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Research Article

IDENTIFICATION OF NOVEL PPARγ MODULATORS / PARTIAL AGONISTS THROUGH VIRTUAL SCREENING WORKFLOW

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ABSTRACT

Thiazolidinedione's (TZDs) being insulin sensitizer's act as agonists of PPARγ used in the treatment of type 2 diabetes but suffered with serious side effects. After understanding the trans-activation mechanism of PPAR receptors and in order to overcome these side effects a new path has been led to new approaches like, PPAR-α/γ dual agonists, PPAR-δ/γ dual agonists, PPAR-pan agonists, selective PPAR-γ modulators (SPPARγMs) / partial agonists. Among them SPPARγMs) / partial agonists attracted due to their selectivity and expression in the selective tissue. The present study aims at identifying novel SPPARγMs) / partial agonists by using VS workflow and molecular docking. Virtual screening workflow is fallowed which consists of several steps like (a) Ligand based anti-pharmacophore screening(b) Ligand based Pharmacophore screening (c) ADME /Toxicity analysis and (d) Molecular Docking. Out of 21,818 molecules subjected to anti pharmacophore model, 4936 molecules qualify for the next step i.e., pharmacophore model screening. Out of these molecules only 12 molecules showed Qfit > 70. Therefore, these molecules were further subjected to ADME /TOX filter step in which 7 molecules passed the step. Further these molecules subjected to docking studies. In the docking studies based on the typical binding modes of the standard partial agonist (INT131) 5 molecules were found to have good binding mode required for a typical partial agonist. The Virtual screening workflow used in the study identifies 5 molecules as partial agonists.

Keywords: Insulin sensitizers, PPARγ partial agonists, Pharmacophore model, Virtual screening

INTRODUCTION

Peroxisome Proliferator Activated Receptors (PPARs) are transcription factor[1], which act by coordinating the activities of multiple pathways involved in metabolism instead of acting through one major target like one enzyme or one pathwa[2]. This unique property of PPARs has created lot of interest for their possible use in a complex metabolic disorder such as type 2 diabetes mellitus (T2DM)[3]. Thiazolidinedione's (TZDs) or glitazones are one such class of anti-diabetic drugs which act by increasing the trans-activation activity of Peroxisome Proliferator Activated Receptors (PPARs)[4]. Further, TZDs reverse insulin resistance without causing hypoglycaemic effect which is major side effect of most widely used anti diabetic drugs such as sulfonylureas. They reduce hepatic output of glucose and increase peripheral uptake, leading to reducing both pre-load and after load on the beta cell. These actions enhance the effectiveness of endogenous insulin, and reduce the amount of exogenous insulin needed to maintain a given level of blood glucose[5]; thus, providing an excellent rationale for the use of glitazonesin T2DM.

Unfortunately, these glitazones used in the clinic today suffer with some serious side effects such as, hepatotoxicity[6], increase in body weight, fluid retention, weight gain, risk of bone fracture[7], bladder cancer[8] and many more. These side effects reported to be due to their non-selective activation of PPARγ in the off-target tissues such as bone and kidney cells. As a result, WHO restricted the use of Rosiglitazone and changed the label claim of Pioglitazone for the risk of bladder cancer[7]. One of the reasons for the failure of these clinically used glitazones is, their time of development. These drugs were developed when there was very little scientific data available on structure and the transcriptional mechanisms of the target peroxisome proliferator activated receptors (PPARs). After understanding the trans-activation mechanism of PPAR receptors has led to the newer approaches to the discovery of development PPAR-α/γ dual agonists, PPAR-δ/γ dual agonists, PPAR-pan agonists, selective PPAR-γ modulators or partial agonists. Among them SPPARγMs attracted many researchers due to their selectivity and expression in the selective tissue.

SPPARγMs hypothesis is based on recruitment of certain differential receptor binding and co-factor recruitment/displacement with specificity to the selective tissue and their expression in favourable target cells. This concept is similar to selective estrogen receptor modulators (SERMs)[9,10]. SPPARγMs provide a target oriented therapeutic profile by maintaining the desired therapeutic benefits and at the same time have minimal adverse effects due to their inability to fully activate the receptor as that of a full agonist[11]. SPPARMs are reported to achieve these effects by selectively recruiting the co-activators to PPAR receptors and thus selectively activating the genes responsible for insulin sensitization, adipogenesis, fluid retention and bone remodelling[12].

In this study virtual screening (VS) work flow is efficiently used in order to identify novel partial agonist. We followed different strategy to screen the compounds instead of screening only from ADMET analysis. Ligand based Pharmacophore based screening was initially implemented followed by usual VS screening. Alternatively this method of screening is defined as Pharmacophore based screening of library of compounds with prescribed generated Pharmacophore from known biological value.

Unfortunately, the glitazones used in the clinic today suffer with some serious side effects such as, increase in body weight, fluid retention, weight gain, risk of bone fracture, bladder cancer, etc.[5, 13-15]. Dose responsive curve of the therapeutic effects and side effect of TZDs coincide each other such a way that increase in dose increases efficacy and also degree of side effect[16] hence multiple activities are appears to be linked[17], as a result they received black box safety warning. One of the reasons for the failure of these clinically used glitazones is, their time of development. These drugs were developed when there was very little scientific data available on structure and the transcriptional mechanisms of the target peroxisome proliferator activated receptors (PPARs). After understanding the trans-activation mechanism of PPAR receptors has led to the newer approaches to the discovery of development PPAR-α/γ dual agonists, PPAR-δ/γ dual agonists, PPAR-pan agonists, selective PPAR-γ modulators or partial agonists. Among them SPPARγ Ms attracted many researchers due to their selectivity and expression in the selective tissue.

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MATERIALS AND METHODS

Virtual screening workflow consists of several steps[18] and was carried out by using Discovery studio software 4.0v (Discovery studio 4.0v., San Diego, CA, USA; http://www.accelrys.com).

Ligand based pharmacopore model: Pharmacophore generation and screening

- ADME/TOX analysis
- Molecular docking

The compounds for initial screening were retrieved from various online databases like ZINC database pub chem in 3:1 ratio and more preferably 75 percent compounds are taken form Zinc database because it contains commercially available natural products and natural-product derivatives. Thus, we could purchase and test *in vitro* the bioactivity of the selected compounds.

Totally, initial dataset contains 21, 818 compounds were processed in this study. The 3D structures of this initial dataset compounds were processed with prepare ligands program (Discovery studio 4.0v., San Diego, CA, USA; http://www.accelrys.com) to remove duplicates, enumerating isomers and tautomers, and generating 3D conformations. This process was carried out with the following parameters (a) clean geometry and the force field used was CHARMm (b) Ionization-pH based, (c) Generate isomers to remove Unknown Stereo atoms and Unknown Stereo bonds, (d) Duplicates removal using SMIRKS Acid Templates, SMIRKS Base Templates and SMARTS Charge Templates. Conformations were built with the cat Conf is the Catalyst conformer generation tool generating in vacuo a maximum number of 255 conformers per structure with energy threshold of 20 kcal/mol.

Anti-Pharmacophore model generation

Pharmacophore generation (Discovery studio 4.0v., San Diego, CA, USA; http://www.accelrys.com) was used for the analysis of the PPAR-gamma structures are Rosiglitazone-2PRG, Pioglitazone-2XKW, Troglitazone-2VN0. Pharmacophore of the three combined structures were generated using biological activity with uncertainty. This pharmacophore (Figure 1A) is formed by 4 sites (two hydrogen-bond acceptors, one hydrogen bond donor and hydrophobic aliphatic sites) that are present in most of the complexes of full agonists analyzed and are therefore assumed to be responsible for the intermolecular interactions that are essential for the activity of PPAR-gamma full agonists. This pharmacophore is named as anti-pharmacophore since we used to exclude these most fit structures.

Pharmacophore model generation

Pharmacophore model generation: Common features of 3 existing PPARγ partial agonist (INT131-3FUR, nTZDpa-2Q5S, SF147-2Q6R) were used to generate pharmacophore model.

The chemical structure of partial agonist compounds along with bound protein was retrieved from structural database (http://www.rcsb.org/) with known biological activity. Pharmacophore model of structures were generated using catalsyst-hypogen algorithm with prescribed uncertainty level. This model (Figure 1 B) was defined as pharmacophore model and it is end results is constructed with three sites (two hydrogen-bond acceptors and hydrophobic aliphatic sites) also, we called this pharamcophore as partial -agonist pharmacophore model which is most used to screen the potential PPAR-gamma partial agonists. Distance matrix formulae were used to compare the anti-pharmacophore features with pharmacophore features (Figure 1 C).

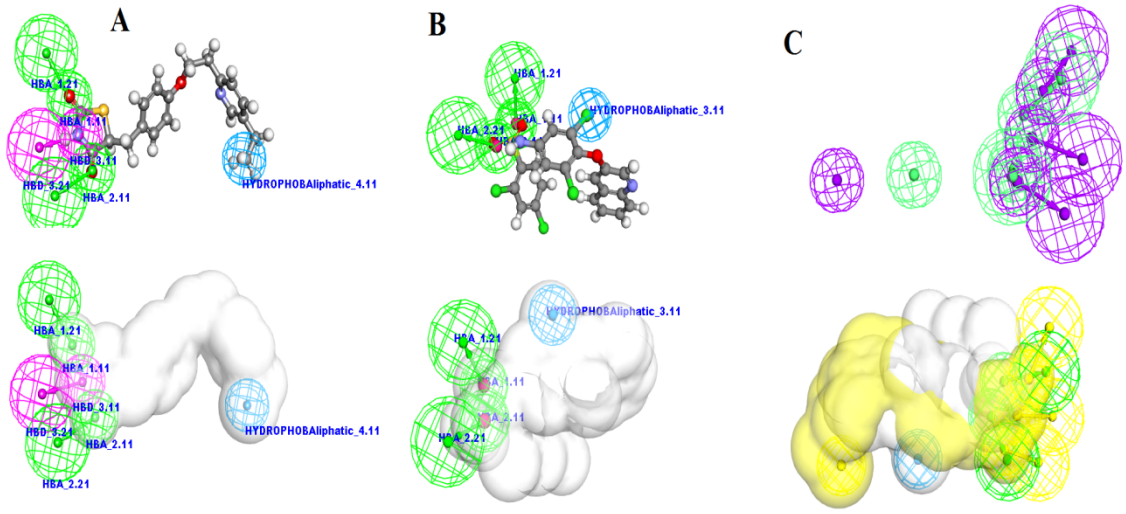

Figure 1: Pharmacophores used for the identification of (A) PPAR-gamma full agonists and (B) PPARγ partial agonists. Hydrophobic and acceptor/donor sites are colored in Cyan, green and pink, respectively. (C) Compared model of anti-pharmacophore and pharmacophore features

Pharmacophore based and ADMET screening

Screening the compounds on the basis of pharmacophore is crucial process. It is projected to carry the Structure Based Pharmacophore tools within Discovery Studio. We screened initial datasets of compounds in anti-pharmacophore model with multiple hypotheses to retrieve the potent compound for biological activity. Further the screening was extended for pharmacophore model with more than one hypothesis. The compounds form second level screening was subjected to further virtual screening using ADMET property. The objective of this screening is to retrieve the biological active compound for further analysis.

Molecular docking

Docking studies of the PPARγ partial agonists C1, C2, C3, C7, C9, C11 and C12 were performed with the Simulation and annealing based docking was carried out using C-docker protocol of Discovery studio software 4.0v (Discovery studio 4.0v., San Diego, CA, USA ;http://www.accelrys.com) on the PPARγ crystal structure 2PRG. The binding site was defined using the define and edit binding tool the sphere is placed 3D coordinate of 49.72 X -36.98Y 19.29 Z with radius of 8.415Å. The docking system was set up with 1000 steps of dynamics with refine orientation to remove bad clashes along with simulation of heating and cooling of 2000 and 5000 steps respectively; whereas target temperature was defined as 300k for both heating and cooling along with default energy threshold for refine docking process. Additionally, CHARMm force filed was applied to each docked pose with momany-rone Ligand Partial charge. The results are screened based on the docking poses along with interaction investigation of docking poses. Discovery studio Visualizer 4.5 (Bio via Discovery studio, Tokyo, Japan; http://www.Dassault Systemes Biovia.com) was used for analyzing and visually investigating the ligand-protein interactions of the best docking poses.

RESULTS AND DISCUSSION

Virtual screening of datasets

The aim of this research work is to identify the novel PPAR-gamma partial agonists. Virtual screening workflow implemented in this study is summarized in Figure 2.

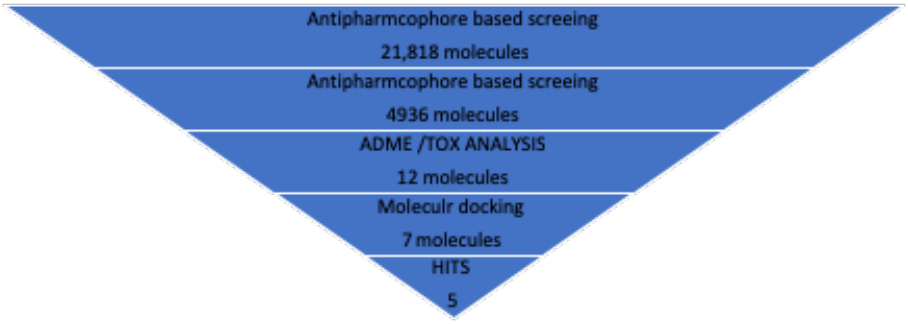

Figure 2: Schematic overview of the VS workflow and the procedure used for selecting the VS hits

The number of compounds that passed each step and the programs used are shown. From an initial set of 21,818 compounds, 12 compounds were identified as putative PPAR-gamma partial agonists by the VS workflow. Five of these compounds after passing ADMET show best docking poses with PPAR-gamma partial agonists.

Application of virtual screening workflow is to identify PPAR-gamma partial agonists was evaluated by applying it to a group of

three known PPAR-gamma full agonists and PPAR-gamma partial agonists. We followed different strategy to screen the compounds instead of screening from ADMET, Pharmacophore based screening was initial implemented followed by usual VS screening method alternatively this method of screening is defined as pharmacophore-based screening of library of compounds with prescribed generated pharmacophore from known biological value. The fitting between the molecules and the pharmacophore was analyzed with the Catalyst program. The compounds are mapped to the pharmacophore and evaluated on the basis of fit value. Anti-pharmacophore and pharmacophore model mapping to features is displayed on Figure 3, initial dataset compounds are first and foremost screened with anti-pharmacophore model now compounds which fits more than fifty percent were drop down, the subset of molecules that did not match the anti-pharmacophore was then used identify the PPAR-gamma partial agonists.

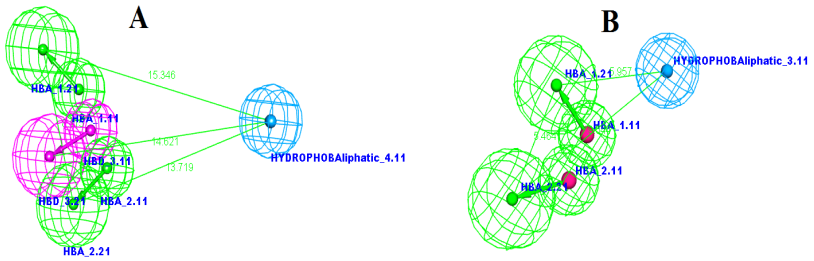

Figure 3: Mapped Pharmacophores (A) PPAR-gamma full agonists and (B) PPARγ partial agonists

Hydrophobic and acceptor / donor sites are colored in Cyan, green and pink, respectively. Subsequently to identify the novel partial agonist pharmacophores obtained using IC50 of known PPAR-gamma partial agonists was used. The subset of molecules that did match and fits more than 70% to the pharmacophore model reveals that identification of novel PPAR-gamma partial agonists. Only 12 compounds satisfied pharmacophore model were screened for ADMET level study using discovery study (ADMET/Topkat tools). The ADMET analysis results of novel 12 PPAR-gamma partial agonists are tabulated in Table 1 and Figure 4.

Table 1: ADMET screening of compounds

Compound No	ZINC ID	Solubility	BBB	CYPD26	Hepatotoxic	Absorption
C1	13259979	3	3	FALSE	FALSE	0
C2	38861161	3	2	FALSE	FALSE	0
C3	13586428	3	2	FALSE	FALSE	0
C4	31932883	3	3	FALSE	TRUE	0
C5	38974587	3	3	FALSE	TRUE	0
C6	68478286	3	2	FALSE	TRUE	0
C7	13849555	2	2	FALSE	FALSE	0
C8	36592901	3	3	FALSE	TRUE	0
C9	31367097	4	3	TRUE	FALSE	0
C10	63059572	3	3	FALSE	TRUE	0
C11	51425645	3	2	FALSE	FALSE	0
C12	59433934	4	3	FALSE	FALSE	0

Solubility levels (2-Low, 3-Good, 4-Optimal), BBB = Blood brain barrier (2-Medium, 3-Low), Absorption (0-Good), Hepatotoxic (TRUE-Toxic, FALSE-Non-toxic), CYPD26 (TRUE-Inhibitor, FALSE-Non-Inhibitor)

Compounds like C1, C2, C3, C7, C9, C11 and C12 are non -toxic, non-inhibitor with good absorption an optimal to low solubility has a competence to across the blood brain barrier from medium to low.

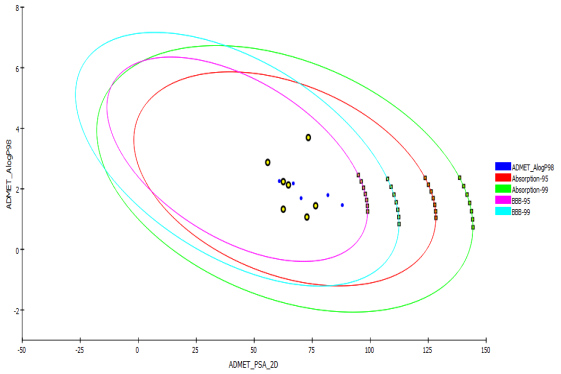

Figure 4: Yellow - Compounds satisfies ADMET property, Blue -Compounds screened out form ADMET property

Our post docking analysis results elicits that, no hydrogen bond interaction and neither makes a hydrogen bond network as extensive as full agonist rosiglitazone with Tyr473 from arm I in AF2 branch I portion of the LBD of PPAR-gamma[19], instead all the 8 compounds formed hydrogen bond interaction with residue Tyr372[20] lie between H3 and the β-sheet, extending from branch II to branch III of the ligand binding pocket.

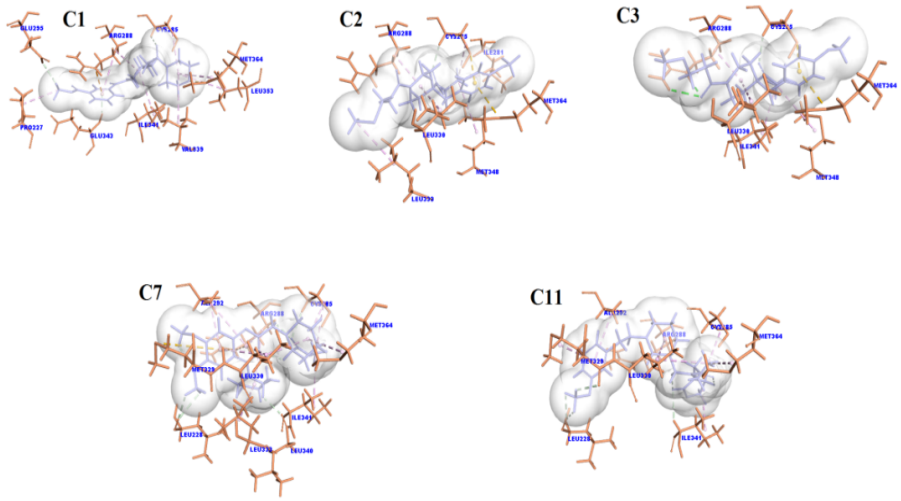

Figure 5: Hydrogen bond interactions of the test compounds with LBD of PPARγ (PDB ID: 3FUR)

DISCUSSION

SPPARγ Ms similar to selective estrogen receptor modulators (SERMs), SPPARMs are reported to achieve effects by selectively activating on the genes responsible for insulin sensitization, adipogenesis, fluid retention and bone remodeling[12,21]. Partial agonists act by partially agonizing PPARγ receptors and also exhibit low trans-activation activity when compared to full agonists[22].

Therefore, in this study such novel SPPARγMs / Partial agonists were identified by using VS workflow where each step it consists was able to identify putative PPARγ partial agonists. Pharmacophore-based virtual screening helped us to enrich active molecules in the hit list compared to a random selection of test compounds. VS workflow is able to predict 8 Hits.

In molecular docking confirmation analysis of existing full agonists extend from the TZD head group to intermolecular hydrogen bonds side chains of PPARγ residues like H323 (2.9˚A), H449 (2.7˚A) and Y473 (2.6˚A) allowing for stabilization of the AF2 surface[23]. Among these the hydrogen bonding with the Tyr473 residue is reported to play a vital role in the stabilization of AF-2 helix through H12, allowing less of an entropic penalty for co-activator binding and thus full transcriptional output which is essential for the recruitment of co-activators necessary for transcriptional activation and these interactions are more prominent for PPARγ full agonists[24,25].

PPARγ partial agonists operate through different structural and mechanistic methods than full agonists rather than simply exhibiting lowered transcriptional output due to suboptimal potency and/or affinity[26]. Partial agonists stabilize the LBD in a distinct manner in comparison to full agonists. Partial agonists were shown to preferentially stabilize other regions of the ligand binding domain, especially the β-sheet region and H3 helix especially interacting with Tyr327 amino acid residue[27-30].

CONCLUSION

In conclusion, among 21,818 compounds, we were able to identify 5 compounds as putative partial agonists with help of virtual screening workflow i.e. both pharmacophore model and molecular docking. Post docking analysis show all 8 compounds interacted with Tyr327 residues as that of existing partial agonists like INT131.

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