1.1 Diabetes Mellitus

Diabetes is one of the major global metabolic disorder characterized by a chronic hyperglycemia and insulin resistance. In 2014, 387 million people suffer from diabetes mellitus globally and number of cases are increasing drastically day by day. Diabetes is responsible for 4.9 million deaths in 2014. Type 2 diabetes mellitus (T2DM) accounts for almost 90% of all cases of diabetes in adults worldwide. India ranks second in highest number of diabetic patients with around 65.1 million people suffering from diabetes. By 2035, this number will be increased up to 109 million as predicted by the International Diabetes Federation (IDF) [1-3].

T2DM which is mainly characterized by insulin resistance and insulin deficiency and main goal behind the treatment is to lower the glycosylated hemoglobin (HbA1c) level below 7% [4]. Conventionally sulfonylureas, meglitinides, thiazolidinediones, biguanides and α-glucosidase inhibitors are used for treatment of T2DM but most of them cause common side effects, hypoglycaemia and weight gain which are major issues on long term treatment. Nowadays, many newer anti-diabetic therapies like 11β-hydroxysteroid dehydrogenase 1 inhibitors, sodium–glucose co-transporter 2 inhibitors, glucagon-receptor antagonists, dipeptidyl peptidase-4 inhibitors, metabolic inhibitors of hepatic glucose, pancreatic-G-protein-coupled fatty-acid-receptor agonists, insulin-releasing glucokinase activators, etc. have been emerged to overcome such side effects [5]. Table 1.1 shows such novel targets and treatments available for the T2DM. Among these, the dipeptidyl peptidase-4 (DPP-4) inhibitors have proven their potential for long term glycemic control globally and so chosen as our research topic.
1.2 The Incretin Pathway

Two incretin hormones, glucagon like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP), play a critical role in the maintenance of glucose homeostasis. In response to food, they are secreted from the intestinal mucosa in the gut and increase the production and release of insulin by promoting the health and function of pancreatic β cells [12-15]. Various actions of GLP-1 that contribute to improved glycemic control have been summarised in Fig. 1.1. [16].

![Fig. 1.1 Actions of GLP-1 in peripheral tissues](image)

Immediately after the release, both incretin hormones are degraded by the enzyme, DPP-4 at their N terminus in the circulation and thereby become inactive. Due to this, they have a very short half-life, 1-2 minutes for GLP-1 while 7 minutes for GIP [17-19]. If the actions of DPP-4 enzyme are inhibited, we can preserve the endogenous activity of incretins for longer duration of time. Orally acting small molecule DPP-4 inhibitors have already proven themselves as effective and well tolerated treatment of T2DM without side effects of weight gain or hypoglycemia. Instead they have many cardiovascular benefits.
1.3 DPP-4 Enzyme

DPP-4 (CD26, DPIV,) enzyme was firstly described in 1967 [20]. It is a multifunctional membrane-anchored serine ectopeptidase belonging to the α,β-hydrolases (family S9B) and sequentially related to the prolyl oligopeptidase (POP) [21]. It is widely distributed in endothelial cells, placenta, liver, adrenal glands, spleen, kidney, lymphocytes and intestine [22]. Its main action is to remove N-terminal dipeptides from substrates containing proline, and to some extent alanine, at the penultimate position as in GIP and GLP-1 (Fig. 1.2) [23]. Other endogenous substrates are insulin-like growth factor-1, substance P, pituitary adenylate cyclase-activating polypeptide, gastrin-releasing polypeptide, peptide YY, various chemokines and neuropeptide Y. Thus, apart from its roll in T2DM, it is also involved in regulation of blood pressure, immune system and neurogenic inflammation [24].

![Fig. 1.2. Point of cleavage for GLP-17-36 amide by DPP-4](image)

1.3.1 Structure

The 110 kDa, 766 amino acids human DPP-4 is a transmembrane glycoprotein which is secreted as a monomer, but for proteolytic activity, forms dimer [25]. It mainly consists of 3 parts, a cytoplasmic tail (residues 1-6), a transmembrane region (residues 7-28), and an extracellular part (29-766). The main two domains of extracellular region are catalytic domain (residues 508-
having a catalytic triad Ser630 – Asp708 – His740 and α/β-hydrolase fold and other eight-bladed β-propeller chain (residues 56-497) which also contributes to the active site (Fig. 1.3a) [23a,25]. The catalytic site lies in a large cavity between the two extracellular domains and can be accessed through two active site openings [23a,26,27].

**Fig. 1.3.** (a) DPP-4 structure: The active site is a large cavity in each subunit, in between the β-propeller domain and the α-helix domain. The eight-bladed β-propeller domain can be seen here as the yellow anti-parallel beta sheets, while the α-helix subunits are in red. (b) Binding site pockets, S1, S2 and S2’ with their amino acids

### 1.3.2 Binding (Active) Site

The DPP-4 enzyme has two, S1 and S2 binding pockets (Fig. 1.3b). The hydrophobic S1 pocket consists of residues, Tyr631, Val656, Trp659, Tyr662, Tyr666, Val711 and catalytic triad (Ser630, Asn710 and His740) [23a]. S2 pocket is a larger cavity having key residues Glu205 and Glu206 dyad and Arg125 while surrounded by Val207, Ser209, Arg358 and Phe357 which makes S2’ extensive sub site. As the interaction increases beyond S1 and S2 sites to S2’ extensive sub site, inhibition of DPP-4 increases. In other related enzymes like DPP-8, DPP-9 and fibroblast activation protein (FAP) of same serine family, this S2’ extensive sub site governs the selectivity towards DPP-4 against them because it has not been clearly defined into their structure [23a, 25, 28-30]. S1 site is almost similar in different DPP isozymes in terms of the residue composition while differs in size [31, 32]. Fig. 1.4 illustrates the shape of binding pocket and key residues involved in protein-ligand interactions.
Fig. 1.4 (a) Solvent-accessible surface of DPP-4 binding site with shape. Surfaces are colored by hydrophobic and hydrogen bonding (HB) properties: HB acceptor (red), HB donor (blue), HB acceptor/donor (magenta), hydrophobic (grey), aromatic hydrophobic (green) (b) Important protein-ligand interactions in a co-crystal structure of DPP-4 and cyanopyrrolidine. Dashed red lines indicate protein-ligand hydrogen bonds and the dashed blue line shows the covalent linkage between the ligand and Ser630. Residues Tyr631, Val56, Trp659, Tyr666, and Val711 lining the S1 pocket in the back are removed for the sake of clarity. Reprinted from Curr. Top. Med. Chem [Bernd Kuhn. et.al. Molecular recognition of ligands in dipeptidyl peptidase IV. Curr. Top. Med. Chem 7 (2007) 609-619] with permission from author.

1.4 DPP-4 Inhibitors

With their own common general advantages and limitations (Table 1.2), at present there are eight DPP-4 inhibitors 1-8 in market (Fig. 1.5) and many are in different phases of drug development [33,34].

These all are more or less similar in their efficacy for DPP-4 inhibition at nanomolar concentration in vitro. The IC$_{50}$ values are ~1 nM for linagliptin Vs. 19, 62, 50, and 24 nM for sitagliptin, vildagliptin, saxagliptin and alogliptin respectively [35]. Also the IC$_{50}$ values for teneligliptin, anagliptin, and gemigliptin are 0.37, 3.8 and 16 nM respectively [36-38]. The comparison of various properties has been described in Table 1.3 [39]. In-vivo, all are giving almost 90% inhibition within 15 min. of administration while around 70–90% inhibition is being sustained till 24h after oral administration of therapeutic dose in humans [39].
Table 1.2. Advantages and limitations of DPP-4 inhibitors

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased risk of hypoglycaemia</td>
<td>Lack of substrate specificity i.e. side effects due to the effect of DPP-4 on</td>
</tr>
<tr>
<td></td>
<td>multiple substrates such as neuropeptides, cytokines or chemokines and also</td>
</tr>
<tr>
<td></td>
<td>have an effect on immunomodulation, cell adhesion and cell movement.</td>
</tr>
<tr>
<td>Orally available</td>
<td>Lack of selectivity against other closely related enzymes DPP-2,DPP-8, DPP-9</td>
</tr>
<tr>
<td></td>
<td>and fibroblast activation protein-α (FAPα)</td>
</tr>
<tr>
<td>Potential for regeneration and differentiation</td>
<td>Other side effects like headache, urinary tract infections, nasopharyngitis</td>
</tr>
<tr>
<td>of pancreatic β-cells</td>
<td>etc.</td>
</tr>
<tr>
<td>Well tolerated</td>
<td></td>
</tr>
<tr>
<td>Weight neutral</td>
<td></td>
</tr>
<tr>
<td>Better glycemic control</td>
<td></td>
</tr>
<tr>
<td>over a longer duration of time</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular benefits (improvement of lipid</td>
<td></td>
</tr>
<tr>
<td>profile, blood pressure, endothelial, and</td>
<td></td>
</tr>
<tr>
<td>myocardial function)</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1.5. Marketed DPP-4 Inhibitors
Table 1.4. Selectivity of marketed drugs against various DPP enzymes

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Selectivity</th>
<th>DPP-2</th>
<th>FAPα</th>
<th>DPP-8</th>
<th>DPP-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitagliptin [52]</td>
<td>High</td>
<td>&gt;1,00,000</td>
<td>&gt;10,000</td>
<td>48,000</td>
<td>&gt;1,00,000</td>
</tr>
<tr>
<td>Vildagliptin [43, 50]</td>
<td>Moderate</td>
<td>&gt;5,00,000</td>
<td>&gt;10,000</td>
<td>&gt;100</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Saxagliptin [52(b), 54]</td>
<td>Moderate</td>
<td>&gt;6000</td>
<td>&gt;1000</td>
<td>&gt;400</td>
<td>&gt;75</td>
</tr>
<tr>
<td>Alogliptin [53]</td>
<td>High</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>Linagliptin [35]</td>
<td>Moderate</td>
<td>&gt;1,00,000</td>
<td>89</td>
<td>40,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>Teneligliptin [36]</td>
<td>Moderate</td>
<td>700</td>
<td>1500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anagliptin [37]</td>
<td>High</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gemigliptin [38]</td>
<td>Moderate</td>
<td>&gt;3000</td>
<td>&gt;3000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.4.2 Longer Acting DPP-4 Inhibitors under Development

Current need of pharmaceutical companies is to discover and develop selective DPP-4 inhibitors with long half-lives that are amenable for once-weekly dosing to improve patients compliance in T2DM. Two such longer acting drugs, Omarigliptin 9 [57] and Trelagliptin 10 (Fig. 1.6) [58] got recent market approval in Japan in September 2015 and March 2015 respectively.

![Fig. 1.6. Longer acting DPP-4 inhibitors under development](image)
1.5 Computer Aided Drug Design (CADD)

Nowadays, Computer Aided Drug Design (CADD) is an indispensable tool of new drug discovery starting from target identification to pre-clinical evaluation of a lead molecules [59]. Almost all new drug molecules are being designed and developed using a rational approach of drug discovery and also resulted into many fruits in the market like dorzolamide, zanamavir, sildenafil, sitagliptin, amprenavir etc. [60]. The main advantage of CADD is to speed up the drug discovery process with reduced cost by minimizing trial and errors. CADD mainly focuses on different structure based drug design (SBDD) methods as well as ligand based drug design (LBDD) methods [59a, 61].

Pharmacophore modelling is one of such tool under both SBDD and LBDD. The concept of pharmacophore was originally developed by Paul Ehrlich during late 1800s [62a]. Later, many other research groups have explained this concept in more or less similar words. Since 1998, it is defined as “The ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response”. [62b]. These features can be labelled as hydrogen bond donor, hydrogen bond acceptor, positive ionisable and negative ionisable, hydrophobic and ring aromatic. A pharmacophore model consists of a different set of these features in a specific 3D orientation which should be validated for desired activity and then later can be used for virtual screening of a chemical database to identify novel hits. The pharmacophore model is also useful to enrich the docking results as well as prediction of ADMET properties [63].

Quantitative structure–activity relationship (QSAR) is an extensively used method under LBDD. It applies statistical methods to evaluate the relationship between the various structural features of ligands and their biological activity. Earlier 2D-QSAR was used to correlate the physicochemical parameters of ligands with biological effects while nowadays various 3D-QSAR methods are adapted to overcome the stereo chemical limitations of 2D-QSAR. The main aim of 3D-QSAR is quantitative predictions of the biologically critical properties of individual structures. It has drastically reduced the trial and error factor involved in the synthesis and further development of a new lead molecules by facilitating the selection of the most promising candidates through their predicted activities before the synthesis. Several success stories of QSAR have attracted the medicinal chemists to investigate the relationships of structural properties with biological activity [64-66].
Chapter 1. Introduction

1.6 Aim and Objectives of Present Work

Looking into the current research of developing novel DPP-4 inhibitors, new hits or leads with novel heterocyclic scaffold are required which can be further modified/optimized to desired candidate.

So, the aim of this research is to design, synthesis and evaluate the new heterocyclic compounds as novel DPP-4 inhibitor hits and so as anti-diabetic agents.

To identify such novel heterocyclic hits which has not been reported for DPP-4 inhibition so far, first objective was to generate the pharmacophore and 3D-QSAR models to get an idea about the crucial ligand features required for DPP-4 inhibition. The validated models were then used for virtual screening of few chemical databases. Few top scored hits were selected for docking and further structural modifications.

Second objective was to design the new heterocyclic molecules based on the results of virtual screening, docking scores, 3D-QSAR contour maps as well as synthetic feasibility and finally to predict their in-silico binding affinity and ADMET properties.

The third objective was to synthesize and characterize the designed compounds which showed promising in-silico results.

The fourth objective was to evaluate their in-vitro DPP-4 inhibition and in-vivo anti-hyperglycemic effect.

1.7. References


Chapter 1. Introduction


Chapter 1. Introduction


Chapter 1. Introduction


when compared to a rapidly-dissociating DPP4 inhibitor.” *BMC Pharmacology* 12.2 (2012): 2-12

(b) Andukuri, Radha, Drincic, Andleja and Rendell, Marc “Alogliptin: A new addition to the class of DPP-4 inhibitors.” *Diabetes Metab Syndr Obes* 2 (2009): 117-126


[56] J. Larsen et al. “Glucagon-Like Peptide-1 infusion must be maintained for 24 h/day to obtain acceptable glycemia in type 2 diabetic patients who are poorly controlled on sulphonylurea treatment.” *Diabetes Care* 24.8 (2001): 1416-1421


Chapter 1. Introduction


