

PPAR γ LBD and its ligand specificity reveal a selection of potential partial agonist: Molecular dynamics based T2D drug discovery initiative

BIDYUT MALLICK^{1,†}; ASHISH RANJAN SHARMA^{2,*}; MANOJIT BHATTACHARYA³; SANG-SOO LEE^{1,*};
CHIRANJIB CHAKRABORTY^{4,*}

¹ Department of Applied Science, Galgotias College of Engineering and Technology, Knowledge Park-II, Greater Noida, 201306, India

² Institute for Skeletal Aging & Orthopedic Surgery, Hallym University-Chuncheon Sacred Heart Hospital, Chuncheon-si, 24252, Korea

³ Department of Zoology, Fakir Mohan University, Vyasa Vihar, Balasore, 756020, India

⁴ Department of Biotechnology, School of Life Science and Biotechnology, Adamas University, Kolkata, 700126, India

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Abstract: PPAR γ is a peroxisome proliferator-activated receptor (PPAR) family protein and is a target for type 2 diabetes (T2D). In this paper, we have performed a molecular docking analysis between ligand molecules (CID9816265, CID11608015, CID20251380, CID20251343, CID20556263, CID624491, CID42609928, and CID86287562) and PPAR γ to determine the ligand specificity. It also helps to understand the ligand-binding domain (LBD) activity of PPAR γ during the binding of the ligand. Further, a molecular dynamics simulation study was performed to determine the ligand binding stability in the PPAR γ LBD. Its ligand specificity informed us about the potentiality of selecting a partial agonist. The study also shows the binding conformation of Ceramicine B having hydrogen bonding affinity with a tricyclic polar head and stabilized the β -sheet region. On the other hand, the tricyclic polar head of nimbolide also formed hydrogen bonding (Ser342), but it shows a lesser degree of stabilization in the β -sheet region. It shows the binding conformation of partial agonist (PPAR γ) in the Pocket-II of PPAR γ LBD, which has a significant role in stabilizing the β -sheet region. It might help to regulate ERK/Cdk5 mediated phosphorylation of Ser245. The study helps us understand the valid pose of a set of ligands confirmation and target protein conformation using docking and molecular dynamics study. This *in silico* study will also help to initiate a drug discovery process of T2D.

Introduction

PPAR (peroxisome proliferator-activated receptors) family proteins belong to nuclear receptor superfamily proteins. The proteins of the superfamily control an extensive range of lipid-associated-genes accountable for lipid metabolism, cholesterol metabolism, lipid transport, and adipose differentiation. These proteins regulate energy homeostasis (Bain *et al.*, 2007; Cox, 2017). PPARs are mainly found in the nucleus. However, PPARs are transported in between cytoplasm and nucleus whenever is needed (Umemoto and Fujiki, 2012). Three diverse family members from PPAR superfamilies are PPAR α , PPAR β , and PPAR γ .

PPAR β is also known as PPAR δ . These diverse family members/subtypes are highly homologous (Kroker and Bruning, 2015). It has been noted that PPAR α is expressed in the tissues and is highly metabolically active, especially in the kidney, heart, liver, and muscle. PPAR α is mainly associated with lipid metabolism (Kersten and Stienstra, 2017; Zoete *et al.*, 2007). PPAR β or PPAR δ is related to different diseases like obesity, diabetes, heart disease, and metabolic hypertension (Feige *et al.*, 2006; Jiang *et al.*, 2019; Yang and Long, 2018). Conversely, PPAR γ is found to be expressed in the adipose tissues. It has been found that PPAR γ can help in the creation of minute insulin-sensitive adipocytes.

Therefore, it has a function as a regulator in insulin sensitization. So, PPAR γ is considered as a target for treating T2D (Type 2 diabetes) (Leonardini *et al.*, 2009; Jiang *et al.*, 2020).

PPAR γ consists of 5 domains, which are named as A to E and are shown in Fig. 1a. These domains are distributed in the

*Address correspondence to: Sang-Soo Lee, 123sslee@gmail.com; Chiranjib Chakraborty, drchiranjib@yahoo.com

[†]These authors contributed equally to this work

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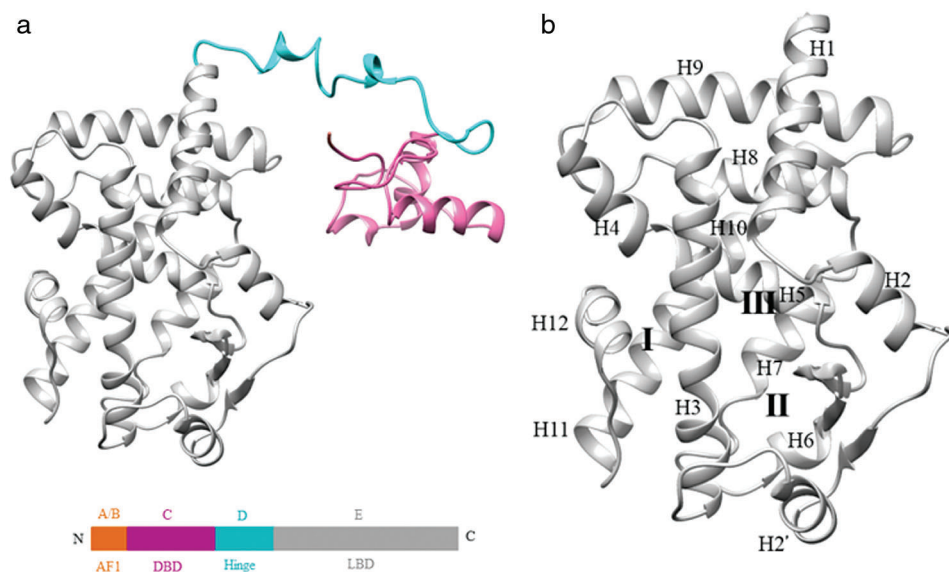


FIGURE 1. (a) Different domain of PPAR γ protein. (b) Ligand binding domain (LBD) of PPAR γ ; Different branches of ligand binding pocket are as mentioned in Roman letter. The 13 α -helices of LBD are also labelled.

direction from N- to C-terminus. These domains are highly conserved and comparable to other nuclear receptor proteins. (i) A and B domain: Both of these domain contains AF1 (activation function 1). It engrosses with the ligand-free coregulator binding (Diezko and Suske, 2013). (ii) C domain: This domain contains DNA binding domain and is highly conserved. It is responsible for DNA binding (Ijpenberg *et al.*, 1997). (iii) Hing domain: This domain helps in the rotation between the domain C and E (the DNA binding domain (C) and the ligand-binding domain (E)). It is a poorly conserved domain. (iv) Ligand binding domain (LBD)/activation function 2 (AF2): It contains a ligand-binding pocket and helps in the ligand binding. This domain is highly conserved (Kroker and Bruning, 2015). Endogenous and synthetic ligands can bind to the ligand-binding pocket (LBP), which is situated within the ligand-binding domain (LBD). The LBP has three ligand-binding branches with different properties and ligand specificity, resulting in a Y-shaped cavity for the ligand to bind. Three branches (I, II & III) are depicted in Fig. 1b. Branch-I is hydrophilic in nature, and molecules with an acidic head easily bind in this pocket, formed by H3, H5, H11 & H12 helices. Ligands (full agonists) binding in Branch-I strongly stabilizes H12 of LBD, which leads to transcriptional activation of PPAR (Gampe, 2000) by allowing the co-activators to dock. H3, Ω -loop, and β -sheet region form Branch-II; this pocket is of hydrophobic character. Ligand binding to this region shows weak interaction with the residues from Branch-I, rather stabilizing the β -sheet region and showing transcriptional activity (Malapaka *et al.*, 2012; Zheng *et al.*, 2013; Delfosse *et al.*, 2015; Amato *et al.*, 2012). Branch-III consists of both hydrophobic and hydrophilic regions, formed by β -sheet, H2, H3, and H5 helices.

It has been noted that full agonists of PPAR γ can attach with the Branch-I of the LBP, and, is responsible for the formation of hydrogen bonds with the different residues such as Ser289, His323, His449, and Tyr473 (Nolte *et al.*, 1998). Thiazolidinediones (TZDs) are an example of full agonists that were used to treat T2D. However, unwanted side effects have been noted during the use of full agonists.

The side effects include fluid retention, fat accumulation, loss of bone density, cancer, augmented risk of heart failure, etc. (Kung and Henry, 2012; Nissen and Wolski, 2007; Rubenstrunk *et al.*, 2007; Tang and Maroo, 2007). Due to the side effects, full agonists are not used for the treatment of T2D. On the other hand, recent studies reported several chemical compounds that bind in the Branch-II of PPAR LBP between H3, Ω -loop, and β -sheet. These compounds are partial agonists (Amato *et al.*, 2012; Choi *et al.*, 2010; Kroker and Bruning, 2015), and does not show full PPAR γ agonism but block the ERK/Cdk5 (cyclin-dependent kinase 5) mediated PPAR γ phosphorylation at Ser245 (Ser273 for PPAR γ 2) (Banks *et al.*, 2015). Blocking of PPAR γ phosphorylation leads to dysregulated expression of a series of genes, including adiponectin and adipisin. It regulates insulin sensitivity with diminished adverse effects (Dunn *et al.*, 2011; Higgins and DePaoli, 2010; Yi *et al.*, 2017). For these reasons, instead of full agonists of PPAR, nowadays partial agonists of PPAR γ are the choice for new generation anti-diabetic drugs. Our recent finding also reports Ceramicine with particular moiety could bind Branch-II of PPAR γ LBD (Mallick, 2018).

Here we report the virtual screening of small molecules from PubChem Database to find the structure of the molecules that will occupy the ligand-binding pocket between H3 and β -sheet (Branch-II). Naphthalene group of a partial agonist SR2067 has been reported to stabilize the β -sheet region of Branch-II employing hydrophobic interaction between helix 3 and the β -sheet (Maltsev and Oswald, 2010). In the present study, we focused on searching for the bicyclic/tricyclic/tetracyclic molecules to find their binding affinity in LBP of PPAR γ . These bicyclic/tricyclic/tetracyclic molecules used for the present study are ((CID9816265, CID11608015); (CID20251380, CID20251343); (CID20556263, CID624491); CID42609928 (Ceramicine B), CID86287562 (Nimbolide)). Further, large molecules with the bicyclic/tricyclic head and different hydrophobic tails have also been searched from the PubChem database to ensure if these molecules can show specific binding modes to act as PPAR γ partial agonists. The present study reveals the ligand specificity of ligand binding pocket of PPAR γ during binding

of the ligand. Its ligand specificity informed us about the potentiality of the selection of partial agonists. Additionally, a molecular interactions study has been executed to determine the ligand-binding affinity with PPAR γ LBD as well as binding stability. This *in silico* study also initiates the drug discovery of T2D.

Materials and Methods

PPAR γ structure and ligand compounds

We have used the X-ray crystal structure of PPAR γ , which is an isoform of this receptor protein. The structure was collected from RCBS Protein Data Bank (PDB) and the PDB ID 2F4B (Mahindroo *et al.*, 2006). The unwanted atoms and bound ligand were removed from the PPAR γ protein structure and minimized using the GROMACS-4.5 software package (Pronk *et al.*, 2013). The structures of ligand compounds (Fig. 2) were collected from the PubChem compound database, and the structure was optimized with the help of Chimera software.

Molecular docking study and virtual screening study

The docking simulation studies of the different ligand molecules in PPAR γ LBD were performed with AutoDock4 (Morris *et al.*, 2009). AutoDock is extensively used docking software. This software uses a Lamarckian genetic algorithm to analyze docking between protein and ligand (Hou *et al.*, 2013). The protein was kept as rigid molecules and the ligand as flexible molecules for generating a protein-ligand complex using AutoDock software. A cubic grid box was created of size 60 Å × 60 Å × 60 Å to encompass all the active site residues of PPAR γ . At the finishing step of each docking process, the lowest energy conformation was considered the best binding conformation between each considered molecule and PPAR γ .

Software LIGPLOT was used to understand the hydrophobic and hydrogen bond contacts between the protein and ligand (Laskowski and Swindells, 2011; Wallace *et al.*, 1995).

Molecular dynamic (MD) simulation

We have performed MD simulations using the GROMACS-4.5.6 package (CDAC server, India) (Hess *et al.*, 2008). All-atom molecular dynamics simulations of PPAR γ protein with each ligand inhibitors were performed using this software package under the GROMOS force field. The force field parameter was laid down as 53A6 (Mallick *et al.*, 2019; Oostenbrink *et al.*, 2004; van Gunsteren *et al.*, 1996).

The PPAR γ protein with each ligand inhibitor complex was handled alone by putting them into a cubic box. All the complexes were solvated by using the SPC216 water model. To electrically neutralize the total charge, Na ions were added to each system by using the genion tool of the GROMACS package. The system replaces water molecules with ions at the most constructive electrostatic potential positions at random. Finally, we performed energy minimization using the steepest descent algorithm with a maximum step size of 0.01 nm. During energy minimization, we have maintained a tolerance of 1000 kJ/mol/nm. The applied system was restrained equilibration, and the parameter was placed as LINCS (linear constraint solver for molecular simulations) constraints for all bonds and at 300 K and 1 bar for 100 ns for heavy atoms (Hess *et al.*, 1997).

Results

Molecular docking study and virtual screening study

We have performed molecular docking of PPAR γ with the different ligand compounds (Bicyclic molecules (CID9816265, CID11608015); Tricyclic molecules (CID20251380, CID20251343); Tetra cyclic molecules (CID20556263, CID624491); CID42609928 (Ceramicine B), CID86287562 [Nimbolide]) [The ligand structure was adopted from PubChem].

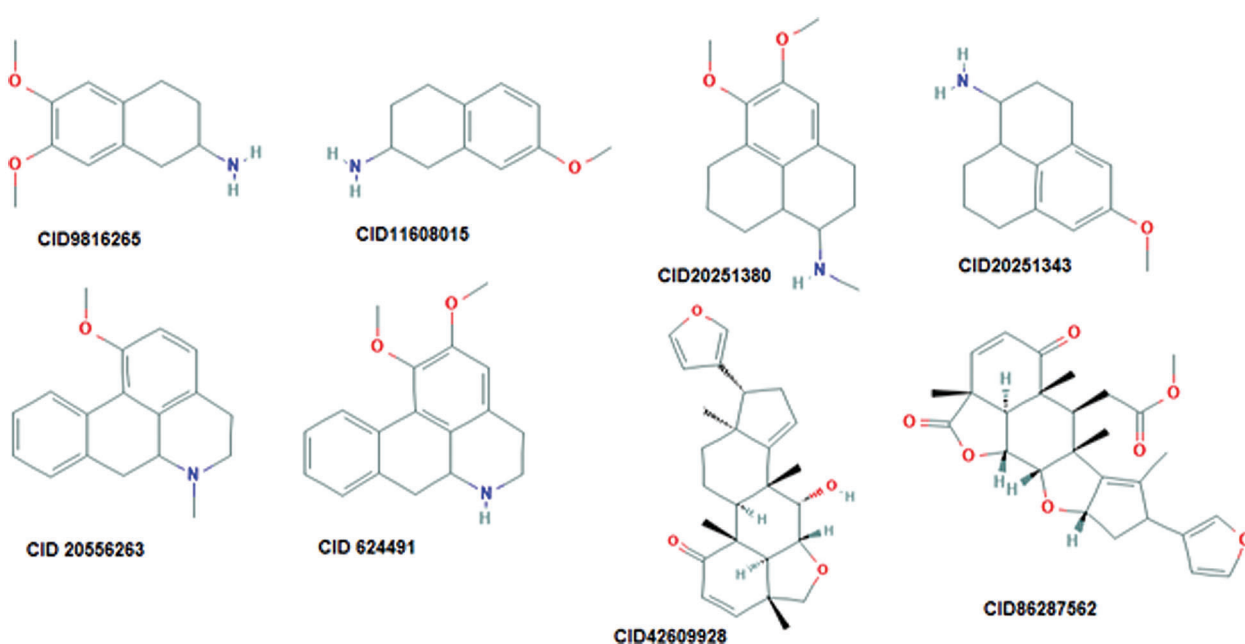


FIGURE 2. The structure of the ligand compounds used for our study (Bicyclic molecules (CID9816265, CID11608015); Tricyclic molecules (CID20251380, CID20251343); Tetra cyclic molecules (CID20556263, CID624491); CID42609928 (Ceramicine B), CID86287562 [Nimbolide]) [The ligand structure was adopted from PubChem].

CID20251343); Tetra cyclic molecules (CID20556263, CID624491); and large molecule with tricyclic head CID42609928 (Ceramicine B), CID86287562 (Nimbolide). The structure of the docking complexes for the selected molecules in PPAR γ LBD is depicted in Fig. 3. Fig. 3a shows ligand-binding pocket in the presence of bicyclic molecules, which are CID9816265 and CID11608015. We have found main binding residues are PHE226, PRO227, LEU228, ARG288, GLU291, GLU295, and GLU343. The molecules CID9816265 and CID11608015 have binding energies of -7.65 kcal/mol and -7.46 kcal/mol for their lowest energy conformation. Fig. 3b shows ligand-binding pocket in the presence of tricyclic molecules, which are CID20251380 and CID20251343. This ligand-binding pocket includes some active binding residues, which are GLU291, ARG288, LEU228, LEU333, GLU343, LEU340, SER342, and ILE341. The lowest binding energy for tricyclic molecules CID20251380 and CID20251343 in PPAR γ LBD are -8.38 kcal/mol and -7.92 kcal/mol, respectively. Fig. 3c depicts the ligand-binding pockets in the presence of tetracyclic molecules, which are CID20556263 and CID624491. This ligand-binding pocket includes some active binding residues, which are GLU291, ARG288, LEU333, PHE287, THR268, GLU343, SER342, and ILE341. The best binding conformation for tetracyclic

molecules CID20556263 and CID624491 has binding energy -8.2 kcal/mol and -8.63 kcal/mol.

The docking complexes structure between CID42609928 (Ceramicine B) and PPAR γ is shown in Fig. 4. Fig. 4a portrays the binding pose of the large molecule Ceramicine B in PPAR γ LBD. The binding energy for the lowest energy conformation was found to be -9.2 kcal/mol. We have noted that molecule CID42609928 has a tricyclic polar head. Fig. 4b displays hydrophobic and hydrogen bond contacts between PPAR γ protein and CID42609928 (Ceramicine B) compound determined by the LIGPLOT program. This ligand-binding pocket includes some active binding residues, which are ARG288, CYS285, PHE287, GLE284, MET384, SER342, GLU343, LEU270, THR268, and ILE281. The LIGPLOT program also shows identical active ligand-binding residues.

The structure of the docking complexes of CID86287562 (Nimbolide) and PPAR γ is shown in Fig. 5. Fig. 5a informs us about the local structure of the docking complex for the large molecule nimbolide with PPAR γ . The lowest energy for binding confirmation is -9.42 kcal/mol. We have noted that molecule CID86287562 has a tricyclic polar head. Fig. 5b shows hydrophobic and hydrogen bond contacts between PPAR γ protein and CID86287562 (Nimbolide)

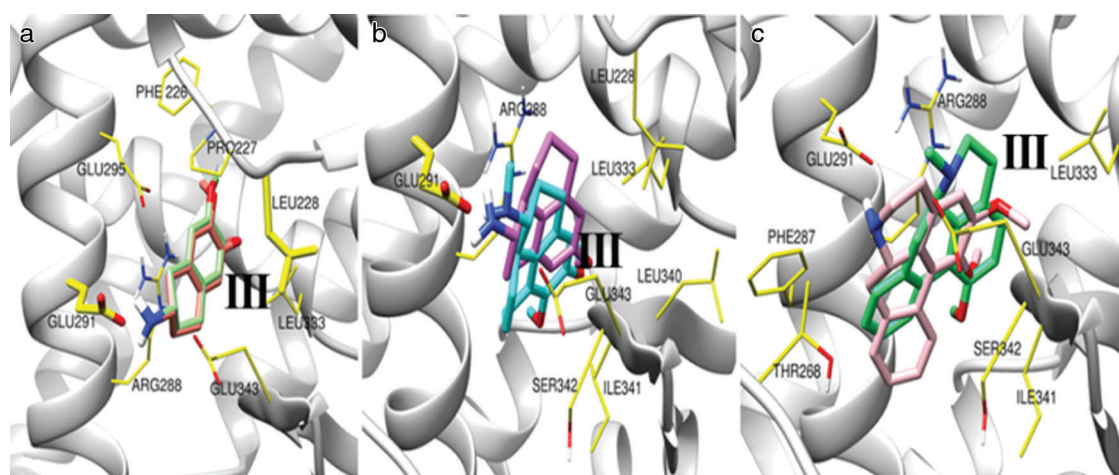


FIGURE 3. The local structure of the docking complexes for selected molecules in PPAR γ LBD.

(a) Ligand binding pocket in the presence of bicyclic molecule CID9816265 (salmon color) and CID11608015 (spring green color). (b) Ligand binding pocket in the presence of tricyclic molecule CID20251380 (cyan color) and CID20251343 (orchid color). (c) Ligand binding pocket in the presence of tetra cyclic molecules CID20556263 (light green) and CID624491 (pink color).

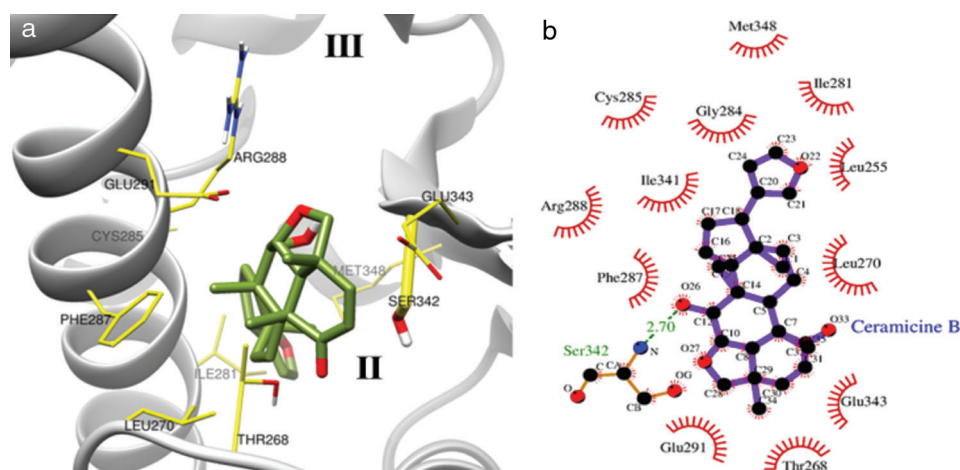


FIGURE 4. The structure of the docking complexes for CID42609928 (Ceramicine B) in PPAR γ LBD. Left (a): The local structure of the docking complex for the large molecule with tricyclic polar head CID42609928 (Ceramicine B) in PPAR γ LBD. Right (b): Hydrophobic and hydrogen bond contacts between PPAR γ protein and CID42609928 (Ceramicine B) compound determined by LIGPLOT program. The figure was taken from Mallick (2018).

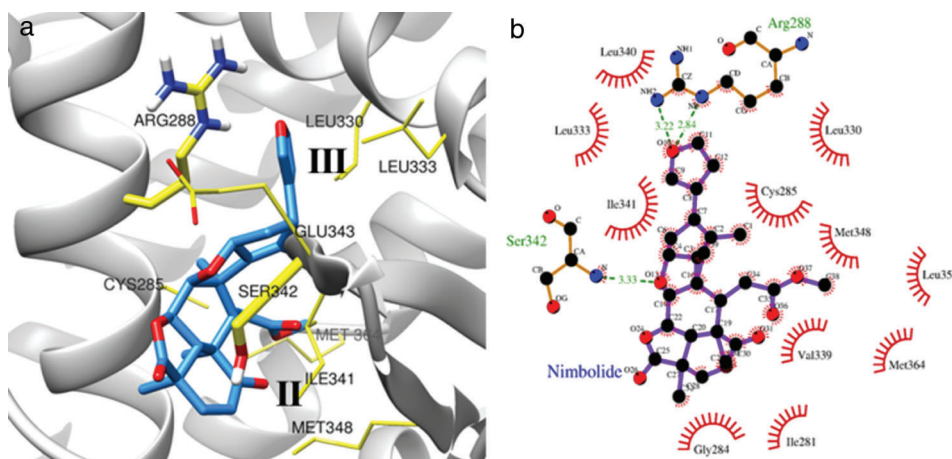


FIGURE 5. The structure of the docking complexes for CID86287562 (Nimbolide) in PPAR γ LBD.

Left (a): The local structure of the docking complex for the large molecule with tricyclic polar head CID86287562 (Nimbolide) in PPAR γ LBD. Right (b): Hydrophobic and hydrogen bond contacts between PPAR γ protein and CID86287562 (Nimbolide) compound determined by LIGPLOT program.

compound as determined by the LIGPLOT program. This ligand-binding pocket includes some active binding residues, which are ARG288, LEU330, LEU333, GLU343, SER342, MET364, ILE341, MET348, and CYS385. The LIGPLOT program also informed the same active ligand-binding residues.

Root-mean-square deviation of the PPAR γ with the different ligands

Binding conformations of CID86287562 (Nimbolide) and CID42609928 (Ceramicine B) in LBD of PPAR γ is similar to the other partial agonist reported in the previous studies (Amato *et al.*, 2012; Motani *et al.*, 2009). Thus molecular dynamics simulation study has been performed for PPAR γ -CID86287562 (Nimbolide) and PPAR γ -CID42609928 (Ceramicine B) complexes to determine their interaction under dynamic conditions. RMSD value of the Ca atoms fluctuations of PPAR γ protein for these complexes is compared with uncomplexed PPAR γ protein in Fig. 6 for 100 ns simulation. The simulation of three molecules shows more or less the same pattern of fluctuations with lower

RMSD value for Ca atoms fluctuations of PPAR γ in complex with CID42609928 (Ceramicine B) molecule. The Ca-RMSD for all three cases shows a steady increase up to 10 ns and then stabilized after 40 ns, during the simulation.

Root-mean-square fluctuation of the PPAR γ with the different ligand

Fig. 7 represents RMSF of Ca atoms of PPAR γ as a function of amino acids for the three considered complexes PPAR γ , PPAR γ -CID86287562 (Nimbolide), and PPAR γ -CID42609928 (Ceramicine B) during 100 ns simulation. The RMSF value over the entire simulation was plotted against residue numbers and has been displayed in this figure. The strength of fluctuation of the PPAR γ -CID42609928 (Ceramicine B) complex has reduced the RMSF value for H3 and β -sheet relative to other considered complexes. Also, displacement of H12 for PPAR γ -CID42609928 (Ceramicine B) complex is comparable to uncomplexed one, whereas highly stabilized RMSF is observed for PPAR γ -CID86287562 (Nimbolide) complex. Thus, RMSF variation indicates a binding mode of CID42609928 (Ceramicine B), stabilizing H3, β -sheet, and Ser245 region more

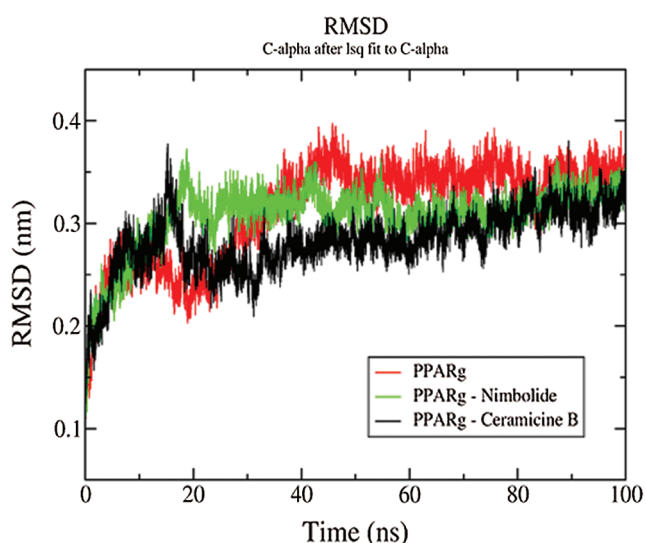


FIGURE 6. The RMSD value of the Ca atoms during 100 ns simulation of PPAR γ (red), PPAR γ -CID86287562/Nimbolide (green) and PPAR γ -CID42609928/Ceramicine B (black) system. RMSD data for PPAR γ and PPAR γ -Ceramicine complex was taken from Mallick (2018).

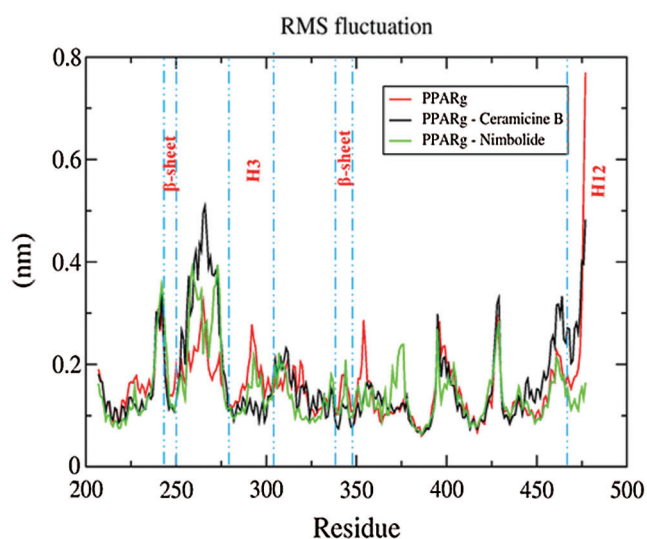


FIGURE 7. The RMSF of Ca atoms as a function of amino acids for PPAR γ (red), PPAR γ - complexed with CID86287562/Nimbolide (green) and PPAR γ - complexed with CID42609928/Ceramicine B (black) system. RMSF data for PPAR γ and PPAR γ -Ceramicine complex was taken from Mallick (2018).

strongly than the binding mode of PPAR γ -CID86287562 (Nimbolide) complex. Stabilization of these regions helps in blocking the ERK/Cdk5-mediated phosphorylation of Ser245. Further, dynamics of H12 of PPAR γ complexed with CID86287562 (Nimbolide) seems affected, whereas it remains unaffected in the binding orientation of CID42609928 (Ceramicine B) molecule, which could reflect H12 independent transcriptional activity of PPAR γ by CID42609928 molecule (Gelman *et al.*, 2007; Lu *et al.*, 2006).

Discussion

Here, we have applied molecular docking studies to find the small molecule that will occupy the ligand-binding pocket between H3 and β -sheet of PPAR γ . A specific configuration of these particular molecules do the job of sandwich filling between Arg288, Glu291 of H3 and Ser342, Glu343 of β -sheet; consequently, the polar atom of the ligands easily forms hydrogen-bonding networks with the residues of the β -sheet, which preferentially will stabilize the β -sheet region of the LBD of PPAR γ protein. Further, large molecules with similar specific configurations also show high binding affinity in the Pocket II of LBD, but the orientation of the molecule changes with the configuration of the hydrophobic tail. Thus, a Molecular dynamics simulation study was performed to establish the orientation of hydrophobic tail of large molecule in Pocket II that will inhibit Ser273 phosphorylation with a lesser degree of H12 stabilization.

Molecular docking was desired to forecast the optimal ligand-receptor complex orientation and conformation using two steps. The first step act through the assembly conformation of ligands in the active site of the protein. The second step helps the positioning of the conformations through a scoring function to forecast binding rigidity for each orientation (Meng *et al.*, 2011).

The LIGPLOT helps to understand the interaction regions and simultaneous interaction points. It also illustrated the interaction of residues through hydrogen bonds and hydrophobic contacts. Finally, the interactions (H bond) are pointed out by a dashed (-) line between the two atoms involved in this interaction. It was shown in our previous study also (Chakraborty *et al.*, 2014). Similarly, hydrophobic interactions are shown through an arc with spokes radiating toward the ligand atoms.

Virtual screening of small molecule uses MTiOpenScreen server, which uses chemical libraries from PubChem Database. It reveals that the bicyclic/tricyclic region of the molecules align between helix 3 and the β -sheet of PPAR γ LBD (results are not shown here). However, the β -sheet plays an essential role in PPAR γ LBD. A recent study suggested that the naphthalene group of a partial agonist SR2067 stabilized the β -sheet region through hydrophobic regions between helix 3 and the β -sheet (Maltsev and Oswald, 2010). Thus, virtual screening in-home PC was performed for the bicyclic/tricyclic molecules with and without polar residues to find their binding affinity in LBD of PPAR γ . Also, large molecules with the bicyclic/tricyclic head have been searched from the PubChem database to ensure if these kinds of molecules can show specific binding modes to act as a PPAR γ partial agonist. A docking

simulation study can predict one molecule's preferred orientation in the active site of a target protein. Therefore, we performed docking simulation to identify the binding affinity and orientation of each considered compound in PPAR γ protein using AutoDock. Bicyclic molecules shown in Fig. 2 with PubChem CID9816265, CID11608015 have been docked in the ligand-binding pocket. Minimum energy docking conformation reveals that the methoxy group of both the molecules CID9816265 and CID11608015 form hydrogen bond with the residue LEU288 and amine group with GLU291. Though both the molecules show hydrophobic interaction with GLU343 of the β -sheet region, the molecules' minimum energy binding conformation is not precisely a sandwich filling conformation between H3 and β -sheet. The minimum energy binding position of these molecules in the LBD is represented in Fig. 3a. The bicyclic molecule CID931 (Naphthalene), lacking polar atom, has the lowest energy binding conformation in parallel to H5 in ligand binding Pocket III. The binding conformation of this molecule is shown in Supplementary Fig. 1a.

Increasing a cyclic ring in the bicyclic molecule changes the orientation to its lowest energy docking conformation in the binding pocket. The binding conformation of the tricyclic molecule CID20251380 and CID20251343 in PPAR γ LBD is depicted in Fig. 3b. Results show that these molecules' minimum energy conformation moves towards the β -sheet to align between H3 and β -sheet. The amine group of these tricyclic molecules also form hydrogen bonding with GLU291 and more residues from the β -sheet region involved in hydrophobic interaction. Increasing another cyclic ring also allows these types of molecules to have minimum energy binding conformation in Pocket II of the LBD (Fig. 3c). In order to check the binding affinity of tetracyclic molecules, compounds with CID20556263 and 624491 were taken from the PubChem database. A bicyclic head characterizes the above-considered molecules with polar residues at the opposite side of the ring (Positions 1, 2, 5, or 6). The side chains of the polar residues GLU291, THR268, GLU343, and α -amino group of SER342 allow these kinds of molecules to have the lowest binding energy in Pocket-II. The nitrogen atom of these molecules aligns towards the side chain of GLU291, while the oxygen atom of the bicyclic head towards the SER342. Thus, the molecules' binding pattern with polar bicyclic head has a sandwich filling pose between H3 and β -sheet in the large "Y" shaped ligand-binding cavity of PPAR γ protein, and polar oxygen could form hydrogen bond interaction with key residue SER342. It is important to mention here, similar molecule with bicyclic head without the polar atom at both the end have lowest energy binding conformation parallel to H5 in ligand binding pocket. The binding conformation of these molecules in the LBP is shown in Supplementary Fig. 1b.

As these fragment-like compounds have a lower binding affinity and can move from the binding site in dynamic conditions, docking simulation studies were performed for large molecules with tricyclic polar head. Large molecule CID86287562 (Nimbolide) and CID42609928 (Ceramicine B) have been considered for our study. These molecules have a tricyclic head with a polar atom at the opposite end and a large hydrophobic tail. The lowest energy binding

mode reveals that the molecules fit in Pocket II in parallel with H3, polar head forms a hydrogen bond with the α -amino group of SER342, as depicted in Fig. 4. Their binding confirmation reveals that all the molecules fit in the Pocket II parallel with H3, polar head forms a hydrogen bond with the α -amino group of SER342. Moreover, the tail region's remaining hydrophobic interaction spreads out to the bottom (binding pose 1) or top (binding pose 2) of Pocket II depending on the molecule's configuration.

Studies have shown that the β -sheet region has a significant role in stabilizing the LBD of PPAR γ , which results in the blocking of ERK/CDK5 mediated phosphorylation. Therefore, β -sheet plays a vital role in acting as a partial agonist of PPAR γ . The β -sheet region is stabilized by means of hydrogen bonding, where an acidic group has a vital role, and most PPAR γ partial agonists act through the β -sheet region to stabilize the complex structure. There is a significant role of acidic groups, which has been shown in β -sheet stabilization. It also has a hydrogen-binding network to the different backbone atoms of the β -sheet, especially the backbone nitrogen of Ser342 (van Marrewijk *et al.*, 2016). Our study shows that the binding conformation of Ceramicine B has hydrogen bonding with the tricyclic polar head and stabilized the β -sheet region. On the other hand, the tricyclic polar head of Nimbolide also formed hydrogen bonding with Ser342, but it shows a lesser degree of stabilization of the β -sheet region. It shows that the binding conformation of partial agonist (PPAR γ) in the Pocket II of PPAR γ LBD has a significant role in stabilizing the β -sheet region and blocking ERK/Cdk5 mediated phosphorylation of Ser245 (van Marrewijk *et al.*, 2016).

Conclusion

This study aims to foresee a valid pose from a receptor configuration and a set of ligand conformation through their scoring-based system depending on their binding affinity. The study could be used as scaffolds to discover more potent compounds or as positive controls for new screens. This study also helps to initiate the drug discovery of T2D. Based on the binding patterns and poses, several future works can be done to improve the biological activity of ligand binding pocket (LBP) inhibitors by increasing the number of the typical hydrogen bonds, molecular volume, and electrostatic interactions. We propose our *in silico* outcomes could be a valuable resource for pharmacologists as well as molecular biologists who are working in the drug development process. Considering these probable structural outcomes, linked to the PPAR γ ligand-binding domain and its ligand specificity, the study will help future researchers to understand the selection of potential partial agonists.

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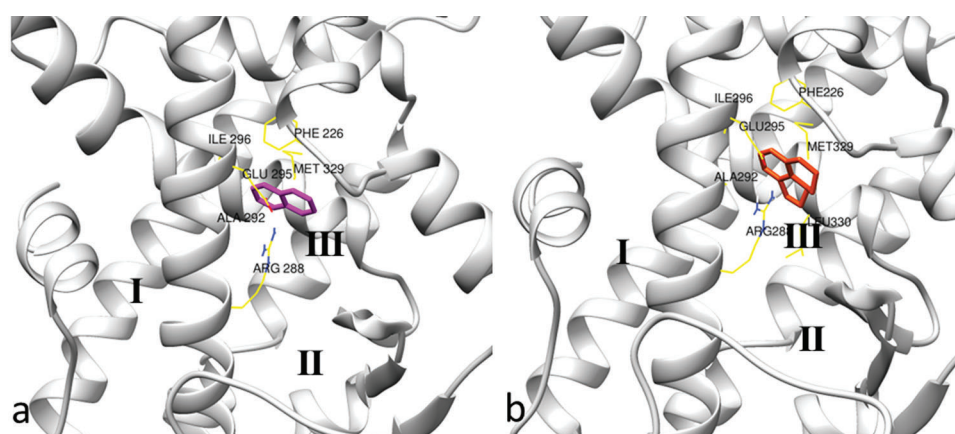
Conflicts of Interest: The authors declare that they have no conflict of interest.

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SUPPLEMENTARY FIGURE 1. Lowest energy binding conformation of (a) bicyclic (CID931/Naphthalene) and (b) Tricyclic (CID 44358650) molecule in the ligand binding pocket of PPAR γ . The binding energy of lowest energy conformation for CID931 & CID 44358650 are -5.52 kcal/mol and -6.77 kcal/mol respectively.