Chapter I:

Introduction

"Imagination is more important than knowledge."-Einstein

1. Introduction

1.1. Metabolic syndrome

The term “Metabolic syndrome” (MS) refers to a cluster of medical disorders such as, hyperinsulinemia, hyperglycemia, dyslipidemia, high blood pressure, insulin resistance and obesity. Metabolic syndrome increases the risk of developing cardiovascular diseases and diabetes. The incidences of metabolic syndrome have reached global epidemic proportions **[1-2]**. Metabolic syndrome is also known as metabolic syndrome X, cardiometabolic syndrome, syndrome X, insulin resistance syndrome, Reaven’s syndrome (named after Gerald Reaven) and CHAOS (in Australia).

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health and is a major risk factor for developing the other metabolic diseases. Obesity as a metabolic disorder was reported in 1947 **[3]** and subsequently described as a syndrome, which comprised of hypertension, hyperglycemia **[4]**. After a gap of four decades, in 1988 a cluster of risk factors for diabetes and cardiovascular diseases were defined and was named as Syndrome X **[5]**. Consequently, patients with metabolic syndrome are at increased risk of micro- and macro-vascular complications (e.g. coronary artery disease (CAD), stroke, renal failure, blindness and lower extremity amputation) as diabetes progress **[6-7]**.

1.2. Diabetes

Diabetes mellitus is a group of metabolic diseases in which hyperglycemia arises as a result of a relative or absolute deficiency of insulin secretion, resistance to insulin action, or both **[8]**. Diabetes is an ailment in which the body does not produce or properly use insulin. Insulin is a regulatory hormone required for energy management. The cause of diabetes continues to be anonymity, although both genetics and environmental factors such as obesity and lack of exercise appear to play roles. Diabetes mellitus is a major and growing public health problem throughout the world, with an estimated worldwide prevalence of 220 million people in 2010 and it is expected to increase to 366 million people by 2030 **[9]**. Many people also have other abnormalities of glucose metabolism (sometimes called “prediabetes”) manifest either as impaired fasting glucose (IFG) levels or as impaired glucose tolerance

(IGT). The criteria for diagnosis of diabetes and prediabetes conditions are summarized in **Table 1**.

Table 1. Diagnostic Criteria for Diabetes Mellitus and Prediabetes conditions

Types of Diabetes	Pre prandial fasting plasma glucose mg/dL (mmol/L)	Post-prendial plasma glucose mg/dL (mmol/L)
Normal	**< 110 (< 6.1)**	**< 140 (< 7.8)**
Impaired fasting glucose (IFG)	**≥ 100 (≥ 6.1) &< 120 (< 7.0)**	**< 140 (< 7.8)**
Impaired glucose tolerance (IGT)	**< 126 (< 7.0)**	**≥ 140 (≥ 7.8)**
Diabetes mellitus	**≥ 126 (≥ 7.0)**	**≥ 200 (≥ 11.1)**

Majority of diabetic patients can be treated with the agents that reduce hepatic glucose production (glucagon antagonist), reduce glucose absorption from gastrointestinal track (GIT), stimulate β-cell function (insulin secretagogues) or with the agents that enhance the tissue sensitivity of the patients towards insulin (insulin sensitizers). The drugs presently used to treat diabetes include α-glucosidase inhibitors, insulin sensitizers, insulin secretagogues and K_{ATP} channel blockers **[10]**. However, almost one-half of diabetic subjects lose their response to these agents, over a period of time and thereby to insulin therapy. Insulin treatment has several drawbacks, it is injectable, causes hypoglycemia and weight gain **[11]**.

1.2.1. Types of diabetes

Although several pathogenic processes are involved in the development of diabetes, the vast majority of cases fall into two main categories: Type 1 diabetes

and Type 2 diabetes. Gestational diabetes, yet another type of diabetes diagnosed in pregnant women.

1.2.1.1. Type 1 diabetes mellitus (T1DM)

Type 1 diabetes occurs usually due to an immune-mediated destruction of pancreatic islet β-cells with consequent insulin deficiency. Although usually having an abrupt clinical onset, the disease process unfolds slowly, with progressive loss of β-cells. In T1DM, >90% β-cells are destroyed by autoimmune-mediated islet cell destruction, and hence T1DM patient rely on insulin injections for survival. T1DM is usually diagnosed in children and young adults, it is also called as juvenile diabetes or insulin-dependent diabetes mellitus (IDDM). Conditions associated with T1DM include hyperglycemia and ketoacidosis (**Figure 1**). T1DM increases risk for many serious complications. Some complications of T1DM includes: heart disease (cardiovascular disease), blindness (retinopathy), nerve damage (neuropathy), kidney damage (nephropathy), foot and skin complications and depression.

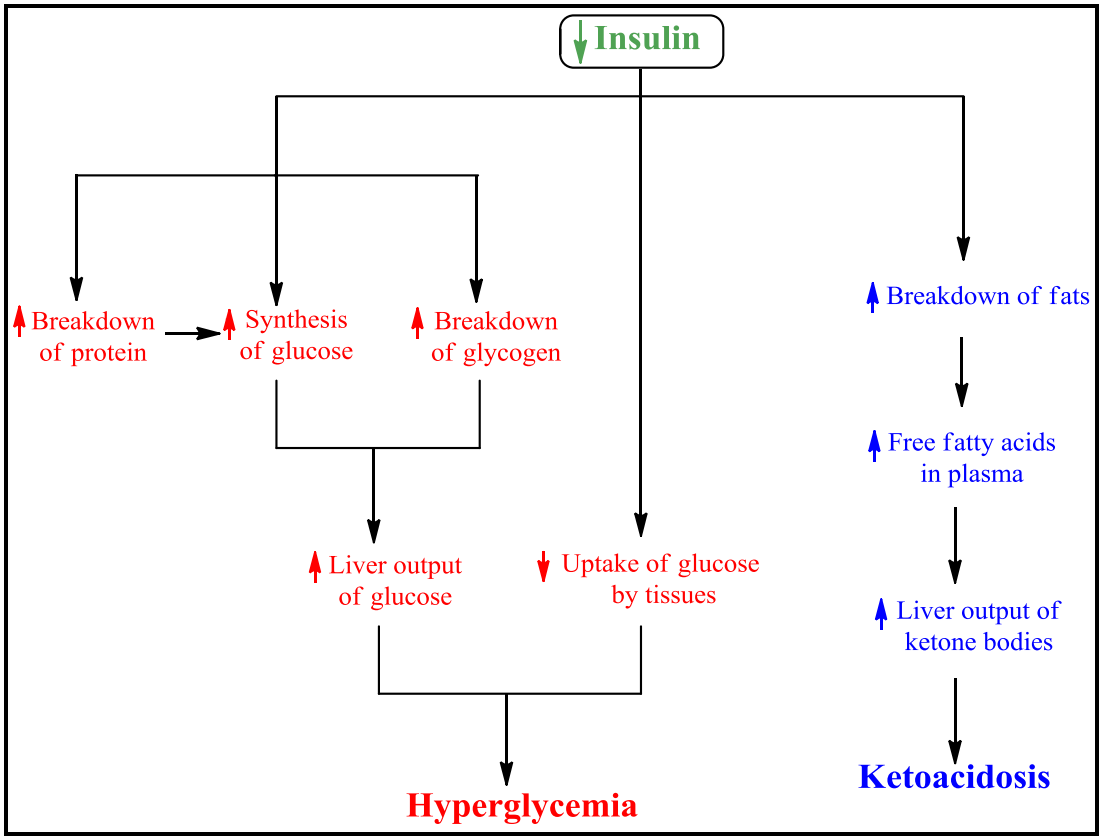


Figure 1. Metabolic Changes in T1DM

1.2.1.2. Type 2 diabetes mellitus (T2DM)

Type 2 diabetes mellitus (T2DM), the most common type of diabetes usually occurs due to insulin resistance, defect in the insulin production or increase in the hepatic glucose production and is usually associated with dyslipidemia, hypertension and obesity **[12]**.

In T2DM, >50% of β-cells are already lost at the time of diagnosis continue to decline throughout the course of T2DM, mainly due to apoptosis **[13]**. As depicted in **Figure 2**, insulin resistance arises as a consequence of multiple factors such as sedentary lifestyle, aging and obesity which results in hyperglycemia, blood pressure elevation, and dyslipidemia. The important contributing factors for T2DM include resistance to insulin, increased hepatic glucose production, decreased insulin-mediated glucose transport into adipose tissues and impaired β-cell function leading to loss of early phase of insulin release.

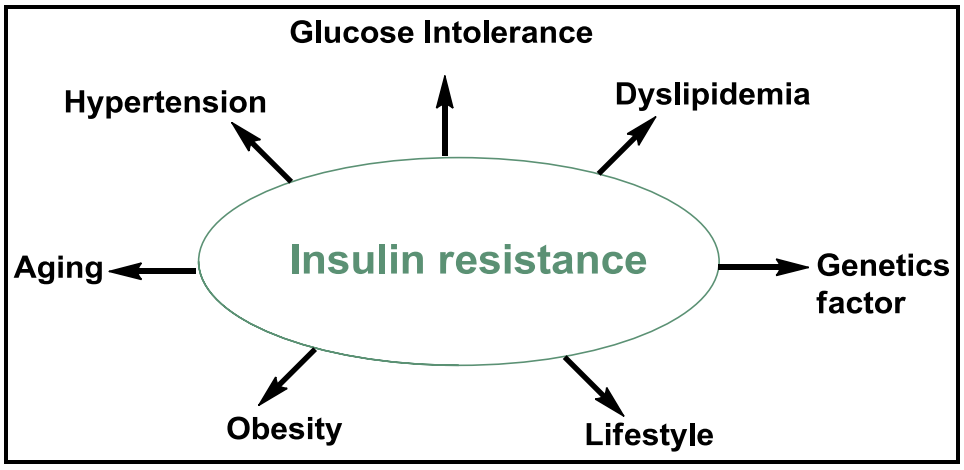


Figure 2. Causes and consequences of Insulin resistance

In T2DM, patients begin with insulin resistance and often treated with various oral antihyperglycemic agents; however, over a period of time, almost one-half of T2DM subjects lose their response to these agents and thereby require insulin therapy **[14]**. The decline in β-cells in T2DM drives the progressive deterioration in glycemic control and develops secondary complications.

1.2.1.3. Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus (GDM) is a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy (especially during third trimester of pregnancy). Gestational diabetes occurs when

the body of a pregnant woman does not secrete excess insulin required during pregnancy leading to increased blood sugar levels.

Gestational diabetes generally has few symptoms and it is most commonly diagnosed by screening during pregnancy. Diagnostic tests detect inappropriately high levels of glucose in blood samples. Gestational diabetes affects 3-10% of pregnancies, depending on the population studied **[15]**. In general, babies born to mothers with gestational diabetes are typically at increased risk of problems such as being large for gestational age (which may lead to delivery complications), low blood sugar, and jaundice. Gestational diabetes is a treatable condition and women who have adequate control of glucose levels can effectively decrease these risks **[16]**.

1.3. Pathogenesis of T2DM

The pathological sequence of T2DM is complex and involves many different elements that act together and make T2DM condition more complex (**Figure 3**). As described earlier, T2DM is characterized by varying degree of insulin resistance and insulin deficiency. It isthought that the earliest defect in the pathogenesis of T2DM is impaired insulin action or insulin resistance.

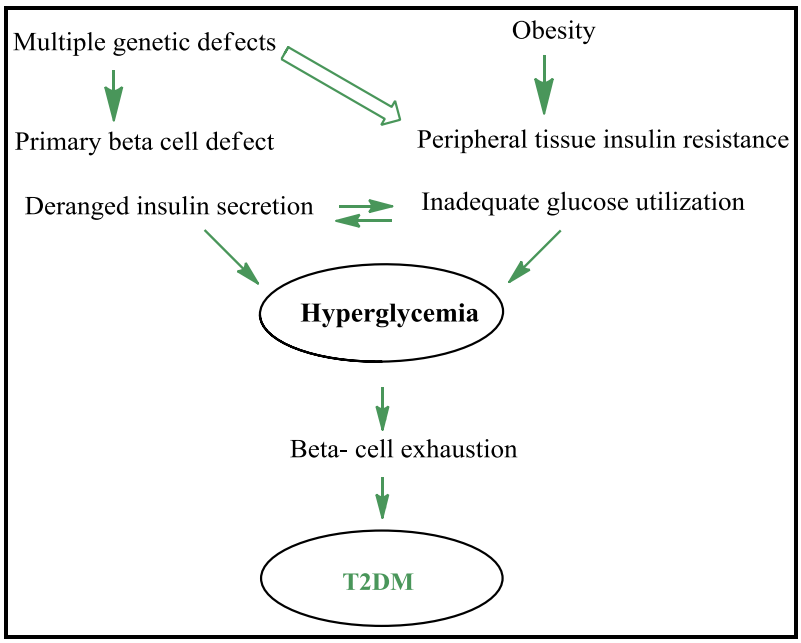


Figure 3. Proposed Pathogenesis of Type 2 Diabetes

Resistance to the action of insulin results in impaired insulin mediated glucose uptake by muscles, incomplete suppression of hepatic glucose output and impaired triglyceride uptake by fat. To overcome the insulin resistance, beta islet cells will increase the amount of insulin secreted.

Along with different factors, both endogenous hormone Glucagon like peptide-1 (GLP-1) and glucagon play an important role in pathogenesis of T2DM **[17]**. GLP-1 (7-36) amide is a product of the preproglucagon gene, which is secreted from intestinal L-cells, in response to the ingestion of food. Endogenous GLP-1 binds to a membrane GLP-1 receptor. As a consequence of this, insulin release from the pancreatic β-cells is increased **[17]**. The major problem of GLP-1 is its shorter half life. Glucagon (29 amino acid peptide) hormone is produced from proglucagon in pancreatic α-cell by prohormone convertase-2(PC2)**[17]**. The main physiological role of glucagon is to stimulate hepatic glucose output, thereby leading to hyperglycemia. Therefore two defects, insulin resistance and insulin deficiency are responsible factors for the development of T2DM.

1.4. Current & Newer therapies for the treatment of T2DM

1.4.1. Current therapies for the treatment of T2DM

The cornerstone of treatment and prevention of T2DM is lifestyle modification through increased physical activity and attention to food intake, particularly among the subjects, where in weight loss is the principal goal. When lifestyle modifications do not result in normalization or near normalization of metabolic abnormalities, pharmacological therapy is required.Based on route of administration, current therapies are divided in two groups, (1) Injectable and (2) Oral therapies.

1.4.1.1. Injectable therapies for the treatment of T2DM

Insulin facilitates glucose entry into adipose tissues, muscles, and liver by stimulating several enzymatic reactions that start at the insulin receptors. The stimulation of an intrinsic tyrosine kinase of the insulin receptor results in an increase in membrane phosphorylation that consequently increases the membrane permeability to glucose through a complicated cascade of intracellular events. Currently available injectable analogues are divided into two groups, (1) insulin analogues and (2) incretin mimetics as shown in **Table 2**. Further, insulin analogues are sub-divided into three groups depending upon their duration of action.

As described earlier, in T1DM >90% β-cells are destroyed and hence T1DM patients rely on insulin injection for survival. While T2DM begins with insulin resistance and over a period of time almost one-half of T2DM patients lose their response to antihyperglycemic agents and thereby require insulin therapy **[13]**.

Table 2. Insulin analogues and incretin mimetics.

Drugs	Source	Trade names
Insulin product **Fast acting:** **Regular**	Recombinant DNA Pork	Humulin R, Novolin R Iletin II
Lispro	Recombinant DNA	Humalog, Humalog, Lispro-PFC
Aspart	Recombinant DNA	Novolog, Flexpen
Glulisine	Recombinant DNA	Apidra
Intermediate acting: **Isophane Insulin**	Pork	Iletin II NPH Purified Pork
Isophane Insulin	Recombinant DNA	Humulin N, Novolin N
Insulin zinc	Pork	Iletin II Lente
Insulin humane zinc	Recombinant DNA	Humulin L, Novolin Ge Lente
Long acting: **Extended insulin human zinc suspension**	Recombinant DNA	Humulin U, Novolin ge Ultralente
Insulin glargine	Recombinant DNA	Lantus
Incretin mimetics **Exenatide**	Saliva of the Gila monster	Byetta
Liraglutide	----	Victoza

The effect of insulin on glucose uptake and metabolism is shown in **Figure 4**. Secreted insulin binds to its receptor, which in turn starts many protein activation cascades. These cascades include translocation of Glut-4 transporter to the plasma

membrane and influx of glucose, glycogen synthesis, glycolysis and fatty acid synthesis. The main action of insulin on cells includes increased glycogen synthesis, increased fatty acid synthesis, increased esterification of fatty acid, decreased proteolysis, decreased lipolysis and decreased gluconeogenesis**[18]**.

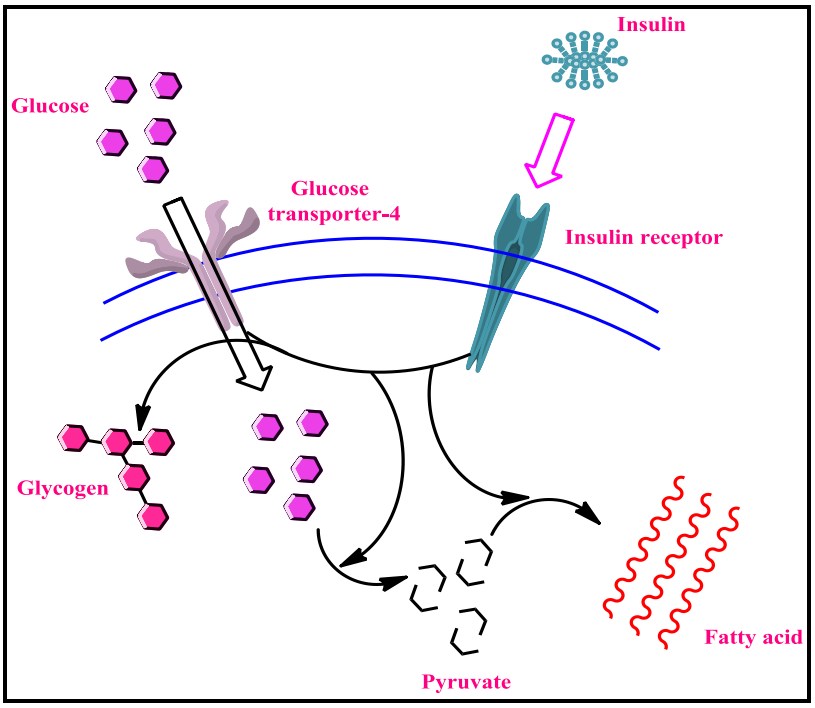


Figure 4. Mode of action of insulin on glucose uptake

Exenatide and liraglutide belong to group of incretin mimetics. Exenatide is a 39-amino-acid peptide, an insulin secretagogues, with glucoregulatory effects. It bears a 50% amino acid homology to GLP-1 and it has a longer half-life. Liraglutide is an acylated human GLP-1 receptor agonist, with a 97% amino acid sequence identity to endogenous human GLP-1(7-37).

Liraglutide is stable against metabolic degradation by both peptidases,dipeptidyl peptidase IV (DPP-IV) and neutral endopeptidases (NEP). Exenatide augments pancreas response **[19]** (i.e. increases insulin secretion) in response to eating meals; the result is the release of a higher, more appropriate amount of insulin that helps lowering the rise in blood sugar. It also suppresses pancreatic release of glucagon in response to eating. Exenatide helps to slow down gastric emptying and reduces liver fat content. While liraglutide acts in a glucose-dependent manner, meaning it will stimulate insulin secretion only when blood glucose levels are higher than normal. It has the potential for inhibiting apoptosis and stimulating regeneration of beta cells. It

decreases appetite and maintains body weight and lowers blood triglyceride levels**[20]**.

These injectable therapies (Insulin &incretin mimetics) have several drawbacks, it is injectable, produces hypoglycemia and causes weight gain, which is believed to be a potential cause for the development of diabetes complications **[21]**. Thus, there is an urgent need to develop some oral antihyperglycemic agents that can complement with the existing injectable therapies.

1.4.1.2. Oral antidiabetic agents for the treatment of T2DM

Before 1995, sulfonylureas were the only oral antidiabetic agents available for the treatment of T2DM. Since 1995, there has been an explosion of introduction of new classes of pharmacologic agents. Currently available oral antidiabetic therapies includes agents which cause insulin production (sulfonylureas, secretagogues); agents which decrease hepatic glucose production (biguanides); agents which act as insulin sensitizers (glitazones) and R-glucosidase inhibitors, which are listed in **Table 3**.

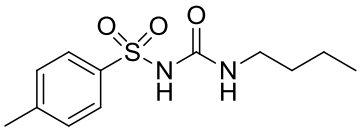
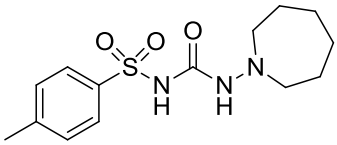
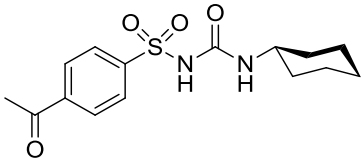
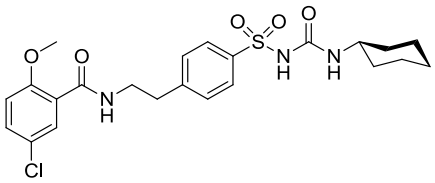
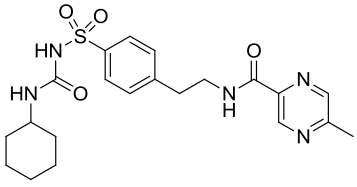
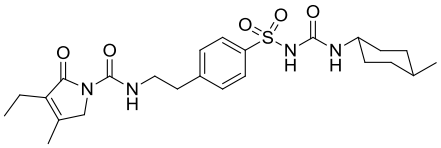
The usual treatment strategy in T2DM is to start with either metformin **[24]** or a secretagogue **[23]**. If adequate control is still not achieved, the second step is to add a complementary drug, i.e. one working by a different pathway. The most common such combination is metformin plus a secretagogue. If adequate glycemic control is still not attained, the choice is to add a third class of oral drugs (e.g. glitazone or glucosidase inhibitors).

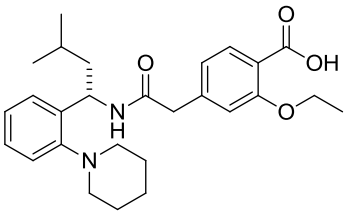
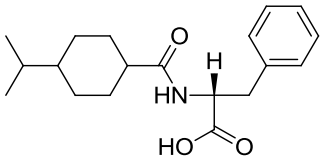
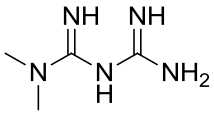
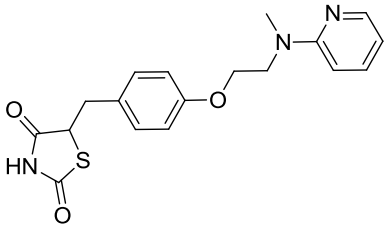
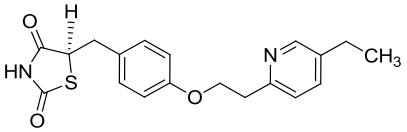
However, most of the oral antihyperglycemic agents are also associated with side effects and adverse events. In case of sulfonylureas, they work by stimulating endogenous release of insulin and exhibit hypoglycemia as the major side effects. The other adverse effect consists of digestive manifestation (nausea, epigastic pain, liver pain) and of hematological manifestations (pancytopenia, autoimmune hemolytic anemia, thrombocytopenia) **[22]**.

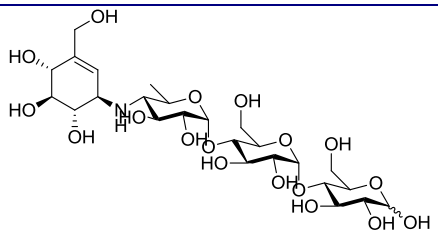
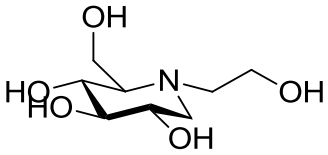
Biguanides reduce hepatic glucose output and increase uptake of glucose by periphery. They are associated with side effects such as digestive manifestations especially epigastric pain and diarrhea. Lactic acidosis is another adverse effect associated with biguanides. The major side effects associated with glitazones are mild edema of the lower limbs, through the loss of elimination of salt and water. The other adverse effect is decrease in hemoglobin, with the appearance of anemia.

Glitazones can also cause hypercholesterolemia and triglycerides disorders. Alpha glucosidase inhibitors exhibit side effects such as weight gain, abdominal bloating, flatulence, abdominal discomfort and diarrhea.

Table 3. Orally administered antidiabetic agents for T2DM treatment

Drug class	Agent(Brand name)	Structure
Sulfonylureas[22]		
First generation	Tolbutamide (Oramide, Orinase)	
	Tolazamide (Tolamide, Tolinase)	
Second generation	Acetohexamide (Demylor)	
	Glyburide (Micronase, Diabeta)	
	Glipizide (Glucotrol)	
Third generation	Glimepride (Amaryl)	
M.O.A- Stimulating insulin production by inhibiting the K_{ATP} channel in pancreatic β- cells		

Non-Sulfonylurea[23] **Secretagogues**	Repaglinide (Prandin)	
	Nateglinide (Starlix)	
M.O.A-enhance insulin secretion in pancreatic β- cells		
Biguanides[24]	Metformin (Glucophage)	
M.O.A- Decreases insulin resistance in liver		
Insulin receptors sensitizers[25]	Rosiglitazone (Avandia)	
	Pioglitazone (Actors)	
M.O.A- stimulates PPAR-γ and PPAR-α, reduces insulin resistance in the liver and peripheral tissues		

Alpha-Glucosidase Inhibitors[26]	Acarbose (Prelose)	
	Miglitol (Glyset)	
M.O.A- Reduces intestinal glucose absorption in G.I tract		

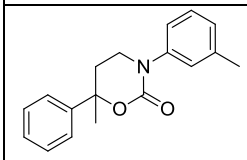
1.4.2. Newer therapies for the treatment of T2DM

As described earlier, most of the injectable and oral therapies exhibit serious side effects and adverse events such as hypoglycemia, GI side effects, lactate production, fluid retention, hepatotoxicity, allergic reaction and cardiovascular effects. Also long term use exhibits side effects such as body weight gain and progressive loss of β-cell function. To overcome the side effects and safety concern of these current drugs, several newer pharmacologic approaches have been developed for the safe and effective treatment of T2DM. Currently several new therapies are in various stages of clinical development for the treatment of T2DM as shown in **Table 4**.

Table 4. New therapies under clinical development for the treatment of T2DM

Target	Structure/ Name	Company	Clinical status	Ref.
GLP-1 agonist*	ZYOG1	Zydus Cadila	Phase I	**[27]**
	PB-1023	PhaseBio	Phase II	**[28]**
	NNC-0113-0987	Novo Nordisk	Phase I	**[29]**
	NOX-G15	Noxxon	Preclinical	**[30]**

Target	Structure/ Name	Company	Clinical status	Ref.
DPP-IV Inhibitors *	Sitagliptin(MK-0431)	Merck	Launched	**[31]**
	Vidagliptin (LAF-237)	Novartis	Launched	**[32-35]**
	Saxagliptin(BMS-477118)	Astra-zeneca/BMS	Launched	**[36-38]**
	Linagliptin(BI-1356)	Boehringer Ingelheim/Eli Lilly	Launched	**[39-40]**
	Alogliptin(SYR-322)	Takeda	Launched	**[41]**
	Anagliptin(SK-0403)	Kowa JW Pharmaceutical	Launched	**[42-43]**
	Gemigliptin(LC15-0444)	LG Life Sciences	Launched	**[44-45]**
	Teneligliptin(MP-513)	Mitsubishi Tanabe Pharma	Launched	**[46-48]**
	Melogliptin(GRC-8200)	Glenmark	Phase II	**[49]**
	Gosogliptin(PF-734200)	SatRx	Phase II	**[50-52]**
	Trelagliptin(SYR-472)	Takeda/Furiex	Phase III	**[53]**
	ARI-2243	Arisaph Pharmaceutical	Phase I	**[54-55]**
	Omarigliptin(MK-3102)	Merck & Co	Phase III	**[56]**
	Evogliptin(DA-1229)	Dong-A	Phase II	**[57-58]**

Target	Structure/Name	Company	Clinical status	Ref.
FBPaseinhibitors**	CS-917	Metabasis Therapeutics	Discontinued(Phase II)	**[59]**
GSK-3 inhibitors**	DM-199	DiaMedica	Preclinical	**[60]**
	DM-204	DiaMedica	Preclinical	**[61]**
11β-HSD-1 inhibitors**	BMS-770767	Bristol-Mayers Squibb	Phase II	**[62]**
	RG-4929	Roche	Phase II	**[63]**
	RG-7234	Roche	Phase I	**[63]**
	BVT-3498	Biovitrum	Discontinued Phase II	**[63]**
	PF-915275	Pfizer	Discontinued Phase I	**[64]**
		Vitae Pharmaceutical	Preclinical	**[65]**
	INCB-13739	Incyte Corporation	Phase IIb	**[66]**
	INCB-20817	Incyte Corporation	Discontinued Phase I	**[67]**
	AZD4017	AstraZeneca	Phase I	**[68]**
	AMG-221	Biovitrum	Phase I	**[69]**

Target	Structure/Name	Company	Clinical status	Ref.

SGLT2 Inhibitors**	Canagliflozin(TA-7284)	Mitsubishi Tanabe Pharma	Under development	**[70]**
	Dapagliflozin(TA-7284)	Bristol-Myers Squibb	Pre-registration	**[71]**
	Empagliflozin(BI-10773)	Boehringer Ingelheim	Phase III	**[72-73]**
	Tofogliflozin(RG-7201)	Roche	Phase III	**[74]**
	Remogliflozin etabonate(KGT-1681)	Kissei	Phase II	**[75-78]**
	Ipragliflozin(ASP-1941)	Kotobuki Pharmaceutical	Phase III	**[79]**
	Luseogliflozin(TS-071)	Taisho	Phase III	**[80-81]**
	SBM-TFC-039	Sirona Biochem	Preclinical	**[82]**
	THR-1474	Theracos	Phase II	**[83]**
	Ertugliflozin(PF-04971729)	Pfizer	Phase II	**[84-86]**
	EGT-0001442	Theracos	Phase II	**[87]**

Target	Structure/ Name	Company	Clinical	Ref.

			status	
PPARγ dual agonist**	Efatutazone(CS-7017)	Daiichi Sanky	Phase II	**[88]**
	Chiglitzar(CS-0038)	Chipscreen Biosciences	Phase III	**[89]**
	Libeglitazone(CKD-501)	Chong Kun Dang	Phase III	**[90-91]**
	PPARγ agonist	Takeda	Preclinical	**[92-93]**
PTP-1B Inhibitors***	Ertiprotafib	Wyeth Pharmaceutical	Discontinued	**[94]**
	TTP-814	Trans Tech Pharma	Phase II	**[95]**
	ISIS-PTP1BRx	Isis Pharmaceutical	Phase I	**[96]**
	PTP1B Inhibitors	Advinus	Preclinical	**[97]**

GLP-1: Glucagon-like peptide 1; **DPP-IV**: Dipeptidyl peptidase IV; **PPARγ:** Peroxisome proliferators-activated receptor gamma; **11β-HSD-1**:11β-hydroxysteroid dehydrogenase type-1; **FBPase**: Fructose 1 6-bisphosphatase ; **GSK-3**: Glycogen synthase kinase-3; **SGLT2**: Sodium-dependent glucose cotransporters; **PTP-1B**: Protein tyrosine phosphatases 1B.***** Injectable; ****** Oral

As described in **Table 4**, currently several new therapies are in various stages of clinical development for the treatment of T2DM. Among these new therapies, DPP-IV inhibitors and PTP-1B inhibitors are most promising. Endogenous dipeptidyl peptidase type IV (DPP-IV) enzyme has been shown to be a key physiological regulator of incretin activity. DPP-IV is a serine protease and *in vivo*, it inactivates both the incretin hormones GLP-1 & Gastric inhibitory peptide (GIP) **[98-100]**, which in-turn stimulates glucose dependent insulin secretion. Thus, inhibition of DPP-IV activity results in increased level of intact bioactive GIP and GLP-1 peptides, which cause an increase in the amount of post prandial insulin release from β-cell of the islets, thereby, it acts as an antidiabetic agent.

Protein tyrosine phosphatase-1B (PTP-1B) enzyme leads to dephosphorylation of insulin receptor and acts as a negative regulator in insulin signaling pathway **[101-102]**. The two different reports suggest that PTP-1B knock-out mice showed improved insulin sensitivity **[103-104]**. Thus, inhibition of PTP-1B could be the most effective and safe target for the treatment of T2DM.

Among the newer therapies, GLP-1 agonists have not been very successful due to little risk of hypoglycemia **[105]**, gastrointestinal side effects **[106]** and their injectable route of administration. Main side effect of PPARγ dual agonist is water retention, leading to edema, an increased risk of coronary heart disease **[107-108]**. Compounds of FBPase inhibitors show hypoglycemia as a major side effect **[73]**. Inhibitors of GSK-3 are associated with side effects such as neuronal disorders **[109]**, while inhibitors SGLT2 and 11β-HSD-1 have low potential for hypoglycemia.

As discuss earlier, inhibitors of DPP-IV and PTP-1B are the most upcoming therapies for T2DM treatment. Many PTP-1B inhibitors are being manufactured and studied. However, the drawbacks of PTP1B inhibitors include their low affinity, selectivity and membrane permeability**[110-111]**.While, treatment of T2DM with DPP-IV inhibition is clinically proven therapy and several DPP-IV inhibitors are in market **[112]**.Long-term inhibition of DPP-IV improves glucose tolerance and preservesislet function in mice**[113]**..Apart from T2DM, DPP-IV inhibitors are also believed to be useful for several other related disease conditions such as diabetic dyslipidemia, conditions of impaired glucose tolerance (IGT), conditions of impaired fasting plasma glucose (IFG), metabolic acidosis and ketosis, appetite regulation and obesity**[114-118]**.

Hence, DPP-IV inhibitors are considered as one of the best validated biological target for T2DM.

Overall among different therapies such as agents which cause insulin secretion, agents which decrease hepatic glucose production and agents which decrease insulin resistance, it can be concluded that the insulin resistance is the most dominant cause for T2DM, hence increasing insulin sensitivity can form a promising therapy for the treatment of T2DM. Various drugs are available that directly or indirectly increase insulin sensitivity but their high cost, selectivity and route of administration are limiting factors. This invites research to develop small molecule based DPP-IV inhibitors which could be safe and cost effective. There are currently eight DPP-IV inhibitors approved worldwide with several more on the way.

Though efficacious and safer treatment are available in the market under DPP-IV tag, considering the seriousness of the growing prevalence of diabetes worldwide, particular research efforts being made from both the academia and the pharmaceutical industry to develop long acting DPP-IV inhibitors. Thereby to meet the regulatory compliances of USFDA for the drug evaluated with regards to their cardiovascular safety as well as to additional effects that DPP-IV inhibition may exert other than antidiabetic effect.

The research presented in this thesis focus on the synthesis, biological evaluation of potent, selective and orally bioavailable DPP-IV inhibitors. In the next section, an overview on DPP-IV inhibitorsare presented.

1.5.Introduction to Dipeptidyl Peptidase IV (DPP-IV) inhibitors

1.5.1.DPP-IV and their importance

The incretin mimeticsglucagon-like peptide 1 (GLP-1) and glucose-dependent gastric inhibitory polypeptide (GIP) are released from the L-cell of intestineupon injestion of food **[119-122]**. Thesehormones regulate insulin secretion in a glucose-dependent manner. GLP-1 has many roles in the human body; it stimulates insulin biosynthesis, inhibits glucagon secretion, slows gastric emptying, reduces appetite and stimulates regeneration of islet β-cells. GIP and GLP-1 have extremely short plasma half-lives, get inactivated by DPP-IV enzyme**[123-124]**.

Dipeptidyl peptidase-IV (DPP-IV), also known as adenosine deaminase complexing protein 2 or CD26 (cluster of differentiation 26) (Enzyme Commission no.: E.C. 3.4.14.5). Itis a serine protease enzyme,which cleaves the N-terminal dipeptide (X-Ala or X-Pro), from target polypeptides, such as chemokines and peptide hormones**[125]**. DPP-IV a multifunctional type II cell surface glycoprotein, is widely expressed in a variety of cell types, particularly on differential epithelial cells of the intestine, liver, prostate tissue, corpus luteum, and kidney proximal tubules**[126-127]** as well as leukocyte subsets**[128]**, such as T-helper lympho- cytes, and subsets of macrophages **[129]**, and a soluble form is reported to be present in plasma and urine **[130]**.Endogenous dipeptidyl peptidase IV (DPP-IV) enzyme has been shown to be a key physiological regulator of incretin activity. In vivo, DPP-IV enzyme inactivates both the incretin hormones (GLP-1 & GIP), which in-turn stimulates glucose dependent insulin secretion. DPP-IV enzyme selectively cleaves first two amino acids (His-Ala) of 29 amino acid GLP-1 peptide and thereby makes it inactive which are eliminated via kidney (**Figure 5**)**[131]**. Potential inhibition of DPP-IV

enzyme may prolong half life of these incretins, thereby helps in glucose homeostasis.

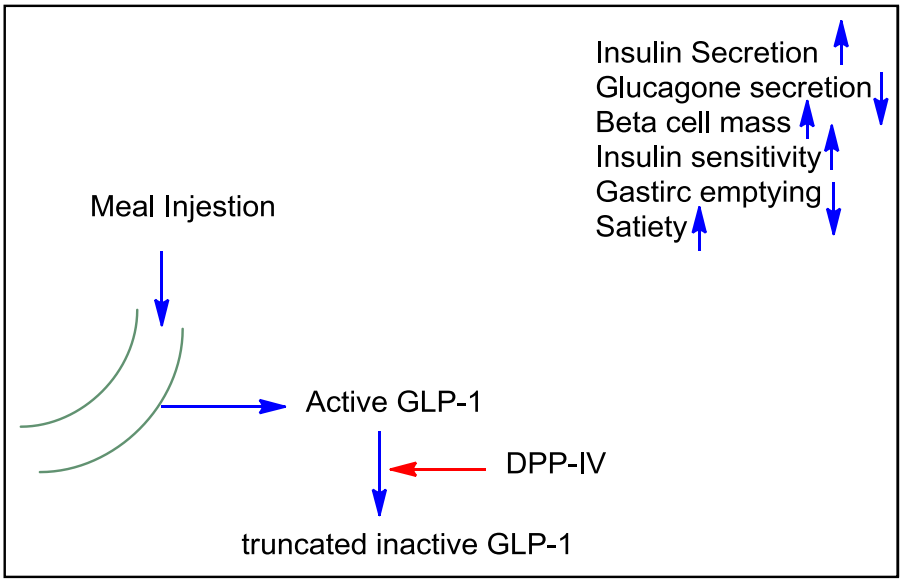


Figure 5. Secretion of GLP-1 after meal ingestion and metabolism by DPP-IV enzyme

The DPP-IV enzyme is a transmembrane glycoprotein, consist of 766 amino acids. It has two subunits and exhibit 85% homology, with its isoforms. The DPP-IV enzyme is consists of three parts; a cytoplasmic tail, a transmembrane region and an extracellular part. The extracellular part is divided into a catalytic domain and an eight-bladed β-propeller domain. The latter contributes to the inhibitor binding site.The extracellular domain of DPP-IV enzyme contains 22 hydrophobic residues of N-terminus which helps to anchor with the cell membrane. The catalytic site of DPP-IV enzyme consists of GWSYG pentapeptide sequence and a catalytic triade S630, Asp708 and His740 forms a binding domain (**Figure 6**).

Structure of DPP-IV enzyme resembles with several isoforms of other protease enzymes, such as DPP-6/8/9, QPP, FAP (fibroblast activation protein)and POP. Potential functions of the various members of the DPP gene family and their relevance with DPP-IV enzyme have been described in the following section.

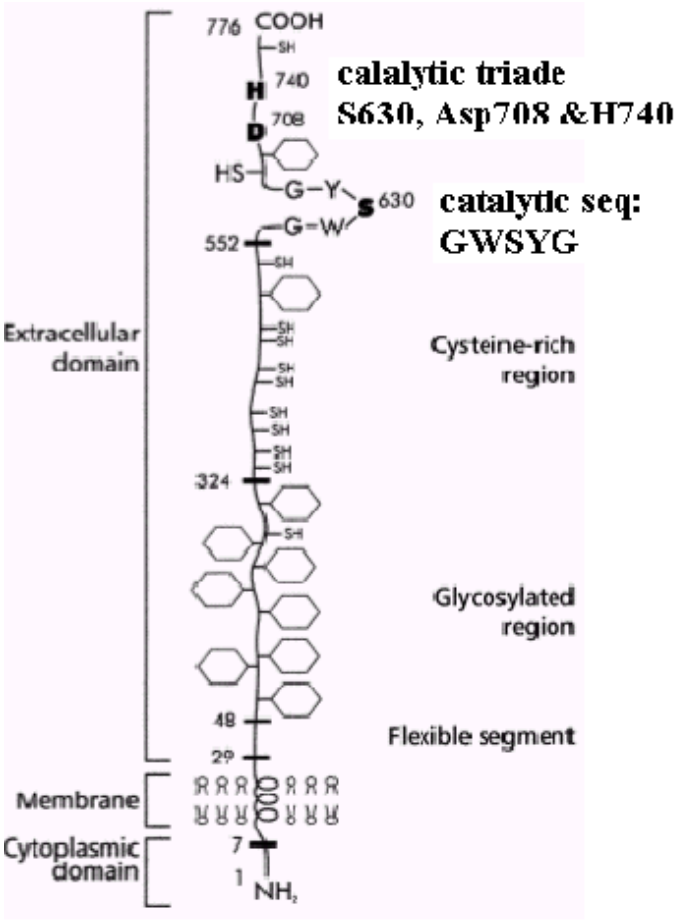


Figure 6: Structure of DPP-IV enzyme

1.5.2.Dipeptidyl peptidase family

The DPP family (family S9), a subfamily of the prolyl oligopeptidase superfamily, includes four enzymes, DPP-IV, FAP, DPP-8 and DPP-9, and two non-enzymes, DPP-IV-like protein-6 (DPP-6, DPL-1 or DPP-X) and DPP-10 (DPL-2)(**Figure 7**)**[132-133]**.

Members of the DPP-IV family preferentially cleave Xaa-Pro- and Xaa- Ala-dipeptides (where Xaa is any amino acid except proline) from theN-terminus of proteins **[134]**. The DPP-IV family differentiates itself from the prolyl oligopeptidase superfamily by the presence of two glutamate residueslocated within the catalytic pocket, which are essential for enzymatic activity **[135]**.

The enzyme FAP, also known as seprase, is the most similar family member to DPP-IV, as it shares a 52% amino acid identity (human enzymes) and similar substrate specificity. Despite these similarities, DPP-IV and FAP differ markedly in their expression patterns. FAP expression is confined predominantly to activated

fibroblasts in diseased tissue, such as fibrotic and epithelial tumours, and invasive cancers, and may be important in wound healing **[136]**.

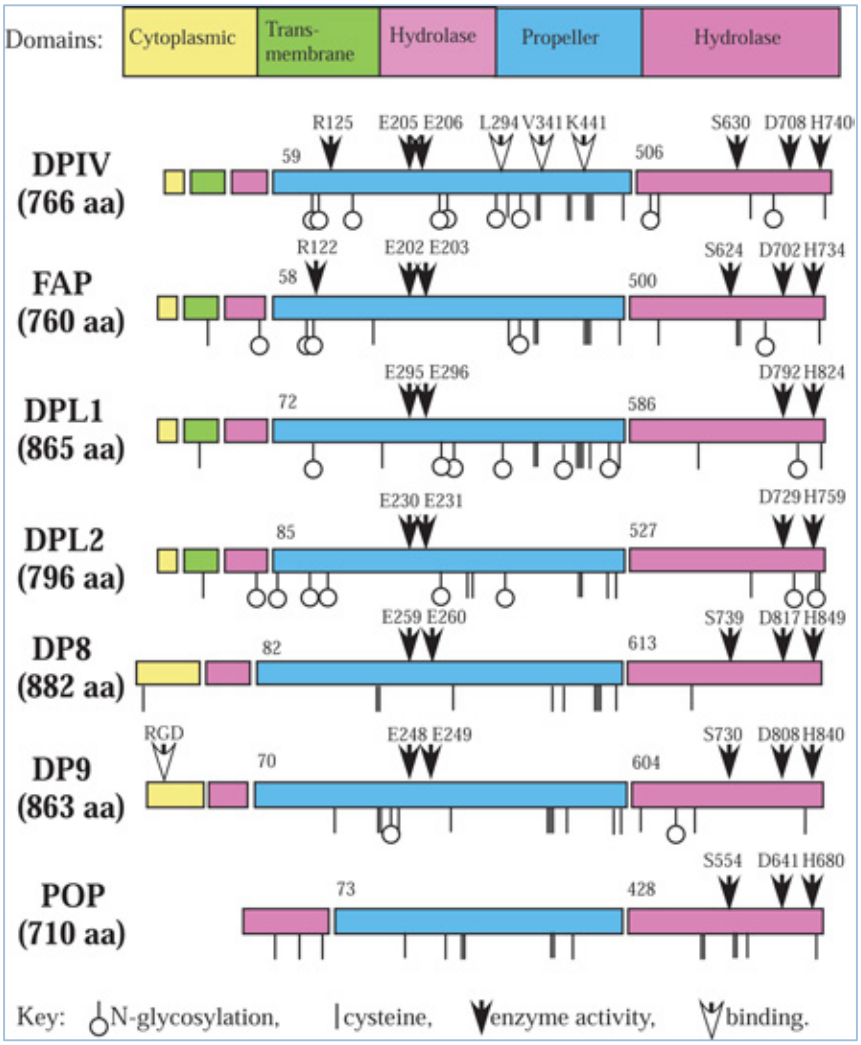


Figure 7: Schematic presentation of the proteins of the DPP family

The other two catalytically active DPP-IV family members, DPP-8 and DPP-9, share 61% amino acid identity with each other, and a 26 and 21% amino acid identity with the protein sequences of DPP-IVand FAP respectively (human enzymes) **[137]**. In contrast with DPP-IVand FAP, which have an extracellular catalytic domain, bothDPP-8 and DPP-9 proteins are localized to the cytoplasm **[137]**. DPP-8 expression is upregulated in activated T-cells. DPP-8 and DPP-9 enzyme activity has been detected in human blood lymphocytes and monocytes **[138]**, High levels ofDPP-9 are found in cancer cells, normal skeletal muscle, the heart and liver. DPP-8 and DPP-9 hydrolyse *H*-Ala-Pro- and *H*-Gly-Pro-derived substrates, although with less efficiency than DPP-IV. *In vitro* peptide substrates of DPP-8 or DPP-9 identified to date include GLP-1, GLP-2, NPY (neuropeptide Y), PYY (peptide YY), SDF-1, IP-10 (interferon-*γ* - induced protein-10) and I-TAC (interferon-inducible T-cell *α*

chemoattractant) **[139-140]**. However, a physiological substrate or role for the DPP activity of either DPP-8 or DPP-9 remains to be demonstrated *in vivo*. However, as the *β*-propeller domain of DPP-IV, DPP-8 and DPP-9 is not as conserved as the *α*/*β*-hydrolase domain and the active sites of DPP-IV, DPP-8 and DPP-9 differ **[141-143]**, hence selective inhibition of DPP-IV may beachieved.

Prolyl oligopeptidase (POP),also known as Prolyl endopeptidases (PEP/PREP)is a group of aminopeptidases and endopeptidases able to hydrolyse the postproline bond, belongs to the DPP gene family. POP bears significant structural homology with the α/β hydrolase fold of DPP-IV**[144]**.POP are involved in the maturation and degradation of peptide hormones and neuropeptides**[145]**. Because of its action on neuropeptides, POP is considered to be involved in processes such as learning, memory, and depression.

Two enzymatically inactive proteins (i.e. DPL1/DPP-6 & DPL2/ DPP-10) closely related to DPP-IV lack catalytic activity due to mutations of thecatalytic serine residue and its neighbouring tryptophan residue, giving a surrounding sequence of Gly-Lys-Asp-Tyr-Gly-Gly instead of the motif Gly-Trp-Ser-Tyr-Gly- Gly.The absence of catalytic activity in DPL1 and DPL2 is also attributed to a number of amino acid substitutions in the catalytic pocket **[146]**.

DPP-II (also known as quiescent cell proline dipeptidase QPP/ DPP-7) shows DPP-IV like peptidase activity although it belongs to another family (family S28) **[147].**

1.5.3.Role of DPP-IV in metabolic diseases

As discussed earlier DPP-IV enzyme rapidly degrades bioactiveincretin hormones GLP-1, and GIP to their inactive metabolites. Both these incretins are important regulator of glucose metabolism. Competitive inhibition of DPP-IV increases the half-life and bioavailability of active incretin hormones, enhancing theirbiological effect (**Figure 8)[148-149]**.

Also, DPP-IV enzyme cleaves many other substrates, such as NPY, GHRH, IL-1 and IL-2, Bradykinin, endomorphin-1 and substance-P (**Table 5**)**[150-151]**. Inhibition of QPP led to reticulocytopenia, while DPP-8/9 inhibition results into thrombocytopenia**[152]**. Thus other than regulating the levels of endogenous incretin hormones such as GLP-1 and GIP, DPP-IV enzyme play crucial role in controlling the lymphocyte and cell growth, T-cell activation, metastasis, inflammation and immune function of body.

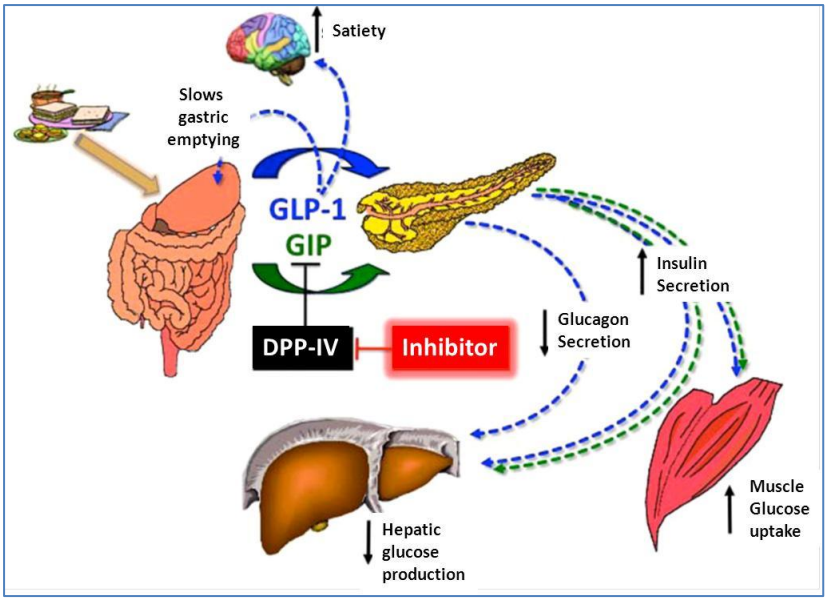


Figure 8: Effect of DPP-IV and its inhibition on physiology of incretin system.

Table 5. Somenatural substrates of DPP-IV

Substrate	N-terminus	Reference
GLP-1	His-Ala-Glu-	**[153]**
GLP-2	His-Ala-Asp-	**[154]**
GIP	Tyr-Ala-Asp-	**[153]**
GRP	Val-Pro-Leu-	**[155-156]**
Substance P	Arg-Pro-Lys-	**[155]**
NPY	Tyr-Pro-Ser-	**[157]**
PACAP38	His-Ser-Asp-	**[156,158]**
IGF-1	Gly-Pro-Glu-	**[159]**
Prolactin	Thr-Pro-Val-	**[155]**
hCG*α*	Ala-Pro-Asp-	**[155]**
GHRF	Tyr-Ala-Glu-	**[153,158]**
LH*α*	Phe-Pro-Asn-	**[159]**
Thyrotropin *α*	Phe-Pro-Asp-	**[159]**
Peptide histidine methionine	His-Ala-Asp-	**[153,158]**
Enkephalins	Tyr-Pro-Val-	**[160]**
Vasostatin-1	Leu-Pro-Val-	**[161]**

Hence, while developing new class of DPP IV inhibitors for the treatment of diabetes, it is essential to consider selectivity of DPP IV inhibitor over other serine protease enzymes and also substrate specificity is crucial, so as to develop safe and effective DPP IV inhibitor based antidiabetic agents.

1.6.Crystal structure of DPP-IV

The seven DPP-IV crystal structures reported till date reflect tremendous global interest in the pharmaceuticaldesign of DPP-IV inhibitors **[162-168]**.Human DPP-IV has a short cytoplasmic tail of 6 amino acids, a 22-amino acid hydrophobic transmembrane region, and a 738-amino acid extracellular domain with ten potential glycosylation sites **[169]**.

The DPP-IV glycoprotein is a homodimer (**Figure 9**). Each monomer subunit consists of two domains, an *α*/*β*-hydrolase domain and a *β*-propeller domain, that enclose a large cavity of approx. 30–45A°in diameter.Dimerization is also observed in solution under various conditions, soluble DPP-IV forms a symmetric assembly as a dimer of dimers, which is required for activity (**Figure 10**). The main DPP-IV structural features includes (A) catalytic or*α*/*β*-hydrolase domain (B) *β*-propeller domain (C) active site and (D) substrate binding site.

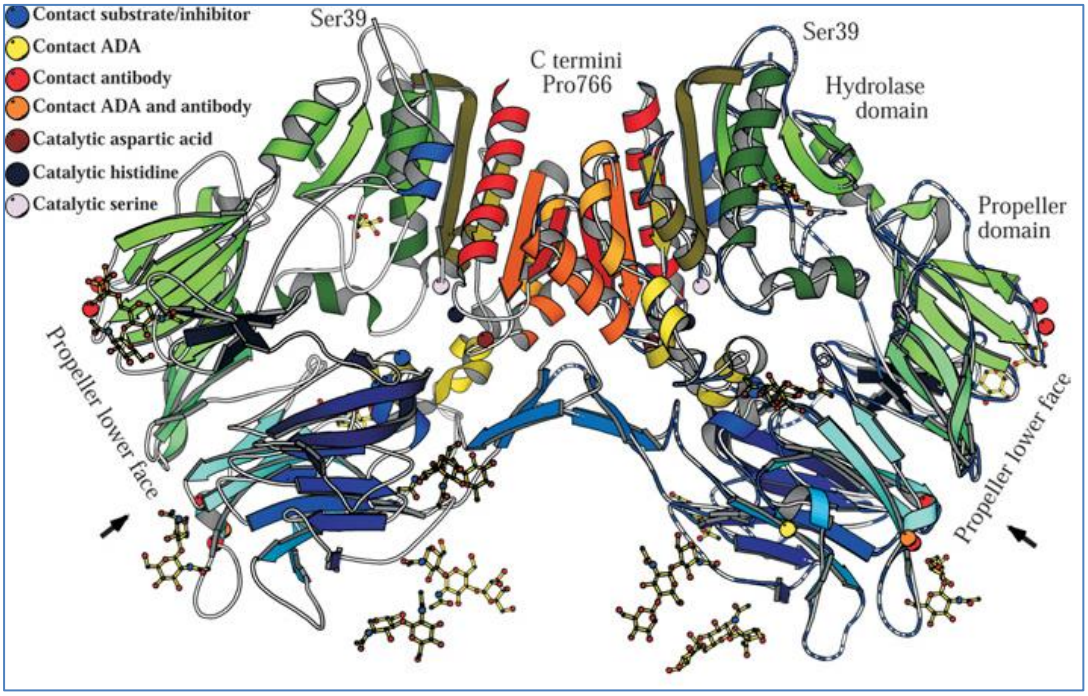


Figure 9:crystal structure of DPP-IV homodimer

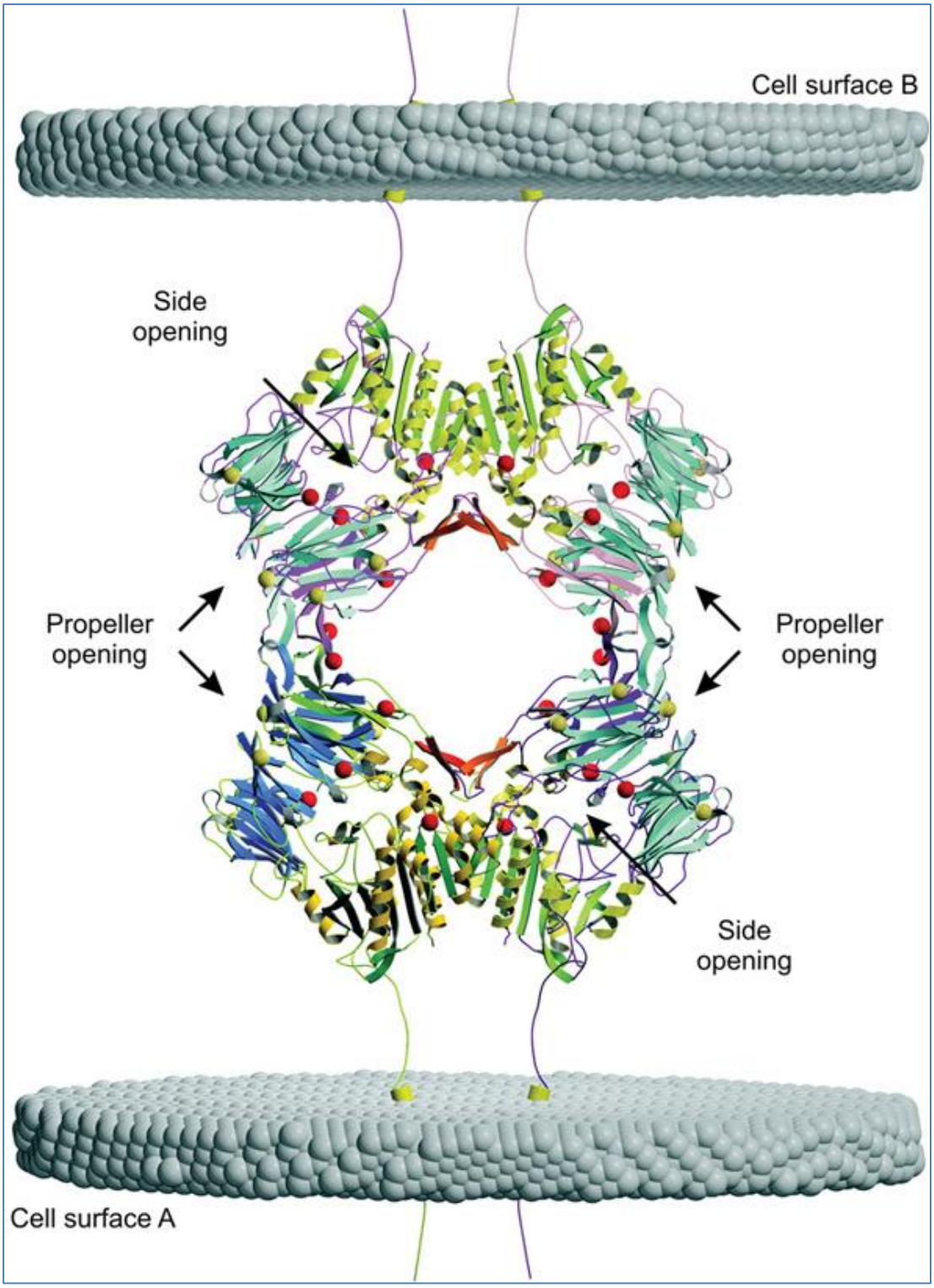


Figure 10:DPP-IV dimer of dimers

(A) **Catalytic domain**: catalytic or*α*/*β*-hydrolase domain is built up of residues Gln508- Pro766and contains a central eight-stranded *β*sheet that is flanked by 12 helices known as the *α*/*β*-hydrolase fold. Superposition of the central *α*helix,

carriesthe catalytic Ser630.The catalytic domain is connected to the βpropeller by an N-terminal 15-residue linker.

(B)***β*-propeller domain**:The βpropeller domain is formed by residues Lys56–Asn497. The preceding N-terminal residues Ser39- Leu55 form a loop structure with a small αhelix at the surface and in close proximity to the first residues of the catalytic domain. The βpropeller domain consists of an 8-fold repeat of a four-stranded antiparallel βsheet motif (**Figure 11**).The βpropeller domainform a significant part of the substrate binding site and are mainly responsible for the substrate specificity.

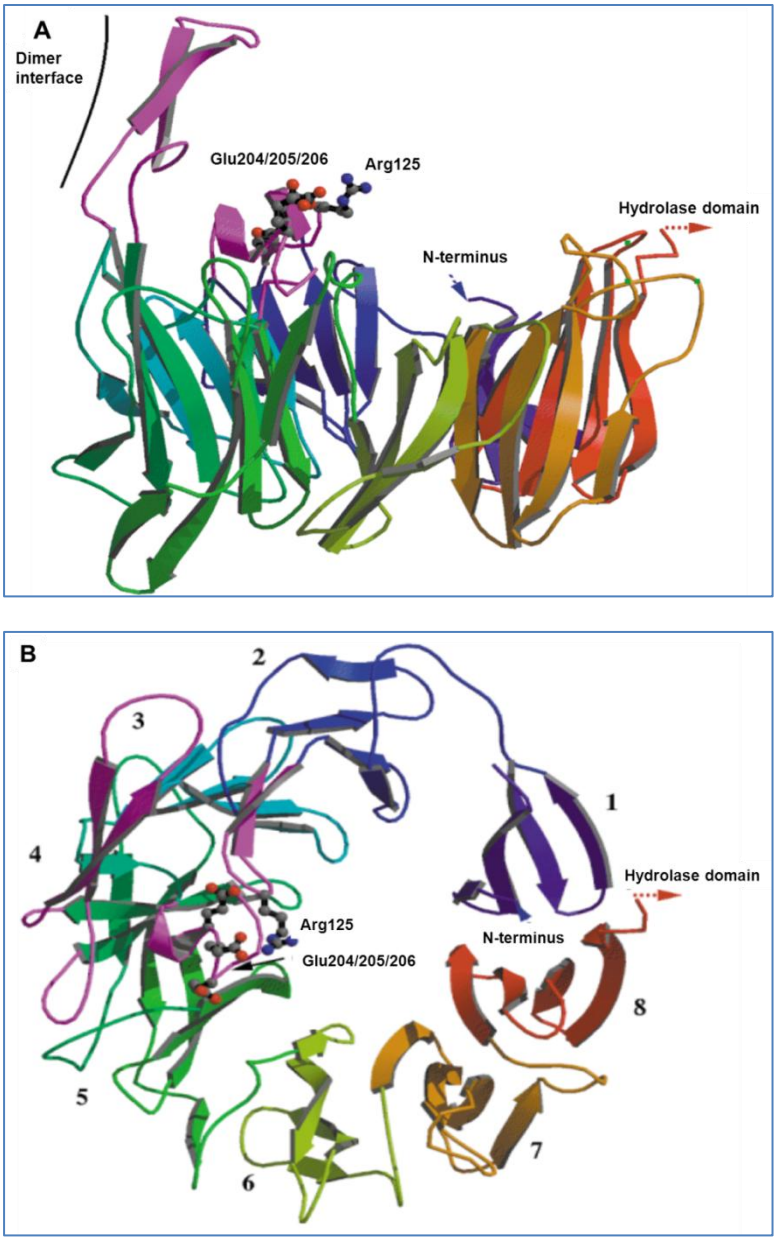


Figure 11:(A) *β* propeller domain of DPP-IV (B) *β* propeller domain of DPP-IV rotated 90°

(C)**Active site**:Active site bears the catalytic triad (Ser630, Asp708, and His740), which is located in a large cavity at the interface of the two domains. Ser630 is found at the tip of a very sharp turn between *β*strand 5 and helix C, called the nucleophile elbow, which is a characteristic of hydrolases of the *α*/*β* type **[170]**. The serine hydroxy group is well exposed to solvent and hydrogen bonded to the catalytic imidazole group of His740 on one side and accessible to the substrate on the other side. His740 is found in the middle of a loop between *β*strand 8 and helix F.One of the oxygen atoms of Asp708 is hydrogen bonded to His740 and completes the catalytic triad. The other oxygen atom of the carboxylate group of Asp708 is coordinated by two main chain NH groups of Val711 and Asn710. Thus, the location and geometry of the triad are very similar to that of the classical serine peptidases **[170]**.

Furthermore, the structure shows that Gly628 and Gly632 are important for the formation of the sharp turn to bring the catalytic residue Ser630 in the correct position. This is in accordance with mutagenesis studies on rat DPP-IV showing that the sequence Gly628-X-Ser630-Tyr631-Gly632 is essential for DPP-IV activity**[171]**.

(D) **Substrate binding site**: Combination of the selected residues of the above three structural motif of DPP-IV forms the substrate binding site. The substrate binding site of DPP-IV is indicated by the bound diprotin A (Ile-Pro-Ile), which is a substrate leading to an apparent competitive inhibition (**Figure 12**)**[172]**.

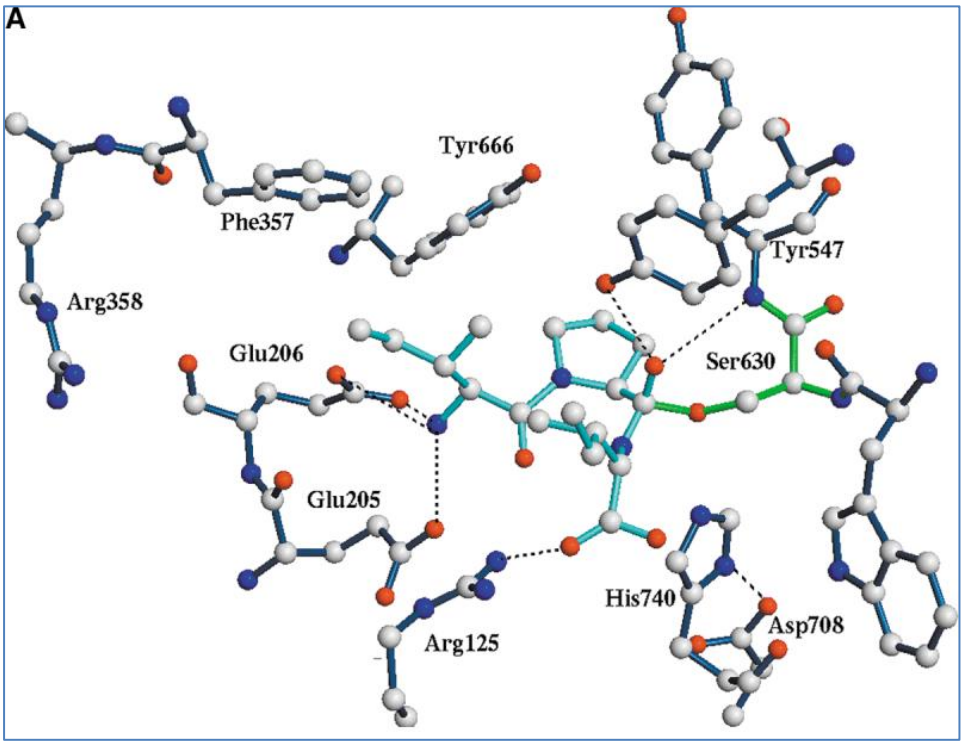


Figure 12: Active site of DPP-IV with substrate Diprotin A.

The ligand is covalently bound to the active site Ser630 of the enzyme in both subunits. The N-terminal Ile (P2) and Pro residues (P1) of ligand are well defined and enable interaction with the substrate binding site **[173]**. The side chain Nof the catalytic His740 is in hydrogen bonding distance to the NH group of P1 and to the oxygenof the Ser630 side chain. The S1 pocket is formed by Val711, Val656, Tyr662, Tyr666, Trp659, and Tyr631, which shape a well-defined hydrophobic pocket that would be filled by proline much better than by alanine. A major contribution to binding to the pyrrolidine ring of Pro is achieved by ring stacking to Tyr662. Essential for substrate binding and catalysis is the N terminus of the substrates, which has to be unprotected and protonated **[174-176]**.

The diprotin A complex shows that the terminal -NH_3^+ group is held very precisely in position by strong interactions with the carboxylates of the double Glu motif, Glu205 and Glu206, as well as the OH of Tyr662. A third glutamate, Glu204 stabilizes this substrate recognition site by a hydrogen bonding network with the backbone NH of Arg125, His126, and Ser127 as well as the hydroxy group of Ser127. This additional structural element in the exopeptidase is very important for substrate selectivity. The importance of the glutamate residues is confirmed by single point mutations that abolish DPP- IV activity **[177]**. Thus, the double Glu motif is a recognition site for the N terminus of substrates and restricts the cleavage to dipeptides, and the S1 pocket provides an optimal binding to proline and alanine residues, leading to a highly specific peptidase.

Hence, detailed structural characteristics of the DPP-IV binding site to identify the molecular interaction that are most important for tight enzyme-inhibitor binding, which indeed leads to selectivity as well as subnanomolar activity is described in the following section.

1.7.Molecular recognition of ligands in DPP-IV

Concomitant with a large variety of published small molecule DPP-IV inhibitors almost one hundred and five co-crystalstructures have been released to the public till April 2014. **[178]**The structural characteristics of the DPP-IV binding site is discussed based upon the available X-ray information (bioinformatics) and pharmacokinetic data together with structure-activity relationship data of the published DPP-IV ligands/inhibitors. This section is divided into several subparagraphs that separately discuss the different interaction motifs used byDPP-IV ligands.

Catalytic Ser630 and Oxyanion Hole: The catalytic machinery of DPP-IV involves a serinenucleophile within the catalytic triad Ser-Asp-His, whosesequential order,

however, is inverse to that found inclassical serine proteases (His-Asp-Ser) **[179]**. Several earlyinhibitors have been developed that use an electrophilicgroup, mainly a nitrile, to interact covalently with Ser630**[180-181]**. The concept of covalent binding to nitriles is wellknown from cysteine protease inhibitors **[182]**. X-ray studiesconfirmed that the nitrile carbon atom changes its hybridizationstate and is in covalent bond distance from the oxygen atom of the Ser630 side chain **[183]**. Theincrease in binding affinity with the additional -CN group issubstantial, leading to an up to 1000-fold tighter binding to the enzyme **[117,184]**. Enzymatic and biophysical studies revealed that the covalent interaction is reversible and that the activity of the enzyme is regenerated upon release of the inhibitor **[184]**. Based upon this phenomenon nitrile group containg inhibitors are classified as reversible inhibitors.

Apart from the transition state mimetics that covalently bind to Ser630, few inhibitors use the oxyanion hole, which is composed of the backbone NH of Tyr631 and the side chain OH of Tyr547, for binding. The only ligands for which this interaction is confirmed by crystal structures are the xanthines and the related pyrimidine-2,4-dione**[185]**, in which a carbonyl group accepts a hydrogen bond from the amide NH of Tyr631. As hydrogen bonding is very sensitive to a correct geometry few chemotypes are apparently able to interact with the hydrogen bond donor arrangement of the oxyanion hole.

S1 Pocket: The specificity pocket S1 is composed of the side chains of Tyr631, Val656, Trp659, Tyr662, Tyr666, and Val711 and it is highly hydrophobic. Overlays of the existing X-ray structures reveal very littlechanges in size and shape of the pocket demonstrating its high specificity for proline residues. The rigidity of this pocket was probed by several groups through modification of the ring size of P1 fragments. The close-up view of the S1 reveals a small hydrophobic niche in the back and suggests to introduce some asymmetry into the P1 fragment to mimic the shape of this pocket. This higher asymmetry can be achieved by introducing a sulfur atom into a 5-membered ring, as illustrated by the thiazolidine, which is approximately 2-fold more active than the corresponding pyrrolidine **[186]**. Hulin *et al*. performed a fluorine scan around the pyrrolidine ring in the cyclohexylglycine amide series and found that the activity highly depends on the position and stereo-configuration of the fluorine substitution **[187]**. A maximal gain in Ki of ~4-fold compared to the unsubstituted pyrrolidine could be achieved. As fluorine occupies little more space than hydrogen this high sensitivity underlines the stringent shape constraints of the S1 pocket.A considerably larger gain in binding affinity compared to the pyrrolidines could be achieved by small substituents on aromatic rings in the S1 pocket **[188-190]**. Slightly bigger substituents than fluorine, such as chlorine or

methyl, are best - in the optimal *para* position - and improve the IC50 compared to the unsubstituted phenyl by a factor 30-40. Substitutions in the *meta* position lead to repulsive interactions with the enzyme and are less favorable. It is noteworthy that also some polar groups such as pyridine and lactam **are** tolerated in the S1 pocket leading to compounds with an overall more balanced polarity pattern.

P2 Amide Recognition: Arg125, Asn710: The carbonyl of the amide bond connecting the N-terminus with the P1 residue in DPP-IV substrates is located in a polar, "electrophilic" environment consisting of the side chains of Arg125 and Asn710. Conversion of the amide to a thioamide leads to a reduction of affinity and replacing the amide by a methylene unit makes the molecule inactive, confirming the favorable electrostatic interaction of the carbonyl dipole with the protein environment **[186]**. Attempts in the cyanopyrrolidine series to mimic the geometry and dipole effect of an amide linker by a *trans*fluoroolefin lead to a reduction of potency **[117]** Merckreported a 3-4 fold tighter binding to DPP-IV substitutingthe phenyl in various β-phenethylamine series with a fluorineat the *ortho*-position **[189, 191]**. While the terminal amidegroup of Asn710 is slightly rotated and not involved in ahydrogen bond with the ligand, a favorable electrostatic interaction between the positively charged Arg125 and the$C^{\delta+}$-$F^{\delta-}$dipole moment remains. Using another *ortho*substituentwith high dipole moment, Takeda reported afavorable interaction of their 2-cyano group with Arg125 inthe pyrimidine-2,4-dione series**[185].** For the aminopyrimidinessmall *ortho*-substitutions (-Me, -Cl,-OMe) on the 6-phenyl moiety lead to 17-28 fold lower IC_{50}values compared to the unsubstituted ring. The consistentgain in affinity with these three substituents indicates thatthe change of torsional angle between the pyrimidine and 6-phenyl rings due to *ortho*-substitution might be acontributing factor for better protein-ligand fit in this series**[188]**.

Overall placing hydrophobic and/or electronegative ligand atoms at a very precise location in the vicinity of Arg125 and Asn710 is rewarded with substantial affinity gains.

N-Terminal Recognition: Glu205, Glu206, Tyr662: Hydrogen bonding interactions with the side chains ofthe two glutamate residues 205 and 206 are, besides the filling of the S1 pocket, the second most important anchor point for inhibitor binding. Secondary and primary amines are recognized by DPP-IV in this region, and for the latter one a third hydrogen bond is formed, typically to Tyr662. This interaction substitutes the binding of the N-terminus of peptide substrates. The positions of the basic nitrogen atoms in the different crystal structures overlap within a sphere of only 1.2 Å, making this a very tight pharmacophore constraint. Destruction of the hydrogen bond network through alkylation of the amino group abolishes activity

[192]. Furthermore, variation of the basicity of the amine in a Roche cyanopyrrolidine series revealed a sharp drop in binding affinity by 2 orders of magnitude when the pKa was lowered from 7.3 to 6.0 **[193]**. An interesting class ofcompounds without basic nitrogen are the carbamoyltriazoles from Eisai**[194]**. Hence presence of primary or secondary amine group in the ligand provides substantial enhancement of the inhibition, However amide linkage or substituted amine group containg substrates can also accommodate this region.

Additional Interactions: Phe 357, Tyr 547 and Arg 358: High nanomolar affinity can be achieved by interactionswith the S1 pocket, the Glu dyad, and the P2 amiderecognition site. However, furtheraffinity gains require either covalent binding to the Ser630residue or the exploitation of additional protein-ligandinteractions. Prime candidates which are used in almost alllow nanomolar DPP-IV inhibitors are the phenyl rings of thetwo residues Phe357 and Tyr547. These are 6-10 Å awayfrom S1 pocket and Glu dyad and exposed to the ligandbinding site. π-π stackinginteractions between each of the two residues with differentaromatic ligand fragments improves the binding affinity of the ligand. Alternative to aromatic-aromatic interactions, hydrophobic contacts between Phe357, Tyr547andlarge aliphatic groups, such as adamantyl can be used to achieve low nanomolar IC_{50} values.

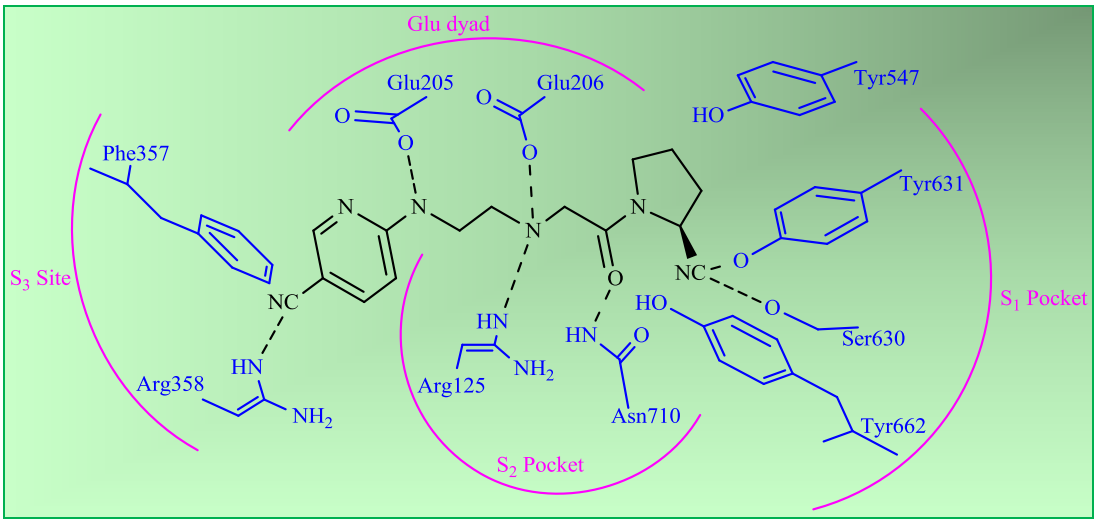


Figure-13: Structural features required for selective DPP-IV inhibitor

Lastly, the presence of Arg358 in close proximity to Phe357 makes the positively charged side chain an additional interaction partner for substituents on ligand aromatic rings. the observed SAR for several series interacting with Phe357 indicates that additional binding free energy can be gained by optimizing the electrostatics in this region. For example, placing electronegative groups such as trifluoromethyl orfluorine next to the positive charge of Arg358 led to a 4-fold increase

in binding in sitagliptin and in thecyanopyrrolidines each,as well as in a potential back up molecule to sitagliptin **[31,183,195]**.

Hence, to achieve subnanomolar potency and selectivity over other serine proteases substrate should have all favourable interaction with above discussed residues of DPP-IV enzyme (**Figure-13**).

1.8.Mechanism of action of DPP-IV inhibitors

As described earlier DPP-IV enzyme selectively cleaves the N-terminal dipeptide from the penultimate position of GLP-1 and GIP thus makes them inactive**[196]**.Competitive inhibiton of the DPP-IV enzyme blocks the degradation of these incretin hormones and extend the duration of action of endogenous GLP-1, thereby stimulating insulin secretion, inhibiting glucagon release and slowing gastric emptying.**[197-198]**.

Inhibition of DPP-IV enzyme activity, using suitable DPP-IV enzyme inhibitor likely to increase the levels (prolong half-life) of endogenous intact and bioactive GIP and GLP-1 peptides which in-turn increase the insulin secretion and decrease the blood glucose, thereby, it acts as antidiabetic agents (**Figure 14**).

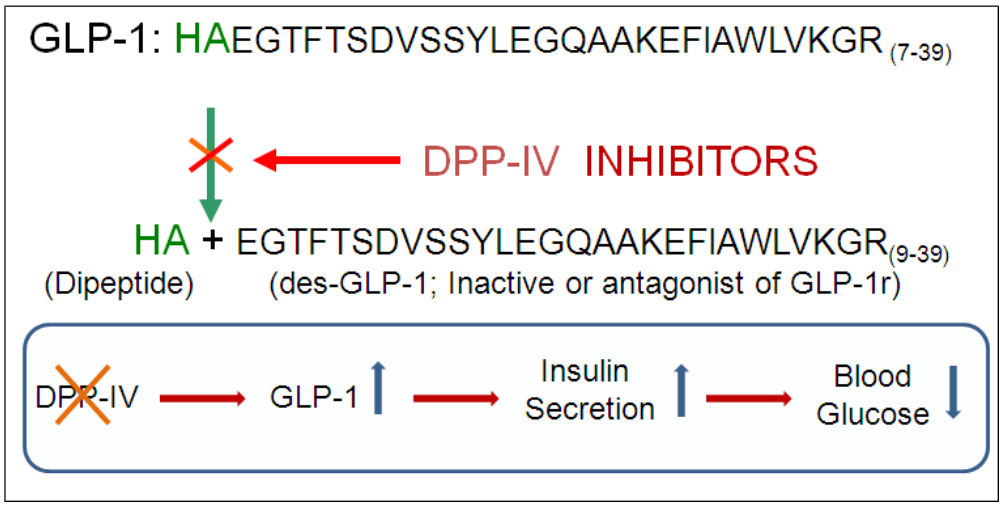


Figure 14. Effect of DPP-IV inhibition

1.9.Challenges in developing potent and selective DPP-IV inhibitors

As discussed earlier in section **1.5.2.** DPP-IV enzyme resembles with several other closely related serine proteases so development of small molecules as selective inhibitors of DPP-IVbecome a major challenge.Although the in vivo function of other members of DPP family, that is, DPP-2, DPP-8, DPP-9 etc. are largely unknown, the physiological effects of their inhibition has been documented in the literature**[199]**.For example, inhibition of DPP-2 has been shown to result in the

apoptosis of quiescent T cells. Selective inhibition of DPP-8/DPP-9 in animals resulted in severe toxic reactions, including alopecia, thrombocytopenia, anemia, enlarged spleen, multiple histological pathologies and increased mortality**[200]**.Notably, it has been shown very recently that inhibition of DPP-8 and DPP-9 did not lead to organ toxicities and mortality in rodents and thus, a mechanism other than DPP-8/DPP-9 inhibition has been suggested to be responsible for the observed toxicities associated with the inhibitors of DPP-8/DPP-9**[201]**.Nevertheless, in view of likely toxic side effects associated with the inhibition of other members of DPP family it has become necessary to design selective inhibitors targeting DPP-IV.

Experimental observation indicated that the S2 pocket of DPP-IV might be similar to that of DPP-8 and consequently inhibitors designed for DPP-IV might show an inhibitory effect on DPP-8 as well. However, extensive SAR work has proved that desired selectivity for DPP-IV inhibition can be achieved via introducing appropriate substituents or groupsthat can attribute all favourable interactions as discussed in the above section **1.7**. Nevertheless, once synthesized, it has therefore become mandatory to determine the DPP-IV selectivity of an inhibitor over closely related other DPPs.

Furthermore, astudy has demonstrated that high levels of GLP-1 should be maintained for 24 h for optimal glycemic control**[202]**.Thus, in addition to focusing on potency and selectivity, development of longacting inhibitors is also desirable that could potentially provide maximal efficacy, particularly in patients suffering from severe diabetes (e.g., HbA1c >9%).

1.10.Overview on DPP-IV inhibitors under recent development

The clinical success of the gliptins following the initial approval of Sitagliptin has stimulated the field todevelop and evaluate additional DPP IV inhibitors in order to obtain drugs with improved properties. Several excellent reviews have been published covering the development of potential new therapeutic DPP IV inhibitors**[203-206]**.

Though BMS discovered Saxagliptin, their continued interest in DPP-IV inhibition as a therapy for type 2 diabetes prompted discovery of the azolopyrimidine based **compound A [207]** and**compound B [208]** as a potent and selective DPP-IV inhibitors(**Figure 15**).

After the development of first FDA approved DPP-IV inhibitor Sitagliptin to treat T2DM, Merck endeavour for batter medication through DPP-IV inhibition led the development of **compound C[209]**which showed good potency (IC_{50}=6nM)and

selectivity but the shortfall with the compound was poor calcium channel and Cyp2D6 selectivity. In continued efforts they develop **compound D [210]**with good pharmacokinetic and pharmacodynamic profile having >1000 fold selectivity for L-type calcium channel and Cyp2D6 with respect to its intrinsic DPP-IV potency.Unfortunately**compound D** showed poor bioavailability in higher animal model (i.e. monkey F%=11%) so it was unable to transform to the clinic for medication and dropped in phase-II.HoweverMerck's indefinite interest in research led the discovery of Omarigliptin **[56]**having all favourable attributes for good medicationand currently it is in phase III clinical trials.

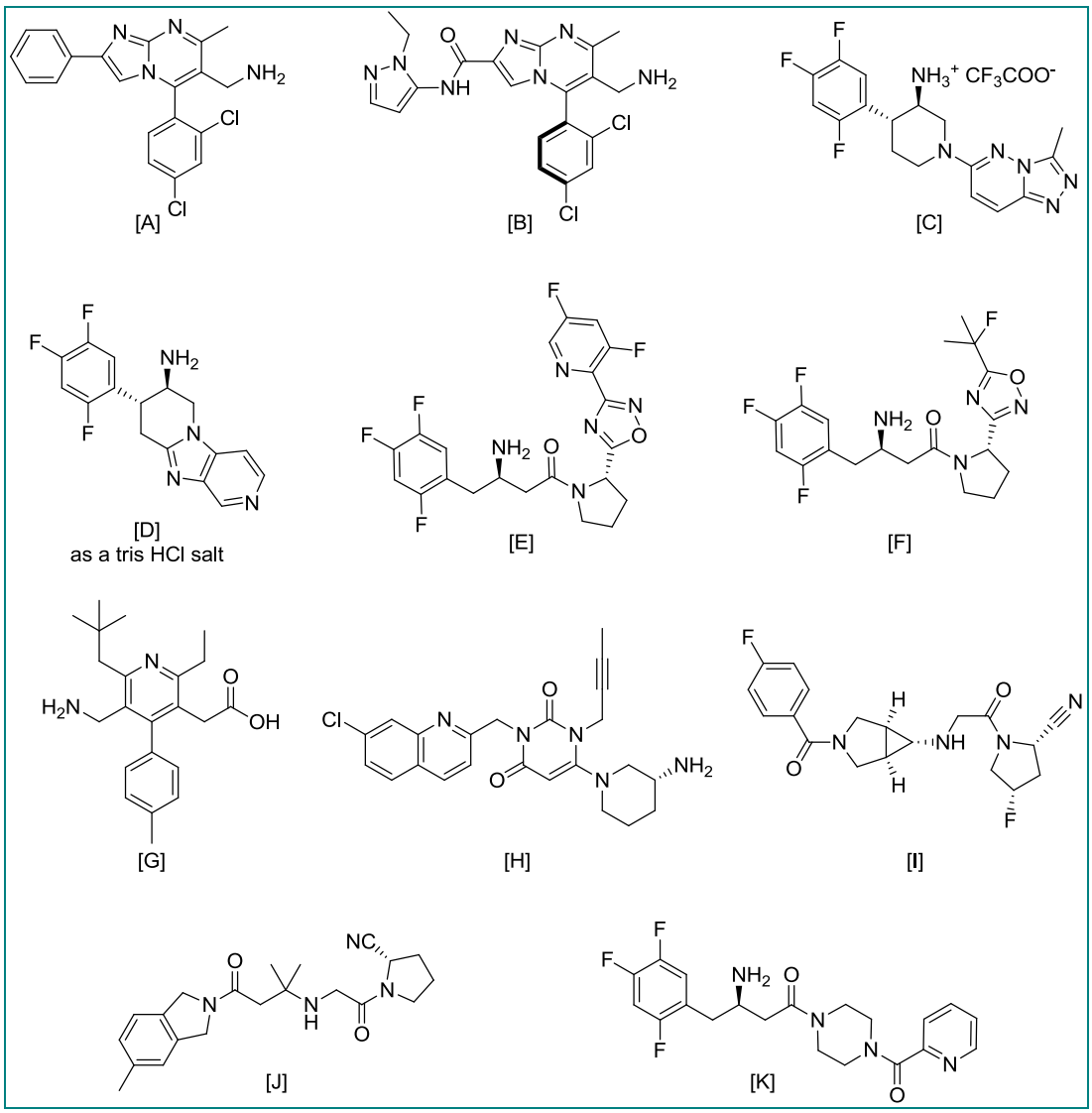


Figure 15. Potent DPP-IV inhibitors under recent development

Santhera Pharmaceuticals from Switzerland reported **compound E** and **compound F**by structural modification in a series of β-homophenylalanine based

DPP-IV inhibitors with good pk profile and efficacy**[211]**. However further evaluation focused on preclinical safety and more comprehensive efficacy profiling is in progress.

Takeda Pharmaceutical from Japan is still in search of even batter treatment for the T2DM though received FDA approval for the drug namely Alogliptin and in phase III clinical trial entity Trelagliptin, they reported **compound G**viz TAK-100 as a potent and selective DPP-IV inhibitor currently in phase I **[212]**.

Recently a group of researchers from Jiangsu Chia-Tai Tianqing Pharmaceutical Co. Ltd. China reported **compound H** derived from Alogliptin through pharmacophore hybridization with low nanomolar potency(IC_{50}=0.4nM)**[213]**.

A group of scientist from Ranbaxy Research Laboratories, India reported **compound I**as a potent, selective and slow binding inhibitor of DPP-IV**[214]**.

Sanwa Kagaku Kenkyusho company from Japan reported isoindiline based **compound J** as a highly potent DPP-IV inhibitor, however due to its high clearance rate further evaluation in this series has been abandoned**[215]**.

A research group from Seoul National University-Korea reported **compound K** as a potent DPP-IV inhibitor showing longer duration of action and no CYP inhibition up to 50µM **[216]**.

Although the bibliography on DPP-IV inhibitors is rich, active research continues on this subject. Currently 35 compounds are in preclinical development having IC_{50}≤6nM. Major players include AstraZeneca Plc., Boehringer Ingelheim GmbH, Bristol-Myers Squibb, Eli Lilly and Company, Merck & Co Inc., Mitsubishi Tanabe Pharma Corp., Novartis AG, Takeda Pharmaceutical Company Limited, Cadila Healthcare Ltd., Phenomix, Lupin limited, L G Life Sciences etc, and have filed no. of patent applictions like WO2006009886, WO2006127530, WO2006039325, WO2006098342, WO2006127287, WO2007053819, US20070082932, WO2007015767, WO2007099385, WO2008119005, WO2008087560, WO2008077597, WO2008130151, WO2008017670, WO2009111239, WO2009082134, WO2009093269, WO2009003681, WO2009045476, WO2009068531, WO2010146597, WO2010029422, WO2011103256, WO2011146358, WO2012118945, WO2013122920, WO2014061031.

Although eight gliptins have reached the market, they have done so recently, and so the long-term adverse effects of these drugs are still unknown. Until now, secondary effectshave been attributed to the off-target repercussion of themolecules. Hence, research has been focused on the discovery of potent, selective and long acting DPP-IV inhibitors with minimal off target activity.

In this regard, knowing the potential of DPP-IV inhibitor target, we also attempted to design novel series of DPP-IV inhibitor and these design strategy is described in next section.

1.11.Introduction to Cytochrome P450 (CYPs) and its importance

The cytochrome P450 system is a group of enzymes, found mainly in the liver and gut mucosa, that plays a crucial role in controlling the concentrations of many endogenous substances and drugs.There are 18 CYP families bearing 43 subfamilies**[217]**. CYPs enzymes are mainly essential for the detoxification and the metabolism of drugs. CYP subfamilies involved in drug metabolism includes CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. However CYP2D6 and CYP3A4 are the major drug-metabolizing enzymes in humans. Diabetic patients are treated with a number of otherdrugs in addition to antidiabetic drugs, including anti-hypertensive and lipid-lowering agents. Notably, more than 50% of these drugs are metabolized by CYP3A4 or CYP2D6 enzymes. Drugs can inhibit (decrease), induce (increase) CYP metabolism or may act as a substratefor CYP enzymes. Inhibition of CYP metabolism will likely increase the affected drug's systemic concentrations, whereas induction of metabolism often reduces systemic concentrations**[218]**.

CYP3A4 or CYP2D6 inhibition and induction is clinically relevant to diabetic patients, especially whentreated with antidiabetic agents such as Sulfonylureas, Metformin and Meglitanides. For example, Sulfonylureas are known substrates of CYP. Thus inducers and inhibitors of CYP can affect the metabolism of Sulfonylureas. Similarly, Repaglinide is metabolized by the CYP3A4 and a serious drug-drug interactions (DDI) may occur when it is coadministered with CYP inhibitor, such as Gemfibrozil (triglyceride lowering agent), as it increases eightfold exposure of Repaglinide. Thus, CYP inhibition/ induction can havesignificant consequences on other antidiabetic drugs that are metabolized by these enzymes, which may result in DDI and idiosyncratic drug toxicity (IDT)**[219-220]**.

Hence, knowing the clinical importance of the CYP enzyme, for particular drug like new chemical entity it is essential to examine its effect on CYP enzyme inhibition.

1.12.Conclusion

Diabetes mellitus is the most prevalent and serious metabolic disorder. Among the T1DM & T2DM, T2DM is one of the major public health challenges of 21st century. Currently available therapies have several drawbackstherefore various new therapies are being developed, among which DPP-IV inhibitors are the most promising approach for the safe and effective treatment of Type 2 diabetes. However achieving selectivity and oral bioavailibity with longer duration of action are major challenges with the design & development of DPP-IV inhibitors. To address these concern, in the next section, we have designed novel series of DPP-IV inhibitors to develop second generation therapies for the treatment of T2DM.