Ligand Based Pharmacophore Modeling and Virtual Screening for Novel Antidiabetics Targeting PPAR-gamma

Partha Sarathi Bairy, Prashant Gahtori, Abhilasha Mishra, Veerma Ram

Abstract: ***A modern sedentary lifestyle with a more calorigenic fast-food diet increasing the prevalence of metabolic syndrome in middle and high-income countries. Peroxisome proliferator-activated receptors (PPARs) are a group of the nuclear receptor, which regulates the metabolic process in physiological systems via influencing gene expressions of cell proliferation, glucose, lipid metabolism, and inflammation. Later one PPAR-γ agonist is a well-established class of pharmacological agents for diabetic control with some promising molecule in the clinical stages. Herein, we have chosen a hybrid indole and azaindole class for developing an effective pharmacophore model. A series of compounds with indole carboxylic acid and hydroxyazaindole core along with their tested biological activity were selected for generating a valid pharmacophore model using Hip-Hop and HypoGen algorithm of Discovery Studio v3.1. A total of 38 numbers of ligand were considered for pharmacophore generation and mapping including test set and training set. Depending upon proper calculative measures the best-validated hypothesis with two hydrophobic, one hydrogen bond acceptor, and one ring aromatic features are set forth for further shortlisting of compounds. A similarity search tool in PubChem structure database with a 70% similarity of best active compounds yields more than four lakhs compounds. The screened drug-like compounds were further shortlisted using 'rules of five' and TOPKAT module. The best-validated HypoGen pharmacophore was utilized for further screening to get the best structures for future in-silico consideration and identifying potential hits for effective diabetes drug discovery research.***

Keywords: PPAR-γ, diabetes mellitus, pharmacophore, ligand, metabolism.

I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major metabolic disorder with an expected projection of 366 million cases in 2030 [1]. Earlier it was concerning for the middle age and older generation but its progressive status of prevalence and abundance makes it “third killer” [2] in the worldwide scenario. According to data and statistics published by World Health Organization, it stands with 8.5% of the total population and the count is in rapid growth especially in low and middle-income countries of America, Asia and Africa [3].

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The reason behind this explosion is probably the modern lifestyle and food habit but complications are serious and not only limited to major organs like heart, brain, kidney, eye, and limbs. Common and popular treatment regimens of DM type II includes oral hypoglycemic drugs either in a single agent or in combination along with proper physical and diet control to balance energy consumption/expenditure ratio. Major evidence of acute and chronic adverse effects of these well known antidiabetic drugs urging the rational research targeting molecular mechanism [4-6]. Thiazolidinediones (TZDs), also known as Glitazones are a relatively newer class of antidiabetic drug acts by targeting Peroxisome proliferator-activated receptor-gamma (PPAR-γ). PPAR-γ is a subfamily of nuclear receptor regulates the metabolic process in physiological systems influencing gene expressions of cell proliferation, glucose, lipid metabolism, and inflammation. This nuclear protein snatching its importance in research fields due to association with other metabolic roles and genetic background. Three-dimensional (3D) structures with proper ligand and DNA binding domains are already available in literatures [7]. Despite their excellent therapeutic potencies, the administration of TZDs is not fully safe with reported adverse effects like weight gain, fluid retention, hepatotoxicity, cardiac hypertrophy, etc. [8]. Troglitazone and Rosiglitazone were withdrawn from the market by proper regulatory authorities later on after their successful clinical uses. So there are several research opportunities to find a safe, potent and effective scaffold which will be a better option in diabetic treatment strategy.

High informative online structural databases with various bioinformatics and machine learning tools are proving materials and methods for innovative research these days. Utilizing this facility various *in-silico* rational design like pharmacophore modeling, molecular docking, quantitative structure-activity relationship (QSAR), homology modeling, fragments based drug design, virtual screening (VS), etc can be performed. Pharmacophore is the minimum steric and electronic structural requirements necessary for a small molecule to show its supramolecular interaction with target protein. An effective pharmacophore model can be gold standard for virtual screening procedure to obtain best actives from huge library of compounds with the help of available features such as hydrogen bond donors (HBDs), hydrogen bond acceptors (HBAs), rings aromatic (RA), hydrophobic areas (Hs), positively/negatively ionizable groups (PIs/NIs), and exclusion volumes (XVOLs) [9].

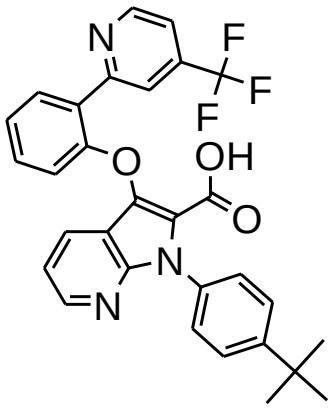
The growing interest in the development of new PPAR-γ agonist along with the shortage of sufficient computer-aided drug discovery reports excited us to explore more study in developing ligand-based three-dimensional (3D) pharmacophore for mining bioactive ligands. In two different studies, Lin C-H *et al* [10] and Dropinski JF *et al* [11] reported chemical structures with their respective EC_{50} values for PPAR-γ agonistic activity. In this present study, we have tried to discover the structural entity through effective pharmacophore modeling and virtual screening to report some potent hybrid molecule with better structural insight using Discovery Studio v3.1 (DS).

II. MATERIALS and METHODS

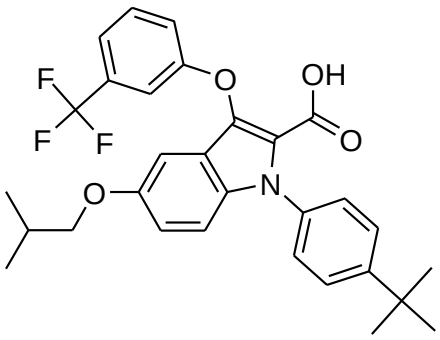
Computer-aided drug design (CADD) is broadly classified in structure based and ligand based drug design (LBDD) where three dimensional (3D) protein structure is the prime prerequisite for structure based strategy. Pharmacophore modeling is well established LBDD strategy that utilizes important steric and electronic features of known small molecules to build a necessary basic molecular skeleton that acts as a filter for a bigger structural library. '3D QSAR based pharmacophore modeling' protocol of Accelrys Discovery Studio predicted and reported many well-validated pharmacophores till now [12-14]. This protocol demands the test and training set of molecules with a well-distributed pattern of bioactivity. The best availability of molecules with tested IC50 values upon the same in-vitro enzyme strain enabled us to proceed for the present study.

A. Data set preparation:

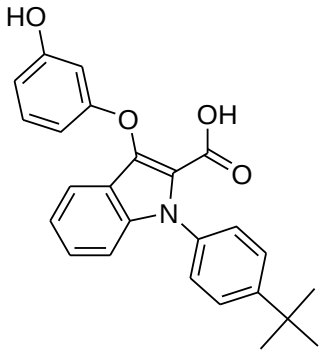
This process aims to obtain effective ligands for further exploitation procedures targeting PPAR-γ. Indole and azaindole derivatives proved to have a diverse class of pharmacological actions [15, 16]. These nucleuses tested for antidiabetic studies also. A total of 38 numbers of compounds with indole carboxylic acid and hydroxyazaindole core along with their tested biological activity against PPAR-γ were chosen to develop pharmacophore [10, 11]. The widely diverse dataset was categorized into four classes according to bioactivity pattern as active, moderately active, less active and inactive. All these molecules were distributed in the training set and test set. The EC50 values ranging 0.001 μM to 0.15 μM for the active set, moderately active sets consist of compounds with EC50 values ranging between 0.151 μM to 0.35 μM, less active set contains the molecules within the EC50 values range of 0.351 μM to 1.0 μM and remaining compounds were kept in inactive category. By this way 12 compounds are in the active set, 09 are in the moderately active set, 10 and 07 are in less active and inactive set respectively. All the ligands were drawn using PubChem Sketcher V2.3 (free online service) and saved in structural data format (.sdf) for further study purposes. They are converted in the 3D format in Accelrys Discovery Studio V3.1 (DS) followed by minimization of their energy using the CHARMM force field [17] prior to submission for establishing pharmacophore. Among these 38 ligands, eight in total consisting of two from each data sets were chosen as a training set to build the models. Remaining 30 ligands acts as a test set for pharmacophore mapping and hypothesis validation.



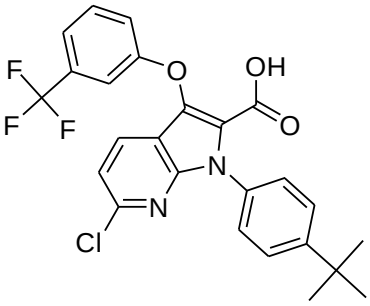
Ligand 40
EC50 (0.034 microM)



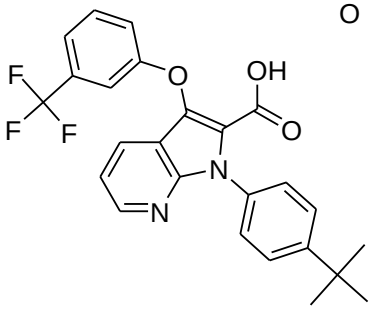
Ligand 35
EC50 (0.091 microM)



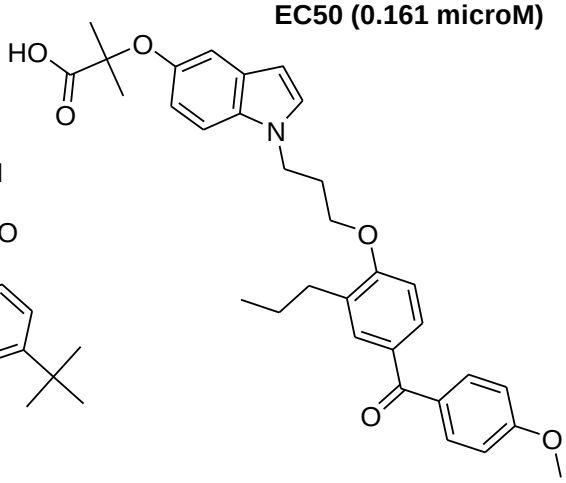
Ligand 20
EC50 (0.161 microM)



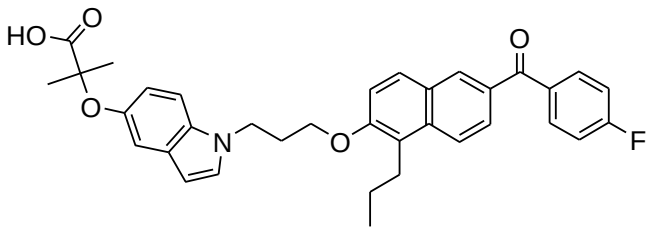
Ligand 41
EC50 (0.176 microM)



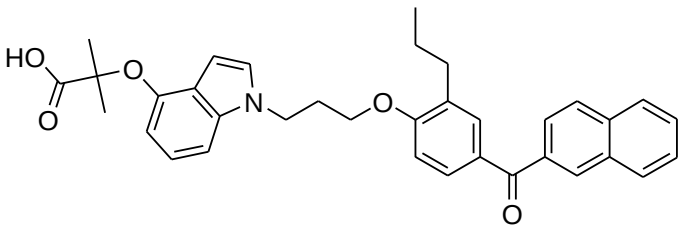
Ligand 39
EC50 (0.464 microM)



Ligand 16
EC50 (0. 47 microM)



Ligand 07
EC50 (2.18 microM)



Ligand 10
EC50 (2.8 microM)

Figure 1: Training set of molecules along with their EC50 values

B. Pharmacophore models generations:

There are several theoretical considerations to develop a pharmacophore but in general, common features pharmacophore and three dimensional quantitative structure activity relationship (3D QSAR) pharmacophore snatching its importance due to theory involve in them. *HipHop* algorithm in Common features pharmacophore uses common structural property like functional groups, electronegativity, ionization donor/acceptor features, etc. to generate a hypothesis. Where *HypoGen* modules of 3D QSAR pharmacophore employ ligand's biological activity as a function of structure to build essential features that contribute to the hypothesis. Herein we have used five features named hydrogen bond donors(HBD), hydrogen bond acceptors (HBA), ring aromatic(RA), hydrophobic (HY) and positive ionization (PI) in *HypoGen* modules to develop pharmacophore hypothesis utilizing energy minimized ligands [18]. The uncertainty value was changed to 2 from default 3 and all other parameters were kept as default. Maximum excluded volumes are set 5 for the run protocol. The best-developed pharmacophore model was selected among the hypothesis based on the statistical parameters like highest total cost, correlation coefficient, and lowest root mean square deviation (RMSD).

C. Hypothesis generation:

Hypothesis generation of DS V3.1 using *the HypoGen* module involves the selection of 8 ligands in training set and remaining 30 as test set. For the purpose of training set molecules, 2 ligands with approximate median values in each activity data set had been chosen to run the protocol as described earlier. The minimum value of 0 and a maximum value of 5 features were selected to build a series of hypotheses for generating the quantitative models. The protocol generates 5 hypotheses as HG1 to HG5 using 3D quantitative structure activity relationship of DS.

Due to the small molecular size of clustered ligands, the inter-feature distance of 2.5 Å was set along with Fisher randomization of the 95% confidence level [12]. All of the hypotheses contain hydrophobicity (HY) and ring aromatic (RA) as common features along with hydrogen bond acceptors (HBA) in three of them.

D. Hypothesis validation:

Pharmacophore validations are powerful parameters to test whether or not models are good enough to predict the active compounds. The 05 numbers of generated hypotheses using the Catalyst/*HypoGen* module of DS were validated according to their respective statistical values in the reports. Three parameters are considered for this purpose as the cost analysis, correlation coefficient, and RMSD values. The hypothesis with good total cost values define close to fixed cost and away from the null cost. For a better hypothesis and predictive model, the cost difference value must be greater than 60 [19]. The highest correlation coefficient indicates the similarity and closeness in the data set with each other. Lower values of RMSD gives better superimposition of structures over pharmacophore models.

Table 1: Ligands with EC50 values and activity distribution pattern for training set

Sl. No.	Ligand Code	hPPAR γ TA EC50 (µM)	Activity group
1	Ligand 40	0.034	ACTIVE
2	Ligand 35	0.091	ACTIVE
3	Ligand 20	0.161	Moderately ACTIVE
4	Ligand 41	0.176	Moderately ACTIVE
5	Ligand 39	0.464	Less ACTIVE
6	Ligand 16	0.47	Less ACTIVE
7	Ligand 7	2.12	INACTIVE
8	Ligand 10	2.8	INACTIVE

hPPAR: human Peroxisome Proliferator Activated Receptor, **TA:** Trans activation, **EC50:** Effective concentration 50. **µM:** micro molar

E. Construction of library with shortlisting:

A widespread structural diversity is available through various databases over the internet from various research findings. These freely available databases provide similar kinds of structures depending upon search tools which are generally used to create a huge library of compounds. PubChem structure database [20] was used to create such a library where the parent compound was aromatic substituted indole 2-carboxylic acid derivative (**Figure 2**). This compound proved to have very good activity with PPAR-γ enzyme assay for antidiabetic effect [21]. The constructed library was screened online using Rules of 5 or Lipinski's rule of five with the help of inbuild command of PubChem. This helps to reduce unwanted virtual chemical space and guide the research in a more rational way [22]. The filtered database now downloaded in a structural data format (.sdf) zip file for further study.

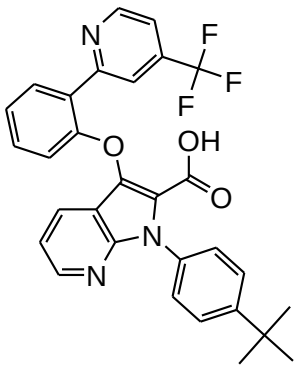


Figure 2: Ligand with best EC50 value utilized for library construction

F. Pharmacophore based screening:

The purpose of this study includes the reduction of enormous virtual chemical space for well-validated hits generation targeting diabetic chemotherapy. The shortlisted library of compounds was subjected to a virtual screening procedure using the best-validated *HypoGen* hypothesis. The library screening protocol was set up in DS by optimizing features parameters as 1 for minimum and 5 for maximum. The entire library was opened in a molecule window and specified them as input ligands for program run. Along with the input library, the validated hypothesis also copied as a screening query. The results were in .sd file format in the output directory for analyzing and reporting purposes. After superimposing the library of structures in the pharmacophore model catalyst generates a hit list with features similarity that provides a fit value score for ranking the molecules within the library.

G. ADMET profiling:

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) profiling is the screening of structural databases based on predictive structural properties. This protocol helps to shortlist a huge library of compounds into the smaller bioactive database with improving pharmacokinetic properties. Along with these attributes, ADMET profiling also helps in minimizing research failures and dead ends in later stages of drug design and development. Now the files are further shortlisted by TOxicity Prediction by Komputer Assisted Technology (TOPKAT) tool of DS v3.1.

This protocol computes and validates the toxic and environmental effects including carcinogenicity, teratogenic and mutagenic effects of chemicals only from their molecular structure [23]. National Toxicology Program (NTP) of the National Institute of Health (NIH) developed these computational algorithms for computing all the predictive toxicities in male and female rat models. Compounds with good LD50 values were shortlisted for further study and compounds with more adverse parameters are screened out.

III. RESULTS AND DISCUSSIONS

A. Pharmacophore modeling:

Compounds with diverse bioactivity profiles distributed well in all the activity classes were selected to build the pharmacophore models using the *HpoGen* algorithm of 3D QSAR methodologies in Accelrys Discovery Studio V3.1. The protocol was run with input features like hydrogen bond donors(HBD), hydrogen bond acceptors (HBA), ring aromatic (RA), hydrophobic (HY) and positive ionization (PI). 05 numbers of *HpoGen* models are generated from 8 training set of the ligand which all contains hydrophobic (HY), ring aromatic (RA) features as common. Additionally, three hypotheses contain hydrogen bond acceptors (HBA) features. All the hypotheses are further mapped with the remaining 30 test set of molecules. Cost analysis, correlation coefficient, and RMSD in the directory of output files are tabulated according to hypothesis and calculated to find the best model. HG1 hypothesis among generated 05 is considered best based on statistical calculations and utilized for further virtual screening protocol. This model comprises one HBA, two HY, and one RA as the pharmacophoric feature with the highest correlation coefficient of 0.986. This pharmacophore model mapped all 30 test set of molecules with optimized inter feature distances. The model showing a very close inter-feature distance of 3.8 Å between RA and HBA (**Figure 3B**). There is a very good distance between two hydrophobic features of 10.091 Å which is most among all inter-feature distances. This high distance will decrease the hydrophobic interactions and will stabilize the model. This kind of stable feature will screen a similar kind of molecule with the same stability. HG1 also achieved an RMSD of 0.348 with the total cost of 87.46 and these parameters make it best among all the generated hypotheses.

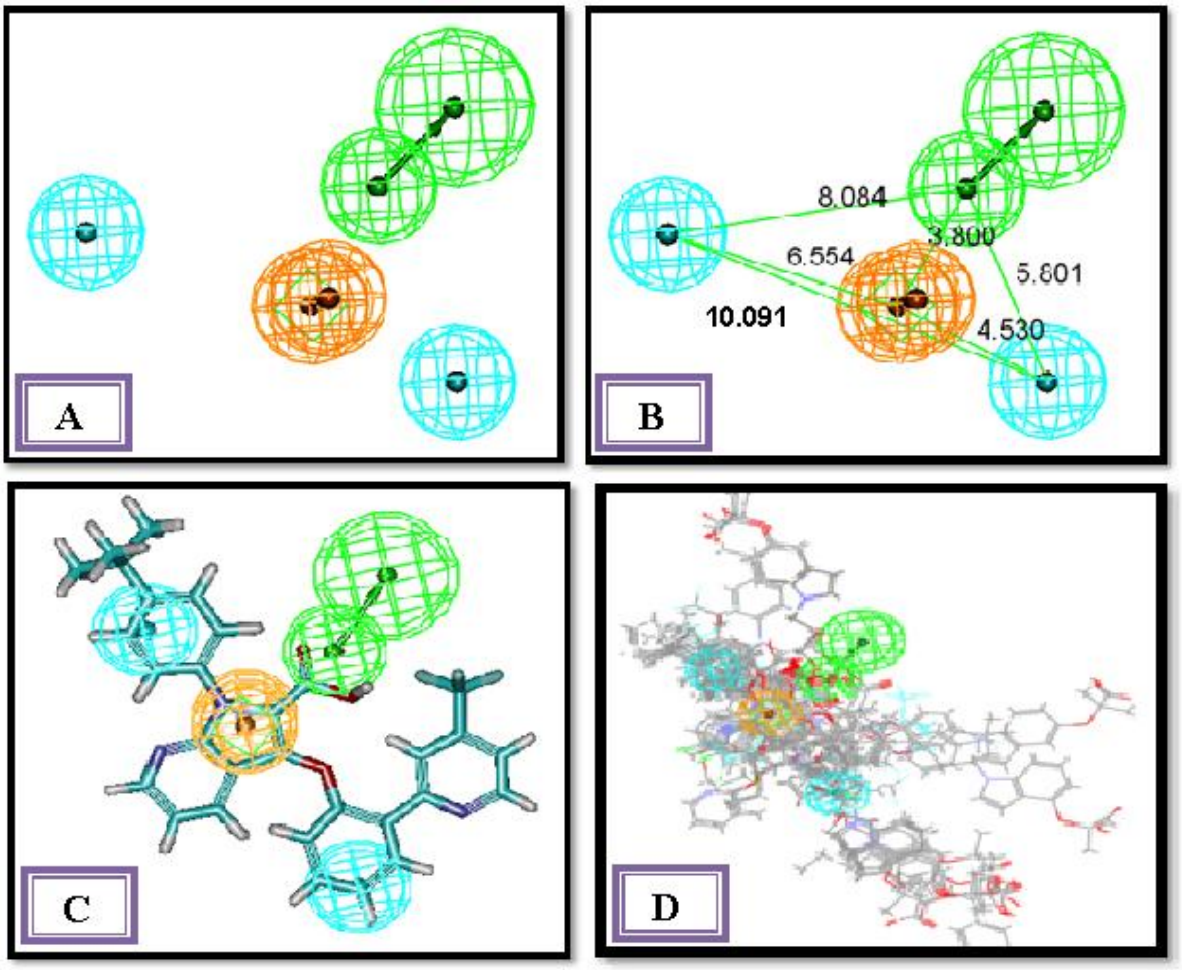


Figure 3: Pharmacophoric visualizations. A: HG1 Pharmacophore features. Sky blue: HY, Light green: HBA, Orange: RA. B: Interfeature distances in angstroms (A^0). C: Mapping of best active ligand among training dataset over the HG1 model. D: Mapping of all the ligands among test datasets over the HG1 model.

Table 2: Statistical results of *HypoGen* hypothesis*

Sl. No	Hypothesis	Features	Correlation	Total Cost	RMSD	Confidence Level	Mapped Compounds	Sensitivity [TP/(TP+FN)]	Specificity [TN/(TN+FP)]	Yield of Actives [TP/n]	Enrichment [(TP/n)/(A/N)]	Accuracy [(TP+TN)/N]
1	HG1	HBA HY HY RA	0.986	87.46	0.348	95%	TP=10 FP=7 FN=8 TN=5	0.56	0.417	0.833	3.33	1.88
2	HG2	HY HY RA RA	0.983	83.56	0.382	85%	TP=09 FP=5 FN=4 TN=3	0.692	0.375	0.75	3	1.5
3	HG3	HBA HY HY RA	0.98	85.68	0.408	95%	TP=10 FP=7 FN=7 TN=3	0.588	0.3	0.833	3.33	1.625
4	HG4	HY HY RA RA	0.979	84.7	0.423	85%	TP=10 FP=7 FN=8 TN=5	0.56	0.417	0.833	3.33	1.88
5	HG5	HBA HY RA RA	0.979	85.7	0.425	95%	TP=10 FP=7 FN=8 TN=3	0.56	0.3	0.833	3.33	1.625

*HBA, hydrogen-bond acceptor; HY, hydrophobicity: RA, Ring aromatic. Calculations are here listed where TP (mapped compound in the active group), FP (mapped compound in moderately active group), FN (mapped compound in less active group), TN (mapped compound in the inactive group) were taken. n= total no of mapped compound of active group in each hypothesis. A= Total no of active compound in a database that is 2 from the active data group. N= total no of entries that is 8 as there 8 no of ligands taken for featuring pharmacophore.

B. Library construction and shortlisting:

PubChem has been a mainstay for the chemical library and bioassay data with the increase in numbers of research publications. This authentic database utilizes cheminformatics data-mining studies to store data regarding each chemical entity for virtual screening studies [24]. A similarity search tool in PubChem structure database with 70% similarity of parent compounds (**figure 2**) yields 4,75,372 compounds. This huge library of structural database further shortlisted using 'Rules of 5' to obtain more drug-like compounds in the structural library. This protocol ruled out approx 50% of the molecules and retains 2,60,526 compounds as the work data. The data file was downloaded in a zip file as a structural data format (.sdf) in the directory before unzipping and submitting in DS.

C. Pharmacophore based screening:

The screening protocol using the pharmacophore model utilizes the consideration of catalyst with or without the flexibility of the approaches. There are two types of pharmacophore screening protocols and we have applied the flexible approaches as a search query. The flexible approach of pharmacophore-based screening in DS allows the searching of best-fit ligands within the scope of the applied energy threshold. The catalyst algorithm of DS searches the potential hits among the input library by checking all of the structural conformations in 3D space in the pharmacophore model for the best fit. As per the optimized features parameters for screening as 1 for minimum and 5 for maximum we have got 2 screened databases. Compounds with 2 features similarity screened 26,629 numbers of molecules and we have got a shorter library of 6,848 numbers of molecules in 3 features matching result file. As per structures concerns, there are almost all of the 3 features query structures in 2 features similarity result file.

D. ADMET profiling:

Our prime aim of this study is to find the best fit ligands targeting PPAR-γ with proper safety profile.

So in this context, both the screened library of compounds further sieved for computed and predictive toxicity profiles. NTP models of toxicity program determine the predictive aqueous solubility, lipophilicity, partition coefficient (logP), carcinogenicity, mutagenicity, hepatotoxicity, irritancy properties on the skin, eye with predictive EC50 and LD50 values. Pharmacophore based screened compounds were further shortlisted with the TOPKAT tool in Accelrys Discovery Studio V3.1. Molecules with best LD50 and lower mutagenic values were shortlisted for further *in-silico* study purposes. As per the final library, we have got 5783 molecules in 2 features list and 1515 numbers of molecules in 3 pharmacophore features matching list. For an obtained list of compounds with 2 features similarity, we have got excellent LD50 values of 10 g/kg and 0.099 as the computed probability of mutagenicity as the highest value. For 3 pharmacophoric similarity features list, LD50 values of 10 g/kg were best and the mutagenic probability of 0.297 was maximum.

IV. CONCLUSIONS

The study provided us well-validated 3D QSAR pharmacophore model with diverse features. HG1 model has a steric feature like ring aromatic (RA) and posses electronic features like HBA. HY as an additional feature in this hypothesis will play a role in both the aspect and balance the lipophilicity in the molecule. A good distance between two similar kinds of hydrophobic features will help to minimize hydrophobic repulsion in the same molecule but will contribute to hydrophobic interaction with targeted protein. HBA features will help in hydrogen bonding and aqueous solubility of the screened drug-like substances. The correlation coefficient of 0.986 indicates the similarity of the pharmacophore and training set of molecules which mapped all the test set in a highly overlapping fashion. 70 % of the best molecular similarity screened a huge library of compounds which enables us to progress further. Toxicity screening protocols screened drug-like compounds with a safer toxicity profile along with higher LD50 values. Pharmacophore based screening gave us a shorter and safer library of molecules. This predictive research model could impact greatly on further continuation in drug design protocols. Further *in-silico* studies like molecular docking from this screened library, their synthesis, and biological evaluation are under process to get communicated.

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