

INSILICO DESIGN OF PYRAZOLE DERIVATIVES AS DPP IV INHIBITORS**S. AMUTHALAKSHMI* AND A. ANTON SMITH**

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ABSTRACT

Objective: The current study was designed to identify a suitable inhibitor agent for DPP IV. Computer-assisted molecular modeling approach has contributed to the successful discovery of several novel antidiabetic DPP IV agents. In this work, we identified a DPP IV inhibitor by docking-based virtual screening method.

Materials and methods: Docking was performed with the Glide. Maestro v9.0 graphical user interface (GUI) workspace was used for all the steps involved in ligand preparation, protein preparation, HTVS and Docking.

Results: The newly designed pyrazole molecules have good score and energy when compared with standard. The compound PYA1 showed good orientation in the active pocket of DPP IV.

Discussion and conclusion: Docking results showed pyrazole derivatives have interaction within the active pocket of DPP IV receptors. These compounds are expected to have good in vivo activity and would represent a potential scaffold as a DPP IV inhibitor.

Keywords: Antidiabetic, Docking, DPP IV, Glide, Pyrazole, Type II diabetes.

INTRODUCTION

Diabetes mellitus is a rapidly increasing metabolic disorder characterized by hyperglycaemia together with disturbances of glucose, lipid and protein metabolism [1]. In people with diabetes, however, the pancreas either produces little or no insulin, or the cells do not respond appropriately to the insulin that is produced [2]. Type 1 diabetes is an autoimmune disease, resulting from selective destruction of the insulin producing β cells in the pancreatic islets [3]. Type 2 diabetes is world wide health crisis increasingly being diagnosed in children and adolescents. The pancreas is usually producing enough insulin, but for unknown reasons the body cannot use the insulin effectively, a condition called insulin resistance.

Diabetes is widely recognized as one of the leading causes of death and disability in the world. Urbanization and the accompanying changes in lifestyle are the main drivers of the development of diabetes. Advancement on the social and economic front in developing countries such as India which have resulted in remarkable lifestyle changes leading to lifestyle related diseases. The evolution of a traditional to modern lifestyle, consumption of diets rich in fat and calories combined with a high level of mental stress has compounded the problem further. There are several studies from various parts of India which reveal a rising drift in the incidence of type II diabetes in the urban areas [4].

Glucagon-like peptide 1 (GLP-1) an incretin hormone is known to act as a mediator of glucose-dependent insulinotropic action, and several clinical studies have shown that this peptide has an antidiabetic action in subjects with type 2 diabetes [5]. The active form of GLP-1 is rapidly inactivated by the plasma DPP-4, which cleaves a dipeptide from the N-terminus [6, 7] limiting its duration of action. On the other hand, GIP is drawn in glucose metabolism by enhancing insulin secretion [8]. Both peptides have short half-lives because of their rapid degradation by DPP-IV. Therefore, inhibiting DPP-IV prolongs the action of GLP-1 [9] and GIP, which, in turn, improves glucose homeostasis with a low risk of hypoglycemia [10].

Sitagliptin was the first DPP-IV inhibitor marketed by Merck & Co. [11], which was followed by the structurally similar vildagliptin (Novartis) [12] and saxagliptin (Bristol-Myers Squibb and AstraZeneca) [13]. The efficacy and safety profile of DPP-IV inhibitors have been promising and advantageous to date. In contrast to other antidiabetic drugs, DPP-IV inhibitors do not have an intrinsic risk of inducing hypoglycemia, and they are body-weight

neutral. Their tolerability profile is good, and no specific adverse reactions have been reported.

The DPP-IV binding site is highly druggable in the sense that tight, specific binding to the enzyme can be achieved with small molecules with drug-like physicochemical properties [14]. The two key binding-site areas for the intermolecular interaction of DPP-IV and reversible inhibitors of non-peptide nature are the lipophilic S1 pocket (formed by Tyr631, Val656, Trp659, Tyr662, Tyr666 and Val711) and the negatively charged Glu205/206 pair [15].

Computational power was applied to the combined chemical and biological space in order to rationalize computer aided drug discovery and development. This strategies for structure based drug discovery offer a valuable alternative to the costly and time consuming process of random screening. Molecular docking studies are designed to predict how small molecules, such as substrates, bind to a receptor of known 3D structures. The goal of the present study was to identify a small molecule as a DPP IV inhibitor. Glide software was used for the docking studies to identify the hit molecules.

MATERIALS AND METHODS**a) Molecular Modeling Studies**

Molecular modeling studies have been carried out using *GLIDE (Grid-based Ligand Docking with Energetics)* software v5.5 developed by Schrödinger running on Red Hat Enterprise Linux 5 (RHEL5) workstation. *Maestro v9.0* Graphical User Interface (GUI) workspace was used for all the steps involved in ligand preparation, protein preparation and docking.

ChemBiodraw Ultra is chemical drawing software developed by Cambridge Pvt. Ltd. The software is user-friendly, provides all details of drawn structures and helped to calculate chemical properties, design professional reports and presentations.

b) Ligand Preparation

The ligands used in this study were prepared using LigPrep [16] module of v2.3 of Schrödinger Suite 2009. *LigPrep* follows OPLS-AA (Optimized Potential Liquid Simulations for All Atoms) force fields for energy minimization.

c) Protein Preparation

The X-ray crystal structures retrieved from PDB database as raw could not be suitable for molecular docking studies. A typical PDB

structure consists only of heavy atoms, waters, Cofactors, metal ions and can be of multimeric. These structures do not have the information about bond orders, topologies or formal atomic charges. So, the raw PDB structure should be prepared in a suitable manner for docking. *Protein Preparation Wizard* of *GLIDE* software was used to process and prepare the protein. This also follows the Optimized Potential for Liquid Simulations-All Atoms (OPLS-AA) force fields for energy minimization.

d) Lipinski Rule of Five

Lipinski rule of 5 helps in distinguishing between drug like and non drug like molecules [17]. It predicts high probability of success or failure due to drug likeness for molecules complying with 3 or more of the following rules

- Molecular mass less than 500 Dalton
- High lipophilicity (expressed as LogP less than 5)
- Less than 5 hydrogen bond donors
- Less than 10 hydrogen bond acceptors
- Molar refractivity should be between 40-130

These filters help in early preclinical development and could help pass up costly late-stage preclinical and clinical failures. In this study, we also calculated all five parameters for all the designed compounds.

e) Docking by Glide

The molecular docking tool, Glide (Schrodinger Inc. U.S.A.) software was used for ligand docking studies in to the Protein DPP IV binding pocket. Glide (Grid-based Ligand Docking with Energetics) is one of the most accurate docking tools available for ligand-protein, protein-protein binding studies. Glide was found to produce least number of inaccurate poses and 85% of Glides binding models had an RMSD of 1.4 Å or less from native co-crystallized structures [18].

Initially a receptor grid, where the ligand has to be docked with the receptor was set by picking the centroid of the co-crystallized inhibitor present at the active site. It creates a grid box and the size of the grid box was limited to 20 Å. The generation of different conformations of the docked complexes (poses) was set to a maximum of 20. Based on the literature we designed 50 derivatives of various pyrazole compounds. The pyrazole derivatives were docked at the active site of 2P8S individually. The poses generated were ranked based on glide score (G-score) and glide energy (G-energy).

G-score takes into account a number of parameters like hydrogen bonds (H-bond), hydrophobic contacts (Lipo), van der-Waals (vdW), columbic (Coul), polar interactions in the binding site (Site), metalbinding term (Metal) and penalty for buried polargroup (BuryP) and freezing rotatable bonds (RotB).

G-score = H bond + Lipo + Metal + Site + 0.130 Coul + 0.065 vdW – BuryP – RotB.

The pose that made the maximum hydrogen bond (H-bond) interactions from pyrazole derivatives – 2P8S docked complexes were considered for further analysis and the results are compared.

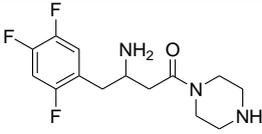
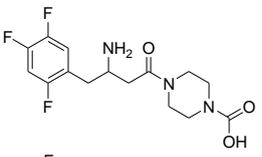
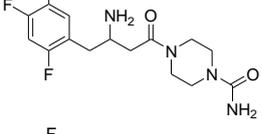
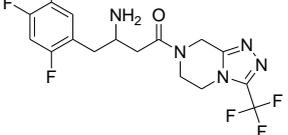
f) Visualization and Analysis

The *PyMol* Molecular Graphics System [19] was used to analyze the hydrogen bond interactions and preparation of high resolution images.

RESULTS

To discover new scaffolds for DPP IV, all the pyrazole derivatives were docked into the DPP IV binding site of 2P8S individually. The physicochemical properties (Table 1) of the resulting virtual screening hits were also analyzed. The results of the three best pyrazole DPP IV inhibitors are presented in the form of G-score and G-energy.

Table 1: Structure and physicochemical properties of top 3 docked compounds with standard sitagliptin

Cpd code	Structure	MW (g/mol)	HA ¹	HD ²	Log P
PYA 1		301.14	2	1	0.57
PYA 2		345.32	1	3	0.54
PYA 3		344.33	2	2	0.17
Sitagliptin (standard)		407.31	1	3	2.2

HA¹ = Number of hydrogen bond acceptor groups

HD² = Number of hydrogen bond donor groups

Two-dimensional representations of the binding models of the top 3 compounds with the crystallographic structure of standard sitagliptin are shown in Fig 1. The β-amino group in the

intermediate chain of sitagliptin (yellow) formed three H bonds with the oxygen atom in the side chain of Glu 206 and nitrogen in Arg 125 in the S1 pocket of DPP IV (red dotted lines).

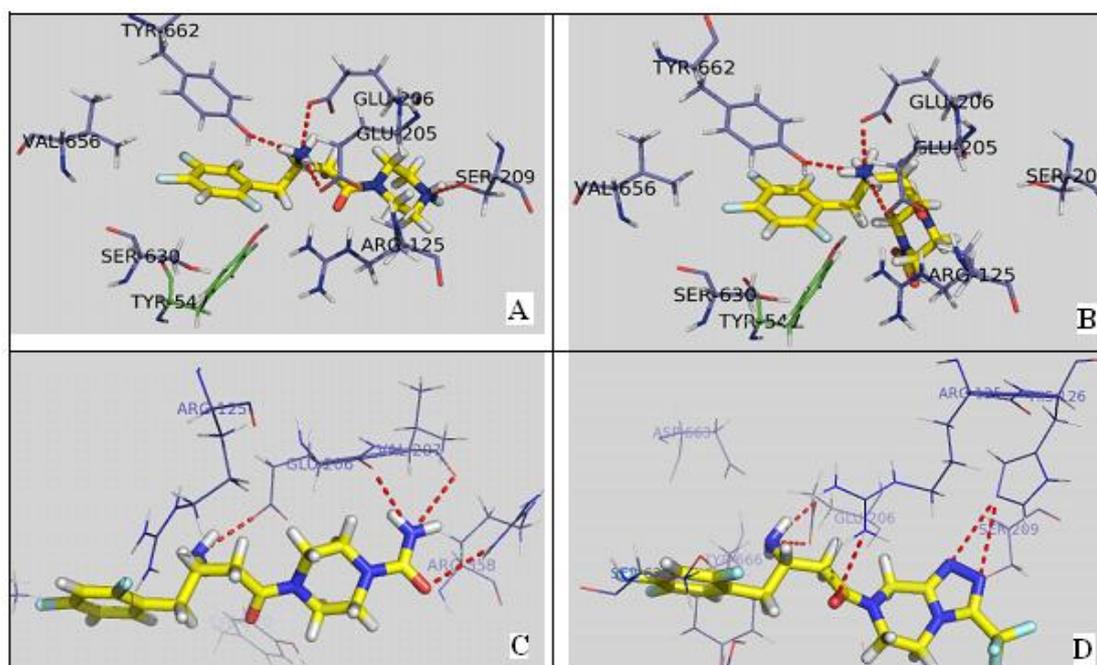


Fig. 1: Docked overlay images of the top 3 pyrazole derivatives viz. PYA1(A), PYA2(B) & PYA3(C) with sitagliptin(D). Hydrogen bonds are shown as red dashes. Non-polar Hydrogen atoms are omitted

The two cyclic nitrogen of the triazolo piperazine ring of sitagliptin made two H bonds with nitrogen of His 126. Similarly the compound PYA1 intermediate amino nitrogen made H bond with Asn 710. The oxygen made two H bonds with Tyr 631 & Arg125. The pyrazole ring nitrogen also showed another H bond with Glu 206. PYA2 also showed three H bonds Glu 206, Tyr 662 & Glu 205. In compound PYA3 the intermediate and the carboxamide nitrogen made H bonds with Glu 206. Additionally H bonds were observed for carboxamide nitrogen and oxygen at the 4th position of pyrazole ring with the oxygen of Ser 209 & Arg 358.

DISCUSSION

For the prediction of results mainly two parameters i.e; G-score, Glide energy are considered. The compounds PYA1-3 showed good G.score (Table 2) and G.energy (PYA1, A2 & A3: -10.80, -54.56; -9.76, -52.41; -9.74, -58.16) than the other pyrazole derivatives when compared with the standard (sitagliptin -8.12, -43.60).

Table 2: Results of extra precision docking studies of Compounds (PYA1- 3) with Standard Sitagliptin

S. No.	CPD Code	Glide Score	Glide Energy (Kcal/mol)
1.	PYA 1	-10.80	-54.56
2.	PYA 2	-9.76	-52.41
3.	PYA 3	-9.74	-58.16
4.	Sitagliptin (Standard)	-8.12	-43.60

The more negative value of G-score indicates good binding affinity of the ligand with receptor. The minimum energy for the formation of complex between ligand and receptor indicates good binding affinity. More H-bonds in the structure shows ligand having good binding mode to receptor. In the present study we aimed to identify to a suitable agent which would have good *in vivo* activity. It is also worth mentioning the three-dimensional similarity of sitagliptin and all pyrazole compounds have a linearity and similar flexibility. Since the selected hits are expected to exhibit good *in vivo* activity, these three compounds are considered for wet lab work.

CONCLUSION

The top 3 compounds with good docking score will be subjected to wet lab work viz., synthesis and evaluation using streptozotocin induced diabetes mellitus and DPP IV inhibitor assay studies. The

results of dry lab work and wet lab work will be analyzed thoroughly to find out correctness of the rational used for the design of novel entities in general and optimization of pharmacophore for inhibition of DPP IV.

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