Middle-East Journal of Scientific Research 16 (5): 580-586, 2013
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2013.16.05.763

Insilico Design of Piperazinone Derivatives as DPP IV Inhibitors in Type II Diabetes Mellitus

Amuthalakshmi Sivaperuman and Anton Smith Arul Gnana Dhas

Department of Pharmacy, Annamalai University,
Annamalai Nagar-608001, Tamilnadu, India

Abstract: Diabetes is a complex metabolic endocrine disorder that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. It is classified into two basic forms Type I and Type II diabetes. Computer-assisted drug design approach has contributed to the successful discovery of several novel antidiabetic agents. Molecular docking continues to be a great promise in the field of computer based drug design. In this study molecular modeling was used to design the drug molecules. Docking results showed six piperazinone derivatives have good interaction with the DPP IV receptors. These compounds showed good orientation and score. This novel compounds will represent a potential scaffold for the design of clinically useful DPP IV inhibitors.

Key words: Docking · DPP IV · Glide · Insilico · Piperazinone · Type II Diabetes

INTRODUCTION

Diabetes Mellitus, better known as type II diabetes, is one of the most onerous chronic diseases and undoubtedly one of the most challenging health problems in the 21st century [1]. It is the fourth or fifth leading cause of death in most high-income countries and there is substantial evidence that it is epidemic in many economically developing and newly industrialized countries [2]. In middle-income countries in particular, the epidemic is hitting younger people and causing death and disability early.

According to the international diabetic federation (IDF) 4.6 million people 20-79 years of age died from diabetes in 2011, accounting for 8.2% of global all-cause mortality of people in this age group. This estimated number of deaths is similar in magnitude to the combined deaths from several infectious diseases that are major public health priorities [3]. Looking at diabetes deaths against spending for diabetes care shows us the impact of a lack of treatment very starkly. The complications of type II diabetes and increasing pervasiveness emphasize the urgent need for new treatment strategies [4].

Novel approaches to glycaemic control includes use of inhibitors of the sodium–glucose co-transporter 2 [5, 6], inhibitors of 11β-hydroxysteroid dehydrogenase 1, agonists of the glucagon-like peptide-1 receptor and inhibition of dipeptidyl peptidase IV (DPP-IV) (increatin based treatments), Protein Tyrosine Phosphatase 1-Beta (PTP-1B) [7], Glycogen phosphorylase [8], Dipeptidyl peptidase IV (DPP IV) [9], Glucokinase [10], Peroxisome Proliferator-activated Receptor (PPAR)-γ [11], 3-hydroxy-3-methylglutaryl(HMG) Co-A Reductase [12].

DPP-IV is an attracting therapy with novel mechanism and improved tolerability [13]. DPP-IV specifically cleaves the active peptide at the alanine residue that is penultimate to the N terminus [14] and transforming them into inactive or even antagonistic species. The two main incretins are Glucose-dependent insulinotropic polypeptide (GIP) and Glucagon-like peptide (GLP-1), released after meals to enhance glucose-stimulated insulin secretion. The deactivation of incretin hormones by DPP-IV makes inhibitors of DPP-IV promising novel scaffolds in the treatment of type 2diabetes. GLP is released from intestinal L-cells after a meal and potentiates both glucose stimulated insulin secretion [15] and inhibition of glucagon secretion [16]. GLP-1 has many actions like increased insulin gene expression and biosynthesis, islet neogenesis and inhibition of pancreatic β-cell apoptosis, leading to increased β-cell mass. GIP, another incretin hormone produced by the K-cells, is located in the duodenum and like GLP-1, is also

Corresponding Author: Amuthalakshmi Sivaperuman,Department of Pharmacy, Annamalai University, Annamalai Nagar-608001,Tamilnadu, India. Tel: +91 9894893066.

extensively involved in glucose metabolism [17]. Since the intact N-terminal ends of both GLP-1 and GIP are essential for biological activity [18-20], cleavage of the N-terminal dipeptide segment by DPP-IV plays an important role in maintaining glucose homeostasis. Thus, shielding incretin hormones from the catastrophic effects of DPP-IV enzyme by the administration of inhibitors will maintain the concentrations of the incretin hormones and prolong their efficient antidiabetic action.

DPP4 inhibition, through the preservation of active GLP-1 levels, offers a number of potential advantages over existing diabetes therapies including minimized risk for hypoglycemia and improved b-cell survival [21]. Clinical studies have shown that DPP4 inhibitors are well tolerated, lower blood glucose levels, improve insulin response to oral glucose and decrease HbA1c levels in patients with type 2 diabetes [22].

Lead discovery is one of the most important components in rational drug design. The shortcoming of traditional drug discovery; as well as the allure of a more deterministic approach to combating disease has led to the concept of "Rational drug design". Insilico drug designing is a form of computer-based modeling whose technologies are applied in drug target identification or drug discovery processes. Unlike the historical method of drug discovery, by trial-and-error testing of chemical substances on animals and matching the apparent effects to treatments, Insilico drug design begins with knowledge of specific chemical responses in the body or target organism and tailoring combinations of these to fit a treatment profile.

MATERIAL AND METHODS

Chem Biodraw Ultra: In order to conduct docking studies the structures of all ligands were prepared using ChemBiodraw Ultra 8.0, chemical drawing software developed by Cambridge Pvt. Ltd. The software is user-friendly, provides all details of drawn structures. It helped in calculate chemical properties, design professional reports and presentations.

Protein Data Bank (PDB): The PDB is the single, global archive for information about the 3D structure of biomacromolecules and their complexes, as determined by X-ray crystallography, NMR spectroscopy and cryoelectron microscopy. The crystal structure of DPP IV in complex with the sitagliptin inhibitor was retrieved from the Protein Data Bank (PDB entry 2P8S) [23].

Protein Preparation: A typical PDB structure file consists of heavy atoms, water molecules, cofactors, metal ions and can be multimeric. The structure generally has no information on bond orders, topologies, or formal atomic charges. 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. The Protein Preparation Wizard module of Maestro was used to prepare the protein. During protein preparation, water molecules and peptide substrate (NAG) were deleted. This follows the Optimized Potential for Liquid Simulations-All Atoms (OPLS-AA) force fields for energy minimization.

Ligand Preparation: The LigPrep process consists of a series of steps that perform conversions, apply corrections to the structures, generate variations on the structures, eliminate unwanted structures and optimize the structures. Many of the steps are optional and are controlled by selecting options in the LigPrep panel by specifying command-line options.

High Throughput Protein Structure-Based Virtual Screening (HTVS): Design of small molecules as selective inhibitors of DPP-4 is a major challenge. Literature reports structurally two distinctive classes (peptidomimetic and non-peptidomimetic) DPP-4 inhibitors. Based on the literature reports we designed 100 compounds of various amino amide non-peptidomimetic heterocyclic derivatives. The High Throughput Virtual Screening (HTVS) of GLIDE *Schrödinger L.L.C* was used as a tool to filter the datasets prepared. Our objective in this is to filter roughly and exclude those compounds which were not expected to bind with DPP IV.HTVS resulted 78 compounds, among these the 26 compounds of piperazinone derivatives which have the Glide score and Glide energy better than the standard drug sitagliptin have been chosen for induced fit docking studies.

Docking by Glide: Docking was performed with the *GLIDE* (*Grid-based Ligand Docking with Energetics*) [24] 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, HTVS (High Throughput Virtual Screening) and Induced Fit Docking (IFD).

For docking, the scoring grids were centered on the crystal structure of compounds using the default bounding box sizes; with an inner box of 10 Å on each side and an outer box of 24Å on each side. Flexible docking with default parameters was used. Glide XP (extra precision) was employed for all docking calculations. The best docked poses were selected as the ones with the lowest Glide Score; the more negative the Glide Score, the more favorable the binding.

Induced Fit Docking (IFD): IFD of the prepared ligands with the prepared proteins was performed using Induced Fit Docking protocol of *GLIDE* v5.5 from Schrödinger Suite 2009 [25]. It is based on *GLIDE* and *Prime* Refinement module. In IFD, both the ligand and the receptor are flexible which enables to dock the ligand at the receptor's binding site to generate multiple poses of the receptor-ligand complex, each including unique structural conformations of the receptor to fit the ligand pose and ranks them by Glide score (G-score) to find the best structure of the docked complex. 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), metal binding term (Metal) and penalty for buried polar group (BuryP) and freezing rotatable bonds (RotB) G-score = H bond + Lipo + Metal + Site + 0.130 Coul + 0.065 vdW – BuryP – RotB. 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. The piperazinone derivatives were docked at the active site of 2P8S individually. The poses generated were ranked based on G-score. The pose that made the maximum hydrogen bond (H-bond) interactions from piperazinone derivatives – 2P8S docked complexes were considered for further analysis and the results are compared.

Visualization and Analysis: The *PyMol* Molecular Graphics System [26] was used to analyze the hydrogen bond interactions and preparation of high resolution images.

RESULTS AND DISCUSSION

Computer-assisted drug design (CADD) approach has contributed to the successful discovery of several novel small molecules as antidiabetic agents.

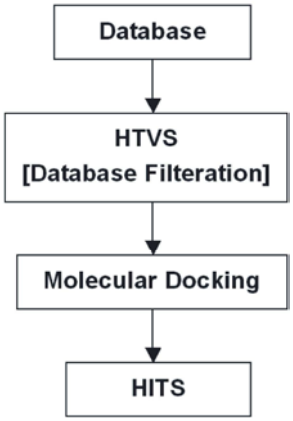


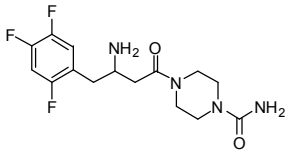
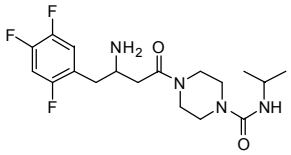
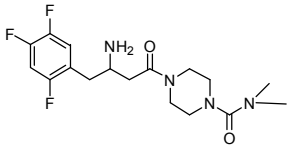
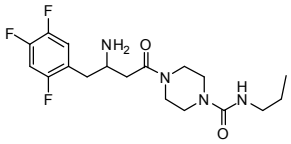
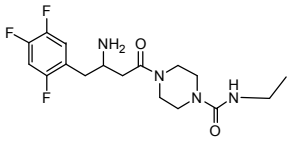
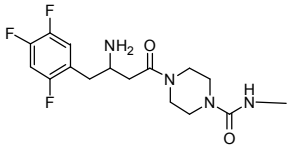
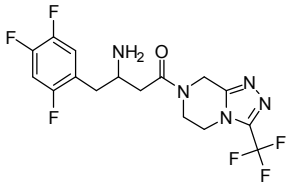
Fig. 1: Schematic representation of the steps adopted in virtual screening and docking

Molecular Docking continues to hold great swear in the field of computer based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. Number of reports citing successful relevance of CADD in developing specific drugs in different therapeutic areas is mounting rapidly.

The 26 piperazinone derivatives were docked into the DPP IV binding site of 2P8S. Two-dimensional representations of the optimized binding models of the top 6 compounds with the crystallographic structure of standard sitagliptin are shown in Figure 2. Designed ligands were pre-filtered for their drug like properties by lipinski's rule. Lipinski's rule of five was calculated for all the six ligand molecules that satisfy the 'rule of - 5' and it was found that all the ligand molecules satisfied the rule for potent promoters (Table 1). The sitagliptin and all other piperazinone derivatives, have similar binding mode. The docking score and the energy were good for all the compounds than the standard (Table 2). The β-amino group in the intermediate chain of sitagliptin (yellow) formed three H bonds with the oxygen atom in the side chain of Tyr 662, Glu 205 and Glu 206 in S1 pocket of DPP IV (yellow dotted lines). All piperazinone derivatives (A1-6 pink) occupied the same binding pockets and similar H bond interactions with Tyr 662, Glu 205 and Glu 206 (red dotted lines) as sitagliptin. A very similar binding model for sitagliptin was previously reported with Glide docking programs [27].

The amino group in the 4th position of the piperazine of the compound A1 formed an additional H bond with the oxygen of Glu 206. A H bond between the carbonyl oxygen at the 4th position of the piperazine of the compound A1 with the backbone NH of Arg358 of S2 pocket in DPP IV receptor was also predicted by Glide. In compounds A2, A4 & A6 H bond between the

Table 1: Structure and physiochemical properties of top 6 docked compounds with standard sitagliptin

Cpd code	Structure	MW (g/mol)	HA[1]	HD[2]	Log P
A1		344.33	3	2	0.1764
A 2		386.41	3	2	2.22
A 3		372.39	3	1	2.07
A 4		386.41	3	2	2.44
A 5		372.39	3	2	1.99
A 6		358.36	3	2	1.54
Sitagliptin		407.31	1	3	2.2

HA[1] = Number of hydrogen bond acceptor groups

HD[2] = Number of hydrogen bond donor groups

Table 2: Results of extra precision docking studies of Compounds (A1- 6) with Standard Sitagliptin

S.No	CPD Code	Glide Score	Glide Energy (Kcal/mol)
1.	A1	-10.01	-52.06
2.	A2	-9.98	-53.33
3.	A3	-9.95	-51.66
4.	A4	-9.90	-51.02
5.	A5	-9.62	-50.47
6.	A6	-9.60	-52.48
7.	Sitagliptin (Standard)	-8.73	-51.44

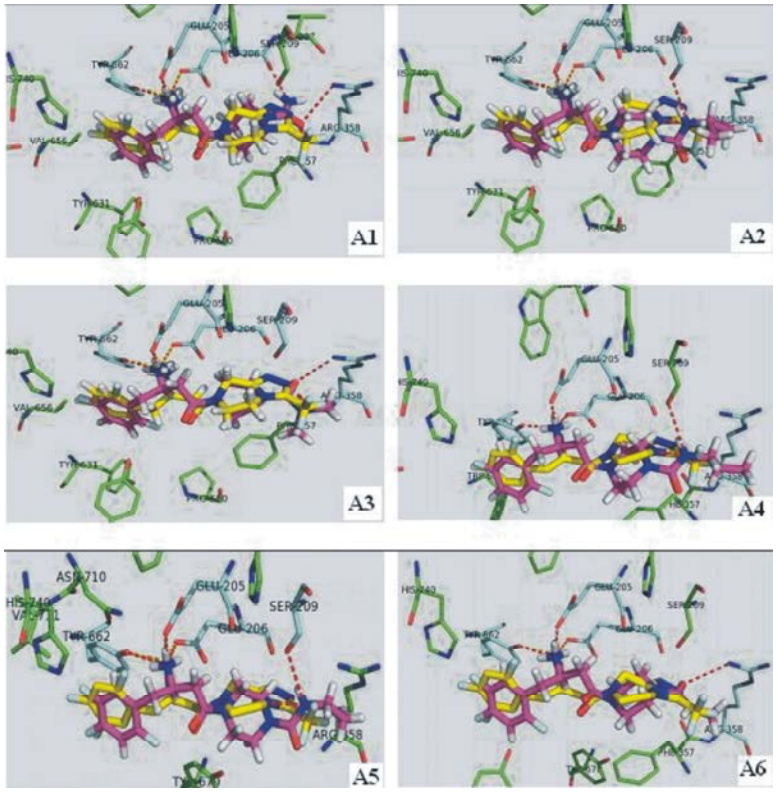


Fig. 2: Docked overlay images of the top 6 piperazinone derivatives (Pink) with sitagliptin (Yellow).Hydrogen bonds are shown as yellow (sitagliptin) and red (piperazinone derivatives) dashes. Non-polar Hydrogen atoms are omitted

carboxamide amino group at the fourth position of the piperazine ring and the oxygen in Ser 209 with the backbone was also observed [Fig 2 image (A2), (A4) and (A6)]. Notably the compounds A1, A3, A5 & A6 the piperazinone ring is close to the Phe 357 of S2 binding pocket and made H bond with the NH of Arg 358. It is also worth mentioning the three-dimensional similarity of sitagliptin and all piperazinone compounds have a linearity and similar flexibility. The selected hits are expected to exhibit good *in vivo* activity. These six compounds are considered for wet lab work.

CONCLUSION

The top 6 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.

ACKNOWLEDGMENTS

The authors thank Dr. Arun Kumar Gupta, Principal, R.K.D.F. College of Pharmacy and Director, Pharmbioresearch Center Pvt Ltd, Indore, India for providing computational facilities and support to carry out this research work.

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