CHAPTER – 2

REVIEW OF LITERATURE
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2.1 DPP-IV and its inhibitors:

The Dipeptidyl Peptidase - IV (DPP-IV) inhibitors enhance the body's own ability to control blood glucose by increasing the active levels of incretin hormones in the body. Their mechanism of action is distinct from any existing class of oral glucose-lowering agents. They control elevated blood glucose by triggering pancreatic insulin secretion, suppressing pancreatic glucagon secretion, and signaling the liver to reduce glucose production. The leading DPP-IV inhibitors have shown clinically significant HbA1c reductions up to 1 year of treatment and offer many potential advantages over existing diabetes therapies including a low risk of hypoglycaemia, no effect on body weight, and the potential, based on animal and in vitro studies, for the regeneration and differentiation of pancreatic β-cells[39]. They are efficacious as monotherapy and also in combination with commonly prescribed antidiabetic agents and are suitable for once-daily oral dosing. Consequently, many DPP-IV inhibitors such as vildagliptin (Galvus; LAF-237), sitagliptin (Januvia; MK-0431), and saxagliptin (BMS-477118) have advanced into late-stage human clinical trials.

According to Avraham Karasika et al, Dipeptidyl peptidase-4 (DPP-IV) inhibitors are a new class of oral antihyperglycemic agents that enhance the body's ability to regulate blood glucose by increasing the active levels of incretins, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). There are numerous DPP-IV inhibitors in development with sitagliptin as the first approved agent for the treatment of patients with type 2 diabetes [40]. Diabetes mellitus (DM) is currently considered to be an epidemic disease. A safe and effective treatment has long been
sought by scientists. Incretin mimetics and dipeptidyl peptidase-4 (DPP-IV) inhibitors represent a new class of agents that have recently been included as antidiabetic drugs. Although only a limited number of studies exist regarding the treatment of DM based on the incretin effect, DPP-IV inhibitors have so far proved to be safe and effective, both when administered alone or in combination with other antidiabetic medication. This review focuses on incretin-effect physiology, as well as the DPP-IV inhibitors, from sitagliptin to the new alogliptin-pioglitazone combination agent, given as monotherapy and in combination with other antidiabetic agents as per the studies made by Georgia Argyrakopoulou and John Doupis [41].

Dipeptidyl-peptidase-4 (DPP-IV) inhibitors are available as oral anti-hyperglycemic drugs for the treatment of type 2 diabetes. Their metabolic effect is mediated through sparing incretin hormones (such as glucagon-like peptide-1, GLP-1) from the rapid degradation by DPP-IV. In turn, GLP-1 improves meal-stimulated insulin secretion by pancreatic β-cells thus reducing hyperglycemia. It has been shown that GLP-1 signaling is also active in the cardiovascular system, where it may exert beneficial effects. However, DPP-IV has several non-incretin substrates, and its immunomodulatory activity is known from decades [42]. DPP-IV physiologically cleaves cytokines, chemokines and neuropeptides involved in inflammation, immunity, and vascular function. Owing to these off-target mechanisms, DPP-IV inhibitors hold promise for cardiovascular protection, but may also face unexpected side effects. Here in, the studies of Neumiller JJ [43], the review available data on the cardiovascular effects of DPP-IV inhibitors, with a special interest in GLP-1-independent mechanisms. The modulation of
endothelial progenitor cells, inflammatory pathway and ischemic response emerges as the major cardiovascular target of DPP-IV inhibitors.

Affinity-based selection strategies have recently emerged as a complement to traditional high throughput screening for the rapid discovery of lead compounds for the large number of protein targets emerging from--omics technologies. Adam GC et al [44] described a method for the ranking of mixtures of ligands by affinity selection and applies it to rank order a set of inhibitors for the enzyme dipeptidyl peptidase IV.

Dipeptidyl peptidase IV (DPP-IV) is a serine protease, a member of the prolyl oligopeptidase (POP) family, and has been implicated in several diseases [45]. Therefore, the development of DPP-IV selective inhibitors, which are able to control the biological function of DPP-IV, is important. We determined the crystal structure of human DPP-IV at 2.6Å resolution. The molecule consists of a unique eight-bladed beta-propeller domain in the N-terminal region and a serine protease domain in the C-terminal region. Also, the large "cave" structure, which is thought to control the access of the substrate, is found on the side of the beta-propeller fold. Comparison of the overall amino acid sequence between human DPP-IV and POP shows low homology (12.9%). According to the works of Rea D, Fülöp V[46], the report the structure of human DPP-IV, especially focusing on a unique eight-bladed beta-propeller domain. We also discuss the way for the access of the substrate to this domain.

Dipeptidyl peptidase IV (DPP-IV, DPP-IV) is a serine protease that releases N-terminal dipeptides. It is a validated drug target for type 2 diabetes and DPP-IV inhibitors are currently evaluated for other therapeutic applications. Various assays are used for
DPP-IV activity measurements in biological samples which were conducted by Matheeussen V et al [47]. Highly sensitive methods are needed to measure also very low activities in inhibited samples.

Altered dipeptidyl peptidase-4 (DPP-IV) activity during the progression of late-stage type 2 diabetes was measured in Otsuka Long-Evans Tokushima fatty (OLETF) rats. Compared with OLETF rats subjected to 30% food restriction, food-satiated OLETF rats exhibited spontaneous hyperphagic obesity, insulin resistance, hyperglycemia, hyperinsulinaemia, and increased plasma DPP-IV activity during the early phase of the experiment (up to ∼30 wk). Subsequently, their plasma DPP-IV activity as well as their body weight, body fat, and plasma insulin concentration declined to control levels during the late phase, resulting in excessive polyuria, proteinuria, dyslipidemia, pancreatic islet atrophy, hyperinsulinaemia, and diabetes, which changed from insulin-resistant diabetes to hypoinsulinemic diabetes secondary to progressive islet insufficiency, and their fasting blood glucose level, remained high. Since plasma DPP-IV activity demonstrated significant positive correlations with body weight and the fasting plasma insulin level but not with the fasting blood glucose level during the late stage of diabetes, body fat and fasting plasma insulin levels may be useful factors for predicting the control of plasma DPP-IV activity [48]. In contrast, pancreatic DPP-IV activity was significantly increased, and the expression of pancreatic insulin was significantly reduced in late-stage diabetic OLETF rats, suggesting that a relationship exists between the activation of pancreatic DPP-IV and insulin depletion in pancreatic islet atrophy. In conclusion, it is suggested that plasma DPP-IV activity changes in accordance with the progression of hypoinsulinemic obesity and pancreatic islet atrophy. DPP-IV activity may play an important role in insulin homeostasis as in the works made by Peters A in his reviews [49].
Dipeptidyl peptidase IV (DPP-IV) deactivates the natural hypoglycemic incretin hormones. Inhibition of this enzyme should restore glucose homeostasis in diabetic patients making it an attractive target for the development of new antidiabetic drugs. With this in mind, the pharmacophoric space of DPP-IV was explored using a set of 358 known inhibitors [50]. Thereafter, genetic algorithm and multiple linear regression analysis were employed to select an optimal combination of pharmacophoric models and physicochemical descriptors that yield self consistent and predictive quantitative structure-activity relationships (QSAR) ($r(2)$ (287)=0.74, F-statistic=44.5, $r(2)$ (BS) = 0.74, $r(2)$ (LOO)=0.69, $r(2)$ (PRESS) against 71 external testing inhibitors=0.51). Two orthogonal pharmacophores (of cross-correlation $r(2)=0.23$) emerged in the QSAR equation suggesting the existence of at least two distinct binding modes accessible to ligands within the DPP-IV binding pocket. Docking experiments supported the binding modes suggested by QSAR/pharmacophore analyses. The validity of the QSAR equation and the associated pharmacophore models were established by the identification of new low-micro molar anti-DPP-IV leads retrieved by in silico screening. One of our interesting potent anti-DPP-IV hits is the fluoroquinolone gemifloxacin (IC (50) =1.12 μM) [51]. The fact that gemifloxacin was recently reported to potently inhibit the prodiabetic target glycogen synthase kinase 3beta (GSK-3beta) suggests that gemifloxacin is an excellent lead for the development of novel dual antidiabetic inhibitors against DPP-IV and GSK-3beta.
2.2 Glucagon-like peptides (GLP):

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are hormones secreted by the enteroendocrine cells of the gut in response to the ingestion of nutrients [52]. These incretin hormones, so called because they increase insulin secretion, are key modulators of pancreatic islet hormone secretion and, thus, glucose homeostasis. The glucoregulatory effects of incretins are the basis for new therapies currently being developed for the treatment of type 2 diabetes mellitus (T2DM). Drugs that inhibit dipeptidyl peptidase-4 (DPP-IV), a ubiquitous enzyme that rapidly inactivates both GLP-1 and GIP, increase active levels of these hormones and, in doing so, improve islet function and glycemic control in T2DM.

According to Abd A. Tahrani, Milan K. Piya and Anthony H. Barnett, [53] Type 2 diabetes mellitus (T2DM) is a global epidemic with increasing impact on individuals and healthcare providers. Available treatments (such as metformin, sulfonylureas, glitazones, and insulin) have proven unsatisfactory in producing a long-lasting impact on glycemic control. In addition, most of these treatments have undesirable side effects such as weight gain and hypoglycemia. As a result, exploring new treatment targets and new therapies is mandatory in order to treat this condition. The incretin pathway, in particular glucagon-like peptide (GLP-1), it plays an important pathological role in the development of T2DM, and treatments targeting the incretin system have recently become available through the reviews of Kania DS in 2011 [54]. These can mainly be divided into two broad categories; GLP-1 agonists/analogs (exenatide, liraglutide), and dipeptidyl peptidase-4 (DPP-IV; the enzyme responsible for rapid inactivation of incretins).
inhibitors (sitagliptin, vildagliptin). Saxagliptin is a novel DPP-IV inhibitor that has recently completed phase 3 studies. Saxagliptin is a potent and specific inhibitor of DPP-IV (in comparison with other dipeptidyl peptidase enzymes) that is given once daily.

The role of dipeptidyl peptidase-IV (DPP-IV) as both a regulatory enzyme and a signaling factor has been evaluated and described in many studies of John Doupis and Aristidis Veves [55]. DPP-IV inhibition results in increased blood concentration of the incretin hormones glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP). This causes an increase in glucose-dependent stimulation of insulin secretion, resulting in a lowering of blood glucose levels. Recent studies have shown that DPP-IV inhibitors can induce a significant reduction in glycosylated hemoglobin (HbA1c) levels, either as monotherapy or as a combination with other antidiabetic agents [56]. Research has also demonstrated that DPP-IV inhibitors portray a very low risk of hypoglycaemia development. This review article focuses on the two leading agents of this category (sitagliptin and vildagliptin), providing an overview of their function along with the latest data regarding their clinical efficacy as antidiabetic agents.

Type 2 diabetes mellitus (T2DM) is a progressive disease warranting intensification of treatment, as beta-cell function declines over time. Current treatment algorithms recommend metformin as the first-line agent, while advocating the addition of either basal-bolus or premixed insulin as the final level of intervention [57]. Incretin therapies, including incretin mimetics or enhancers, are the latest group of drugs available for treatment of T2DM. These agents act through the incretin axis, are currently recommended as add-on agents either as second-or third-line treatment, without
concurrent use of insulin. Given the novel role of incretin therapy in terms of reducing postprandial hyperglycemia, and favorable effects on weight with reduced incidence of hypoglycemia, we explore alternative options for incretin therapy in T2DM management. Furthermore, reviews of Campbell RK suggested [58] that some evidence alludes to incretins potentially increasing beta cell mass and altering disease progression, we propose introducing these agents earlier in the treatment algorithm. In addition, we suggest the concurrent use of incretins with insulin, given the favorable effects especially in relation to weight gain.

2.3 Anti-Hyperglycemic Agents

The single-tablet combination of vildagliptin and metformin addresses key defects of type 2 diabetes for improved glycemic control. By inhibiting the dipeptidyl peptidase-4 (DPP-IV) enzyme, vildagliptin raises the levels of the active incretin hormones, glucagon like peptide 1 and glucose-dependent insulinotropic peptide. This leads to increased synthesis and release of insulin from the pancreatic beta cells and decreased release of glucagon from the pancreatic alpha cells. The combination tablet also contains metformin, which addresses insulin resistance. The complementary mechanisms of action of the two agents in combination have been shown to provide additive and sustained reductions in hemoglobin A\textsubscript{1c} compared with metformin monotherapy. In active-controlled trials, the vildagliptin-metformin combination has been shown to produce equivalent reductions in hemoglobin A\textsubscript{1c} to pioglitazone-metformin and glimepiride-metformin combinations, without significant risk of hypoglycemia and without causing weight gain. In clinical trials, the overall incidence of any adverse event was similar in
patients randomized to vildagliptin plus metformin and placebo plus metformin [59]. Available data support the use of vildagliptin in combination with metformin as a promising second-line treatment for the management of type 2 diabetes.

According to Ahrén, Bo et al [60] Sulfonylureas (SUs) are commonly used as add-on to metformin in treatment of type 2 diabetes in patients who are insufficiently controlled by metformin alone. They have good efficacy and have been shown to prevent microvascular complications. However, treatment with SUs is also associated with a high frequency of hypoglycemia, increased body weight, and a high risk of secondary failure. During recent years, dipeptidyl peptidase-4 (DPP-IV) inhibitors have emerged as alternatives to SUs. They show similar efficacy as SUs but with lower risk of hypoglycemia, and reduction or no change in body weight, and if confirmed in humans, they may preserve islet function and thereby minimize the risk for secondary failure. Their limitation at present is the lack of long-term (>5 years) experience on durability and safety. Overall, therefore, the conclusion emerges that SUs are less desirable than DPP-IV inhibitors in management of hyperglycemia in type 2 diabetes.

A new chemical class of potent DPP-IV inhibitors structurally derived from the xanthine scaffold for the treatment of type 2 diabetes has been discovered and evaluated by Matthias Eckhardt, et al [61]. Systematic structural variations have led to 1 (BI 1356),

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\text{IC}_{50} = 3900 \text{ nM} \quad \text{IC}_{50} = 1 \text{ nM}
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a highly potent, selective, long-acting, and orally active DPP-IV inhibitor that shows considerable blood glucose lowering in different animal species. It is currently undergoing clinical phase IIb trials and holds the potential for once-daily treatment of type 2 diabetics.

Linagliptin (BI 1356) is a novel, orally available inhibitor of dipeptidyl peptidase-4 (DPP-IV). Linagliptin improves glycaemic control in type 2 diabetic patients by increasing the half-life of the incretin hormone glucagon-like peptide-1 (GLP-1). Linagliptin is expected to be used as monotherapy or in combination with other antihyperglycaemic agents as described in the works made by Graefe-Mody EU et al [62]. This study was conducted to investigate potential pharmacokinetic or pharmacodynamic interactions between linagliptin and metformin.

2.4 Structural Studies of DPP-IV

The three-dimensional structure of rat DPP-IV/CD26, as determined by cryo-TEM and single particle analysis at a resolution of approximately 14A. The reconstruction confirms that the protein exists as a dimer, as predicted earlier. Since there are structural analogies to the serine peptidase POP, docking calculations of the two structures were performed. Although the docking showed a similar spatial organization (catalytic domain, beta-propeller, distal opening, central cavity), the detailed comparison revealed clear discrepancies. The most marked difference is a second (lateral) opening in DPP-IV/CD26, which would enable direct access to the catalytic site [63]. We therefore assume that substrate selectivity and binding rate are most probably driven by different mechanisms in DPP-IV/CD26 and POP.
According to Jiang YK [64] Three-dimensional quantitative structure-activity relationship (3D-QSAR) and molecular docking studies were carried out to explore the binding of 73 inhibitors to dipeptidyl peptidase IV (DPP-IV), and to construct highly predictive 3D-QSAR models using comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA). The negative logarithm of IC (50) (pIC (50)) was used as the biological activity in the 3D-QSAR study. The CoMFA model was developed by steric and electrostatic field methods, and leave-one-out cross-validated partial least squares analysis yielded a cross-validated value (r(2)(cv)) of 0.759. Three CoMSIA models developed by different combinations of steric, electrostatic, hydrophobic and hydrogen-bond fields yielded significant r(2)(cv) values of 0.750, 0.708 and 0.694, respectively. The CoMFA and CoMSIA models were validated by a structurally diversified test set of 18 compounds. The entire test compounds were predicted accurately using these models. The mean and standard deviation of prediction errors were within 0.33 and 0.26 for all models. The comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) contour plots of pyrrolidine based analogues are used to analyze the structural requirements of a DPP-IV active site. [65] Analysis of CoMFA and CoMSIA contour maps helped identify the structural requirements of inhibitors, with implications for the design of the next generation of DPP-IV inhibitors for the treatment of type 2 diabetes.

Probing with tool molecules and by modeling and X-ray crystallography the binding modes of two structurally distinct series of DPP-IV inhibitors led to the discovery of a rare aromatic fluorine H-bond and the spatial requirement for better biaryl
binding in the DPP-IV enzyme active site [66]. These newly found binding elements were successfully incorporated into novel DPP-IV inhibitors.

As per the works of Gao YD [67] Molecular modeling was used to improve potency of the cyclohexylamine series. In addition, a 3-D QSAR method was used to gain insight for reducing off-target DPP-8/9 activities. Some compounds which were synthesized and found to be potent DPP-IV inhibitors; in particular two of them designed to be highly selective against off-target DASH enzymes while maintaining potency on DPP-IV.

Diabetes Mellitus Type 2 is one of the common diseases and found worldwide. The cause of this abnormality is due to lack of insulin production. Glucagon like peptide-1 (GLP-1), product of the glucagon gene is one of the prime components which stimulates the β cell proliferation and inhibits β cell death. Dipeptidyl Peptidase IV (DPP-IV) acts as protease degrading incretins which stimulates the secretion of insulin for normalizing sugar level. Therefore, inhibiting DPP-IV would decrease incretins degradation and in turn will stimulate the insulin secretion. The objective of this study was to search for an inhibitor with minimum or no harmful effects [68]. The sequence coding for DPP-IV was retrieved and remodeled Insilco and DOCKING studies found that methyl amine is one of potential inhibitors of DPP-IV. The Insilco study for activity and drug likeness of the ligand molecule found it to be non mutagenic, non irritant, non tumorogenic and have no effect on fertility.
2.5 Antihyperglycemic Drugs

According to Gram J, [69] determining the effect of treatment with insulin apart compared with NPH insulin, together with metformin/placebo and rosiglitazone/placebo. The hypothesis was that combined correction of major pathogenetic defects in type 2 diabetes would result in optimal glycemic control to evaluate the efficacy and safety of add-on insulin glargine versus rosiglitazone in insulin-naïve patients with type 2 diabetes inadequately controlled on dual oral therapy with sulfonylurea plus metformin [70].

No satisfactory effective treatment is available yet to cure diabetes mellitus. Though, synthetic drugs are used but there are several drawbacks [71]. The attributed antihyperglycemic effects of many traditional plants are due to their ability for the management of diabetes mellitus. There are lots of chemical agents available to control and to treat diabetic patients, but total recovery from diabetes has not been reported up to this date. In addition to adverse effects, drug treatments are not always satisfactory in maintaining euglycemia and avoiding late stage diabetic complications. Alternative to these synthetic agents, plants provided a potential source of hypoglycemic drugs and are widely used in several traditional systems of medicine to prevent diabetes. Several medicinal plants have been investigated for their beneficial effect in different type of diabetes. According to the systematic reviews conducted by Pandey A et al [72], the other alternative therapies such as dietary supplements, acupuncture, hydrotherapy, and yoga therapies less likely to have the side effects of conventional approaches for diabetes.