 inhibitors to be used either as a monotherapy or in combination with other antidiabetic agents for the treatment of type-2 diabetes, as well as metabolic syndrome. Omarigliptin (Marizev®(Japan)] is a smallmolecule dipeptidyl peptidase-4 (DPP-4) inhibitor developed by Merck for the oral treatment of type 2 diabetes (T2DM). Unlike the majority of other approved agents of
its class, which are usually administered once daily, omarigliptin can be administered once weekly. Once weekly omarigliptin has received its first global approval in this indication in Japan for use in adults. Phase III clinical development of the product is underway in several other countries [187].

Table 1.4 DPP-4 inhibitors (approved and under development) [84]

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Development stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitagliptin (Januvia)</td>
<td>Meark&amp; Co</td>
<td>Approved in 2006</td>
</tr>
<tr>
<td>Vildagliptin (Galvus)</td>
<td>Novartis</td>
<td>Approved in 2008</td>
</tr>
<tr>
<td>Saxagliptin (Onglyza)</td>
<td>Bristol-Mayers-Squibb</td>
<td>Approved in 2009</td>
</tr>
<tr>
<td>Linagliptin (Tradjenta)</td>
<td>BI and Eli Lilly and Company</td>
<td>Approved in 2011</td>
</tr>
<tr>
<td>Aloglaptin (SRY-322)</td>
<td>Takeda</td>
<td>Approved in 2013</td>
</tr>
<tr>
<td>Dutoglaptin (PHX1149)</td>
<td>Phenomix</td>
<td>Dropped from the market</td>
</tr>
<tr>
<td>Omarigliptin (MK-3102)</td>
<td>Mearck</td>
<td>Approved in Japan 2015</td>
</tr>
<tr>
<td>PSN9301</td>
<td>OSI Pharmaceuticals</td>
<td>Phase 2</td>
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<tr>
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<td>Sanofi-Aventis</td>
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<td>TS-021</td>
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<tr>
<td>SYR-619</td>
<td>Takeda</td>
<td>Phase 1</td>
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</tbody>
</table>

1.1.5.1 Comparative binding site DPPs

Dipeptidyl peptidase -4/CD26 is a type II integral membrane protein consisting of a hydrophobic N-terminal domain, a transmembrane region, and a C-terminal domain containing the catalytic triad that acts on oligopeptides by selectively removing N terminal dipeptides [65]. The DPP-4 exists as dimer (asymmetric) and each monomer consists of 766 amino acids, in which 6 amino acids in the the cytoplasm (residues 1-6), 22 residues in the transmembrane region (residues 7-28), 295 residues in the glycosylated region (residues 29-323) and 215 residues in the catalytic region (residues 552-776) [66].The N-terminal domain comprises of eight-bladed β-propeller domain and C-terminal domain comprises of catalytic domain (α/β-hydrolase domain). The dimer interface consists of Ile237-Thr251 in the N-terminal and Ala717-Thr736 in the C-terminal. The catalytic triad consists of Ser630, His740 and Asp708 which forms stable
carbaminic acid adducts with the carbonyl carbon of the inhibitors [67]. The β-propeller domain, α/β-hydrolase domain and ligand binding site were shown in Fig. 1.2.

![Secondary Structure of DPP-4 enzyme and binding site](image)

**Fig.1.2** Secondary Structure of DPP-4 enzyme and binding site (orange circle). [84]

The DPP-4 active site located at the interface of β-propeller and the α/β-hydrolase domain. The DPP-4 α/β-hydrolase is highly conserved with DPP8 and DPP9 include S1 pocket residues (Tyr547, Ser630, Tyr631, Val656, Trp659, Asp663, Tyr666, Asn710, Val711 and His740) and negatively charged glutamic acid motive, EE-Helix (Glu205, Glu206), which is inserted into the first sheet of the fourth propeller blade. The S2-loop (Agr358 and Phe357) is present between the propeller blades 5 and 6 connected to EE-Helix contributing S2-pocket of DPP-4 (Fig. 1.3) [68].
DPP-4 enzyme selectively binds with substrates having proline at the P1-position, many DPP-4 inhibitors have 5-membered heterocyclic rings which mimic proline, e.g. pyrrolidine, cyanopyrrolidine, thiazolidine and cyanothiazolidine by covalent bonding with catalytic residue Ser630 [69]. Generally, the nitrile group form bonding but can also be boronic acid or diphenylphosphonate. In 1994, scientists from Zeria Pharmaceuticals revealed cyanopyrrolidines with a nitrile functional group form an imidate with the catalytic serine and responsible for stability issues due to reactions with the free amino group of the P2-amino acid. Further inhibitors without the electrophilic group have also been developed, but these molecules have shown toxicity due to affinity to other dipeptidyl peptidases, e.g. DPP-2, DPP-8 and DPP-9 [70].

1.2 Drug design and molecular modeling

The drug discovery process involves the identification of the lead structure followed by the synthesis of its analogs, their screening to get candidate molecule(s) for drug development. Despite advances in understanding of biological systems, drug discovery is still a long process with low rate of new therapeutic discovery. It has been estimated that out of 10000 compounds synthesized in the laboratory only one achieves clinical use as a drug. The whole process takes around 10-15 years and an investment of nearly $800 million today.
The use of computers and computational methods permeates all aspects of drug discovery today and forms the core of drug design. High-performance computing, data management software and internet are facilitating the access of huge amount of data generated and transforming the massive complex biological data into workable knowledge in modern day drug discovery process. The use of complementary experimental and informatics techniques increases the chance of success in many stages of the discovery process, from the identification of novel targets and elucidation of their functions to the discovery and development of lead compounds with desired properties. Computational tools offer the advantage of delivering new drug candidates more quickly and at a lower cost [71].

Drug design also sometimes referred to as rational drug design is the inventive process of finding new medications based on the knowledge of the biological target. The drug is most commonly an organic small molecule which activates or inhibits the function of a bio-molecular such as a protein which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves design of small molecules that are complementary in shape and charge to the bio-molecular target to which they interact and therefore will bind to it.

Drug design frequently but not necessarily relies on computer modeling techniques. This type of modeling often referred to as computer-aided drug design. In-silico methods can help in identifying drug targets via bioinformatics tools. They can also be used to analyze the target structures for possible binding/active sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities, further optimize the molecules to improve binding characteristics (Fig 1.4) [72].

1.2.1 Types of drug designing

1. Ligand based drug design.

2. Structure based drug design.

3. Fragment based drug design.

1.2.1.1 Ligand based drug design
Chapter 1

Background Overview

The ligand based (indirect) approach uses the information gathered from a set of ligands acting on a particular target (receptor or enzyme) to identify important structural and physicochemical properties responsible for variation in the observed biological activity. To be used most effectively, one should have compounds with high, low and intermediate activities spread over a wide range. Here, an assumption is made that all the compounds interacts with the receptor in a similar manner. The 2D and 3D QSARs, molecular similarity search and pharmacophore modeling, virtual screening etc. are the ligand based design techniques [73].

1.2.1.2 Structure based drug design:

The process of structure-based drug design is an iterative one and often proceeds through multiple cycles before an optimized lead goes into phase I clinical trials. The first cycle includes the cloning, purification and structure determination of the target protein or nucleic acid by one of three principal methods: X-ray crystallography, NMR, or homology modeling. This approach involves analyzing features of the receptor active site and the spatial relationships among them. Then, the negative image of this configuration is used to construct a pharmacophore model. Using computer algorithms, compounds or fragments of compounds from a database are positioned into a selected region of the structure. These compounds are scored and ranked based on their steric and electrostatic interaction actions with the target site and the best compounds are tested with biochemical assays [74].

1.2.1.3 Fragment based and Denovo drug design

Fragment-based discovery has gathered considerable momentum in the pharmaceutical and biotechnology arenas in recent years. In fact, these approaches can be considered to be the same in many respects. First, both techniques start with small chemical building blocks and attempt to find novel drug-like molecules with the desired properties. The general view is that de novo work starts with smaller building blocks, although there is no clear definition regarding when the size scale for a de novo building block stops and a fragment begins. Next, the initial molecular building blocks that have desired properties (preferably activity at an early stage of a project) are either elaborated upon (growing), directly connected (joining), or connected by a linker (linking). This process can be iterated until one or more molecules with the desired properties are obtained.
Because of this overlap we will in many cases interchange the terms “fragment-based” and “de novo”[75].

![Drug design strategies](image)

**Fig. 1.4** Drug design strategies

### 1.2.2 Drug design Techniques

#### 1.2.2.1 Pharmacophore modeling

The identification of a pharmacophore, that is the group of atoms in a specific arrangement which are responsible for the bioactivity of a series of chemical entities, is one of the most widely applied molecular modeling approaches for both discoveries of new scaffolds. Thus, a pharmacophore capturing this compound feature should be able to identify from a database novel compounds that binds to the same site of the protein as the known compounds do. The process of deriving pharmacophore is known as pharmacophore mapping, consist of three steps (1) Identifying common binding element that are responsible for the biological activity; (2) Generating potential conformations that active compound may adopt; and (3) Determining the 3D relationship between pharmacophore element in each conformation generated.
Chapter 1

Background Overview

There are several programs PHASE, Hip Hop, Hypogen, Disco, Gaps, flo, APEX, and ROCS, that can automatically generate potential pharmacophore from a list of known inhibitors [76].

1.2.2.2 QSAR

The Quantitative Structure Activity Relationship (QSAR) paradigm is based on the assumption that there is an underlying relationship between the molecular structure and biological activity. On this assumption QSAR attempts to establish a correlation between various molecular properties of a set of molecules with their experimentally known biological activity.

There are two main objectives for the development of QSAR:

1) Development of predictive and robust QSAR, with a specified chemical domain, for prediction of activity of untested molecules.

2) It acts as an informative tool by extracting significant patterns in descriptors related to the measured biological activity leading to understanding of mechanisms of given biological activity. This could help in suggesting design of novel molecules with improved activity profile.

Most often the QSAR methods are categorized into following classes, based on the structural representation or the way by which the descriptor values are derived:

1D-QSAR correlating activity with global molecular properties like pKa, log P etc.

2D-QSAR correlating activity with structural patterns like connectivity indices, 2D-pharmacophores etc., without taking into account the 3D-representation of these properties

3D-QSAR correlating activity with non-covalent interaction fields surrounding the molecules

4D-QSAR additionally including ensemble of ligand configurations in 3D-QSAR

5D-QSAR explicitly representing different induced-fit models in 4D-QSAR

6D-QSAR further incorporating different solvation models in 5D-QSAR

QSAR's most general mathematical form is:

\[ \text{Activity} = f (\text{physiochemical and/or structural properties}) \]
Chapter 1

Background Overview

For QSAR analysis, a dataset of a series of synthesized molecules tested for its desired biological activity is required. For a QSAR to be valid and reliable, the activity of all of the chemicals covered must be elicited by a common mechanism. The quality of the model is totally dependent on the quality of the experimental data used for building the model.

Comparative Molecular Moment Analysis (CoMMA) is one of the unique alignment-independent 3D-QSAR methods, which involves the computation of molecular similarity descriptors based on the spatial moments of molecular mass (shape) and charge distributions up to and including second order as well as related quantities [77].

1.2.2.3 Molecular docking

The computational process of searching for a ligand that is able to fit both geometrically and energetically into the binding site of a protein is called molecular docking. Molecular docking helps in studying drug/ligand or receptor/protein interactions by identifying the suitable active sites in protein, obtaining the best geometry of ligand-receptor complex and calculating the energy of interaction for different ligands to design more effective ligands. The target or receptor is either experimentally known or theoretically generated through knowledge based protein modeling or homology modeling. The molecular docking tool has been developed to obtain a preferred geometry of interaction of ligand-receptor complexes having minimum interaction energy based on different scoring functions viz. only electrostatics, sum of steric and electrostatic and Dock Score [78].

Three options for docking are available.

i. Rigid docking where a suitable position for the ligand in receptor environment is obtained while maintaining its rigidity

ii. Flexible docking where a favored geometry for receptor-ligand interaction is obtained by changing internal torsions of ligand into the active site while receptor remains fixed

iii. Full flexible docking where the ligand is flexed via its torsion angles as well as the side chain of active site residues (selected active site residues within a user specified radius around the ligand) are flexed.
Chapter 1  

Background Overview

One key aspect of molecular modeling is calculating the energy of conformations and interactions using methods ranging from quantum mechanics to purely empirical energy functions. Molecular docking energy evaluations are usually carried out with the help of a scoring function. Developing these scoring functions is a major challenge in structure based drug design. Efficiency and accuracy of geometric modeling of the binding process to obtain correct docking solutions depends on scoring function. Usually scoring functions are based on force fields that were initially designed to simulate the function of proteins (based on enthalpy). Some scoring functions used in molecular docking have been adapted to include terms such as solvation and entropy [79].

1.2.2.4 Virtual screening

Medicinal Chemistry has developed modern strategies such as Virtual Screening (VS) and High-Throughput Screening (HTS) to explore the huge chemo-diversity in an efficient (and bio-ethically compatible) manner. Although is highly unlikely that in silico and in vitro tools will ever totally replace in vivo assays, ligand- and structure–based VS strategies have been used to filter the ever-growing chemical space, in order to rationally reduce the number of compounds to synthesize/evaluate. Two general and fundamental approaches have been developed: structure based VS (SBVS) and ligand-based VS (LBVS). In SBVS, the search of new leads is guided by the knowledge of the 3D structure of the biological target. The procedure, called molecular docking, involves a quantitative analysis of the molecular recognition events between the ligands and the targeted binding site. To this end, the compounds from a virtual library are docked to the structure of the active site of the target protein (by adjusting their conformation to bind to the selected receptor) and the free energies of binding are calculated [80].

Alternatively, ligand-based VS (LBVS) can be performed when there is little or no information available on the molecular target. LBVS methodologies usually involve two fundamental steps: first, structural features significant to elicit biological activity should be studied on a set of ligands which are known to bind the molecular target of interest. Once those critical features have been identified it is possible to apply them in the search of chemical entities that satisfy those requirements in virtual libraries of chemical compounds. In the limit, LBVS may be applied even if a single known-ligand has been identified, through similarity-based VS.
Chapter 1  

Background Overview

The use of sequential ligand-based filters followed by structure-based filters in VS is justified by the optimization of computational resources to accelerate the processes, increasing the computational requirements of the selected methodologies progressively in each step. When this strategy is applied, the molecular docking usually constitutes one of the last stages, and it is used to provide more information about the interaction between the best ranked ligands and the binding site. Although it is usually assumed that target-based leads to more accurate and reliable predictions (as has been stated in previous paragraphs) recent evidence from systematic studies shows that ligand-based methodologies frequently present best enrichment factors while both SBVS and LBVS performances are highly variable from target to target [81].

1.2.3 Drug design protocol for DPP-4 inhibitors

To identify a rapid and accurate system to discover new DPP-4 inhibitors hit to lead approaches can be a greater tool for exploration of novel scaffold. 3D pharmacophore models can provide a useful tool for designing novel DPP-4 inhibitors. Pharmacophore models provide the basic key chemical features of compounds with DPP-4 inhibitory. These models can be further used for virtual screening of large database or fragment designing. Lead optimization has for example been used to screen for small primary fragments that could be placed in S1 and S2 sites of DPP-4 [82]. The data from the 3D structure of the target protein a ligand-protein complexes is used to design new ligands with improved binding affinities. After synthesis and testing, the underlying hypotheses on the structure-activity relationships are modified and a new design cycle starts. In optimal cases ligands with nanomolar affinities result after several design cycles, but that other important properties like bioavailability or metabolic stability of a drug are neglected. In current lead-finding activity were mainly directed towards affinity and selectivity rather than molecular properties, metabolic liabilities. To use a combination of thoughtful design with one-at-time synthesis and the preparation of intelligently designed arrays of compounds in an automated fashion is integral to the way research and allows faster provision of higher quality development of DPP-4 inhibitors candidates [83].
Chapter 1  
Background Overview

1.3 Review of Literature

Type–2 diabetes is becoming a global epidemic, with the incidence and prevalence of the condition as well as related risk factors, such as obesity, continuously rising worldwide. Diabetic patients have seen a new ray of hope in the form of new drug classes, such as DPP-4 Inhibitors. Patients are increasingly gaining access to new drugs that offer novel benefits. It also includes a strong safety profile of early DPP-4 inhibitor drugs, lower side effects and weight-neutral effect of these drugs, lower risk for cardiovascular complications, and convenience associated with the use of DPP-4 inhibitors. The global market for DPP-4 inhibitors is forecast to reach US$14.8 billion up to 2022. The increased interest of the pharmaceutical industry in DPP-4 inhibitors reflects their market attractiveness and patent application. DPP-4 inhibitors have become a new hope of researchers that will affect approximately 8-10% of the adult population of new diabetes therapy [85]. Up to date there are more than 20 different DPP-4 inhibitors developed with available favorable clinical and preclinical studies with different chemical structures, including amino acid amide, carbocyclic, alkylamine, pyrrolidine, pyridine, and xanthine derivatives.

1.3.1 Classification of DPP-4 inhibitors

Although, a number of DPP-4 inhibitors have been accounted, still great need exists for novel DPP-4 inhibitors which are useful in diabetic medication. The development of selective DPP-4 inhibitors is a big task due to another member of DPP group like DPP-2, DPP-8, DPP-9, etc. which may produce side effect as severe toxic reaction, alopecia, thrombocytopenia, anemia and increased mortality. In addition development of long acting inhibitors is also desirable that could provide maximum efficacy for diabetic patient. In respect of current clinical developments mainly two substance classes’ standout whose representatives are under investigation in man.

Based on structural features, two unique classes of DPP-4 inhibitors were identified, for example, peptidomimetic and non-peptidomimetic DPP-4 inhibitors. The peptidomimetic class is further subdivided into (a) glycine-based (α-series) and (b) β-alanine-based (β-series) inhibitors (Fig. 1.5).

Based on the X-ray crystal structure of DPP-4 and computer modeling studies two key interactions have been suggested between the enzyme and α-series inhibitors, 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 β-series but the hydrogen bond to the carbonyl oxygen was not well defined [86a].

**Fig 1.5** Classification of DPP-4 inhibitors [84]

1.3.1.1 Peptidomimetics DPP-4 inhibitors

The Peptidomimetic series can be further subdivided into (a) glycine-based inhibitors (α-series) and (b) β-alanine-based inhibitors (β-series). In the case of α-series, pyrrolidine derivatives have been widely explored due to the specificity of DPP-4 for substrates having an amino at C-2. Depending on the presence of a substituent, e.g., C-2 of the pyrrolidine ring, the α-series can be further subdivided into two classes, e.g., (a) irreversible (when R=diphenylphosphonate ester[-P(O)(OPh)2] or O acylhydroxamic acid (CONHOCOR)) and (b) reversible (when R=boronic acid [B(OH)2], nitrile (CN) or hydrogen) inhibitors. Notably, the 2-cyanopyrrolidine-based inhibitors that belong to the reversible class of the α-series have been studied most extensively. The β-series was generally developed from a lead obtained via high-throughput screening (HTS) and a number of inhibitors based on a β-amino amide backbone have been reported [86b].
Due to DPP-4’s specificity for substrate pyrrolidine derivatives have been widely explored as DPP-4 inhibitors. Thus, many DPP-4 inhibitors resemble the cleavage product of P2–P1 dipeptidyl substrate where the P-1 site contains a proline mimic. Nitrile in the position of the scissile bon of the peptide substrate is important for higher potency. The presence of the nitrile on the five-membered ring provides reversible and nanomolar inhibition of DPP-4 and chemical stability adequate for oral administration [86c]. 2(S)-cyanopyrrolidine moiety has been found to be an integral part of many DPP-4 inhibitors (Fig. 1.6.1) [87]. Where

- Nitrile group forms reversible covalent bonds with the catalytically active serine hydroxyl group (Ser630).
- Hydrogen bonding network forms between the protonated amino group and a negatively charged region of the protein surface Glu205, Glu206 and Tyr662.

One of the potent, selective and stable inhibitors in this class was reported as cyclohexylglycine-(2S)-cyanopyrrolidine (Table 1.5, 1) [88]. The new DPP-4 inhibitor class developed by Novartis N-substituted-glycyl-2-cyanopyrrolidines which was found to be potent, selective and short-acting DPP-4 inhibitor [89]. (Table 1.5, 2) Later identified that DPP-4 inhibitors generally are not very stable compounds; therefore, chemical stability has improved by incorporating a steric bulk. Two cyanopyrrolidines that have been most pronounced, vildagliptin and saxagliptin, were created in this manner. Vildagliptin was first synthesized and discovered by Novartis in May 1998 (Table 1.5, 3) [90]. Researchers examined adamantly derivatives to be very potent. The adamantyl group worked as a steric bulk and slowed intramolecular cyclization with increasing chemical stability. Replacing the 3-hydroxy group of adamantan moiety by other substituent’s led to the identification of a new series of potent DPP-4 inhibitor (Table 1.5, 4) [91]. As bulky group were well tolerated at P-2 site different modification were done by gem dimethyl substitution (Table 1.5, 5) [92]. Bridging carbon and nitrogen with small ring (Table 1.5, 6) [93] and tert-butyl- substituent (Table 1.5, 7) [94] at p-2 site which showed enhanced potency and selectivity.

With the increased steric bulk of the N-terminal amino acid side chain led to increased stability. To increase stability the transrotamer with a cis-4,5-methano substitution of the pyrrolidine ring, results in intramolecular van-der-Waals interaction, thus preventing intramolecular cyclization. Because of this increased stability, the researchers continued their investigation on cis-4,5-
methanocyanopyrrolidines and encountered with a new adamantyl derivative which showed impressive ex vivo DPP-4 inhibition in rat plasma[95]. Further hydroxylation on the adamantyl group improved chemical stability and better microsomal stability, called saxagliptin (Table 1.5, 8) [95].

Further optimization was done by 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 (Table 1.5,9) [96]. 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. 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-4. (Table 1.5, 10-11) [97,98]. Further optimization by substitution of 2-hydroxy-1,1-dimethylethyl side chain (Table 1.5, 12) [99], five member heterocycle (Table 1.5, 13) [100], introducing an additional fluoro group at the C-4 position of chiral 2-cyano-4-fluoropyrrolidine moiety (Table 1.5,14) [101] and incorporating conformationally restricted N-(aryl or heteroaryl)-3-azabicyclo[3.1.0]hexane moiety in 2-cyanopyrrolidine (Table 1.5, 15) [102] has led to the identification of a potent and stable DPP-4 inhibitor. Sakashita et al carried out a modification at C-4 position of pyrrolidine and develop new potent inhibitors 1-(γ-substituted prolyl)-(S)-2-cyanopyrrolidine [103]. Compounds bearing (S) stereochemistry were more potent than the antipode (Table 1.5, 16) [103]. 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 (Table 1.5, 17) [104]. By introducing ring-constraint a series of substituted pyrrolidine-2,4-dicarboxylic acid amides were designed which showed good in vitro DPP-4 inhibition with selectivity over DPP-2, DPP-8 and FAP enzymes.33c Compounds(Table 1.5, 18)[105].

Modification of the C-5 position of the P2 pyrroldine also afforded potent inhibitors of DPP-4 (Table 1.5, 19) [106]. Recently, cis-3-amino-4-(2-cyanopyrrollidide)-pyrrolidines have been shown to be a unique scaffold for the development of potent DPP-4 inhibitors (Table 1.5, 20) [107]. Achiralcis-2, 5-dicyanopyrrolidine template (Table 1.5, 21) [108] was identified as an achiral inhibitor of DPP-4 that showed selectivity over DPP-2, DPP-3, DPP-8, DPP-9, APP and FAP. 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. In place of two cyano group on prrolidine ring one is substituted with alkyne provided superior selectivity profiles (Table 1.5, 22) [84, 109].

Table 1.5: Pyrrolidilnes derivative DPP-4 inhibitors [84]

<table>
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<tr>
<th>S. N.</th>
<th>Compound</th>
<th>DPP-4 activity</th>
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<td>5.</td>
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<td>$IC_{50}=15 \text{ nM}$</td>
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<td>Ki</td>
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<td>IC\text{$_{50}$} = 0.0017 \text{uM}</td>
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Non-Nitrile Pyrroolidines derivatives

Removal of nitrile moiety from 2-cyanopyrrolidine derivative is an obvious solution to the intramolecular cyclization related problem of this class of compounds. The 3-fluoropyrrolidine analogues obtained by replacing the pyrrolidine ring by 3-fluoropyrrolidine moiety were shown to have activity equal or superior to that of the parent compounds. (Table 1.6, 1) [110]. Replacing the 4-fluorophenyl group by a heterocyclic moiety and cyclohexyl ring yielded the compound (Table 1.6, 2-4) [111-113]. Cyclohexylglycine-based inhibitors of DPP-4 lacking an electrophile, 2,4-difluorobenzenesulfonamide had good pharmokinetic properties but it was not selective against DPP-8.(Table 1.6,5)[114]. Replacing the 4-fluorophenyl group by polar heterocycles such as methylpyridone resulted in identification of a potent DPP-4 inhibitor (Table 1.6,6) [115]. By replacing the central phenyl group with a heterocycle and subsequent SAR work
led to the identification of a novel series of oxadiazole-based amides as potent DPP-4 inhibitors (Table 3,7) [116]. Highly fluorinated pyrrolidine derivative of cyclohexylglycine amides were explored as a new class of DPP-4 inhibitors (Table 1.6, 8) [117]. A boronic acid moiety at the 2-position of the pyrrolidine ring has shown to be effective for the inhibition of DPP-4 (Table 1.6, 9) [118]. Another series, that is, N-Alkyl Gly-boro-Pro derivatives were evaluated for DPP inhibitory properties and a representative compound (Table 1.6,10) was found to be a potent but a moderately selective inhibitor of DPP-4[119,186].

Table 1.6: Non-nitrile Pyrrolidilnes derivative DPP-4 inhibitors [84]

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Compounds</th>
<th>DPP-4 Activity</th>
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<td>5</td>
<td><img src="image5.png" alt="Image" /></td>
<td>IC_{50} = 48 nM</td>
<td>114</td>
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</table>
Non Pyrrolidines derivatives

Fumihiko Akahoshi et al., describe [(S) -c-(arylamino) prolyl] thiazolidine compounds as a novel series of potent and stable DPP-4 inhibitors [123]. They are the thiazolidine analogs of [(S)-c (arylamino) prolyl]-(S)-2-cyanopyrrolidine but with the electrophilic nitrile removed to improve chemical stability in aqueous solution. The thiazolidide is more effective than the corresponding pyrrolidide and free from intramolecular cyclization, they are chemically stable, named P32/98, (Table 1.7, 1) [120]. This compound class, produced improved glucose tolerance in diabetic patients and healthy volunteers in clinical trial, but exhibited only modest inhibitory activity.

Further optimization includes [(S) -c-(arylamino) -L-prolyl] thiazolindine derivatives containing a 4β-(amino) -L-prolyl moiety (Table 1.7, 2) [121]. Other representative compounds in this series
modified by sulfur-containing hetero-aromatic moiety of N-(3-methyl-1,2,4-thiadiazol-5-yl) piperazine (Table 1.7,3) showed a longer duration of plasma DPP-4 inhibition, high oral bioavailability [122]. Modifications of the β-amino amide backbone 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 [123] and the piperazine series, [124]. Further lead optimization was carried out by Merck company to obtain inhibitors having improved metabolic stability and PK properties piperazine moieties were replaced with metabolically robust heterocycles particularly fused heterocycles. A series of β-amino amides bearing triazolopiperazines were identified as potent, selective, orally active DPP-4 inhibitors named sitagliptin (Table 1.7, 6). Compound 6 has been approved by the USFDA for the treatment of type 2 diabetes. X-ray crystallography studies revealed binding mode of sitagliptin with DPP-4 complex: (Fig. 1.6, 2) [125]
1. The trifluorophenyl group invades the S1-pocket
2. The trifluoromethyl group interacts with the side chains of residues Arg358 and Ser209.
3. The amino group generates a salt bridge with Tyr662 and the carboxylated groups of the two glutamate residues, Glu205 and Glu206.
4. The triazolopiperazine group clashes with the phenyl group of residue Phe357.

Further studies were carried out to optimize these compounds for the treatment of diabetes.

The 4-fluorobenzyl-substituted compound (Table 1.7, 7) was notable for its superior potency but showed poor oral bioavailability in mice [126]. 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 compound 7 in complex with DPP-4 enzyme. Also, the superior DPP-4 potency of compound 7 was thought to be due to the additional water molecule-bridged hydrogen bonding interaction between 4- fluorophenyl and Ser630.

Moving the nitrogen from the 2- to the 3-position in the triazolopiperazine ring of 6 yielded a new family of compounds and subsequent modification of the 2-position as well as the 8-position of the resulting bicyclic ring provided potent, selective and orally active DPP-4 inhibitors (Table 1.7, 8) [127].
Based on the observation that modification of the piperazine moiety of improved the DPP-4 potency by several folds, an imidazopiperidine analog (Table 1.7, 9) [128] 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 (Table 1.7, 10) [129].

Recently, a series of β-aminoacyl-containing cyclic hydrazine derivatives derived from moderately effective pyrazolidine-based DPP-4 inhibitors (Table 1.7, 11) [130]. One member of this series 12 showed potent in vitro activity, good selectivity and in vivo efficacy in mouse models [131]. 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 His126 and Ser209 and the carbonyl group of Glu205[132].

To identify a follow-up compound, a new series was developed by replacing the triazolopiperazine ring of 6 with various heterocycles. Accordingly, 1,4-diazepan-2-one derivative 14. 14 bound to the active site of DPP-4 indicated that the basic amino 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 groups filled the hydrophobic area above the side chain of Phe357 [133]. A further optimization with different substitution on the seven-membered ring led in several highly potent and selective, orally bioavailable and efficient DPP-4 inhibitors (Table 1.7, 15) [84,134].

**Table 1.7: Non Pyrrolidines derivative DPP-4 inhibitors [84]**

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Compounds</th>
<th>DPP-4 activity</th>
<th>References</th>
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1.3.1.2 Non-Peptidomimetics DPP-4 inhibitors

A high throughput screening programme identified DPP-4 inhibitors related to xanthines include purine, uracil, imidazole, pyrimidine, and pyridine analogs. 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 [86b].

Takeda San Diego (formerly Syrrx) recently disclosed aloglaptin (SYR-322) which bears a central uracil moiety (Table 1.8, 1) [135] In spite of their distinct structural features these inhibitors interacted well with the DPP-4 active site and serves as a core to direct key peripheral groups in a direction important to maintain several points of contact needed for potent DPP-4 inhibition [84].

Xanthine derivatives

A high throughput screen of the Abbott library of compounds for potential DPP-4 inhibitors revealed several xanthine analogs exhibiting low micromolar potency for the enzyme [126]. Systematic structural modification on the xanthine scaffold provided compound (Table 1.8, 2). By using a buty-2-nyl group a potent candidate, called BI-1356 or Linagliptin is discovered which is currently approved by the US FDA on 2 May 2011 for treatment of type II diabetes and holds the potential for once-daily treatment of T2D (Table 1.8, 3) [137].

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 inhibitor (Table 1.8,4) [138].

X-ray crystallography has shown that xanthine type binds the DPP-4 complex in a different way than other inhibitors (Fig.1.6, 3) [138].
The aminopiperidine substituent at C-8 of the xanthine scaffold occupies the S2 subsite. Its primary amine formed a network of charge-reinforced hydrogen bonds to Glu205, Glu206 and Tyr662.

The 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 p-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 pi-stacking its phenyl ring with the pyrrol ring of the amino acid side chain [84].

**Benzimidazole based inhibitors**

A series of potent benzimidazole-based inhibitors derived from a weak inhibitor of DPP-4, for example, 2-phenylbenzylamine has been reported (Table 1.8, 5) [139]. The ortho- position on the phenyl ring that appeared to be most promising for gaining affinity and possibility for interaction with nearby Arg125. There also seemed to be a small hydrophobic pocket at the para-position. Substituent’s larger than a methyl group at R1 seemed possible, provided that these larger groups would be able to pack along the backbone of Glu206 and Val207 while directing towards Arg358. Substitution at R2 seemed promising due to the possibility of creating an interaction with Ser209. Finally, derivatization at R5 looked highly promising due to the close proximity of Ser630 (Fig.1.6, 5) [140].

**Cyclohexylamine and aminopiperidine analogs**

Skapin G et al suggested that Central β-amino butyl amide moiety of sitagliptin could be replaced with a cyclohexylamine group to provide a rigid analog (Table 1.8, 6) [141]. A further replacement of the central cyclohexylamine in 3 with a 3-aminopiperidine (Table 1.8, 7), aminotetrahydropyran (Table 1.8, 8) provided potent, selective and orally bioavailable DPP-4
inhibitors [142-143]. Aminopiperidine- based natural product isolated from culture broth of Streptomyces sp. MK251-43F3 showed potent inhibition of DPP-4. (Table 1.8, 9) [144].

In another effort, starting with the HTS by Abbott laboratories identified cyclohexene-constrained phenethylamine (ABT-341) (Table 5, 10) as a candidate for clinical development [145] which is a potent and selective DPP-4 inhibitor having 2D similarity with sitagliptin. However, the 3D-structure is quite different (Fig. 1.6, 4) [146].

- The X-ray study indicated that the trifluorophenyl group of 10 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 was oriented towards a water molecule positioned for a bridging hydrogen-bonding interaction with the side chain of Arg669.
- A favorable hydrophobic interaction of the heterocycle with the side chain of Phe357 was observed.

A further SAR afforded a series of pyrrolidine-constrained phenethylamines as novel and potent DPP4 inhibitors (Table 1.8, 11) [147]. Expanding the five-membered pyrrolidine ring to a six membered one led to the identification of a series of piperidinone and piperidine-constrained phenethylamines as novel and potent DPP-4 inhibitors (Table 1.8,12)[148]. X-ray crystallographic data showed that halogenatedphenyl 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 13 as a model compound for optimization, a new series of DPP-4 inhibitor based on 2-aminobenzo[a]quinolizine was developed (Table 1.8, 14) [149-150].

**Quinazolinone/ pyrimidinedione derivatives**

By using structure-based design further lead was optimized by substituting aminopiperidine and cyanobenzyl groups on quinazolinone ring (Table 1.8, 15) [151]. 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 Val656, Tyr631, Tyr662, Trp659, Tyr666 and Val711) and interacted with Arg125. The carbonyl group participated in hydrogen bonding to the
backbone NH of Tyr631, and the quinazolin moiety was pi-stacked with Tyr547 (Fig. 1.6, 6) [151].

The Quinazolinone based structure has the necessary groups to interact with the active site on the DPP-4 complex. Quinazolinone based compounds interact effectively with the DPP-4 complex, but suffered from low metabolic half-life. Further optimization leads by replacing the quinazolinone with a pyrimidinedione and developed a potent inhibitor of DPP-4 named aloglaptin (Table 1.8, 16) that exhibited greater than 10,000-fold selectivity over the closely related serine proteases DPP-8 and DPP-9 [152].

Table 1.8: Non peptidomimetics DPP-4 inhibitors [84]
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<th>Chemical Structure</th>
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<td>( \text{IC}_{50} &lt; 10 \text{ nM} )</td>
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</table>
Fig. 1.6. Binding mode of different DPP-4 inhibitors [84]
1.3.2. Other DPP inhibitors

1.3.2.1 Selective Leads of DPP-8 and DPP-9

Inhibition of two closely related monomeric cytosolic proteases DPP-8 and 9 enzymes was reported by Lankas et al. to be associated with severe in vivo toxicity in animal models and to provoke attenuation of T-cell proliferation in human in vitro models [61a]. Later, Burkey et al found that at high dose, the toxicities of a selective DPP-8/DPP-9 inhibitor that were reported. To date no evidence of non-specific enzyme inhibition has been found, however, with current DPP-4 inhibitors at clinically relevant doses. Toxicities claimed to be associated with DPP-8 and DPP-9 inhibition in animals have not been observed in clinical trials of vildagliptin or sitagliptin for up to 1 year in duration. In addition, another study claims the observation that DPP-8 and DPP-9 are up-regulated in experimentally induced asthma and that these peptides specifically respond to the inflammatory stimulus [153]. Structurally distinct and selective inhibitors verify whether the reported toxicity observations are directly related to DPP-8/9 inhibition and to further study the clinical relevance of potential enzyme involved in pathologies [154].

The highest degrees of sequence homology between DPP-8 and DPP-9 on one side and DPP-4 on the other, makes selective DPP-8/9 inhibitor design a challenging task that hitherto has been the subject of only a limited number of publications [155,156].

Veken P. V. et al reported the DPP-8/9 inhibitors which were developed starting from allo-Ile-isoidoline as a lead [157]. Two types of structural modifications were investigated in order to obtain compounds with a maximally optimized DPP-8/9 potency-selectivity profile and to explore the possibility of discerning between DPP-8 and DPP-9 with a amino acylisoindoline derivatives [158].

- The first modification type consists of introducing substituents on the phenyl ring of isoindoline in order to allow for a steric and electronic scan of the P1 pocket of DPPs.
- Secondly, modified P2 site with different P2 residues, Isoleucine, lysine, and e-N-Z-protected lysine, three amino acids that were identified as useful building blocks for DPP8/9 inhibitors; along with 4-aminoproline were selected for this purpose.
- allo-isoleucine clearly out performs isoleucine as a P2 fragment in inhibitors of both DPP8 and 9, a 10-fold increase in DPP-8/9 potency and selectivity toward DPP II can be observed (Table 1.9, 1).
• In terms of DPP8/9 potency, introduction of a 4-substituent (Table 1.9, 2) proved to be less favorable than introducing the same substituent at the 5-position (Table 1.9, 3).
• Further, decrease in potency toward DPP-8 and 9 with increasing substituent sizes for both DPP8 and DPP-9, but not for DPP II (Table 1.9, 4).
• Side chains of Ile, allo-Ile, Lys, and Lys (Z) can be selected as useful P2 fragments for dipeptide-derived DPP-8 or DPP-9 inhibitors (Table 1.9, 5).
• Introduction of mono- or di aryl substitution (Table 1.9, 6, 7) or naphthyl (Table 1.9, 8) increased potency for DPP8/9; however, significant DPP II inhibition was also observed.
• Substitution of the allo-Ile moiety by a 4-aminoprolyl residue afforded some compounds with high nM activity against DPP II (Table 1.9, 9-13).

Table 1.9 DPP-8 and DPP-9 inhibitors [84]

<table>
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<td>&gt;50</td>
<td>0.111</td>
<td>0.2</td>
</tr>
<tr>
<td>---</td>
<td>----------------------</td>
<td>--------</td>
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<td>-------</td>
<td>-----</td>
</tr>
<tr>
<td>7</td>
<td><img src="image" alt="Structure 7" /></td>
<td>0.365</td>
<td>&gt;62.5</td>
<td>0.666</td>
<td>2.39</td>
</tr>
<tr>
<td>8</td>
<td><img src="image" alt="Structure 8" /></td>
<td>0.316</td>
<td>178</td>
<td>0.043</td>
<td>0.169</td>
</tr>
<tr>
<td>9</td>
<td><img src="image" alt="Structure 9" /></td>
<td>3.4</td>
<td>&gt;250</td>
<td>2.9</td>
<td>13.1</td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="Structure 10" /></td>
<td>1.3</td>
<td>&gt;100</td>
<td>0.91</td>
<td>5.77</td>
</tr>
<tr>
<td>11</td>
<td><img src="image" alt="Structure 11" /></td>
<td>41</td>
<td>&gt;500</td>
<td>24</td>
<td>67</td>
</tr>
<tr>
<td>12</td>
<td><img src="image" alt="Structure 12" /></td>
<td>3.5</td>
<td>&gt;1000</td>
<td>5.9</td>
<td>29.5</td>
</tr>
</tbody>
</table>
### 1.3.2.2 DPP-II Inhibitors

The most of compounds reported to have DPP II inhibitory activity were originally described as DPP-4 inhibitors. Pyrrolidinyl boronic acids such as Val-boroPro (Table 1.10,1) [159-160] pyrrolidinyl nitriles such as Ala-Pyr-2-CN (Table 1.10,2),[161] and aminoacylpyrrolidines and thiazolidines (Table 1.10,3-4) [162,163] show indeed a higher potency towards DPP-4. The inhibitory potential of these compounds for DPP-4 is well described and recently reviewed. Thioamide analogues of the latter compounds such as Ala-[CSN]-Pyrr (Table 1.10,5) and Ala-[CS-N]-Thia (Table 1.10,6) are the only DPP II–DPP-4 inhibitors described to have some selectivity towards DPP II [164].

Koen Augustyns et al explored Dab-Pip as a first potent and selective inhibitor for DPP II. Selectivity with respect to DPP-4 that is much higher than reported for the thioamides [165]. Dab-Pip (Table 1.10,7) with an IC\(_{50}\)=0.13 mM and a selectivity index of more than 7000 is the most active and by far the most selective DPP II inhibitor reported to date. Dab-Pip promises to be a useful compound to establish the physiological and biochemical role of DPP II as well as its potential as a therapeutic target. Its high selectivity will enable to differentiate between DPP II and DPP-4 in biological systems. Dab-Pip will also serve as a lead compound for the further development of DPP II inhibitors [84].

**Table 1.10 DPP II inhibitors [84]**

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Compound</th>
<th>DPPII/QPP inhibition</th>
<th>DPP inhibition</th>
<th>IV</th>
<th>SI</th>
<th>Ref.</th>
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<tr>
<td>13</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>0.29</td>
<td>&gt;250</td>
<td>0.29</td>
<td>2.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chemical Structure</td>
<td>Ki (nM)</td>
<td>IC₅₀ (nM)</td>
<td>Kᵢ (nM)</td>
<td>R²</td>
<td>p-value</td>
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<td>-----------</td>
<td>---------</td>
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<td>---------</td>
</tr>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Chemical Structure 1" /></td>
<td>125</td>
<td></td>
<td>2</td>
<td>0.016</td>
<td>160</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Chemical Structure 2" /></td>
<td>110</td>
<td>0.2</td>
<td>-</td>
<td>161</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Chemical Structure 3" /></td>
<td>24.7</td>
<td>0.218</td>
<td>0.0088</td>
<td>162</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="Chemical Structure 4" /></td>
<td>8.17</td>
<td>0.126</td>
<td>0.015</td>
<td>163</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><img src="image5.png" alt="Chemical Structure 5" /></td>
<td>1.43</td>
<td>47.6</td>
<td>33</td>
<td>164</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><img src="image6.png" alt="Chemical Structure 6" /></td>
<td>0.277</td>
<td>7.88</td>
<td>28</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><img src="image7.png" alt="Chemical Structure 7" /></td>
<td>0.13</td>
<td>&gt;1000</td>
<td>&gt;7592</td>
<td>166</td>
<td></td>
</tr>
</tbody>
</table>
1.3.2.3 FAP inhibitors

Fibroblast activation protein alpha (FAPalpha) is highly expressed in epithelial cancers and has been implicated in extracellular matrix remodeling, tumor growth, and metastasis. [166]. Natural substrates for FAP still have not been identified. FAP is not explicated by normal mature somatic tissues and benign epithelial tumors but is explicated on stromal fibroblasts in more than 90% of carcinomas of the breast, colon, ovarian, bladder and pancreas, making this a very specific target for potential anti-tumor agents [167]. Expression of FAP has also been concerned with the invading potential of melanoma cells and its presence has been associated with metastasis of colorectal tumors. Its presence has been shown to increase tumor formation in animal models [168].

The only FAP inhibitor explored to date is ValboroPro (PT-IOO, Talobostat which has been accounted to show potent anti-tumor activity in mice, slowing growth of syngeneic tumors and causing regression and rejection of tumors after oral administration. This compound is currently undergoing clinical trials as a treatment for cancer [169] which is a non-specific inhibitor of dipeptidyl peptidases, has been shown to be a potent inhibitor of DPP-4 [84].

1.3.3. Clinical progress

The DPP-4 inhibitors are orally active, small molecular weight drugs that inhibit >90% of plasma DPP activity for over 24 hours in vivo. After these promising preclinical studies, the first clinical proof-of-concept was obtained using the short-acting Novartis inhibitor, NVP-DPP728 [170]. When given twice or three times daily for four weeks in patients with relatively mild T2DM (mean HbA1c of 7.4%), both fasting and prandial glucose levels were lowered significantly, resulting in a reduction in HbA1c of 0.5%; despite the fall in glycemia, fasting and post-prandial insulin levels were sustained. NVP-DPP728 appeared to be well tolerated, with only minor adverse events being reported. However, some of these symptoms (pruritus and nasopharyngitis) seem to be drug- rather than class-specific, because they were not reported for another inhibitor, LAF237 (vildaglaptin), also developed by Novartis [171]. NVP-DPP728 has now been dropped in favor of vildaglaptin, which is longer-acting and suitable for once-daily administration.
In different clinical trials vildagliptin was tested both as monotherapy and in combination with other anti-diabetic agents like metformin, TZD, SU or insulin, and was found to improve glycemic control. Vildagliptin is weight-neutral and has a very low hypoglycaemic potential, explained by its remarkable ability to enhance both \( \alpha \)-cell and \( \beta \)-cell sensitivity to glucose. Therefore, vildagliptin offers a clinically important outcome when added to metformin with a twice daily dose regimen, taking advantage of its tight binding and slow dissociation characteristics that lead to a sustained overnight effect [172a].

Vildagliptin, at 50 and 100 mg, is safe and tolerable with incidence of adverse events similar to placebo group. In a large meta-analysis, vildagliptin was not associated with an increased risk of adjudicated CCV events relative to all comparators in the broad population of type 2 diabetes including patients at increased risk of CCV events [172b]. In second meta-analysis found that vildagliptin was not associated with increased risk of hepatic events or hepatic enzyme elevations indicative of drug-induced liver injury, pancreatitis, infections or skin-related toxicity [173c]. The most common adverse events associated with vildagliptin are headache, nasopharyngitis, cough, constipation, dizziness, and increased sweating [88d].

**Sitagliptin** developed by Merck is an orally-bioavailable selective DPP-4 inhibitor that was disclosed through the optimization of a class of \( \beta \)-amino acid- derived DPP-4 inhibitors. It lowers DPP-4 activity in a sustained manner following once daily administration, maintains the circulating levels of intact GIP and GLP1 following meals in both acute and chronic studies and reduces blood glucose levels without signs [173].

Sitagliptin, an oral once daily and highly selective DPP-4 inhibitor, was evaluated in clinical trials as a monotherapy, or as an add-on therapy with existing anti-diabetic agents like metformin. Sitagliptin provided effective fasting and postprandial glycemic control in a wide range of patients with T2DM. Markers of \( \beta \)-cell function also improved with Sitagliptin treatment [174]. It was generally well tolerated with an overall incidence of adverse experiences comparable to placebo, a low risk of hypoglycemia or GI disturbances, and a neutral effect on body weight [175]. Other reported side effects associated with the use of Sitagliptin include stuffy or runny nose, sore throat, upper respiratory tract infection, stomach pain, diarrhea and headache. Sitagliptin is superior to vildagliptin in terms of DPP-4 selectivity, and if any resulting off-target effects with the inhibition of other DPP subtypes prove to be associated with adverse events, then
Sitagliptin may have a competitive edge over vildagliptin. Sitagliptin was generally well tolerated and provided effective glycemic control in patients with T2DM and moderate to severe renal insufficiency, including patients with end stage renal disease (ESRD) on dialysis [176,84].

**Saxagliptin** (BMS-477118; (S)-3-hydroxyadamantylglycine-L-cis-4,5-methanoprolinenitrile) is a Nitrile-containing DPP-4 inhibitor. It is a potent inhibitor of DPP-4 (inhibition constant, $K_i=0.6-1.3$ nM) that displays slow-binding properties. Thus, kinetic studies have suggested that inhibition of DPP-4 by saxagliptin is a two-step process that involves the formation of a reversible covalent enzyme inhibitor complex, in which there is a slow onset of inhibition and a slow rate of inhibitor dissociation, resulting in the enzyme slowly equilibrating between the active and inactive forms. Saxagliptin is metabolized in vivo to form an active metabolite (BMS-510849), which is twofold less potent than the parent molecule. It appears that this metabolism is largely mediated via the cytochrome CYP3A in the liver; in subjects with liver failure, plasma concentrations of the metabolite are reduced (7%-33% lower) with increasing severity of hepatic impairment, while at the same time exposure to the parent drug increases (10%-77% higher) [177].

The combination of Saxagliptin with SU or TZD was well tolerated over the course of the studies, and significantly more number of patients were able to achieve the ADA recommended target (HbA1c < 7%). A NDA was submitted to the U.S. FDA and a Marketing Authorization Application to the European Medicines Agency (EMEA) in 2008. Phase 3 trials assessing the safety and efficacy of Saxagliptin involving more than 4,000 patients revealed minor adverse effects like urinary tract infection, nasopharyngitis, upper respiratory tract infection, influenza, diarrhea, back pain, headache, cough and hypertension. BMS and Astrazeneca have proposed the name ONGLYZA™ and await the approval from FDA and EMEA [178]. Adding saxagliptin to metformin XR provided superior glycaemic control compared with up titrating metformin XR without the emergence of additional safety concerns [179]. Saxagliptin 5-mg once-daily add-on therapy improves glycemic control in T2D patients on insulin alone or combined with metformin and is generally well-tolerated [84,180].

**Alogliptin** (code named SYR-322) is an orally administered, anti-diabetic drug in the DPP-4 inhibitor class, developed by Takeda Pharmaceutical Company. Alogliptin has been evaluated as monotherapy for 26 weeks in patients with poorly controlled diabetes mellitus at doses of
12.5mg or 25mg daily, which achieved reductions in HbA1c levels of 0.56% and 0.59%, respectively, and seemed to be well tolerated. Alogliptin, used at doses of 12.5 mg and 25mg once daily, also lowered patients’ blood glucose levels when it was added to existing therapy in patients who had responded inadequately to metformin alone. Patients experienced a 0.6% reduction in HbA1c level and a 1mmol/l reduction in fasting glucose level after 26 weeks of adding alogliptin to metformin therapy [181].

In December 2007, Takeda submitted a New Drug Application (NDA) for alogliptin to the United States Food and Drug Administration (USFDA) after positive results from Phase III clinical trials. In September of 2008, the company also filed for approval in Japan, winning approval in April 2010. The company also filed a Marketing Authorization Application (MAA) elsewhere outside the United States, which was withdrawn in June 2009 needing more data. The first USFDA NDA failed to gain approval and was followed by a pair of NDAs (one for alogliptin and a second for a combination of alogliptin and pioglitazone) in July 2011. In 2012, Takeda got a negative response from the USFDA on both of these NDAs [182].

Linagliptin (BI 1356, Ondero) is a novel, orally DPP-4 inhibitor currently in development by Boehringer Ingelheim. Unlike the other inhibitors, linagliptin is extensively protein-bound (>80% of the therapeutic dose). Because DPP-4 is expressed in various tissues but soluble DPP-4 is also present in plasma, binding to soluble DPP-4 may influence the PK of linagliptin [183]. High affinity, but readily saturable binding of linagliptin to its target DPP-4 accounted primarily for the concentration-dependent plasma protein binding at therapeutic plasma concentrations of linagliptin. As the DPP-4 binding capacity is saturated already at low doses, accumulation of linagliptin in tissues is unlikely, despite the long persistence of low amounts in the body. Linagliptin (10 mg/day) and metformin (850 mg three times daily) administered alone and concomitantly or co-administration of linagliptin had no apparent effect on metformin exposure. Linagliptin has shown no clinically significant pharmacokinetic interactions with commonly prescribed oral glucose lowering agents (metformin, pioglitazone, glibenclamide) and with drugs commonly used in patients with cardiac disorders (warfarin, digoxin). Apart from it Linagliptin showed an excellent tolerability, weight neutrality, showed no increased risk of drug-drug interactions and, importantly, there was no increased risk of hypoglycaemia attributed to linagliptin use in monotherapy, or combination therapy with metformin or pioglitazone. The
significant efficacy results together with the favorable safety profile shown by the drug linagliptin prove to be a Novel DPP-4 inhibitor (Table 2.7) [84,184].

**Dutogliptin** is a small molecule DPP-4 inhibitor that prevents DPP-4 from breaking down the GLP-1, thereby increasing the levels of this hormone in the digestive tract and the blood. The increased levels of GLP-1 stimulate insulin production by the pancreas β-cells and reduce glycogen production by the pancreas, both of which result in reduced blood glucose levels. In a double-blind, randomized, 12-week, 422 patient Phase 2b clinical trial, dutogliptin satisfied all primary and secondary endpoints, including statistically significant reductions in HbA1c when administered once-daily in combination with metformin and glitazone for the treatment of Type 2 diabetes. The trial evidenced dutogliptin’s excellent safety and tolerability profile [185]. Phenomix and partner Forest Laboratories started conducting several Phase 3 pivotal trials. In April 2010, Phenomix announced phase 3 data showing that dutogliptin’s efficacy is comparable to that of the currently approved DPP-4 inhibitors sitagliptin (Merck’s Januvia) and saxagliptin (BMS/AZ’s Onglyza) and dropped from the market [84].

**Table 1.11** Main clinically relevant pharmacokinetics differences between the five dipeptidylpeptidase-4 inhibitors [84,182]

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Sitagliptin MK-0431</th>
<th>Vildagliptin LAF237</th>
<th>Saxagliptin BMS-477118</th>
<th>Alogliptin SYR-322</th>
<th>Linagliptin BI 1356</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapeutic dose (mg/day)</td>
<td>100</td>
<td>2 × 50</td>
<td>5</td>
<td>12.5–25</td>
<td>5</td>
</tr>
<tr>
<td>Half-life</td>
<td>Long</td>
<td>Short</td>
<td>Short (but active metabolite)</td>
<td>Long</td>
<td>Very long</td>
</tr>
<tr>
<td>Administration</td>
<td>Once daily</td>
<td>Twice daily</td>
<td>Once daily</td>
<td>Once daily</td>
<td>Once daily</td>
</tr>
<tr>
<td>Active metabolite</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Fraction bound to protein (%)</td>
<td>Intermediate</td>
<td>Low</td>
<td>Very low</td>
<td>Rather low</td>
<td>High</td>
</tr>
</tbody>
</table>
Renal excretion | Predominant | Intermediate | Predominant | Predominant | Low
---|---|---|---|---|---
Dose reduction with renal impairment | Yes (25–50 mg) | No | Yes (2.5 mg) | Probably yes | Probably no
Drug – drug interactions | No | No | Yes | No | No
Dose reduction with CYP3A4 inhibitors | No | No | Yes (2.5 mg) | No | No

Omarigliptin (MK-3102) is a potent, long-acting oral antidiabetic drug of the DPP-4 inhibitor class used for once-weekly treatment of type 2 diabetes and currently under development by Merck & Co.[186] It inhibits DPP-4 to increase incretin levels (GLP-1 and GIP), which inhibit glucagon release, which in turn increases insulin secretion, decreases gastric emptying and decreases blood glucose levels. Omarigliptin) 25 mg and 12.5 mg tablets were approved by Japan's Pharmaceuticals and Medical Devices Agency (PMDA) on 28th Sept 2015 [187,188]. Japan was the first country to have approved omarigliptin. However, Merck has announced that the company will not submit marketing application in the US and Europe [189].

1.4. Critical issue

The biology of DPP-4 is an extremely complex field, and it is difficult to predict the consequences of long-term administration of DPP-4 inhibitors in humans. Several factors preclude a straightforward interpretation of experimental results. First, some biological actions of DPP-4 are unrelated to its enzymatic activity and thus are not necessarily affected by DPP enzyme inhibitors. Second, not all in vitro substrates of DPP-4 are necessarily biologically relevant substrates in vivo. Also, some biological effects, associated with DPP-IV-like enzymatic activity, may actually be related to other enzymes of DPP-4 family rather than to DPP-4 itself. The effect of nonselective inhibitors may therefore differ from that of selective DPP-4 inhibitors. The elucidation of several new members of the DPP-4 group will have consequences for the development of DPP-4 inhibitors. Compounds previously thought to be specific for DPP-4 could in fact be inhibitors of other members of the DPP-4 enzyme group. A number of DPP-4
inhibitors have recently been tested for selectivity to DPP-4, FAP, DPP 8, DPP 9 and DPP II enzymes. The DPP 8/9-selective inhibitor produced alopecia, thrombocytopenia, reticulocytopenia, multi organ histopathological changes, enlarged spleen and mortality in rats. This inhibitor also produced gastrointestinal toxicity in dogs. Furthermore, the DPP II-selective inhibitor produced reticulocytopenia in rats. However, investigation of the DPP-4 selective inhibitor demonstrated no apparent toxicity. DPP-4 is a pleiotropic enzyme which cleaves and generally inactivates a wide range of peptides that contain proline, alanine or serine that are penultimate to the N-terminus. Many of these are regulatory peptides that are involved in metabolic, vascular, neural, immunological and other physiological control processes. Thus, the action of DPP-4 inhibitors is fundamentally different to that of incretin hormones, acting on diverse substrates rather than through specific receptors on target tissues. This has prompted concerns relating to possible adverse effects of DPP-4 inhibitors.

DPP-4 exerts mainly stimulating effects, while its relation to malignancies is highly variable. Therefore, long-term inhibition of this enzyme could have serious side effects including immune dysregulation or increased risk of cancer. Although the data on the effects of DPP-4 inhibitors in humans are scarce, the increased risk of infections and the tendency towards a higher incidence of some tumours fall in line with experimental evidence suggesting the possibility of their adverse immunological and oncological effects [84].

1.5 References

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Chapter 1

Background Overview


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Chapter 1

Background Overview


Chapter 1  

Background Overview


Chapter 1  

Background Overview


Chapter 1  

Background Overview


Chapter 1  

Background Overview


Chapter 1

Background Overview


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Chapter 1  

Background Overview


