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Research Article

IN SILICO SCREENING OF POTENT PPARGAMMA AGONISTS AMONG NATURAL ANTICANCER COMPOUNDS OF INDIAN ORIGIN

HEMANTH GURULA[1], THOLCOPIYAN LOGANATHAN[1], YAONGAMPHI VASHUM[1], SUDHA PANNERSELVAM[1], UMASHANKAR VETRIVEL[2], SHILA SAMUEL[1]*

[1]Department of Biochemistry, VRR Institute of Biomedical Science (Affiliated to University of Madras), Kattupakkam, Chennai - 600 056, Tamil Nadu, India. [2]Centre for Bioinformatics, Vision Research Foundation, Sankara Nethralaya, Nungambakkam, Chennai - 600 006, India. Email: shilasamuel72@gmail.com

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ABSTRACT

Objective: Naturally occurring anticancer compounds of Indian origin are well-known for potential therapeutic values. A better understanding of the intermolecular interactions of these compounds with peroxisome proliferator-activated receptor gamma (PPARγ) is essential, as its activity is reported in many of the cancers involving colon, breast, gastric, and lung. By this study, it is attempted to perform an *in silico* screening of natural anticancer compounds of Indian origin with PPARγ ligand binding domain (LBD). The potential anticancer leads ranked in this study will also exert an additional advantage of PPARγ activity modulation. As PPARγ is also an important nuclear hormone receptor that modulates transcriptional regulation of lipid and glucose homeostasis and also a key target for many of the anti-diabetic medications, the compounds ranked by this study will also be utilized for other related therapeutic effects.

Methods: This study features *in silico* screening of compounds from Indian Plant Anticancer compounds database against PPARγ LBD main performed Schrodinger glide virtual screening and docking module to delineate potential PPARγ agonists. Finally, the most potential lead was also subjected to molecular dynamics simulation to infer the stability of complex formation.

Results: The results reveal that majority of the top ranking compounds that interact with LBD was found to be flavonoids, and all these compounds were found to interact with key residues involved in PPARγ agonist interactions.

Conclusion: The leads from this study would be helpful in better understanding of the potential of naturally occurring anticancer compounds of Indian origin toward targeting PPARγ.

Keywords: Peroxisome proliferator-activated receptor-gamma, Agonists, Docking, Natural compounds, Anticancer.

INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) belong to the steroid receptor superfamily and comprise three different isoforms: PPARα, PPARβ/δ, and PPARγ [1,2]. PPARs have a DNA-binding domain and ligand binding domain (LBD). PPARs are triggered by both endogenous ligands like unsaturated fatty acids, low-density lipoproteins, eicosanoids, and prostaglandins, and the synthetic agonists include the fibrates, thiazolidinediones (TZDs) and glitazones. PPARs bind to ligand and form heterodimers with a different ligand-activated nuclear receptor, the retinoid X receptor (RXR). The PPAR-RXR heterodimer binds to peroxisome proliferator response elements (PPREs) in the promoter region of the corresponding target genes and the different transcriptional cofactors initiate the transcription process, thus accelerating gene expression [3,4].

The nuclear receptor PPARγ is the master regulator of adipogenesis and the pharmacological target of the TZD class of insulin sensitizers. As metabolic regulators, PPARs control the expression of genes involved in adipocyte differentiation [5,6], lipid and glucose metabolism [7,8] and as well as inflammation in immune cells and cell proliferation [9-11]. Apart from the known metabolic actions, PPARγ has also been shown to be overexpressed in numerous human cancers including breast, bladder, prostate, colon, and thyroid [12,13]; PPARγ agonists exhibit antitumor activities [14]. It was also proposed to induce apoptosis in some malignant cell lineages [15]. *In-vitro* and *in-vivo* studies have revealed antiproliferative and proapoptotic actions of PPARγ agonists indicating that PPARγ could be a promising therapeutic target for the treatment of cancers [16,17].

The prehistoric Indian medicinal system was entirely based on the natural products derived from a medicinal plant in various forms such as juice, decoction, and crude extracts. Phytochemical compounds extracted from these plants are expansively studied for their mode of action and therapeutic efficacy. In India, a great number of plant species had been screened for their pharmacological properties but still vast wealth of rare species is unexplored [18].

Biological products from natural plants are an excellent source for the development of new drugs. The traditional use of plant preparations gives strong indications for the pharmacological effects of their ingredients. Natural compounds have their advantage over chemical inhibitors by being a safer substitute and ensure lesser/no side effects. However, faith in the traditional medical practices slowly faded away for want of scientific evidence. Recently, there is an enormous interest, shown on the traditional medicine, for the development of new drugs on scientific lines.

There are numerous plant-derived compounds with proven anticancer activity like topotecan and irinotecan, etoposide derived from epipodophyllotoxin [19], camptothecin derivatives [20], and paclitaxel (taxol) [21]. Other key molecules include vinca alkaloids (vinblastine, vincristine) [22] and flavopiridol, a pyridoindole alkaloid derived from leaves of *Ochrosia* species [23]. The modern trend of anticancer drug development research is largely based on the exploration of potential phytochemicals. Indian sub-continent is renowned for its plant biodiversity and ethanobotanical tradition with highly valuable medicinal properties. Vetrivel *et al.,* have developed a comprehensive database called Indian Plant Anticancer Database (INPACDB) which

features anticancer phytochemicals of Indian plant origin. All the compounds indexed in the database of the above study is well-curated with literature cross references, chemical properties, mode of action, chemical descriptors, atomic coordinates, pictures of the respective plants, cancer targets, and its origin. Many scientists have used this database for screening anticancer compounds of Indian plant origin and gained valuable insights [24].

A substantial amount of research was carried out to explore the PPARγ activating potential of a wide range of natural products originating from medicinal plants. Natural products prove to be a rich source for the discovery of novel PPARγ ligands and many structurally diverse agonists of this receptor were recently identified from traditionally used medicinal plants, initiating the view to consider modulation of the activity of this nuclear receptor through medicinal plant species [25]. Gurula *et al.*, have performed virtual screening to identify potential PPARγ agonists from SeaWeed Metabolite Database [26].

This study presents new research efforts and views on the search for the agonists of the nuclear receptor PPARγ with anti-carcinogenic effects using *in silico* screening from a wide range of natural products originating from traditionally used Indian medicinal plants.

METHODS

Protein structure data collection and preparation

The atomic coordinates of LBD main of PPARγ (225-462 region) were extracted from the human RXR-alpha and PPARγ LBD bound with 9-cis retinoic acid and rosiglitazone and coactivator peptides (PDB ID: 1FM6). Further, the extracted region was optimized using protein preparation wizard module of Schrodinger suite (Schrödinger, LLC, 2012, New York, NY, 2012), wherein, the structural defects were fixed, the hydrogen atoms were added and optimized, proper bond orders were assigned and tautomeric forms and ionization states were fixed to the optimum. Finally, the fixed structure was energy minimized using optimized potentials for liquid simulation (OPLS) 2005 force field to achieve optimal geometry (Fig. 1 and 2).

Small molecule data collection and refinement

The complete ligand datasets of INPACDB (288 structures which include tautomeric forms, conformers and stereoisomers) in MOL format were procured through email request to the database team [24] and were further geometry optimized using lig prep module (Schrödinger, LLC, New York, NY, 2012) to fix the ionization states, stereochemical errors and to assign proper ring conformation at a pH range of 7±2. In addition, the compounds which possess reactive functional groups and others which do not comply with Lipinski's rule of five [27] (partition coefficient, clog p≤5, H-bond donors ≤5, H-bond acceptors ≤10, molecular weight ≤500) were omitted during the optimization process. Finally, the ligands with optimal geometry devoid of undesirable features as discussed above were utilized for high-throughput virtual screening (HTVS) and Docking studies.

In silico virtual screening of INPACDB compounds

The *in silico* virtual screening and docking of INPACDB compounds against PPARγ were performed using glide HTVS option of Schrodinger suite (Schrödinger, LLC, 2012, New York, NY software). As a first step, the entire LBD domain was fixed as a grid box, as this domain is large and it homes diverse types of small molecules. The van der Waals radius scaling was set to 1.0, so as to soften the non-polar region of receptor and rest of other atoms were left free of scaling. Finally, the optimized small molecules were successively docked to the LBD, ensuring flexible sampling with <300 atoms and 50 rotatable bonds. A total of 10 energetically favorable conformations were selected out of 1000 poses generated per docking; among these, the best poses were decided based on the glide docking score and were confirmed to be the optimal docked complex.

During the screening process, successive elimination of ligand hits was carried out based on the significance of glide docking at three stringent

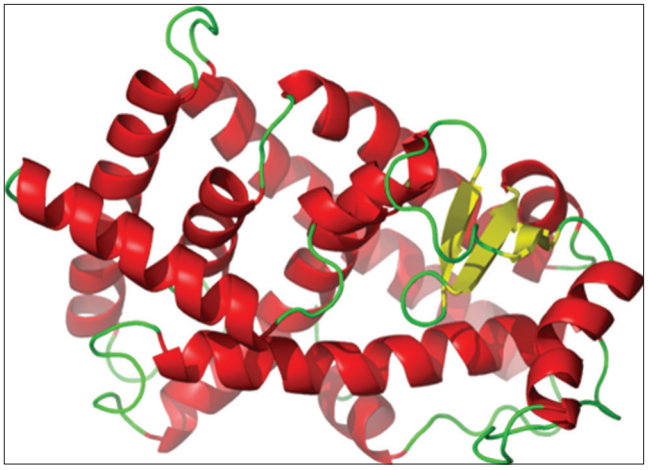

Fig. 1: The crystal structure of peroxisome proliferator-activated receptor-gamma ligand binding domain, helices represented in red, loops in green and beta strands in yellow (PDB ID: 1FM6)

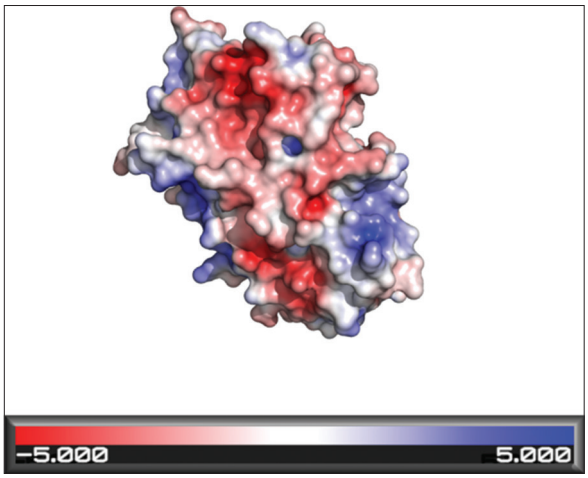

Fig. 2: The electrostatic surface of peroxisome proliferator-activated receptor-gamma ligand binding domain (PDB ID: 1FM6) (positively charged regions are shown in blue color and negatively charged regions are shown in red color)

modes using Schrödinger suite: HTVS (100%) of best hits passed to standard precision (SP) (80% of best hits from SP passed to extra precision [XP]). From the results of XP step, top 10 hits were shortlisted based on the glide docking score.

Graphical visualization and analysis

The results of virtual screening were visualized in Schrodinger Maestro Interface (Schrödinger, LLC, 2012, New York, NY software). The two-dimensional interaction maps for the top 10 hits shortlisted compounds were generated using Schrodinger Maestro. Further, these maps were investigated for intermolecular interactions such as H-bond formation, pi-cation contacts, and pi-pi stacking and other residue contacts were also tabulated. Finally, the tabulated data were compared with the previous studies which depicted the significant amino acids of PPARγ involved in agonist contacts. The hits which showed similar interactions to that of well proven PPARγ agonists were concluded as most potential lead compounds.

Molecular dynamics (MD) simulation studies of top ranking LBD - hesperetin docked complex

The topmost docked complex (LBD-hesperetin) was further validated for the stability of complex formation using MD simulation. The MD

simulation was run using Desmond package, which features explicit solvent MD (developed by D. E. Shaw Research, New York, NY) with built in OPLS 2005 force field. The solvated system was built for simulation using single point charge water model in a cubic box with the dimension of 10Å × 10Å × 10Å and desirable electrically neutral system for the simulation was built with 0.15 M NaCl (physiological concentration of monovalent ions). Subsequently, the system was relaxed by energy minimization using hybrid method of the steepest descent and the limited-memory Broyden-Fletcher-Goldfarb-Shanno (LBFGS) algorithms. Martyna-Tobias-Klein barostat method and LBFGS vectors method were implemented to run the simulation at a constant temperature and pressure of 300 K. The short- and long-range Coulombic interactions were analyzed using a cutoff value 9.0 Å. A smooth particle mesh Ewald method was used for handling long-range Coulombic interactions. The complete production run of the system was carried out for 5 nano seconds with a sampling interval of 1 pico second. The final MD trajectories were analyzed using maestro interface.

RESULTS AND DISCUSSION

Inferences from *in silico* virtual screening and docking studies

Human PPARγ is well-documented to be one of the most crucial drug targets in the treatment of Type II diabetes, and it is also proven to be a potential anticancer target [1,28]. In this study, we have performed virtual screening of LBD of PPARγ against INPACDB compounds. During the ligand optimization and filtration process, among all the INPACDB datasets (288 structures which include tautomeric forms, conformers and stereoisomers), only 51 passed the Lipinski's rule [27] and all the stereochemical checks. Further, these compounds were subjected to computational docking to LBD of PPARγ. The docked molecules were ranked according to a binding affinity with LBD. Out of all the compounds that were identified from virtual screening, the top 10 hit with a glide docking score <−6.0 kcal/mol at the glide XP mode were shortlisted as potential lead molecules. Further, these top scoring PPARγ-ligand complexes were visualized in Schrodinger maestro interface and the corresponding intermolecular interactions were tabulated (Table 1 and Fig. 3). As per the previous studies, Ser 289, His

Table 1: The top 10 ranking agonists for PPARγ among INPACDB compounds with corresponding residue interactions, bond length, glide energy score and medicinal plant details as shortlisted from virtual screening

INPACDB Acc.no/compound/ plant	Interaction type	Protein-ligand interaction	Bond length (Å)	Glide score (kcal/mol)
ACD0106/hesperetin/*C. limonum*	Pi-Pi stacking	Edge to face His 449. Ring A	4.70	−8.617
	Hydrogen bond (side chain)	His 449 (4435)...O (22)	1.83	
		Arg 288 (2873)...O (42)	2.74	
ACD0075/luteolin/*A. graveolens*	Pi-Pi stacking	Edge to face Arg 288...Ring A	4.09	−8.419
		Edge to face Tyr 327...Ring C	5.27	
	Hydrogen bond (backbone)	Leu 340 (1068)...O (18)	1.86	
	Hydrogen bond (side chain)	Arg 288 (2877)...O (22)	2.36	
ACD0070/apigenin/*C. minima*	Pi-Pi stacking	Edge to face Arg 288...Ring C	5.0	−7.232
		Edge to face Tyr 327...Ring A	5.30	
	Hydrogen bond (backbone)	Leu 340 (1068)...O (18)	1.86	
	Hydrogen bond (side chain)	Arg 288 (2877)...O (22)	2.34	
CD0105/naringenin/*C. limonum*	Pi-Pi stacking	Edge to face Arg 288...Ring C	4.98	−7.77
		Edge to face Tyr 327...Ring A	5.26	
	Hydrogen bond (backbone)	Leu 340 (1068)...O (18)	1.88	
	Hydrogen bond (side chain)	Arg 288 (2877)...O (22)	2.34	
ACD0046/acacetin/*C. zawadskii*	Pi-Pi stacking	Edge to face Arg 288...Ring C	5.0	−6.859
		Edge to face Tyr 327...Ring A	5.29	
	Hydrogen bond (backbone)	Leu 340 (1068)...O (41)	1.86	
	Hydrogen bond (side chain)	Arg 288 (2877)...O (38)	2.36	
ACD0001/genistein/*G. max*	Pi-Pi stacking	Edge to face Arg 288...Ring C	4.96	−6.685
	Hydrogen bond (backbone)	Leu 340 (1068)...O (24)	1.83	
	Hydrogen bond (side chain)	Tyr 327 (3185)...O (37)	1.86	
ACD0081/kaempferol/ *I. balsamina*	Pi-Pi stacking	Edge to face His 449...Ring A	5.31	−6.641
		Edge to face Tyr 327...Ring A	5.23	
	Hydrogen bond (backbone)	Leu 340 (1068)...O (18)	2.43	
	Hydrogen bond (side chain)	Arg 288 (2877)...O (30)	2.73	
ACD0006/wedelolactone/*E .alba*	Pi-Pi stacking	Edge to face Arg 288...Ring A	5.13	−6.402
ACD0041/ellagic acid/ *P. granatum*	Pi-Pi stacking	Edge to face Arg 288...Ring A	5.26	−6.386
	Hydrogen bond (backbone)	Leu 340 (1068)...O (26)	1.70	
ACD0049/resveratrol/*V. vinifera*	Hydrogen bond (backbone)	Ser 342 (3300)...O (30)	1.87	−6.169

PPARγ: Peroxisome proliferator-activated receptor-gamma, INPACDB: Indian plant anticancer database, *C. Limonum: Citrus Limonum, A. graveolens: Apium graveolens, C. minima: Centipeda minima, C. zawadskii: Chrysanthemum zawadskii, G. max: Glycine max, I. balsamina: Impatiens balsamina, E. alba: Eclipta alba, P. granatum: Punica granatum, V. vinifera: Vitis vinifera*

323, His 449 and Tyr 473 [29,30], Arg 288 [31] Tyr 327 [32] are important residues of PPARγ that participate in intermolecular interactions with well-established agonists PPARγ such as macelignan, pioglitazone, dehydro-Di-isoeugenol, netoglitazone, and rosiglitazone [33]. Phe360 and Phe363 were also observed to contribute toward stable binding of many of the PPARγ agonists [30]. Thus, these residues contact information was kept as a scaffold to validate the top scoring ligands from INPACDB.

Among the top ten ranking compounds, the top most scoring ligand was found to be Hesperetin, a flavanoid from *Citrus limonum* [34], which showed a glide docking score of −8.617 kcal/mol. This compound interacted with His 449 of LBD by pi-pi stacking with an edge to face orientation of the aromatic rings along with a hydrogen bond. It also formed a hydrogen bond with Arg 288. Furthermore, the top second ranking was found to be Luteolin which is a flavone from the plant *Apium graveolens* [35], this compound showed pi-pi stacking interaction with edge to face orientation of the aromatic rings Arg 288 and Tyr 327. It also showed two hydrogen bonding interactions with Leu 340 and Arg 288. The three successive ranking compounds in order: Apigenin, a flavanoid from *Centipeda minima* [36], Naringenin from *C. limonum* [37], Acacetin from *Chrysanthemum zawadskii* were also found to be flavanoids similar that of Luteolin and also exhibited synonymous interactions to LBD residues (Fig. 3).

The sixth compound was genistein, is also flavanoid from *Glycine max* [38] and it showed pi-pi stacking interactions with Arg 288 and hydrogen bonding with Leu 340 and Tyr 327. The next ranking compound was to be kaempferol from *Impatiens balsamina* [39] which formed edge to face pi-pi stacking interactions with His 449 and Tyr 27, stabilized by two hydrogen bonds formed with Leu 340 and Arg 288. The eighth ranking compound was found to be Weldelactone, which is a coumestan type of compound is a phytoestrogen from *Eclipta alba* [40] showed only single pi-pi stacking interaction with Arg 288.

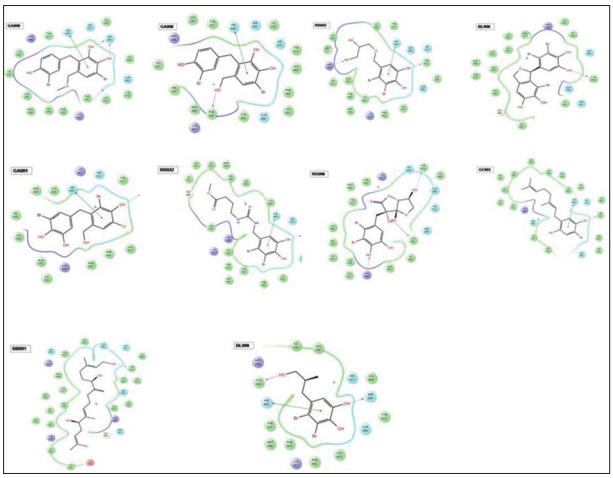

Fig. 3: The two-dimensional molecular interaction maps of the top 10 docked complexes

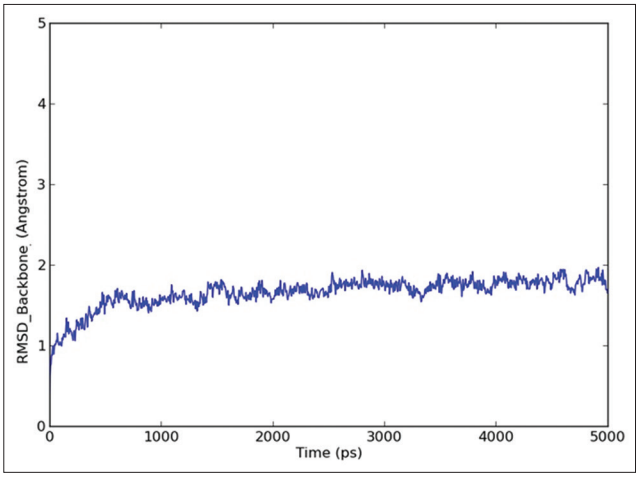

Fig. 4: The backbone root mean square deviation plot of the top ranking docked complex (ligand binding domain-hesperetin)

The ninth ranking compound was Ellagic acid from *Punica granatum* which is a phenolic antioxidant (Mandal and Stoner), it showed a pi-pi stacking interaction with Arg 288 and a single hydrogen bond interaction with Leu 340. The tenth ranking compound was found to be Resveratrol from *Vitis vinifera* and it showed a single hydrogen bond with Ser 342. Furthermore, the top ranking docked complex (LBD - Hesperetin complex) was subjected to MD simulation and the backbone root mean square deviation plot was analyzed, wherein, the plot showed deviation within 1Å which is suggestive of the stable complex formation (Fig. 4).

The results of this study pose the potential of naturally occurring compounds of Indian origin to be potential PPARγ agonists, as it can be noticed that the most of the compounds were found to be flavonoids showing significant molecular interactions with Arg 288, Tyr 327 [31,32] and also with His 449 [29] which have been well proven to be PPARγ agonist interacting residues. In this study, the receptor grid selection for this docking was fixed in an impartial manner, such that the complete LBD was ascertained as the receptor cavity without adding any reference information on agonist interacting residues. This method was implemented to imitate the native probing mode of ligand binding by exhaustive conformational search. Eight out of ten top ranking were found to flavonoids mostly interacting with Arg 288 with is shown to play an important role, as it aids in salt bridge formation by PPARγ with fatty acids [31]. Thus, the outcome of this study will aid in the selection of optimal flavanoids for PPARγ agonist activity inferred through molecular docking studies.

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