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IN SILICO BASED VIRTUAL SCREENING FOR BIOACTIVE PPAR-γ AGONISTS

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Abstract:
*The predominance of multifactorial diseases has become an epidemic in the tropical and sub-tropical areas all over the world. According to the reports of the World Health Organization (WHO), around 250 million people are currently living with diabetes and this number is expected to be more than 366 million by 2030. Although, there are a number of naturally occurring and synthetic agents (glitazones) that activate PPARγ, the identity of the true natural ligand of this receptor is still unknown; this may be the reason for their lethal side effects. In silico virtual screening has presently become an integral part of contemporary drug research. A variety of computational tools are being developed and refined to effectively employ fast screening methods to yield potent hits. In recent past, an explosive growth in the successful applications employing a wide range of methods, such as molecular docking, pharmacophore modelling, etc. Efforts are also being made to employ the drug likeliness of a given compound. Hence in the present investigation, it was hypothesized worthwhile to discover a new prototype of molecule to facilitate our search for novel PPARγ agonists by using computer aided molecular docking studies. In conclusion, we could identify the potential ligands **4**, **16** and **22** with predicted PPPAγ ligand binding domain agonistic activity equal to 0.1 µg/mL.*

Keywords: *Diabetes mellitus (DM), PPARγ, In silico, Virtual screening.*

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INTRODUCTION:

Drug discovery and development is an interdisciplinary, expensive and time consuming process. Scientific advancements during the past two decades have changed the way pharmaceutical research generate novel bioactive molecules. Advances in computational techniques and in parallel hardware support have enabled in silico methods, and in particular structure-based drug design method, to speed up new target selection through the identification of hits to the optimization of lead compounds in the drug discovery process. Genomics, proteomics, bioinformatics and, chemoinformatics have gained immense popularity and have become an integral part of the industrial and academic research, directing drug design and discovery. Virtual screening emerged as an important tool in our quest to access novel drug like compounds. (Van Drie, 2007.; Kier, 1967.; Mason, J. S, et al., 2001).

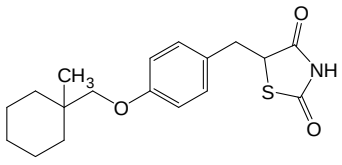
Virtual screening can be done in two ways: ligand-based or protein-based. With the availability of the 3D structure of a biological target, it is feasible to use a structure-based approach to evaluate and predict the binding mode of a ligand within the active site of the receptor with docking methods. Now it is a popular technique used for increasing the speed of drug designing process. This was made possible by the availability of many protein structures which helped in developing tools to understand the structure function relationships, automated docking and virtual screening. Furthermore, when no 3D structural information about target proteins with their receptor site is available ligand-based design is applied. The ligand-based approach starts with a group of ligands binding to the same receptor with the same mechanism. Today four different strategies based on the prior knowledge of the targets 3D structure and the ligands binding to it are predominant. (Wermuth C. G, et al.,1998.; Allen F. H. 2002.; Mayer D, et al., 1987).

Type 2 diabetes is a chronic disease characterized by insulin resistance in the liver and peripheral tissues accompanied by a defect in pancreatic β-cells. The insulin resistant state at the peripheral level causes impaired glucose utilization leading to hyperglycemia, which may also play a role in the etiology of a wide spectrum of metabolic disorders such as obesity, hypertension, atherosclerosis, neuropathy, nephropathy, retinopathy, etc. (Smith A, et al.,2000.; Saltiel, A.R. 2001).

Between 1997 and 1999, a new class of drugs called 'glitazones' was approved by the US FDA for the treatment of type 2 diabetes. The most extensively studied therapeutic utility for PPARγ agonists has been in the treatment of type 2 diabetes. PPARγ agonists have been shown to enhance the sensitivity of target tissues to insulin and to reduce plasma glucose, lipid, and insulin levels in animal models of type 2 diabetes as well as in humans. The molecular target of glitazones was reported to be the peroxisome proliferator-activated receptor-γ (PPAR-γ). Troglitazone, the first agent of this class was approved by US FDA for the treatment of type 2 diabetes. (Fujiwara T, et al.,1988.; Stevenson R. W, et al.,1990.; Cantello, B. C. C, et al.,1994).

The Peroxisome proliferator-activated receptors (PPARs) are subfamily of nuclear hormone receptors include distinct genes that code for several PPAR isoforms denoted: PPARα, β/δ and γ. Expression of PPARs is tissue dependent. PPARα is highly expressed in liver, cardiacmyocytes, enterocytes and proximal tubule of the kidney. PPARβ/δ is ubiquitously expressed, where as PPARγ is highly expressed in adipose tissue and the immune system.(Liu, L. S, et al.,1998).

PPARγ is involved in the regulation of expression of myriad genes that regulate energy metabolism, cell differentiation, apoptosis and inflammation. Although originally discovered as a pivotal regulator of adipocyte differentiation, the roles that this transcription factor play in physiology and pathophysiology continue to grow as researchers discover its influence in the function of many cell types. Thiazolidinediones (TZDs) or glitazones are a class of PPARγ agonists (insulin sensitizers) which include ciglitazone (1), troglitazone (2), pioglitazone (3), and rosiglitazone (4). (Shoda T, et al.,1982.; Willson, T. M, et al.,2000).



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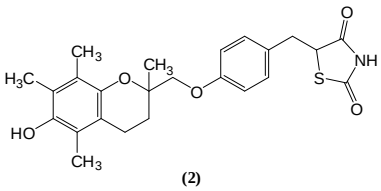

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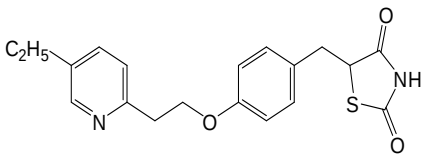

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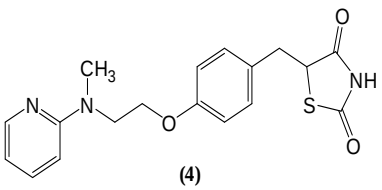


(4)

Diabetes mellitus (DM) is a growing problem that threatens human health all over the world. According to WHO reports, around 250 million people are currently living with diabetes and this number is expected to be more than 366 million by 2030. Due to major side effects of currently available drugs for the treatment of type 2 diabetes predominantly thiazolidinediones (TZDs) as PPARγ agonists (insulin sensitizers), the search for new PPARγ agonists endowed with more favorable druggable properties is still a major pharmaceutical challenge. However, eventual findings of risk for muraglitazone (risk of bone fracture), ciglitazone (hepatotoxicity), troglitazone (hepatotoxicity), rosiglitazone (congestive heart failure), and pioglitazone (bladder cancer) have significantly decreased the future scope of TZDs for clinical use in many countries (US, Europe, Italy, France). As a result, there is a strong demand for identification of replacement for TZDs therapy with conserved insulin sensitizing properties in the treatment of type 2 diabetes. Therefore in the present investigation, we proposed to highlight on the discovery of non-thiazolidinedione PPARγ agonists by using in silico based virtual screening protocols, which might surmount the problem, associated with the known TZDs.

Computational software requirements

Computer aided drug discovery softwares along with graphical user interface (GUI) were utilized for molecular modeling, energy minimization, molecular docking and virtual screening protocols.

Table 1: List of software applications used in the present study

S.No	Activity (will be performed)	Software (will be used)	License type (will be obtained)	Source
1).	Molecular modeling (2D-Drawing)	Accelrys Draw	Academic License GPU (General Public User) License	Open Source
2).	Molecular modeling (2D-3D Conversion)	Open Babel	Academic License GPU (General Public User) License	Open Source
3).	Molecular modeling (Molecular Mechanics & Energy Minimization)	ArgusLab v 4.0	Academic License GPU (General Public User) License	Open Source
4).	Molecular Docking & Virtual screening	Molegro Virtual Docker v 5.0	1 Month Academic License	Commercial

Table 2: Citations for software applications used in the present investigation

S.No	Software	Citation
[1].	**Accelrys Draw**	Draw, A. (2011). Accelrys Software Inc. San Diego.
[2].	**Open Babel**	OLBoyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. J Cheminf, 3, 33.
[3].	**Argus lab**	Thompson, M. A. (2004). ArgusLab 4.0. 1. Planaria Software LLC, Seattle, WA.
[4].	**Molegro Virtual Docker v 5.0**	Molegro, A. P. S. (2011). MVD 5.0 Molegro Virtual Docker. DK-8000 Aarhus C, Denmark.
[5].	**Protein Data Bank (PDB)**	Bank, P. D. (1971). Protein Data Bank. Nature New Biol, 233, 223.

Computational hardware requirements

The minimum central hardware system configuration include Intel (R) Core (TM) 2Duo Central Processing Unit (CPU), 2.5 GHz, 1 TB hard disk, 2 KV Power Backup, WinXP or higher operating system was used for running all the selected computer aided drug discovery softwares. All softwares were well compatible with the selected system configuration.

X-ray crystallographic structure of protein target (PPARγ)

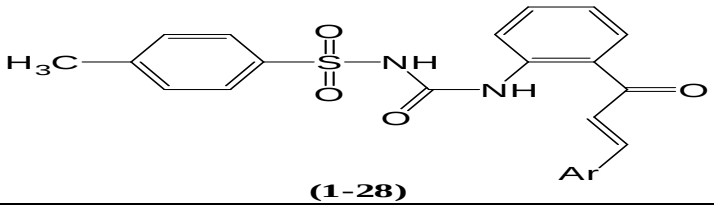
X-ray crystallographic data of PPARγ Ligand Binding Domain (LBD) was obtained from Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). The protein data bank code (PCB ID: 3CS8) deposited by Li, et al., 2008.

METHODS:

General procedure for the construction of ligand data base and validation

The chemical structures of the selected ligands as shown in Table 3 (Test set) which earlier synthesized, characterized and reported by Avupati, et al., 2012 were initially modelled as 2D chemical structures using Accelrys Draw software and transformed into 3D chemical structures using Open Babel software and created a 3D ligand database of 28 (Test set) and subjected for energy minimization using ArgusLab v 4.0 software. The minimization was executed until the root mean square gradient value reached a value smaller than 0.0001 kcal/mol. Such energy minimized structures were considered for molecular docking studies (virtual screening). The corresponding docking engine compatible 'MDL MOL' file format has been adapted to ligand database by using integral option (save as /MDL MOL).

Table 3: Ligand database of 28 compounds (Test Set) selected for molecular docking studies against PPARγ LBD of 3CS8 (Avupati, et al., 2012).



(1-28)

Ligand Code (Avupati, et al., 2012)	Ar	Ligand Code (Avupati, et al., 2012)	Ar
1	C_6H_5	**15**	3-$NO_2C_6H_4$
2	4-MeC_6H_4	**16**	5-OH,2-$NO_2C_6H_3$
3	4-$NMe_2C_6H_4$	**17**	3-FC_6H_4
4	3-$OMeC_6H_4$	**18**	4-FC_6H_4
5	4-$OMeC_6H_4$	**19**	2-ClC_6H_4
6	3,4-di$OMeC_6H_3$	**20**	4-ClC_6H_4
7	2,4-di$OMeC_6H_3$	**21**	2,4-diClC_6H_3
8	3,4, 5-tri$OMeC_6H_2$	**22**	3-BrC_6H_4
9	2-OHC_6H_4	**23**	4-Allyl-OC_6H_4
10	3-OHC_6H_4	**24**	Phenylethene-yl
11	4-OHC_6H_4	**25**	Pyrrol-2-yl
12	3-OEt,4-OHC_6H_3	**26**	Pyridin-3-yl
13	3-OMe,4-OHC_6H_3	**27**	Pyridin-4-yl
14	2-$NO_2C_6H_4$	**28**	Anthracen-9-yl

General procedure for the protein target selection and validation

The selection of PPARγ Ligand Binding Domain (LBD) for molecular docking studies was carried out based upon several factors such as structure should be determined by X-ray diffraction spectroscopy, and resolution should be between 2.5-3.0 A^o, it should contain a co-crystallized ligand; the selected protein should not have any protein breaks in their 3D structure. On the other hand, we further consider Ramachandran plot statistics as the important filter for protein selection with none of the residues present in disallowed region. Finally the resultant protein target was prepared for molecular docking simulation in such a way that all heteroatoms (i.e., nonreceptor atoms such as water, ions, etc.) were removed.

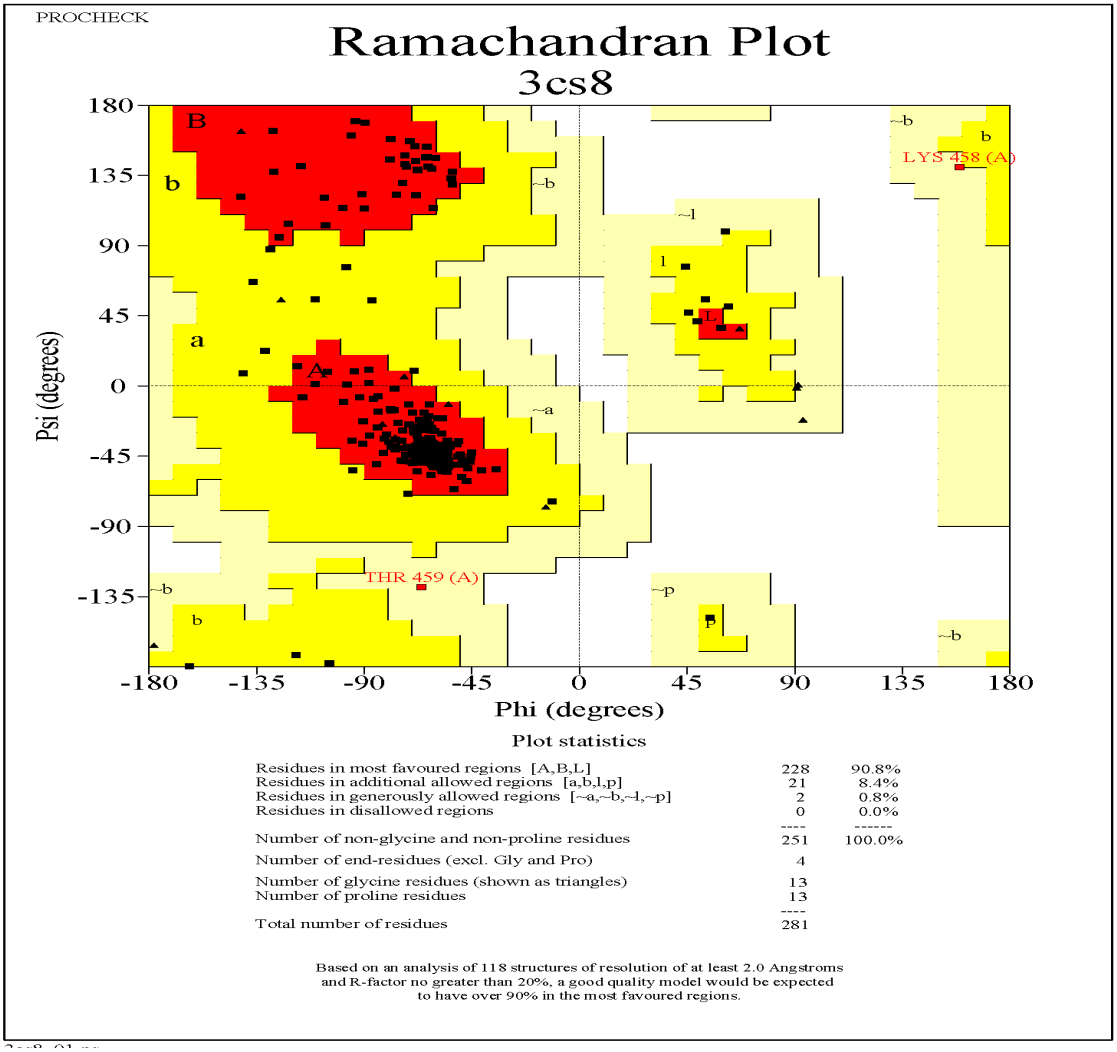


Fig 1: Ramachandran plot statistics of the selected PPARγ target protein (3CS8)

General procedure for the software validation

Molegro Virtual Docker v 5.0 validation was performed by using X-ray structure (3CS8) deposited with co-crystallized ligand was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). The Root Mean Square Deviation (RMSD) between the X-ray co-crystallized ligand and docked conformation was 1.07 A^{o} indicated that the parameters for docking simulation was good in reproducing X-ray crystal structure.

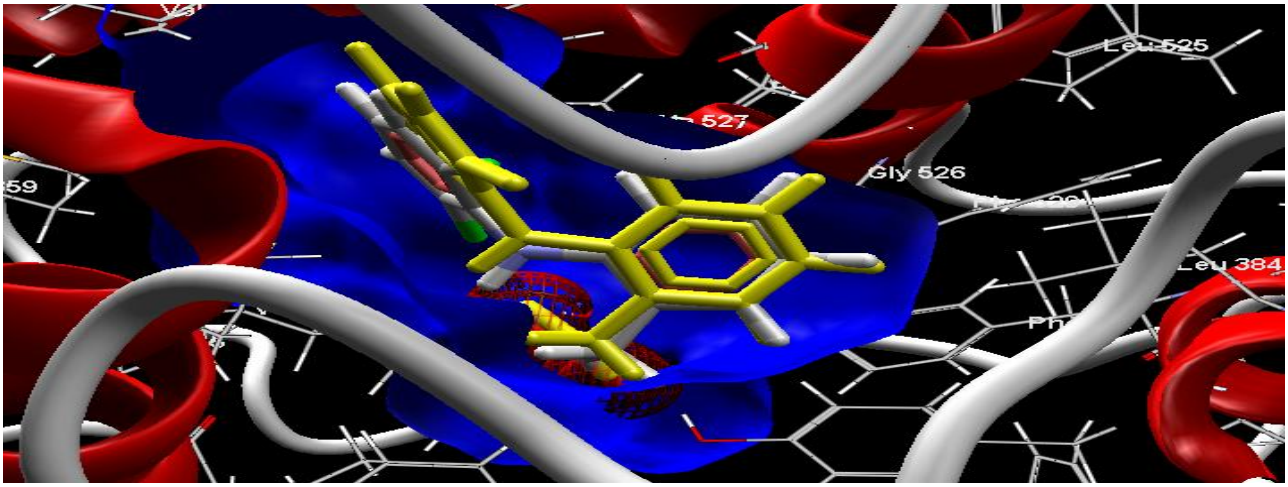
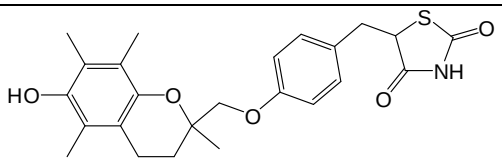
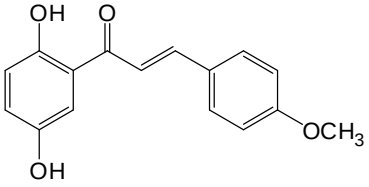
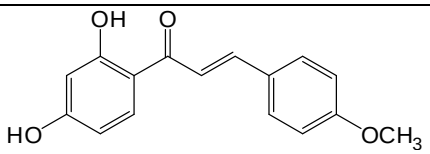
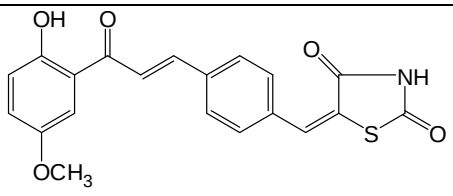


Fig 2: Superimposed binding orientation of docked conformer (yellow) and crystal ligand (white) within the active binding site region of 3CS8

General procedure for the virtual screening

- Our approach to creating a ligand database (Training set) and (Test set) was similar to the methods outlined previously by my research supervisor **Dr. VasudevaRao (Avupati, et al., 2012)**.
- A set of criteria was applied to remove pharmaceutically undesirable molecules.
- Subsequently, Ligand–protein inverse docking approach has been applied as a useful tool in facilitating the lead identification through molecular docking studies.
- In this approach, we docked a database of 28 modeled ligands (Test set) into site of crystallographic ligand binding domain of PPARγ target protein was attempted to find putative binding ligands. Further these compounds were analyzed for their binding mode, binding interaction and binding energy of putative stable ligand conformations.
- The predicted PPARγ ligand binding domain activity of 28 modeled ligands (Test set) has been carried out based on the standard curve developed by mapping correlation plots between experimentally observed PPARγ ligand binding activity (µg/mL) and corresponding Moldock Scores (kcal/mol) of the experimental compounds (Training set).
- A set of bioactive PPARγ agonists with observed Ligand Binding Domain (LBD) agonistic activity were taken from the PPARγ LBD activity reported by **Jung, et al., 2006.**

Table 4: PPARγ Ligand Binding Domain (LBD) activity data reported by Jung, et al., 2006.

S.No	Ligand Code (Jung, et al., 2006)	Chemical structure	PPARγ LBD activity (µg/mL)	Fold activation (%)
1	2 (troglitazone)		1	22
2	5		0.5	38
3	12		10	16
4	20		0.5	24

Statistical analysis

The GraphPad Prism (Version 5.0) was used in data analysis. Bivariate or multivariate statistical analytical tools have been utilized to develop a working prediction model.

Table 5: Ligands (Training set) displaying PPARγ Ligand Binding Domain (LBD) activity data reported by Jung, et al., 2006, binding affinities, H-bonds and H-bond interacting residues with 3CS8.

S.No	Ligand Code (Jung, et al., 2006)	PPARγ LBD activity (µg/mL)	Moldock score (kcal/mol)	No. of. H-Bonds	H-Bond Forming Residues
1	**2 (Troglitazone)**	1	-127.184	3	Gln 271, Gln 283 and Arg 280.
2	**5**	10	-104.466	5	His 449, Cys 285 and Ser 289.
3	**12**	10	-113.913	4	Pro 227, Glu 343, Leu 340 and Gly 344.
4	**20**	0.5	-147.347	5	Cys 285, Arg 288 and Glu 259.

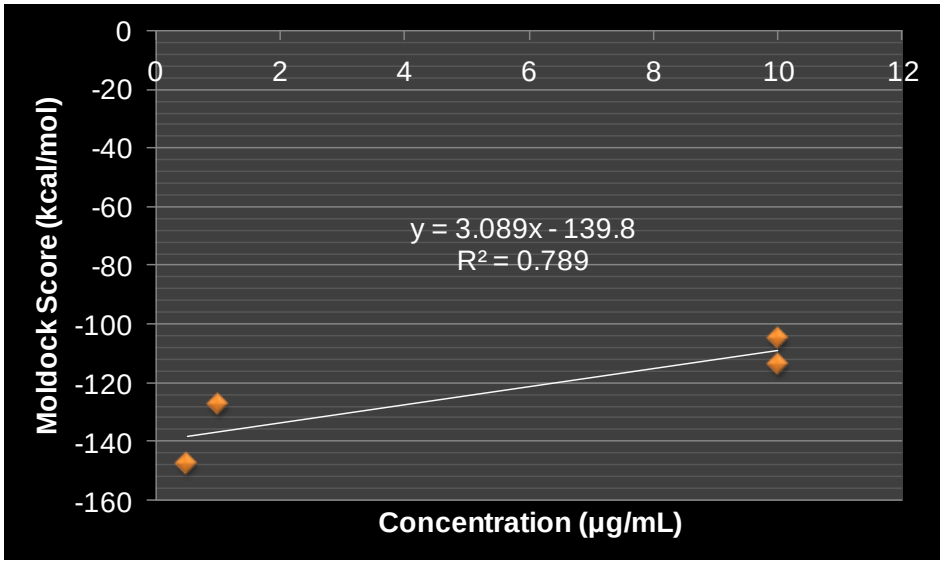


Fig 3: Standard graph for determination of predicted PPARγ Ligand Binding Domain (LBD) activity of the selected 28 ligands (Test set).

Concentration (µg/mL) = $\frac{\text{(Moldock Score) + 139.8}}{3.089}$

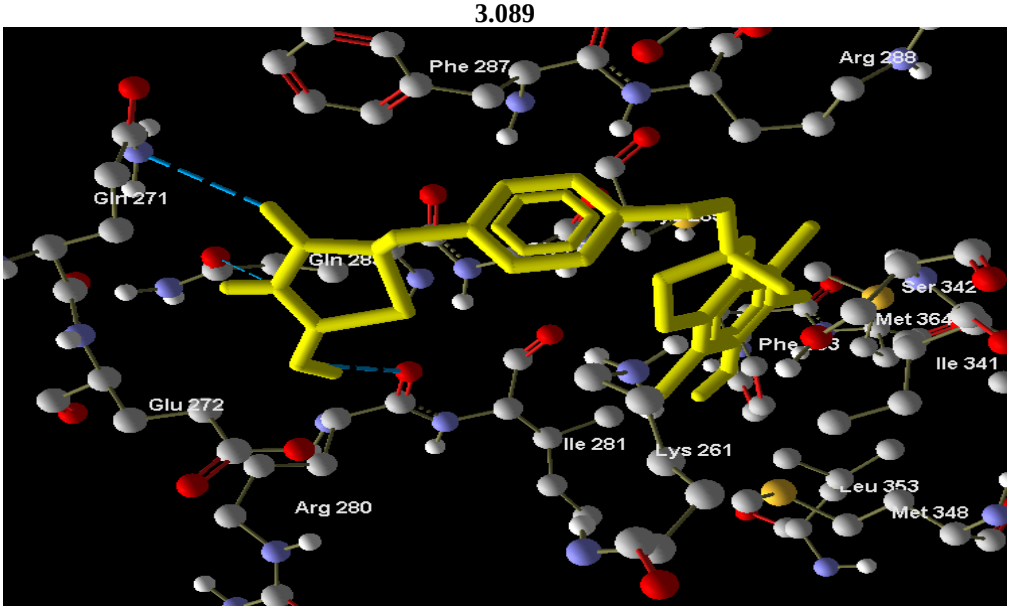


Fig 4: Hydrogen bond interactions (blue dashes) of predicted stable binding mode of 2 (troglitazone) (yellow).

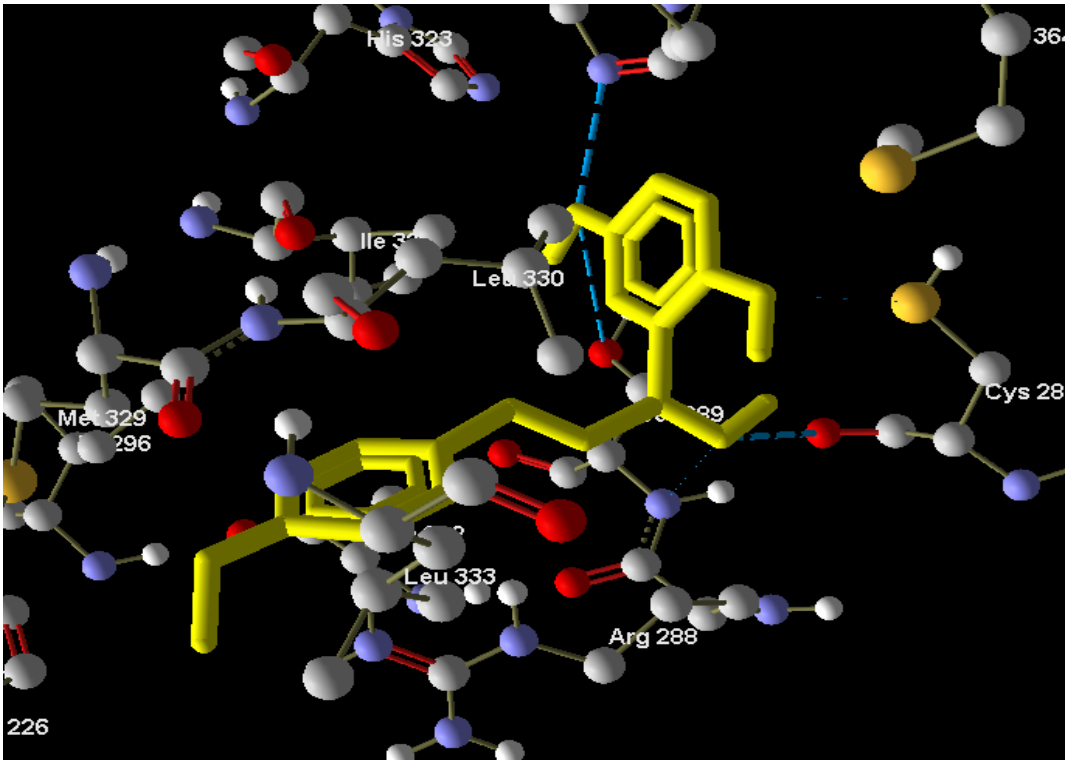


Fig 5: Hydrogen bond interactions (blue dashes) of predicted stable binding mode of 5 (yellow).

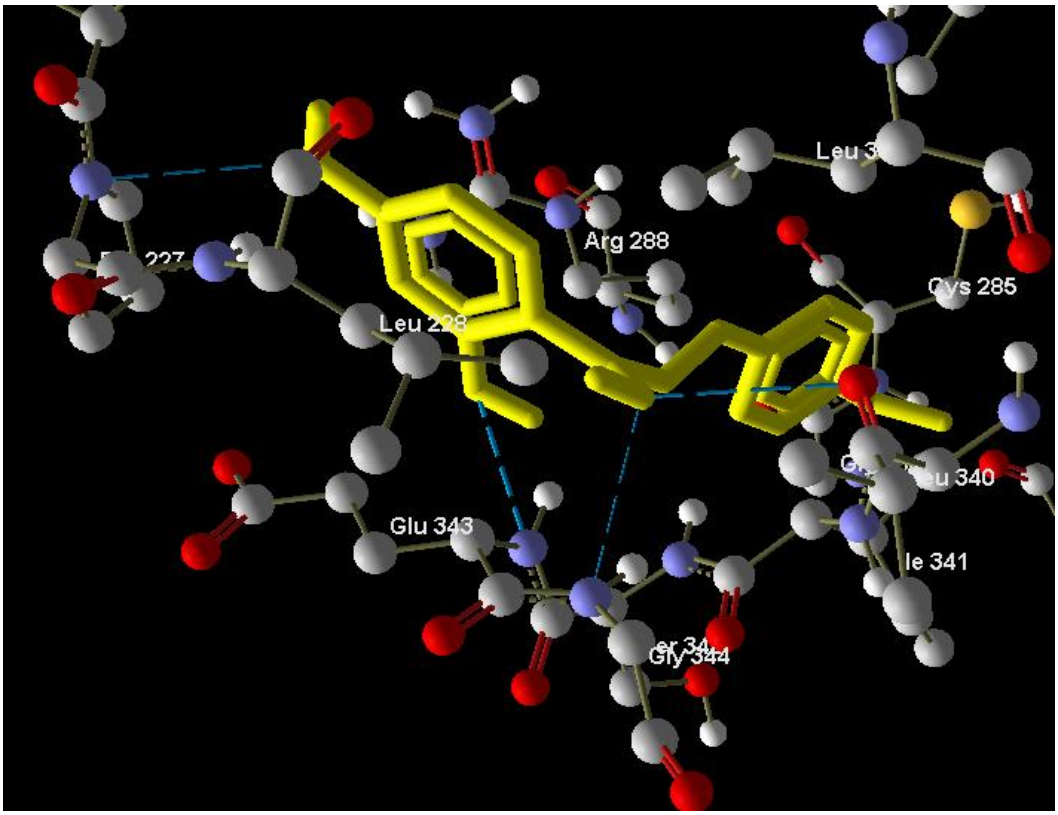


Fig 6: Hydrogen bond interactions (blue dashes) of predicted stable binding mode of 12 (yellow).

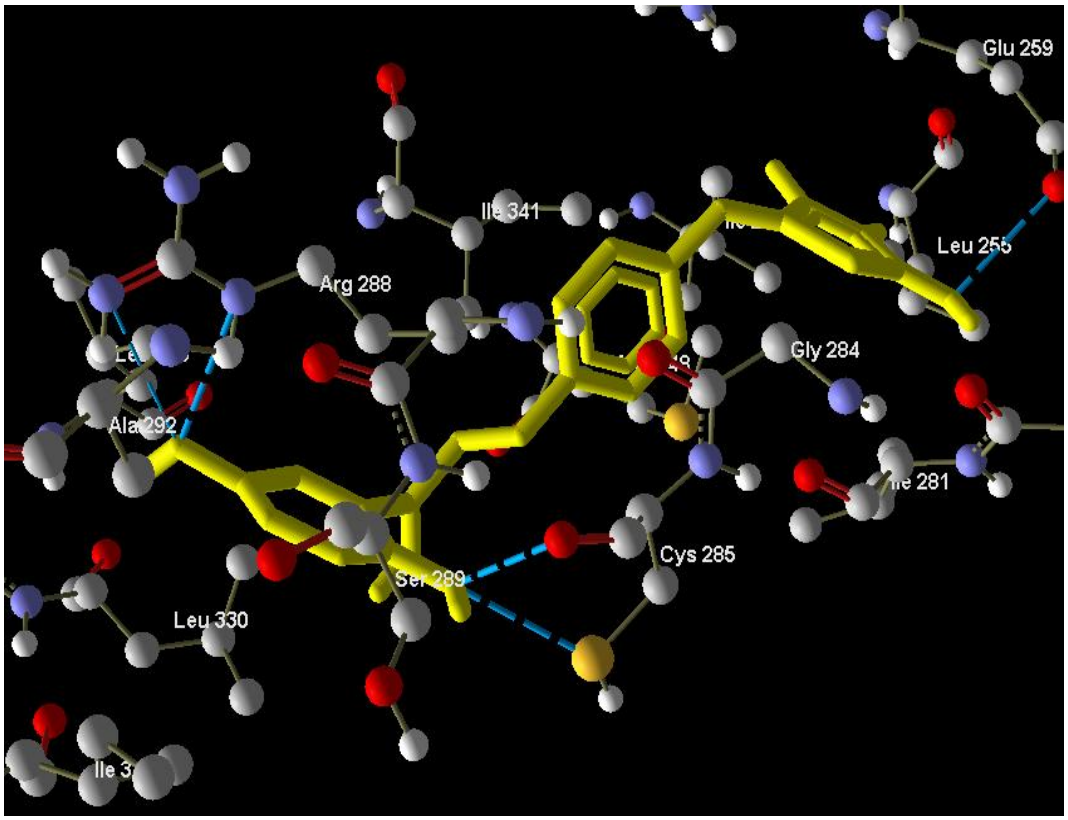


Fig 7: Hydrogen bond interactions (blue dashes) of predicted stable binding mode of 20 (yellow).

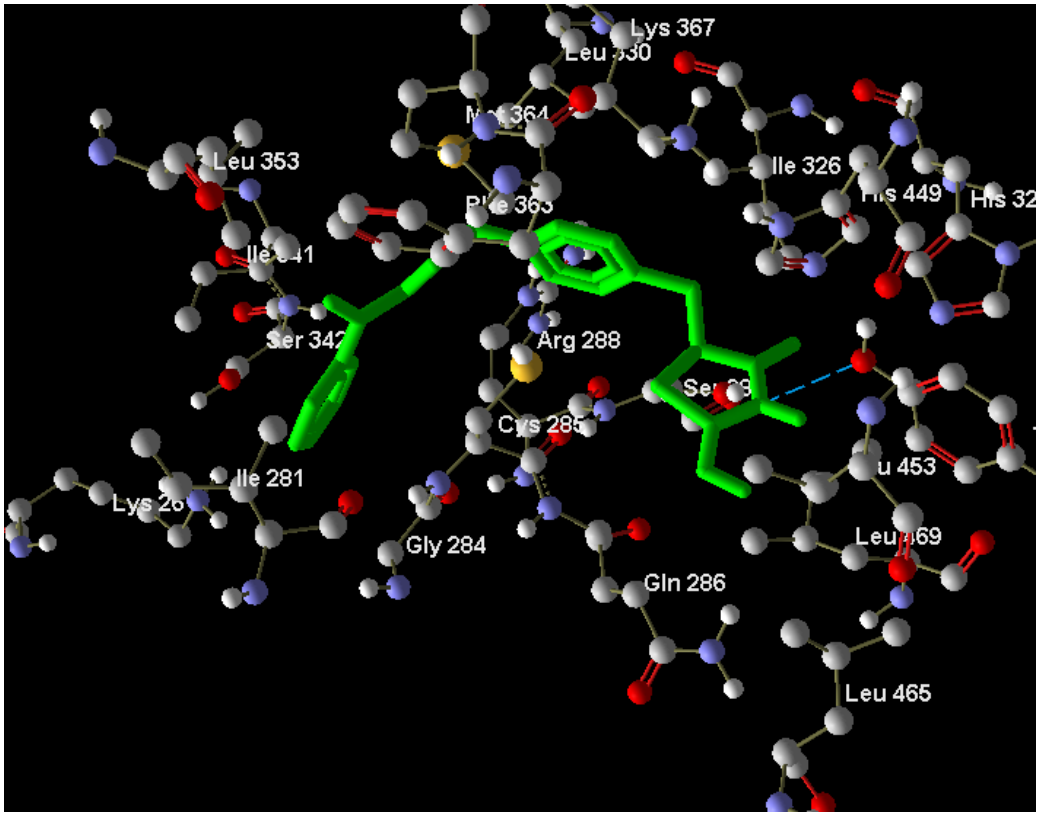


Fig 8: Hydrogen bond interactions (blue dashes) of predicted stable binding mode of rosiglitazone (BRL-503) (green).

Table 5: Ligands (Test set 1-14) displaying binding affinities, H-bonds and H-bonds interacting residues with 3CS8.

Ligand Code (Avupati, et al., 2012)	Ar	MolDock Score (kcal/mol)	No. of. H-Bonds	H-Bond Forming Residues
1	C_6H_5	-132.231	1	Leu 228
2	4-MeC_6H_4	-131.478	2	Gln 283 and Arg 280
3	4-$NMe_2C_6H_4$	-140.879	1	Gln 283
4	3-$OMeC_6H_4$	-139.469	8	Lys 261, Ser 342, Gly 284, Arg 280 and Glu 272
5	4-$OMeC_6H_4$	-133.579	4	Lys 261, Ser 342, Gly 284 and Arg 280
6	3,4-di$OMeC_6H_3$	-128.042	3	Cys 285, Lys 261 and Glu 272.
7	2,4-di$OMeC_6H_3$	-138.347	1	Arg 280.
8	3,4, 5-tri$OMeC_6H_2$	-151.54	3	Arg 288, Leu 340 and Cys 285.
9	2-OHC_6H_4	-131.628	6	Glu 259, Gln 283, Arg 280 and Ile 281.
10	3-OHC_6H_4	-132.366	6	Glu 272, Gly 284, Ser 342, Lys 261 and Glu 259.
11	4-OHC_6H_4	-125.136	5	Glu 259, Glu 272, Ser 342 and Arg 288.
12	3-OEt,4-OHC_6H_3	-131.352	3	Cys 285, Ser 342 and Lys 261.
13	3-OMe,4-OHC_6H_3	-134.854	6	Ser 342, Lys 261, Leu 340, Glu 343, Glu 295 and Pro 227.
14	2-$NO_2C_6H_4$	-115.768	4	Ser 342, Lys 261, Cys 285 and Ile 281.
CL*	-	-127.830	1	Tyr 473

*3CS8 co-crystallized ligand (Rosiglitazone)

Table 6: Ligands (Test set 15-28) displaying binding affinities, H-bonds and H-bonds interacting residues with 3CS8.

Ligand Code (Avupati, et al., 2012)	Ar	MolDock Score (kcal/mol)	No. of. H-Bonds	H-Bond Forming Residues
15	3-$NO_2C_6H_4$	-139.474	5	Arg 280, Gln 283, Glu 272 and Lys 261.
16	5-OH,2-$NO_2C_6H_3$	-137.982	5	Gln 283, Gly 284, Lys 261 and Glu 259.
17	3-FC_6H_4	-137.038	1	Gln 283
18	4-FC_6H_4	-136.764	3	Leu 228, Arg 288 and Gly 244.
19	2-ClC_6H_4	-125.422	4	Ser 342, Lys 261, Ile 281 and Arg 288.
20	4-ClC_6H_4	-157.103	3	Leu 330, Arg 288 and Glu 343.
21	2,4-diClC_6H_3	-139.47	3	Ser 342, Lys 261 and Arg 280.
22	3-BrC_6H_4	-137.512	1	Gln 283.
23	4-Allyl-OC_6H_4	-143.325	5	Cys 285, Ile 281, Ser 289 and Ser 342.
24	Phenylethene-yl	-132.311	3	Arg 288, Leu 340 and Glu 343.
25	Pyrrol-2-yl	-135.63	1	Gln 283.
26	Pyridin-3-yl	-119.79	6	His 449, Tyr 473, Ser 289, Cys 285 and Arg 288.
27	Pyridin-4-yl	-129.382	3	Glu 343, Leu 340 and Cys 285.
28	Anthracen-9-yl	-149.266	2	Cys 285 and Leu 340.
CL*	-	-127.830	1	Tyr 473

*3CS8 co-crystallized ligand (Rosiglitazone

Table 7: Predicted PPARγ ligand binding activity (µg/mL) of the selected 28 ligands (Test set) based on the standard curve.

Ligand Code (Avupati, et al., 2012)	Ar	Predicted PPARγ ligand binding activity (µg/mL)
1	C_6H_5	2.4
2	4-MeC_6H_4	2.6
3	4-$NMe_2C_6H_4$	-0.3 (Inconsistent prediction)
4	3-$OMeC_6H_4$	0.1
5	4-$OMeC_6H_4$	2.0
6	3,4-di$OMeC_6H_3$	3.8
7	2,4-di$OMeC_6H_3$	0.4
8	3,4, 5-tri$OMeC_6H_2$	-3.8 (Inconsistent prediction)
9	2-OHC_6H_4	2.6
10	3-OHC_6H_4	2.4
11	4-OHC_6H_4	4.7
12	3-OEt,4-OHC_6H_3	2.7
13	3-OMe,4-OHC_6H_3	1.6
14	2-$NO_2C_6H_4$	7.7
15	3-$NO_2C_6H_4$	7.7
16	5-OH,2-$NO_2C_6H_3$	0.1
17	3-FC_6H_4	0.5
18	4-FC_6H_4	0.8
19	2-ClC_6H_4	0.9
20	4-ClC_6H_4	4.6
21	2,4-diClC_6H_3	-5.6 (Inconsistent prediction)
22	3-BrC_6H_4	0.1
23	4-Allyl-OC_6H_4	0.7
24	Phenylethene-yl	-1.1 (Inconsistent prediction)
25	Pyrrol-2-yl	2.4
26	Pyridin-3-yl	1.3
27	Pyridin-4-yl	6.4
28	Anthracen-9-yl	3.3
Rosiglitazone	-	-3.0 (Inconsistent prediction)

DISCUSSION:

With respect to the molecular modeling study, software Molegro Virtual Docker (MVD) v 5.0 (www.molegro.com) along with Graphical User Interface (GUI), MVD tools was utilized to generate grid, calculate dock score and evaluate conformers.

Among all the entries of PPARγ proteins deposited in RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do), 3CS8 was selected for molecular docking analysis, where the residues were bonded more closely to the rosiglitazone agonist, co-crystallized with PPARγ. In this crystal structure, the Ligand Binding Domain (LBD) forms a homodimer in which both monomers have nearly identical Co conformations. The structure of the ‘‘A’’ monomer of the LBD homodimer was selected for docking studies.

Molecular docking was performed using MolDock docking engine of software. The scoring function used by MolDock is derived from the Piecewise Linear Potential (PLP) scoring functions. The active binding site region was defined as a spherical region which encompasses all protein within 15.0 A^o of bound crystallographic ligand atom with a size of X: 18.89 Ao, Y: 2.16 Ao and Z: 30.05 A^o axes, respectively. Default settings were used for all the calculations. Docking was performed using a grid resolution of 0.30 A^o and for each of the 10 independent runs; a maximum number of 1500 iterations were executed on a single population of 50 individuals. The active binding site was considered as a rigid molecule, whereas the ligands were treated as being flexible, i.e. all non-ring torsions were allowed. This approach seems to be consistent with the structural similarity observed between rosiglitazone and compounds under investigation. The predicted binding energies and the corresponding hydrogen bond forming residues for the ligands 1-28 were summarized in Tables 5 and 6.

According to the crystallographic structure of rosiglitazone in the Ligand Binding Domain (LBD) of PPARγ, the thiazolidinedione ring revealed some specific interactions with neighboring amino acid residues of the LBD. These interactions include hydrogen bonding with amino acids Cys 285, His 449 and Tyr 473 as reported in the literature.

The binding profile of ligands 1-28 were compared with the profile of the rosiglitazone molecule. The Figures under chapter 5 summarizes the binding modes and hydrogen bond interactions of the ligand database 1-28 selected for the study. According to the docking models, all ligands were predicted to bind into the LBD, thus sharing a very similar binding mode. Interestingly, we found the docking scores of 1-28 (Test set), Training set (2, 5, 12, 20) were favorable for the most stable conformations (most negative docking energies ranging from -157.103 to -104.466 kcal/mole comparable to that of the crystallographic ligand (rosiglitazone) with 127.830 kcal/mole and it could be the remarkable in silico confirmation to evaluate these compounds for their in vitro and in vivo antidiabetic activity.

The docking analysis of the ligands 1-28 in the present study revealed the PPARγ activating process, termed "dock and lock" as seen in case of some compounds. Example of compounds that exhibit the PPARγ activities are 6 (formed hydrogen bond with Cys 285, Lys 261 and Glu 272), 8 (formed hydrogen bond with Cys 285, Arg 288 and Leu 340), 12(formed hydrogen bond with Cys 285, Ser 342 and Lys 261), 14 (formed hydrogen bond with Cys 285 Ser 342, Lys 261 and Ile 281), 23 (formed hydrogen bond with Cys 285, Ile 281, Ser 289 and Ser 342). 26 (formed hydrogen bond with Cys 285, His 449, Tyr 473, Ser 289 and Arg 288), 27 (formed hydrogen bond with Cys 285, Glu 343 and Leu 340) and 28 (formed hydrogen bond with Cys 285 and Leu 340). All the above stated compounds are binding covalently to Cys 285 within the PPARγ LBD which is important interaction to exhibit the PPARγ activity. Thus these compounds suggested that the formation of a covalent bond with Cys 285 causes a conformational transmission to the receptor surface. We consequently found α,β-unsaturated ketone group (chalcone moiety) is the fundamental moiety of naturally occurring ligands to exhibit PPARγ agonistic activity as reported earlier and also the same observation is valid in the ligands 1-28 simulated in the present investigation, as they also consist of similar α, β-unsaturated ketone moiety in their general structure.

A close look into the H-bond interactions with amino acids such as Arg 288, Ser 289, Pro 227, Glu 343, Leu 340, Gly 344 and Glu 259 respectively were identified through molecular docking studies of a series of chalcones which earlier experimentally proved to be as potential PPARγ agonists at varied dose concentrations ranging from 0.5-10µg/ml. Among the database, compounds 8, 10, 11, 13, 16, 18, 19, 20, 23, 24, 26, 27 and 28 showed H-bond interactions with the above mentioned residues. Since both the classes (test test and training set) are belongs to chalcone family, the interactions identified in case of training set which have also been significant in case of test set. Hence, based on this hypothesis we could have hypothesized these compounds as leads. Moreover, similar study has also been made with Troglitazone which was an approved drug by USFDA as PPARγ agonist in the treatment of type 2 diabetes but due to its severe side effects this drug was withdrawn in early 2000s. Our investigations branched forward to determine the leads with possible side effects. Subsequently, we studied H-bonds interactions of Troglitazone so as to enable us to determine the comparative hypothesis between the H-bond interactions formed by Troglitazone and test.

Therefore, compounds 2, 3, 4, 5, 7, 9, 15, 16, 17, 21, 22 and 25 were virtually hypothesized to be having major side effects, which earlier reported in the clinical use of Troglitazone. The hypothesis has been confirmed based on their relative comparisons of H-bond interacting residues, Gln 271, Gln 283, and Arg 280 formed by Troglitazone were also seen in some of the compounds as mentioned earlier (Table 5). This could be the evidence for further confirmations of our virtual hypothesis.

Subsequently, we have determined the predicted biological activity i.e. predicted PPARγ LBD agonistic activity using a Beer-Lambert linear relationship, a regression equation was plotted between PPARγ agonistic activity (experimentally observed) and Moldock scores (in silico observations). This relationship was validated with correlation coefficient r^2 = >0.78 indicating the significance as prediction model. Further, the Moldock scores (kcal/mol) of all the ligands (1-28) were substituted in the standard equation, extrapolated and derived predicted PPARγ LBD agonist activity (µg/mL) of each ligand selected for the study (Table 7).

CONCLUSION:

From these studies, we could successfully able to understand the predicted mechanism of action as well as their predicted PPARγ ligand binding domain agonistic activity (µg/mL). Among all tested, ligands 4, 16 and 22 were appeared to be most stable compounds. The score values are -139.469 (Ligand 4), -137.982 (Ligand 16) and -137.512 (Ligand 22). Thus these compounds appeared to be the potential leads.

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REFERENCES:

1.Alberton, E. H., Damazio, R. G., Cazarolli, L. H., Chiaradia, L. D., Leal, P. C., Nunes, R. J., ... & 987Silva, F. R. M. B. Influence of chalcone analogues on serum glucose levels in hyperglycemic rats. Chemico-biological interactions,2008;171(3): 355-362.

2.Allen, F. H. The Cambridge Structural Database: a quarter of a million crystal structures and rising. Acta Crystallographica Section B: Structural Science,2002; 58(3):380-388.

3.Avupati, V. R., Yejella, R. P., Guntuku, G., & Gunta, P. Synthesis, characterization and in vitro biological evaluation of some novel diarylsulfonylureas as potential cytotoxic and antimicrobial agents. Bioorganic & medicinal chemistry letters, 2012;22(2): 1031-1035.

4.Buckingham, J., Glen, R. C., Hill, A. P., Hyde, R. M., Martin, G. R., Robertson, A. D., ... & Woollard, P. M. Computer-aided design and synthesis of 5-substituted tryptamines and their pharmacology at the 5-HT1D receptor: discovery of compounds with potential anti-migraine properties. Journal of medicinal chemistry,1995; 38(18):3566-3580.
5.Cantello, B. C., Cawthorne, M. A., Cottam, G. P., Duff, P. T., Haigh, D., Hindley, R. M., ... & Thurlby, P. L. [[. omega.-(Heterocyclylamino) alkoxy] benzyl]-2, 4-thiazolidinediones as potent antihyperglycemic agents.Journal of medicinal chemistry, 1994;37(23):3977-3985.
6.Enoki, T., Ohnogi, H., Nagamine, K., Kudo, Y., Sugiyama, K., Tanabe, M., ... & Kato, I. Antidiabetic activities of chalcones isolated from a Japanese Herb, Angelica keiskei. Journal of agricultural and food chemistry, 2007;55(15):6013-6017.
7.Fujiwara, T., Yoshioka, S., Yoshioka, T., Ushiyama, I., & Horikoshi, H. (1988). Characterization of new oral antidiabetic agent CS-045: studies in KK and ob/ob mice and Zucker fatty rats. Diabetes, 1988;37(11):1549-1558.
8.Greer, J., Erickson, J. W., Baldwin, J. J., & Varney, M. D. Application of the three-dimensional structures of protein target molecules in structure-based drug design. Journal of medicinal chemistry, 1994;37(8):1035-1054.
9.Hsieh, C. T., Hsieh, T. J., El-Shazly, M., Chuang, D. W., Tsai, Y. H., Yen, C. T., ... & Chang, F. R. (2012). Synthesis of chalcone derivatives as potential anti-diabetic agents. Bioorganic & medicinal chemistry letters, 22(12), 3912-3915.
10.Jung, S. H., Park, S. Y., Kim-Pak, Y., Lee, H. K., Park, K. S., Shin, K. H., ... & Lim, S. S. (2006). Synthesis and PPAR-. GAMMA. Ligand-Binding Activity of the New Series of 2'-Hydroxychalcone and Thiazolidinedione Derivatives. Chemical and pharmaceutical bulletin, 54(3), 368-371.
11Kawakami, Y., Inoue, A., Kawai, T., Wakita, M., Sugimoto, H., & Hopfinger, A. J. (1996). The rationale for E2020 as a potent acetylcholinesterase inhibitor.Bioorganic & medicinal chemistry, 4(9), 1429-1446.
12.Keenan, R. M., Weinstock, J., Finkelstein, J. A., Franz, R. G., Gaitanopoulos, D. E., Girard, G. R., ... & Samanen, J. M. (1993). Potent nonpeptide angiotensin II receptor antagonists. 2. 1-(Carboxybenzyl) imidazole-5-acrylic acids. Journal of medicinal chemistry, 36(13), 1880-1892.
13.KIER, L. B. (1967). Molecular orbital calculation of preferred conformations of acetylcholine, muscarine, and muscarone. Molecular pharmacology, 3(5), 487-494.
14.Koga, H., Itoh, A., Murayama, S., Suzue, S., & Irikura, T. (1980). Structure-activity relationships of antibacterial 6, 7-and 7, 8-disubstituted 1-alkyl-1, 4-dihydro-4-oxoquinoline-3-carboxylic acids. Journal of medicinal chemistry,23(12), 1358-1363.
15.Lewis, J. D., Ferrara, A., Peng, T., Hedderson, M., Bilker, W. B., Quesenberry, C. P., ... & Strom, B. L. (2011). Risk of bladder cancer among diabetic patients treated with pioglitazone interim report of a longitudinal cohort study. Diabetes care, 34(4), 916-922.
16.Li, Y., Kovach, A., Suino-Powell, K., Martynowski, D., & Xu, H. E. (2008). Structural and biochemical basis for the binding selectivity of peroxisome proliferator-activated receptor γ to PGC-1α. *Journal of Biological Chemistry*,*283*(27), 19132-19139.
17.Lim, S. S., Jung, S. H., Ji, J., Shin, K. H., & Keum, S. R. (2001). Synthesis of flavonoids and their effects on aldose reductase and sorbitol accumulation in streptozotocin-induced diabetic rat tissues. Journal of pharmacy and pharmacology, 53(5), 653-668.
18.Lipscombe, L. L., Gomes, T., Lévesque, L. E., Hux, J. E., Juurlink, D. N., & Alter, D. A. (2007). Thiazolidinediones and cardiovascular outcomes in older patients with diabetes. Jama, 298(22), 2634-2643.
19.Liu, L. S., Tanaka, H., Ishii, S., & Eckel, J. (1998). The New Antidiabetic Drug MCC-555 Acutely Sensitizes Insulin Signaling in Isolated Cardiomyocytes 1.Endocrinology, 139(11), 4531-4539.
20.Mason, J. S., Good, A. C., & Martin, E. J. (2001). 3-D pharmacophores in drug discovery. Current pharmaceutical design, 7(7), 567-597.
21.Mayer, D., Naylor, C. B., Motoc, I., & Marshall, G. R. (1987). A unique geometry of the active site of angiotensin-converting enzyme consistent with structure-activity studies. Journal of computer-aided molecular design, 1(1), 3-16.
22.Nicolle, E., Souard, F., Faure, P., & Boumendjel, A. (2011). Flavonoids as promising lead compounds in type 2 diabetes mellitus: molecules of interest and structure-activity relationship. Current medicinal chemistry, 18(17), 2661-2672.
23.Park, H. G., Bak, E. J., Woo, G. H., Kim, J. M., Quan, Z., Kim, J. M., ... & Cha, J. H. (2012). Licochalcone E has an antidiabetic effect. The Journal of nutritional biochemistry, 23(7), 759-767.
24.Parker, J. C. (2002). Troglitazone: the discovery and development of a novel therapy for the treatment of Type 2 diabetes mellitus. Advanced drug delivery reviews, 54(9), 1173-1197.
25.Penumetcha, M., & Santanam, N. (2012). Nutraceuticals as Ligands of PPAR.PPAR research, 2012.
26.Piccinni, C., Motola, D., Marchesini, G., & Poluzzi, E. (2011). Assessing the association of pioglitazone use and bladder cancer through drug adverse event reporting. Diabetes care, 34(6), 1369-1371.
27.Saltiel, A. R. (2001). New perspectives into the molecular pathogenesis and treatment of type 2 diabetes. Cell, 104(4), 517-529.
28.Seo, W. D., Kim, J. H., Kang, J. E., Ryu, H. W., Curtis-Long, M. J., Lee, H. S., ... & Park, K. H. (2005). Sulfonamide chalcone as a new class of α-glucosidase inhibitors. Bioorganic & medicinal chemistry letters, 15(24), 5514-5516.

29.Shoda, T., Mizumo, K., Imamiya, E., Sugiyama, Y., Fukits, T & Kawamatsu, Y. (1982). Studies on antidiabetic agents. II. Synthesis of 5-(4-(1-methylcyclohexylmethoxy)-benzyl) thiazolidine-2, 4-dione (ADD-3878) and its derivatives. Chemical and Pharmaceutical Bulletin, 30(10), 3580-3600.
30.Shukla, P., Singh, A. B., Srivastava, A. K., & Pratap, R. (2007). Chalcone based aryloxypropanolamines as potential antihyperglycemic agents.Bioorganic & medicinal chemistry letters, 17(3), 799-802.
31.Smith, A., Fogelfeld, L., & Bakris, G. (2000). New therapies in diabetes-thiazolidinediones. Emerging Drugs (null), 5(4), 441-456.
32.Stevenson, R. W., Hutson, N. J., Krupp, M. N., Volkmann, R. A., Holland, G. F., Eggler, J. F., ... & Kreutter, D. K. (1990). Actions of novel antidiabetic agent englitazone in hyperglycemic hyperinsulinemic ob/ob mice. Diabetes, 39(10), 1218-1227.
33.Van Drie, J. H. (2007). Monty Kier and the origin of the pharmacophore concept. Internet Electron. J. Mol. Des, 6(9), 271-279.
34.Waki, H., Park, K. W., Mitro, N., Pei, L., Damoiseaux, R., Wilpitz, D. C., ... & Tontonoz, P. (2007). The small molecule harmine is an antidiabetic cell-type-specific regulator of PPARγ expression. Cell metabolism, 5(5), 357-370.
35.Wang, L., Waltenberger, B., Pferschy-Wenzig, E. M., Blunder, M., Liu, X., Malainer, C., ... & Atanasov, A. G. (2014). Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARγ): a review.Biochemical pharmacology, 92(1), 73-89.
36.Wermuth, C. G., Ganellin, C. R., Lindberg, P., & Mitscher, L. A. (1998). Glossary of terms used in medicinal chemistry (IUPAC Recommendations 1998). Pure and Applied Chemistry, 70(5), 1129-1143.
37.Willson, T. M., Brown, P. J., Sternbach, D. D., & Henke, B. R. (2000). The PPARs: from orphan receptors to drug discovery. Journal of medicinal chemistry, 43(4), 527-550.
38.Wlodawer, A., & Vondrasek, J. (1998). INHIBITORS OF HIV-1 PROTEASE: A Major Success of Structure-Assisted Drug Design 1. Annual review of biophysics and biomolecular structure, 27(1), 249-284.