The search for dipeptidyl peptidase iv (DPP4) inhibitors for the treatment of type 2 diabetes: an in-silico study

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Abstract. Dipeptidyl Peptidase IV (DPP4) enzyme is a dissolved plasma enzyme found in the intestinal, kidney and liver capillaries that degrade the Glucagon Like Peptide-1 (GLP-1), which is crucial in regulating blood glucose levels. Thus, DPP4 inhibition was considered as an important strategy to combat diabetes. In this research, a pharmacophore modeling and molecular docking was conducted to identify the potential hits of DPP4 inhibitors. The pharmacophore features consisted of three hydrogen bonds acceptors and one positive ion with Area Under Curve of Receiver Operating Characteristic (AUC-ROC) were 0.72 and GH score of 0.592. Screening on the ZINC database resulted in 1151 hit molecules, in which all molecules were subjected to molecular docking to explore their binding interactions. The binding energies of all ligand were between \(-5.08\) and \(-10.56\) kcal/mol, in which four hit molecules, i.e. The four best hit molecules in term of binding orientation and binding energy were Lig_1418/zinc215387739, Lig_37/zinc7983247, Lig_1432/zinc100998449, and Lig_1037/zinc104157322, exhibited better affinities than that of cognate ligand (ABT341, \(E=\,-9.98\) kcal/mol), which indicated their potentials as novel DPP4 inhibitors.

1. Introduction

Diabetes mellitus (DM) is a degenerative disease which become the most challenging health problem public in recent days. According to the International Diabetes Federation, its global prevalence affected more than 400 million people in 2017 and it is estimated to be about 600 million in 2045 [1]. Diabetes was characterized by the increased blood glucose levels as a result of improperly insulin function, and around 90% people was diagnosed by type 2 diabetes.

One of the molecular targets to improve hyperglycemic type 2 diabetes is dipeptidyl peptidase 4 (DPP4). DPP4 is a serine protease that is highly expressed in tissues and body fluids which deactivate the hormone glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [1–3]. GLP-1 is an important incretin hormone that helps insulin secretion and suppress the formation of glucagon, gastric emptying retardation, induction of satiety and stimulate the regeneration and differentiation of cells \(\beta\) pancreas. However, GLP-1 has a very short half-lives for their catalytic activity by the enzyme DPP4. Therefore, inhibition of the enzyme DPP4 is considered as a new therapeutic target to restore glucose homeostasis by increasing more GLP-1 and GIP, and thereby increasing the secretion of insulin which can improve hyperglycemia in diabetes mellitus type 2 [4–7].

Computational drug design has been a crucial approach in the discovery of new hit molecules against a wide variety of therapeutic targets [2,3,8]. It includes structure based and ligand-based drug design. In the present work, we used pharmacophore modeling which is a ligand-based drug design to develop a pharmacophore model which can be used to screen new molecular hits against a large
number of small molecules. In addition, molecular docking protocol was employed to explore the binding orientation of ligand with DPP4.

2. Methods
A pharmacophore was developed using structure of ABT341, which is a cognate ligand of DPP4. The pharmacophore model was then validated against 1079 actives and 6931 decoys, which originated from a Directory of Useful Decoy: Enhanched (Dude) (http://www.dude.docking.org/) [9]. This task was completed employing LigandScout Advanced 4.3 [10]. Virtual screening was done against ZINC database employing Pharmit (http://pharmit.csb.pitts.edu) [11].

The Molecular docking was conducted using iDock [12] software. Previously, structure of DPP4 was taken from the Protein Data Bank database (http://www.rcsb.org/pdb/) with PDB ID 2I78. The macromolecule was prepared by removing water molecules and Kollman charge was assigned using AutoDockTools 1.5.6 [13–16] software. The results of docking then visualized using Discovery Studio Visualizer.

The active side of the protein made to follow the natural ligand binding to the width of a grid of 40 x 40 x 40 on the X Y Z with spacing 0.375 Å. Another parameter used followed a default value of center x = -22.175, center and center y = -2.11 z = 63.989. Grid box (grid) was loaded with adjusting the active site on the protein.

3. Results and Discussion
The pharmacophore features of ABT341 consisted of 10 features which were three hydrophobic (yellow), one positive ion (blue colour), three hydrogen bond acceptors (red arrows), and 3 hydrogen bond donors (green arrows). Figure 1 showed the pharmacophore features of ABT341.

Further, pharmacophore models were developed using combination of those features and the following table 1 are good pharmacophore models based on the values of Area Under Curve of Receiver Operating Characteristics (AUC-ROC) which was above 0.5.
**Table 1.** The selected models of pharmacophore.

| Code   | Model of Pharmacophore       | Features                                      | Value of AUC\textsubscript{100%} |
|--------|------------------------------|-----------------------------------------------|----------------------------------|
| KIQ-1  | ![Image](image1.png)         | 1 positive ion, 3 hydrogen bond acceptors, 3 hydrogen bond donors | 0.57                             |
| KIQ-2  | ![Image](image2.png)         | 2 hydrophobics, 1 hydrogen bond acceptor, 3 hydrogen bond donors | 0.54                             |
| KIQ-3  | ![Image](image3.png)         | 1 hydrophobic, 1 hydrogen bond acceptor, 3 hydrogen bond donors | 0.62                             |
| KIQ-4  | ![Image](image4.png)         | 1 positive ion, 3 hydrogen bond acceptors     | 0.72                             |
| KIQ-5  | ![Image](image5.png)         | 1 hydrophobic, 1 positive ion, 3 hydrogen bond acceptors, 3 hydrogen bond donors | 0.50                             |

With five models generated, KIQ-4 with AUC\textsubscript{100%} 1.00 is 0.72 was chosen for its highest AUC score. The high AUC-ROC indicated that the model should be able to distinguish the actives from decoys [16]. Figure 2 displays the ROC curve.
Furthermore, virtual screening performed using the selected model of pharmacophore model retrieved 1,151 hit molecules. They are each docked against DPP4. Redocking of ABT341 gave binding energy of $-9.98$ kcal/mol, with RMSD 1.661 Å. The docked pose of ABT341 suggests two hydrogen bonding interactions with Tyr547 and three hydrophobic interactions with Tyr666, Tyr662 and Phe357. Pei et al. (2007) showed that ABT-341 is a robust and selective inhibitor of DPP4, having trifluorophenyl group which occupy S1 pocket [17]. In addition, the carbonyl oxygen oriented toward the water molecules that are positioned to bridge hydrogen interaction with Arg669 side chain (figure 3).

On the other hand, 1151 docked gave affinities in the range of $-5.08$ and $-10.56$ kcal/mol. The top four molecules include Lig_1418/zinc215387739 (E=$-10.56$ kcal/mol), Lig_37/zinc7983247 (E=$-10.39$ kcal/mol), Lig_1432/zinc100998449 (E=$-10.24$ kcal/mol), and Lig_1037/zinc104157322 (E=$-10.17$ kcal/mol). The affinities were higher than that of ABT341. Figure 4 shows the molecular structures of top hits.

**Figure 2.** The Area Under Curve of Receiver Operating Characteristic curve (AUC-ROC) of KIQ-4.

**Figure 3.** The docked (green) and X-ray conformation (gray) of ABT341 with RMSD 1.661Å. The hydrogen bonds were displayed in green colour.
The molecular structures of hit molecules.

Figure 4. The molecular structures of hit molecules.

The hydrogen bond (hbond) and hydrophobic interactions were noticed in the interaction of each hit. Hbond with Arg125 observed in crystallographic structure was reproduced in the binding of Lig_1418/ZINC215387739 and Lig_1432/ZINC100998449. In addition, hbond with Asn710 was also observed in the binding of Lig_1418/ZINC215387739. Lig_1037/ZINC104157322 interacted through Hbonds with Tyr547 and Arg669, while hbond interactions were established with Arg358 and His740 in the binding of Lig_37/ZINC7983247. The poses of lig_1418, Lig_1432/ZINC100998449, Lig_1037/ZINC104157322, and Lig_37/ZINC7983247, are depicted in figure 5.

Figure 5. The poses of Lig_1418/ZINC215387739, Lig_1432/zinc100998449, Lig_1037/zinc104157322, and Lig_37/zinc7983247. The hydrogen bond (hbond) interactions were showed in green colors.
4. Conclusion
In the present work, a pharmacophore modeling protocol was employed to identify the potential hit of DPP4. The built pharmacophore model was then utilized to screen new potential hit of DPP4 in the ZINC database which contained more than 13 million compounds. It was found that one molecule (lig_1418) had stronger affinity than that of native ligand (ABT341), which may be used for further experimental study.

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