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Original Article

VIRTUAL SCREENING STUDIES OF SEAWEED METABOLITES FOR PREDICTING POTENTIAL PPARγ AGONISTS

HEMANTH GURULA[1], THOLCOPIYAN LOGANATHAN[1], THIAGARAJAN KRISHNAMOORTHY[1], UMASHANKAR VETRIVEL[2], SHILA SAMUEL[1*]

[1]VRR Institute of Biomedical Science (Affiliated to University of Madras), Kattupakkam, Chennai 600056, India, [2]Centre for Bioinformatics, Vision Research Foundation, Sankara Nethralaya, Nungambakkam, Chennai 600006, India
Email: shilasamuel72@gmail.com

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ABSTRACT

Objective: Peroxisome Proliferator-Activated Receptor-gamma (PPARγ) is a crucial nuclear hormone receptor, which modulates the transcriptional regulation of lipid and glucose homeostasis. It plays a crucial role in many of the metabolic and inflammatory systems. It is a key target for many of the anti-diabetic medications. Perturbation of PPARγ activity is also observed in many of the cancers involving colon, breast, gastric and lung. Thus, it is considered to be the hub molecule for targeting many of these cellular disorders. Seaweed metabolites have been well documented to be novel structural entities with a broad spectrum of pharmacological values. However, it is yet to be utilized for screening PPARγ agonists.

Methods: In this study, virtual screening of PPARγ Ligand Binding Domain (LBD) was performed against the datasets from SeaWeed Metabolite Database (SWMD) using Schrodinger Glide High Throughput Virtual Screening module to identify potential PPARγ agonists. Further, the most potential lead was also subjected to molecular dynamics simulation to infer the stability of complex formation.

Results: The results have revealed that bromophenolic compounds from the genus *Avrainvillea* to interact with documented key residues of LBD involved in agonist interactions. Many other metabolites from the genus *Rhodomela, Leathesia, Bifurcaria, Osmundaria, Cymopolia* also showed significant interactions with LBD of PPARγ.

Conclusion: The insights from this study will pave the way for further exploration of lead compounds from seaweed metabolites targeting PPARγ.

Keywords: PPARγ, Agonists, Seaweed, Metabolite, Docking.

INTRODUCTION

PPARγ is a crucial molecule which actively modulates the transcriptional regulation of lipid and glucose homeostasis and is also reported to regulate the pathways involved in the differentiation of adipocytes [1, 2]. This protein harbors a large binding pocket, thereby allowing interaction of a diverse range of small molecules. The binding of small molecules to PPARγ binding pocket leads to conformational change in the Activation Function-2 (AF-2) domain, which in turn recruits co-regulatory factors, thereby regulating gene transcription.

This protein also exists in a hetero dimeric form in complex with retinoic X receptor alpha (RXRα), which forms an essential base for numerous PPARγ-DNA interactions [3]. PPARγ is a prominent molecular target for anti-diabetic medications like Thiazolidine-diones (TZDs). It is also found to play a key role in many of the metabolic and inflammatory mechanisms. Dys regulation of PPARγ is also observed in many of the cancers involving colon, breast, gastric and lung. PPARγ agonists treated tumor cells were also shown to respond positively by adopting growth inhibition and apoptosis [4, 5]. Though numerous synthetic PPARγ agonists are available, the compounds of natural origin are highly preferred for safe therapeutic applications. Hence, biologically safe and potential agonists are essential for the effective treatment of cancer conditions, as these patients are already challenged with cytotoxic drugs and radiation.

Marine chemicals have been documented to be novel structural entities with a broad spectrum of pharmacological values [6]. Among marine organisms, seaweeds are extensively studied and cataloged for its metabolite contents [7]. Davis & Vasanthi (2011) have created a chemical structure repository, namely, SWMD which currently features around 1055 compounds encompassing 25 descriptive fields and mostly from the Red algae of the genus *Laurencia* (*Ceramiales, Rhodomelaceae*) [8]. Hence, in this study, it is attempted to computationally screen the reported active metabolites from SWMD for compounds which potentially act as PPARγ agonists inferred through *in silico* binding affinity analysis, as similar studies on this database have yielded potential leads [9, 10].

PPARγ ligand binding domain

Ligand binding domain (LBD) spans at the C-terminal of all the peroxisome proliferator-activated receptors (PPARs), structurally comprising of thirteen alpha helices and a four-stranded beta sheet. This Y-shaped domain, which is divided into Arm I, Arm II and a charge-clamp, ranging about 1400 cubic angstroms in size, favor interactions with the multitude of structurally discrete ligands [11]. Arm I harbors conserved polar residues: Ser, Tyr and His are found to be conserved in all the PPARs and these amino acids form hydrogen bond network with the carboxylate group of fatty acids and other ligands [12]. This conserved network also aids to hold the AF2-helix in the active conformation, which mediates the formation of co-activator binding pocket in the C-terminal region. Moreover, the hydrogen bonding network of Glu324, Arg397, Arg443, and Tyr477 (in PPARγ) was also shown to stabilize the AF-2 helix towards active conformation upon binding of small molecules [13]. The Arm II is extremely hydrophobic, thereby enabling the binding of the hydrophobic tail of fatty acids through van der Waals interactions. Though LBD is conserved among all the PPAR isotypes (around 80%), the rest of the regions account (around 20%) for the ligand selectivity. For instance, PPARγ shows selectivity to fatty acids with larger carboxylate head groups as it possesses His323 in the place of Tyr314 as found in PPAR alpha [13].

MATERIALS AND METHODS

Protein structure data collection and preparation

The crystal structure coordinates of the Human retinoid X receptor-alpha (RXR-alpha) and PPARγ ligand binding domains bound with 9-cis retinoic acid and rosiglitazone and co-activator peptides (PDB ID: 1FM6) was downloaded from Protein Data Bank. Further, the coordinates of LBD (225-462 region) was parsed and extracted

manually. The Protein Preparation Wizard module of Schrodinger suite (Schrödinger, LLC, 2012, New York, NY, 2012) was used to correct the structural defects, and to add and optimize hydrogen atoms, to assign bond orders and also to selectively assign tautomerization and ionization states for the extracted LBD coordinates. Subsequently, the corrected structure was geometry optimized by energy minimization using Optimized Potentials for Liquid Simulation (OPLS) 2005 force field (fig. 1, fig. 2).

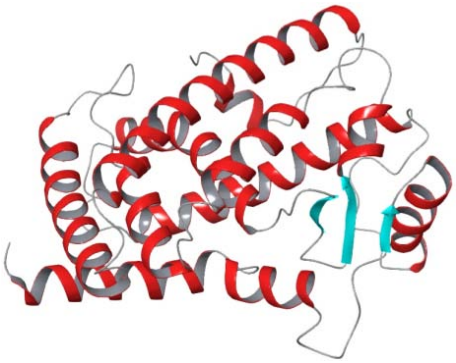

Fig. 1: The structure of PPARγ ligand binding domain (PDB ID: 1FM6)

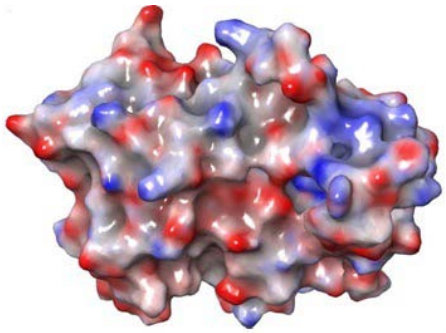

Fig. 2: The electrostatic surface of PPARγ ligand binding domain (PDB ID: 1FM6) (positively charged regions are shown in blue color and negatively charged regions are shown in red color)

Ligand preparation

The complete ligand datasets (1055 compounds with different conformers) in MOL format was downloaded from SWMD and was optimized for ionization states, tautomers, stereochemical errors and ring conformations, under a pH range of 7±2, using Lig prep module (Schrödinger, LLC, New York, NY, 2012). Moreover, the compounds with reactive functional groups and those which do not follow Lipinski's rule of five [14] (partition coefficient, Clog P≤ 5, H-bond donors ≤ 5, H-bond acceptors ≤ 10, molecular weight ≤ 500) were excluded during the optimization process. Finally, the optimized ligands were used for High-Throughput Virtual Screening and Docking studies.

High-throughput virtual screening (HTVS) and docking

The virtual screening of SWMD ligands against PPARγ was set using Glide HTVS option of Schrodinger suite (Schrödinger, LLC, 2012, New York, NY software). The complete LBD domain was set as a grid box as this domain is large and it binds to diverse types of ligands. 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. Further, the optimized small molecules were sequentially docked to the LBD, ensuring flexible sampling with less than 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 finalized based on the Glide Docking Score and was confirmed to be the optimal docked complex.

In this screening process, sequential reduction of ligand hits was carried out based on the significance of glide docking at three stringent 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.

Molecular data visualization and analysis

The virtual screening results were visualized in Schrodinger Maestro Interface (Schrödinger, LLC, 2012, New York, NY software). The two-dimensional (2D) interaction maps for the top 10 hits shortlisted based on significant molecular interactions and Glide Docking Score were produced using Schrodinger Maestro. Further, the 2D maps generated were analyzed for intermolecular interactions like H-bond formation, pi-pi stacking, pi-cation contacts and other residue contacts were duly tabulated. The tabulated data was compared to documented studies which portray 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 simulation of the top ranking docked complex

The top ranking docked complex (LBD-Avrainvilleol methyl ether complex) was subjected to Molecular Dynamic (MD) simulation to evaluate the stability of the complex formation. The MD simulation was performed using Desmond, which is an explicit solvent molecular dynamics program (developed by D. E. Shaw Research, New York, NY) with built-in OPLS 2005 force field. The system was built for simulation using SPC water model as solvent in cubic box with the dimension of 10Å x 10Å x 10Å distance and desirable electrically neutral system for simulation was built with 0.15M (physiological concentration of monovalent ions) NaCl in 10 Å buffer maestro 9.3. Further, the system was relaxed by energy minimization using a 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 300K. The short-range and long-range Coulombic interactions were analyzed using a cut-off 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 performed for 5 nano seconds with a sampling interval of 1 pico second. Finally, the MD trajectories were analyzed using maestro interface.

RESULTS AND DISCUSSION

Outcome of virtual screening and docking studies

Human PPARγ, one of the most crucial drug targets for the treatment of diabetes mellitus, also an emerging anticancer target [4, 5] was virtually screened against the compounds in the SWMD. During the ligand filtration process, out of 1,055 compounds from SWMD, only 656 passed the Lipinski's rule [14] and all the stereochemical checks. Further, these compounds were computationally docked to LBD of PPARγ and were ranked in accordance to its binding affinity with LBD of PPARγ. Among all the compounds that were identified from virtual screening, the top ten compounds with a glide docking scoreless than-6.0 kcal/mol at the Glide XP mode were shortlisted as potential leads. The ten top scoring PPARγ-Ligand complexes were visually inspected and the interactions were tabulated (table 1, fig. 3). As per the documented studies, Tyr473, His449, His323 and Ser289 are crucial residues of PPARγ [1, 15] involved in interactions with well proven agonists like Dehydro di isoeugenol, Macelignan, Pioglitazone, Netoglitazone, and Rosiglitazone [17]. Moreover, interactions with residues, Phe360 and Phe363 were also observed to be formed by many of the of PPARγ agonists [16]. Hence, keeping these residue contacts information as a reference, the top scoring ligands of this study were further validated.

Table 1: The top lead agonists for PPARγ with corresponding residue interactions, Bond Length, Glide Energy Score and seaweed details as shortlisted from virtual screening

SWMDAcc. No/Metabolite Name/Seaweed	Interaction Type	Interacting Residues	Bond length (Å)	Glide Docking Score (kcal/mol)
GA009/Avrainvilleol methyl ether/*Avrainvillea rawsonii*	pi-pi stacking	Edge to face His 449. Ring B	4.46	-9.137
	Hydrogen bond (side chain)	Ser 289 (673). .O(38)	1.92	
		Ser 289 (673). .O(36)	1.82	
GA008/Avrainvilleol/*Avrainvillea rawsonii*	pi-pi stacking	Edge to face His 449. Ring B	4.67	-8.266
	Hydrogen bond (backbone)	Phe 363 (1254). O(30)	2.35	
RR052/3,4-dibromo-5-((2,3-dihydroxypropoxy)methyl) benzene-1,2-diol/*Rhodomela confervoides*	pi-pi stacking	Edge to face His 449. Ring A	4.61	-8.100
	Hydrogen bond(side chain)	Tyr 473 (2143). .O(35)	1.84	
		Arg 288(2873). .O(19)	1.97	
BL006/(+)-3-(2,3-dibromo-4,5-dihydroxyphenyl)-4-bromo-5,6-dihydroxy-1,3-dihydroisobenzofuran/*leathesia nana*	Hydrogen bond (backbone)	Cys 285	1.88	-8.081
GA001/5'-hydroxyisoavrainvilleol/*Avrainvillea nigricans*	pi-pi stacking	Edge to face His 449. Ring B	4.60	-7.924
	Hydrogen bond(side chain)	Ser 289 (673). .O(38)	1.86	
RR032/Methyl N'-(2,3-dibromo-4,5-dihydroxybenzyl)-γ-ureidobutyrate/*Rhodomela confervoides*	pi-pi stacking	Edge to face His 449. Ring A	4.70	-7.737
	Hydrogen bond(side chain)	Ser 289 (673). .O(44)	1.84	
RO006/Rhodomelol/ *Osmundaria colensoi*	Hydrogen bond(side chain)	Arg 288(2873). .O(23)	2.25	-7.674
		Tyr 473 (2147). .O(41)	2.46	
	Hydrogen bond (backbone)	Cys 285 (634). O(37)	1.71	
GC002/Cymopol/*Cymopolia barbata*	pi-pi stacking	Edge to face His 449. Ring A	4.56	-7.670
	Hydrogen bond(side chain)	Tyr 473 (2147). .O(39)	1.99	
BB001/4α-Acetyldictyodial/*Bifurcaria bifurcata*	Hydrogen bond(side chain)	Tyr 473 (2147). .O(49)	1.97	-7.577
BL009/2-methyl-3-(2,3-dibromo-4,5-dihydroxyphenyl)-1-propanol/ *Leathesia nana*	pi-pi stacking	Edge to face His 449. Ring A	4.78	-7.560
	Hydrogen bond(side chain)	Ser 289 (673). .O(30)	1.71	
	Hydrogen bond (backbone)	Cys 285 (634). O(16)	1.79	

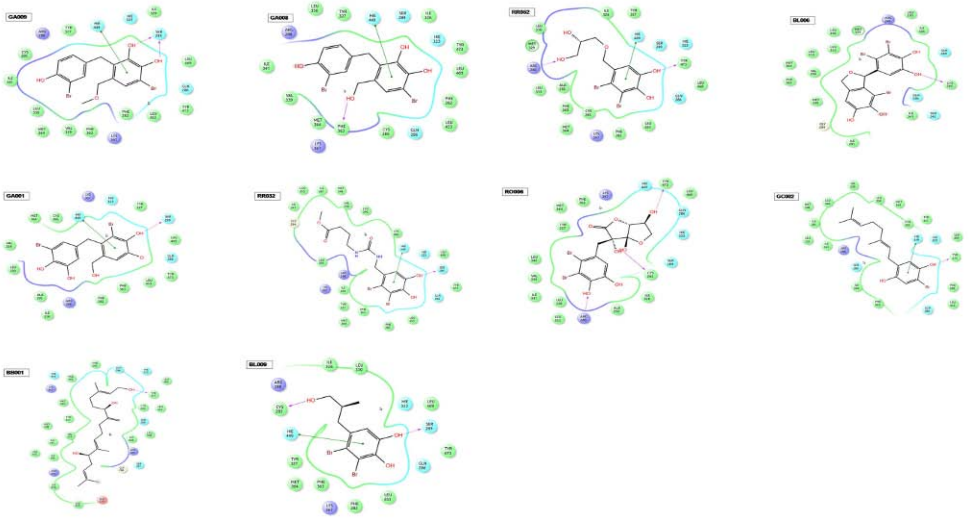

Fig. 3: The 2D molecular interaction maps of the top 10 docked complexes

In this present study, the top most scoring ligand with significant Glide Docking Score (-9.137kcal/mol) was found to be Avrainvilleol methyl ether, a bromophenolic metabolite from *Avrainvillea rawsonii*. This was found to interact with His449 by pi-pi stacking with an edge to face interaction of the aromatic rings and also it formed two hydrogen bonds with Ser289. Moreover, the top second ranking compound from *Avrainvillea rawsonii* was Avrainvilleol, which does not have methyl ether group and this compound also formed similar pi-pi stacking interaction with His449, however, does not form hydrogen bonds with Ser289, instead it showed a hydrogen bond with the backbone of Phe363 (glide docking score-8.393 kcal/mol).

The top third ranking compound was 3,4-dibromo-5-((2,3-dihydroxypropoxy)methyl) benzene-1,2-diol, a metabolite from *Rhodomela confervoides* and it also showed pi-pi stacking with His449 edge to face mode, stabilized by two hydrogen bonds with Tyr473 and Arg288. The fourth ranking compound namely, (+)-3-(2,3-dibromo-4,5-dihydroxyphenyl)-4-bromo-5,6-dihydroxy-1,3-dihydro-iso-benzo-furan from *Leathesia nana* showed a single hydrogen bond with Cys285, with a Glide Docking Score of-8.081 kcal/mol, and no other interactions were observed. The fifth ranking compound was 5'-hydroxyisoavrainvilleol form *Avrainvillea nigricans* which was similar to that of Avrainvilleol metabolites listed as top two hits and also formed similar interactions with His449 and Ser289, however, showed a lower docking score compared to the top two hits.

The sixth compound was from *Rhodomela confervoides* (RR032/Methyl N'-(2,3-dibromo-4,5-dihydroxybenzyl)-γ-ureido-butyrate and it also showed pi-pi stacking interactions with His449 and hydrogen bonding with Ser289. The seventh ranking compound was found as Rhodomelol from *Osmundaria colensoi* which is also bromophenolic of origin and was found to stably form hydrogen bonding interactions with Arg288, Tyr473 and Cys285. The next ranking compound was Cympolol from *Cympolia barbata* which showed an edge to face pi-pi stacking interactions with His449 and Tyr473. The ninth ranking compound was found as 4α-Acetyldictyodial from *Bifurcaria bifurcata* which showed only a single hydrogen bond interaction with Tyr473. The tenth ranking compound, 2-methyl-3-(2,3-dibromo-4,5-dihydroxyphenyl)-1-propanol from *Leathesia nana*, showed two hydrogen bonding interactions with Ser289 and Cys285. It also formed pi-pi stacking interactions with His449, as observed in few of the other top-ranking compounds. Moreover, the top ranking docked complex (LBD-Avrainvilleol methyl ether complex) was subjected to MD simulation and the backbone RMSD (Root Mean Square Deviation) plot was analysed, wherein, the plot showed deviation within 1Å which is suggestive of the stable complex formation (fig. 4).

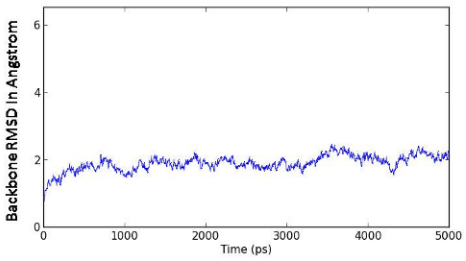

Fig. 4: The backbone RMSD plot of the top ranking docked complex (LBD-Avrainvilleol methyl ether complex)

These interesting results strongly reinforce agonistic activities of the shortlisted metabolites from seaweed, as it can be noticed that majority of these compounds formed strong molecular interactions with Tyr473, His449, Ser289 [1, 15] and also with Phe363 which has been well documented as favourable agonist interacting interfaces of PPARγ agonists. As discussed above, the receptor grid selection for this docking study was formulated in an unbiased manner, as the entire LBD was assigned as the receptor cavity without assigning the information on documented residues of PPARγ involved in ligand interactions.

By this, it was intended to mimic the native exploratory mode of ligand binding by exhaustive binding conformation search. Six out of ten top ranking of seaweed were found to be bromophenolic compounds and these compounds were highly in favor of forming pi-pi stacking interactions with His449 and also favored hydrogen bonding with Ser289 which are proven hotspot residues for PPARγ agonist interactions [15]. Moreover, three bromophenolic compounds were from the genus *Avrainvillea* and this seaweed might be of interest for the examination of hidden medicinal properties. The insights from this study will pave the way for the exploration of a treasure trove of seaweed metabolites which still remains unraveled for PPARγ agonists.

CONFLICT OF INTERESTS

Declared None

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