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Research Article

NOVEL DRUG DISCOVERY FOR DIABETES TYPE-2 BY PHARMACOPHORE, VERTUAL SCREENING AND DOCKING OF PPARγ

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ABSTRACT

Diabetes mellitus (DM) is a metabolic syndrome that constitutes a major health problem most drugs currently employed in the treatment of type 2 diabetes targeting the peroxisome proliferator-activated receptor (PPARγ) improving increasing prevalence of metabolic disorders, such as type 2. The 3 PPAR isoforms (alpha, delta/beta and gamma) are known to control many physiological functions including glucose absorption, lipid balance, and cell growth and differentiation. The peroxisome proliferator-activated receptors (PPARs) are involved in the regulation of lipid and glucose metabolism. Peroxisome proliferator-activated receptor gamma (PPARγ) has become an attractive molecular target for drugs that aim to treat diabetes mellitus type 2, and its therapeutic potency against skin cancer and other skin diseases is also currently being explored.

We have developed a virtual screening procedure based on ligand-based pharmacophore construction based on known drugs of type 2 diabetes and protein-ligand docking to discover novel scaffolds of (PPARγ) partial agonists. A dataset consisting of approximately 5,00,000 small molecule natural compounds were downloaded in (SDF) format from ZINC database. It was used as an input in the Generate phase database panel of phase. The structures were cleaned and different conformations were generated for each compound along with defining the pharmacophore sites points for each. The database prepared was then used to screen potential diabetes mellitus type 2. Structure and ligand based approach of drug designing is used for analysis for that some inhibitor molecules have been taken for docking by using the software GLIDE (Grid-based Ligand Docking with Energetics), run under Schrodinger's Job Control facility by taken known protein structure from PDB (1NYX) .The predicted inhibitors are quite novel compared with the Known (PPARγ) inhibitor. The work provides insight for molecular understanding of (PPARγ) and can be used for development of anti-diabetes drugs.

Keywords: PPARγ, ZINC database, Schrodinger, PDB.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome that constitutes a major health problem. It is estimated that 246 million people worldwide have diabetes and 380 million people will be afflicted with diabetes by 2025 [1]. In addition, 3.8 million people died each year from diabetes [2]. DM is characterized by abnormally high levels of plasma glucose, known as hyperglycemia, in the fasting state or after the administration of glucose during an oral glucose tolerance test. DM is caused by a relative or absolute deficiency in insulin secretion, a resistance to insulin secretion or both [3]. The World Health Organization recognizes two distinct clinical forms of diabetes (Figure 1), type 1 diabetes (T1DM) and type 2 diabetes (T2DM). T1DM, also referred to as the juvenile variety of DM, results from an absolute deficiency of insulin due to the destruction of insulin-

producing pancreatic β-cells. T2DM is a multifactorial disease that is characterized by insulin resistance associated with not only hyperinsulinaemia and hyperglycemia but also atherosclerosis, hypertension and an abnormal lipid profile [4]. T2DM accounts for 90-95% of the diagnosed cases of DM [8]. Genetic and environmental factors, increased height and weight development, increased maternal age at delivery, and exposure to some viral infections have also been linked to the risk of developing T1DM. Several risk factors have been associated with T2DM, including obesity, changes in diet and physical activity, age, insulin resistance, a family history of diabetes and ethnicity [5]. Changes in diet and physical activity related to rapid development and urbanization have led to a sharp increase in the number of people developing diabetes [6].

T1DM and T2DM require careful monitoring and control. Without proper management, they can lead to very high blood sugar levels, which can result in long-term damage to various organs and tissues. The major chronic complications of diabetes are cardiovascular disease, which is the primary cause of death in people with diabetes [7, 8]; nephropathy, which can result in total kidney failure and the need for dialysis or kidney transplant, neuropathy, which can ultimately lead to ulceration and amputation of the toes, feet and lower limbs; and retinopathy, which is characterized by damage to the retina of the eye and can lead to a loss of vision [9]. Both T1DM and T2DM are chronic conditions that typically cannot be cured. However, all forms of diabetes have been treatable since the development of readily available insulin in 1921. The enhancement of insulin secretion by pancreatic islet β-cells is a major goal for the treatment of T2DM. Anti diabetic drugs or hypoglycemic agents are medications that work to lower blood glucose concentrations (i.e., the amount of sugar in the blood). There are different classes of anti diabetic drugs, and their selection depends on the nature of the diabetes and the age and situation of the person, as well as other factors. Antidiabetic drugs exert their useful effects through (1) increasing insulin levels in the body, (2) increasing the body's sensitivity (or decreasing its resistance) to insulin, or (3) decreasing glucose absorption in the intestines [10].

Notably, insulin, which is used to treat T1DM patients (for whom the hormone is no longer produced internally), is also occasionally used for patients with T2DM when other medications fail to adequately control blood glucose levels. However, hypoglycemia and weight gain are common side effects. Thus, new approaches are needed to treat T2DM. One of the desirable approaches to achieve this goal would be to identify agents that promote/enhance glucose (nutrient)-dependent insulin secretion. Extensive research has been conducted on the molecular targets for T2DM, including PPARγ, protein tyrosine phosphatase-1B (PTP1B), DPP-IV, glycogen synthase kinase-3 (GSK-3), pyruvate dehydrogenase kinase (PDHK), cannabinoid receptors, fructose-bisphosphatases, and β3-adrenoceptor (β3-AR), in an attempt to develop newer antidiabetic agents [11,12]. These therapeutic targets are important, and most of them are suitable for an in silico analysis.

PEROXISOME PROLIFERATE-ACTIVATED RECEPTOR GAMMA (PPARγ):

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor super family that regulate the gene expression of proteins involved in energy, glucose and lipid metabolism, the proliferation and differentiation of adipocytes and the sensitivity of insulin [13]. They function as cellular sensors that activate transcription in response to the binding of natural or synthetic ligands. Three receptor subtypes, PPARα, PPARβ/δ and PPARγ, have been identified. Although the three subtypes share a high level of sequence and structural homology, they exhibit differences in tissue expression and physiological function [14]. PPARα is found in the liver, kidney, heart, and muscle. It is important for the uptake and oxidation of fatty acids and lipoprotein metabolism. PPARα is the target of lipid lowering fibrates. PPARγ is localized in fat, large intestine, and macrophages. It plays an important role in adipocyte differentiation. PPAR β/δ is expressed in most cell types. Agonists of PPARα and PPARγ are currently approved for use in treating dyslipidemia and T2DM, respectively [15]. PPARβ/δ agonists play important roles in dyslipidemia, cancer treatment, and cell differentiation within the central nervous system.

PPARγ agonists: TZDs are an important class of synthetic PPARγ agonists. TZDs are anti diabetic agents that target adipose tissue and that improve insulin sensitivity. They are currently used in the treatment of T2DM. Despite the clinical

benefit of these drugs, the use of TZDs has been associated with adverse effects, including weight gain, increased adipogenesis, renal fluid retention, and possible increased incidence of cardiovascular events. Therefore, new PPARγ ligands with enhanced therapeutic efficacy and reduced adverse effects are needed. A promising new group of such ligands are selective PPARγ modulators (SPPARγMs) [16, 17]. These compounds act as partial agonists of PPARγ and display different binding properties when compared with full agonists. There is another type of synthetic PPAR agonists called dual PPARα/γ and pan PPARα/γ/β/δ ligands. They were developed in an attempt to achieve multiple therapeutic benefits; however, these compounds have encountered multiple safety issues that have thus far not been resolved [18].

PPARγ mechanism:

PPARs function through the formation of heterodimers with the retinoid X receptor (RXR) and dock to the promoter regions of genes, which regulates transcription in a ligand-dependent manner through the differential recruitment of co-activators and co-repressors [19]. PPARγ can considered a rheostat for insulin sensitivity that responds to an integrated nutritional status conveyed through multiple signals sensitive to the dietary and endocrine status [20]. Like other nuclear receptors, PPARs are modular in structure and contain the following functional domains: a N-terminal region, a DNA-binding domain (DBD), a flexible hinge region, a ligand binding domain (LBD) and a C-terminal region. The DBD contains two zinc finger motifs, which bind to specific sequences of DNA, known as hormone response elements, when the receptor is activated. The LBD has an extensive secondary structure that consists of 13 α-helices and a β-sheet (see Figure 3A) [21]. Natural and synthetic ligands bind to the LBD and either activate or repress the trans-activation activity of the receptor.

Because of their importance as pharmaceutical targets for regulating the fatty acid metabolism and ant-diabetic drugs and because they provide an interesting example of receptors interacting with other molecular partners in a ligand-dependent manner, the structure of the PPAR LBD has been intensively studied at the atomic level. Since the first experimental X-ray structures of PPARγ were obtained in 1998 [22], numerous structures have been determined for PPARα, PPARγ and PPARδ in both the ligand and apo forms, with or without a co-activator or a co-repressor, and in the presence or absence of RXR.

PPARγ is thought to be activated by full agonists via a molecular switch in the most carboxy terminal helix, H12, of the LBD. H12 forms part of the ligand-dependent activation domain AF-2 that closes on the ligand-binding site in response to ligand binding. The resulting active form can bind to several co-activator proteins that activate the cellular transcriptional machinery. Full agonists occupy the large binding site of PPARγ in a U conformation and are generally formed by a polar head and a hydrophobic tail. The polar head forms a net of hydrogen bonds with the Ser 289, His 323, His 449 and Tyr 473 PPARγ side chains. This net of hydrogen bonds is responsible for the conformational change of H12 and the activation of PPARγ [23]. Partial agonists, however, activate PPARγ using a H12-independent mechanism. The key interactions between partial agonists and the (LBD) of PPARγ are different, since partial agonists do not use the net of hydrogen bonds used by full agonists to bind to PPARγ. This causes a reduction in the degree of H12 stabilization that affects the recruitment of co-activators and that decreases the transcriptional activity of PPARγ [24]. With only minor differences, most of the currently described partial agonists interact with the LBD of PPARγ through a hydrogen bond with Ser342 and several hydrophobic interactions (Figure 3C). These hydrophobic interactions are similar to those used by full agonists. A new mechanism has been recently suggested by which partial and full PPARγ agonists may improve insulin sensitivity independent of receptor agonist. This mechanism consists in blocking the phosphorylation of PPARγ [25] and may explain how partial agonists can exhibit similar or higher antidiabetic effects than full agonists and the differing side-effect profiles of both types of agonists. These partial agonists may then achieve comparable efficacy in insulin sensitization through a similar inhibitory effect on PPARγ phosphorylation whereas the differences in their agonist potency could be linked to differences in side effects [26].

In practice, the choice of employing CADD approaches is usually determined by the availability of experimentally determined 3D structures of the target proteins. Thus, there

are two major types of drug design: ligand-based drug design and structure-based drug design. If protein structures are unknown, various methods of ligand-based drug design can be employed, such as quantitative structure activity relationship (QSAR) and pharmacophore analysis. If the target structures are known, structure-based approaches can be used, such as molecular docking, which employs the 3D structures of the targets to design novel active compounds with improved potency. As more structures are becoming available, the prediction accuracy will likely improve [27].

In modern computational biology, pharmacophore based approach is used to delineate the essential features of one or more molecules with the same biological activity. The pharmacophore based modeling of ligands is a well-established approach to quantitatively discover common chemical features among a considerable number of structures. Pharmacophore mapping can be used in designing the inhibitors in several ways, including justification of activity trends in molecules, searching of databases to find new chemical entities and to identify important features for activity [28]. In this study, the pharmacophoric features of the inhibitors of PPARγ have been developed. The pharmacophore based modeling has been carried out to identify the best features, such as hydro-gen bond acceptor, donor, aromatic ring, and aliphatic chain for PPARγ, from the existing drug which facilitates the drug activity for the identification of novel inhibitors from the natural compound database. Furthermore, docking and molecular dynamics (MD) simulation were performed to analyze the binding affinity of the identified natural compounds.

MATERIAL AND METHODS

Ligand preparation:

A data set of ligand molecules having PPARγ inhibitory activity were collected from the known drug bank. (Balsalazide, Indomethacin, Icosapent, Nateglinide, Mesalazine, Telmisartan, Rosiglitazone, Sulfasalazine, Repaglinide, Ibuprofen, Glipizide, Pioglitazone, Mitiglinide, Bezafibrate, Diclofenac and roglitazone are the ligand taken from the drug bank.) Quantitative pharmacophore was generated for the molecules based on the diversity of their chemical structure and biological activity against human PPARγ inhibitors. The PPARγ inhibitors used in this study were further energy minimized using Ligprep module of Schrödinger software. [29] The conformations of the above structures were generated using the MMFFs force field, with an implicit GB/SA solvent model. A maximum of 1000 conformers were generated per structure by a preprocess minimization of 1000 steps using ConfGen algorithm. During the search, hydrogen-bonding interactions were suppressed to facilitate con-formations in which the ligand bonds to the receptor, and not just conformations with internal hydrogen bonding, as this is essential for the model.

Pharmacophore model generation:

The quantitative pharmacophore model was built using the Phase software [30]. The diverse dataset were used to generate the pharmacophore model. A set of pharmacophore features for the PPARγ inhibitors were produced using create sites option, which creates the site points for each con-former of the above ligands. A default setting having acceptor (A), donor (D), hydrophobic (H), negative (N), positive (P), and aromatic ring (R) features were used to create pharmacophore sites. Pharmacophore hypotheses common for the set of active ligands were generated using these pharmacophore features. Common pharmacophore are identified from a set of variants, which is a set of feature type that defines a possible pharmacophore using a tree based portioning algorithm. The common pharmacophore hypotheses from the active ligands were scored by setting the root mean square deviation (RMSD) value below 1.0, the vector score value to 0.5. Higher survival score resembles better mapping of the pharmacophore with the active molecules. Apart from the survival score, fitness score was also used to confirm the quality of pharmacophore hypothesis [31].

Database screening:

The developed Pharmacophore hypothesis was further screened for the ZINC database of natural compounds holding approximately 5,00000 small molecule natural compounds was downloaded in (SDF) format from ZINC database. It was used as an input in the Generate phase database panel of phase. The structures were cleaned and different conformations were generated for each compound along with defining the pharmacophore sites points for each. The database prepared was then used to screen potential diabetes mellitus type II and screen for potent inhibitor for PPARγ. The criteria for finding out the hits was that all the

five out of five pharmacophoric features must match with default inter feature distance. ZINC database was scanned for geometric arrangements of pharmacophore sites that match the above generated hypothesis within a tolerance applied to the intersite distances. Molecules with fitness score above 1 were taken for docking studies.

Glide ligand docking:

Glide module was used to perform docking of the ligands to PPARγ with crystal structures, the PDB ID: 1NYX was chosen, and may be use-full starting point for drug design. Protein was prepared using protein preparation wizard which assigns bond order and adds hydrogen. The active site of the protein was defined using default parameters of receptor grid generation. Ligands were energy minimized using the Ligprep module. Docking was performed using XP docking mode (Extra Precision). The results of the docking were then quantified in terms of the Glide score and Glide energy.

RESULTS AND DISCUSSION

Pharmacophore model generation:

This study is aimed to screen inhibitors for PPARγ which plays a major role in Diabetes mellitus. Ligand based drug designing approach is employed to identify the novel molecules against PPARγ. The PPARγ inhibitors used to generate the pharmacophore model and ligands taken for this study include Balsalazide, Indomethacin, Icosapent, Nateglinide, Mesalazine, Telmisartan, Rosiglitazone, Sulfasalazine, Repaglinide, Ibuprofen, Glipizide, Pioglitazone, Mitiglinide, Bezafibrate, Diclofenac and roglitazone. Only the active compounds are considered when developing common pharmacophore hypotheses. Using tree based partition algorithm with maximum tree depth of five, a list of 10, four featured hypotheses from the variant list were generated. All these variants were selected to find the common pharmacophore among the diabetes mellitus (DM) inhibitors.

Table 1: The best 5 common pharmacophore hypotheses with survival active scores.

Hypothesis	Survival Active	Site	Matches
ADHR.22	3.138	0.67	4
ADHR.85	3.124	0.73	4
ADHR.70	3.103	0.68	4
ADHR.60	3.081	0.71	4
ADHR.20	3.045	0.7	4

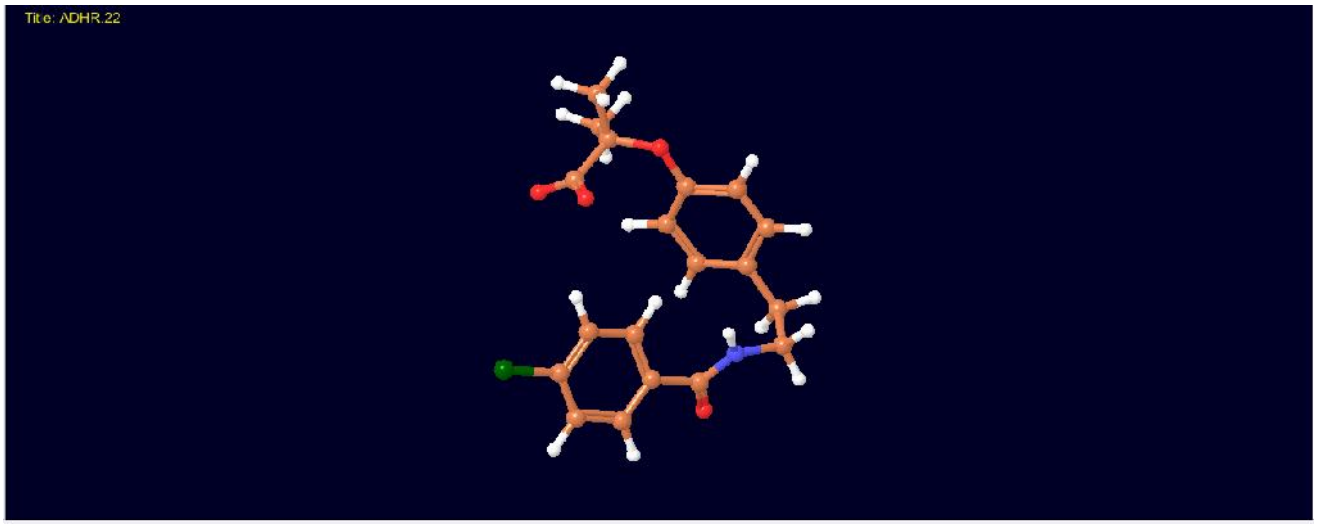

Figure 2: The best generated pharmacophore model ADHR.22, developed using Phase module.

Table 2: The low energy conformations of each ligand were selected for docking against protein.

File name	No of structure	No of hits	No of hits taken
3_p0.1.sdf	500000	28000	251

Table 3: Few Fitness scores and matched ligand sites for the ligand based hits.

Title	Num Sites Matched	Matched Ligand Sites	Fitness	Potential Energy-OPLS-2005
ZINC31820319	4	A(3) D(8) H(9) R(14)	-0.72524	143.165
ZINC77488580	4	A(2) D(6) H(7) R(11)	-0.70322	185.526
ZINC95453515	4	A(2) D(5) H(6) R(9)	-0.24513	146.035
ZINC25763854	4	A(5) D(6) H(7) R(10)	-0.72942	232.833
ZINC54774978	4	A(3) D(8) H(9) R(11)	-0.39903	87.3125

Table 4: Glide docking XP score, Vander wall score, XP H-bond etc, for ligand based hits.

Ligands	G Score	Lipophilic Vander wall	H-Bond	Electro	Low MW	Rot Penal
ZINC22018264	-9.76	-6.17	-0.7	-0.25	-0.29	0.19
Telmisartan	-9.63	-6.89	-1.63	-0.51	0	0.2
ZINC00146021	-9.07	-4.56	-1.63	-0.37	-0.5	0.1
ZINC27533573	-8.75	-8.78	-0.43	0.08	0	0.3
ZINC70925337	-8.72	-7.06	-0.65	-0.08	-0.03	0.31
ZINC04235689	-8.56	-7.59	-0.22	-0.24	-0.08	0.33
ZINC35592833	-8.51	-5.3	-1.05	-0.26	-0.34	0.33
ZINC25763854	-8.34	-6.06	-0.7	-0.26	-0.1	0.29
ZINC35592840	-8.2	-5.65	-0.97	-0.34	-0.37	0.35
ZINC59513055	-8.01	-5.6	-0.96	-0.19	0	0.22
ZINC81622109	-7.93	-5.2	-1.05	-0.41	-0.42	0.53
ZINC64705034	-7.9	-5.86	-0.44	-0.22	-0.41	0.3

Table 5: Hydrogen Bond Analysis.

Ligands	Amino acid	No of H-bond	H-bond distance(A)
ZINC22018264	LEU340	1	1.766
Telmisartan		2	1.947, 2.085
ZINC00146021	LEU 340, ILE 281	2	2.046, 1.920
ZINC27533573	TYR327	1	2.253
ZINC70925337	SER342, ARG288	2	2.130, 2.621
ZINC04235689	HIP323	1	2.042
ZINC35592833	ARG288, SER342	2	1.925, 2.087
ZINC25763854	CYS285	1	1.952
ZINC35592840	ILE281, GLU343	2	2.305, 2.139
ZINC59513055	TYR327	1	1.857
ZINC81622109	CYS285, ARG288	2	2.012, 1.751
ZINC64705034	SER342	1	2.119

A summary of statistical data of best 5 common pharmacophore with survival score is listed in Table 1.

Pharmacophore hypothesis named ADHR.22 has the best survival score of 3.138. The pharmacophore features present in this hypothesis have one accepter, one donor, one hydrogen bond, and one ring aromatic regions which are shown in Fig. 2.

Pharmacophore screening:

The best hypothesis ADHR.22 was used as a 3D query for retrieving the 5,00,000 active molecules from ZINC database using Phase software. The virtual screening of ZINC database has yielded several hits (Table-2) by matching the predicted hypotheses ADHR.22 to the ligand sites of the ZINC database and ranks the molecules based on the fitness score. The fitness scores and matched ligand sites for the few ZINC molecules were listed (Table 3). Apart from ZINC molecules, the known highly active from the dataset were taken to compare the binding modes with the receptor PPARγ.

Docking of ligands to PPARγ:

XP ligand docking was performed for the ligand molecules using the Glide module from the Maestro package. The best compounds were selected based on the glide score and its interaction with amino acid residues. Interestingly known active ligands were found to have lesser docking score compared to novel natural compounds. The 245 hits obtained from databases were docked with the PPARγ protein to predict their binding affinities and PPARγ inhibiting activity. Twelve compounds including one approved drug were shortlisted from the huge list of hits using extra precision docking of these small molecules against the active site of PPARγ. By this docking study we came to know that most of our designed ligands are interacting to various proteins with sufficient selectively and specificity. The docking analysis is done and the results are presented in the form of table given in (Table 4) and hydrogen bond interactions between receptor and ligands, given in the (Table 5).

CONCLUSION

PPARγ is an important protein in the process of improving, increasing prevalence of metabolic disorders, such as type 2 diabetes. Pharmacophore modeling for the PPARγ inhibitors was performed and four featured pharmacophore hypotheses were developed. A four point pharmacophore with one accepter, one donor, one hydrogen bond, and one ring aromatic regions including high survival score were predicted. This pharmacophore hypothesis is further used to screen the natural ZINC compound database for the identification of potential PPARγ inhibitors. From in silico studies, twelve compounds including one known approved drug were shortlisted from the huge list of hits using extra precision docking of these small molecules against the active site of PPARγ. These compounds were shown to possess high binding affinity for the PPARγ active site. Thus, these compounds can be considered as potential PPARγ inhibitors for the treatment of diabetes.

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