ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 4, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

SCREENING AND MOLECULAR DOCKING STUDIES OF NEW NATURAL AGONISTS AGAINST PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-ALPHA TARGETED TO TREAT OBESITY

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Received: 12 February 2016, Revised and Accepted: 16 February 2016

ABSTRACT

Objective: Obesity was considered as a serious health concern apart from the age group in today's population globally. The percentage of obese people in the world's population is increasing at a faster rate, and health issues arising due to obesity are gradually increasing. Our present insilico study was aimed to screen out natural molecules against the peroxisome proliferator-activated receptor (PPAR), especially alpha aids in triggering the obesity.

Methods: Several targets for treating obesity were identified, and one among such promising target was PPAR. Using the insilico applications such as natural database was screened and the molecules were further evaluated based on their docking score parameter with the receptor.

Results: The docking methodology suggested that two molecules zinc02091671 and zinc02137525 were found to reproduce the similar type of interactions such as that of the known inhibitor and crystal ligand.

Conclusion: The reported two molecules were found to be promising agonists based on the computational studies and can be advanced the *in vitro* based evaluation.

Keywords: Obesity, Peroxisome proliferator-activated receptor, e-pharmacophore, QikProp, Docking.

INTRODUCTION

Accumulation of excess fat inside the body was clinically identified as obesity. This may be due to eating behaviors, lifestyle, heredity, low physical exercise, etc. Obesity was designated as a chronic metabolic disease that can lead to other various health problems such as diabetes, cardiac problems, cancer, arthritis, etc. It became a serious health problem in all the nations and also the rate of the population with obesity were increasing day by day at an alarming rate [1,2]. Therefore, it is necessary to discover new drugs for the treatment so that a healthy life can be retained. Targets prone to obesity were several. Until today, drugs targeting the obesity were very few, and some were withdrawn from the market due to their side effects. To overcome the side effects, natural compounds were targeted to treat obesity. Peroxisome proliferator-activated receptor (PPAR) was one among the major targets for obesity treatment has drawn attention in developing the new drugs. This receptor belongs to the nuclear receptor subfamily and plays a key role in treating metabolic processes including fatty acid β-oxidation, hypertension, insulin resistance, and atherogenic dyslipidemia apart from obesity [3-5]. The PPARs are a group of three isoforms, namely, PPAR gamma, PPAR alpha, and PPAR delta, which were encoded by different genes [6]. These isoforms are ligand-regulated transcription factors, and their functions are arbitrated by changing the gene expression.

Among the three isoforms, PPAR alpha was targeted for the treatment of obesity [7,8] because it is predominantly expressed in adipose tissue. Hyperplasic is characterized by the increase in the adipocyte number, and hypertrophy is characterized by the increase in adipocyte volume are the two major conditions observed in obesity [9]. Through catabolizing the fat, PPAR alpha regulates the energy balance in the cell. Increase in fatty acid oxidation and decrease in the triglycerides levels in plasma are the key for adipose tissue hypertrophy and hyperplasia, resulting in the decrease in the body weight [10,11]. This hypothesis was supported from the mice studies reported that deficiency in the PPAR alpha exhibited abnormalities in the levels of plasma triglycerides and also cholesterol metabolism and finally leading to obese [11].

Computational approaches, such as molecular docking, pharmacophore modeling, and structural bioinformatics, have been proved as useful tools in predicting the structural insights of receptors and also the mode of binding by ligands inside the binding pocket provoking the researchers in developing new drugs toward various diseases [12]. In our study, we have applied various insilico methods such as e-pharmacophore, QikProp for ADME analysis, and Glide-based docking bundled in the Schrodinger suite. The study was initiated with e-pharmacophore modeling and continued by screening the natural database to retrieve the new natural agonists for PPAR alpha. Isolated molecules were further screened out through the QikProp; obtained molecules were then docked into the receptors active pocket using the Glide module to analyze the interactions between PPAR alpha and molecules. Thus, this study may provide useful active site binding residue insights for designing and developing new drugs for the obesity.

METHODS

Hypothesis generation

Pharmacophore-based screening includes structure and ligand based, and they are standard computational approaches in the drug designing. e-Pharmacophore from the scripts is a methodology which combines the advantages of ligand and structure-based pharmacophore protocols [13,14]. Pharmacophore sites generation was carried out using the pose viewer file of the docked complex as an input. The pharmacophores are hydrogen bond donor (D) displayed as projected points, hydrogen bond acceptor (A) symbolized as vectors, aromatic ring (R) as a ring, positive ionizable (P), and negative ionizable (N). Explicit matching is required in this script for the generation of energetically favorable sites. By calculating enrichment factor (EF) and goodness of hit (GH) score using the below formulae generated pharmacophore hypothesis was validated.

$$E = (Ha/Ht)/(A/D) \quad (1)$$

$$GH = ([Ha\ (3A+Ht)]/4HtA)(1-[Ht-Ha]/[D-A]) \quad (2)$$

Where, E represents EF, GH means GH, D was the total compounds in the dataset, A denotes total number of actives in the dataset, total hits as Ht, and last Ha as active hits.

Database screening

Zinc, a natural and non-commercial database, contains 1.6 million molecules were used as the database in our study. The database was converted from 2D to 3D dataset using the canvas minimization tool from the Schrodinger suite. Hypothesis generated by the e-pharmacophore was used to screen out the molecules possessing the pharmacophores properties present in the hypothesis. Phase module [15] was used to perform this activity and finally screened molecules were continued for further procedures.

ADME-based screening

For calculation of ADME properties of the screened molecules, QikProp module in Schrodinger suite was executed for additional screening [16]. This module helps in predicting the properties such as human oral absorption, Lipinski rule of five, central nervous system (CNS) activity, log BB, octanol/water, and water/gas and helps in further screening of the molecules. Properties namely Human oral absorption, CNS, molecular weight, and Lipinski's rule of 5 were considered as major properties in this study as a second level of the screening process. The ranges of these properties are like Human oral absorption to be measured on a scale of >80% and <25% as high and poor. CNS activity on a scale of −2 (inactive) to 2 (active), molecular weight to be in between 130.0 and 725.0 K, and finally Lipinski's rule of 5 violations maximum is 0.

Protein preparation and grid generation

The 3D coordinates of PPAR alpha were retrieved from the protein data bank (pdb id:1k7l) having a resolution of 2.5 Å [17]. Initially, the pdb structure was prepared with the help of protein preparation wizard [18] by implying properties such as adding hydrogen's to justify bond orders, zero bond order to metal atoms, adding the missing side chains and backbone if any using prime, capping the termini, and removal of crystallized free water molecules beyond 5 Å know as desolvation. Following the hydrogen bonds present in the protein structure were optimized and minimized under force field OPLS 2005 [19,20].

Ligand preparation

Molecules obtained from the above screening protocols were prepared with using the LigPrep module under the OPLS 2005 force field.

Ligand docking

For ligand docking studies, we have applied Glide extra precision (XP) module of Schrodinger suite. This module is a grid-based ligand docking method with energetics results in the favorable interactions between a molecule and protein. First, a grid box was generated at the centroid of the ligand through the assistance of receptor grid generation protocol. With the receptor grid, prepared molecules were docked using the XP docking protocol [21-23]. The interactions in the receptor-ligand complex were calculated based on their energy and quality of geometric contacts. Using the Ligplot interactions in the receptor-ligands complexes were analyzed. After docking, complexes were taken for molecular dynamic studies to study the interactions exhaustively.

Hardware used

The entire work was executed using the Schrodinger Suite 2014-3 in the HP Z600 workstation.

RESULTS AND DISCUSSION

e-Pharmacophore hypothesis generation

Ligand- and structure-based screening protocols have their importance in virtual screening protocol in drug discovery. The e-pharmacophore technique accomplishes the advantages of both ligand- and structure-based screenings. This energetically optimized structure-based pharmacophores (e-pharmacophore) approach was employed in our study from the scripts of the Schrodinger suite for generating pharmacophores. This technique brings together the rapidity of pharmacophore screening with the energy terms from Glide XP module. To generate e-pharmacophores, a pose viewer file was required. For that, pdb structure (1k7l), which was using for this study was split into the receptor and ligand separately. Using Glide XP module, the crystal ligand was redocked into the receptor active pocket and obtained pose viewer file was considered for generating e-pharmacophores. The technique generated a six featured hypothesis HNRRRR; one H-bond

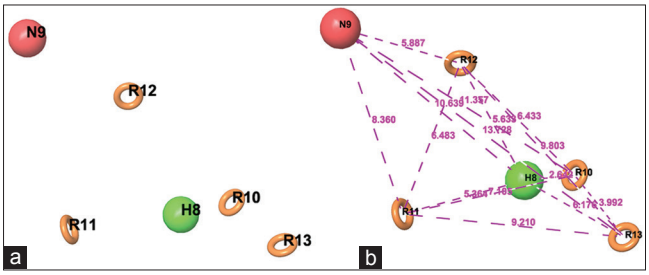

Fig. 1: Hypothesis generated by e-pharmacophore (a) six featured HNRRRR hypothesis (b) intra distances between the features of the hypothesis

Table 1: QikProp analysis of finally screened 19 molecules

Molecule	CNS	mol_MW	Donor HB	Accpt HB	QPlogPo/w	QPlogBB	Percent human oral absorption	Rule of five
ZINC02092279	−2	388.419	1	5.25	4.318	−1.188	87.675	0
ZINC02092359	−2	402.446	1	5.25	4.675	−1.227	89.971	0
ZINC02091671	−2	402.446	1	5.25	4.842	−1.297	91.424	0
ZINC85878789	−2	485.452	4	9.5	2.655	−2.782	56.304	0
ZINC02135452	−2	488.496	3.25	7.5	3.201	−1.937	66.067	0
ZINC02138703	−2	496.903	3.25	7.75	2.924	−2.322	58.256	0
ZINC02102606	−2	458.47	2.25	6.75	3.564	−1.454	74.291	0
ZINC02128790	−2	474.469	3.25	7.5	2.976	−2.045	61.828	0
ZINC02105418	−2	472.496	2.25	6.75	3.821	−1.884	71.176	0
ZINC02137525	−2	412.441	1	5	4.991	−1.132	92.147	0
ZINC02137060	−2	398.414	1	5	4.567	−1.052	89.604	0
ZINC02158001	−2	472.496	2.25	6.75	3.966	−1.756	78.148	0
ZINC02137716	−2	488.496	3.25	7.5	2.966	−2.416	56.001	0
ZINC02137714	−2	488.496	3.25	7.5	2.943	−2.512	54.376	0
ZINC02153223	−2	498.534	2.25	6.75	4.275	−1.664	78.161	0
ZINC02151654	−2	388.419	1	5.25	4.38	−1.09	90.145	0
ZINC04046435	−2	388.419	1	5.25	4.527	−1.231	89.488	0
ZINC04044233	−2	398.414	1	5	4.678	−1.044	90.306	0
ZINC02129181	−2	486.48	3.25	7.75	2.825	−2.927	50.44	0

CNS: Central nervous system

acceptor, one negative ionizable, and four ring features. The generated hypothesis model was represented in the Fig. 1.

Four rings (R) were generated, three from the benzene rings and one representing oxazole ring. The other features H-bond acceptor on the c-27 and N feature on the c-38 of the crystal ligand GW409544 (2-(1-methyl-3-oxo-3-phenyl-propylamino)-3-{4-[2-(5-methyl-2-phenyl-oxazol-4yl)ethoxy]-phenyl}-propionic acid). Further, the hypothesis was verified for its reliability using EF and GH. Hypothesis produced 32.5 EF and 0.81 GH proving that this hypothesis was good enough in picking the actives. With this hypothesis, virtual screening of the natural molecule database was initiated.

Virtual screening of database

Zinc natural molecules as a database were used in screening of agonist for PPAR alpha targeting the obesity. The hypothesis was imported to the advanced pharmacophore screening protocol present in the Phase module. Screening of database molecules was carried by imposing must match 6 out of 6 as chosen as default. That means the inclusion of all six pharmacophore features was taken into consideration for screening the molecules. From the large natural database, 57 molecules were screened out by this protocol. These molecules were advanced to QikProp filter to check their pharmaceutical related properties.

QikProp

This tool helps in screening out the molecules which are violating the pharmaceutically relevant properties such as CNS activity, Lipinski's rule of five, log BB, octanol/water and water/gas log Ps, and log S of molecules and thereby reducing the cost and time of the drug development process. In this study, two properties CNS activity and Lipinski's rule of five were taken as a major criterion in screening out 57 molecules. On this basis, 19 molecules have shown CNS as

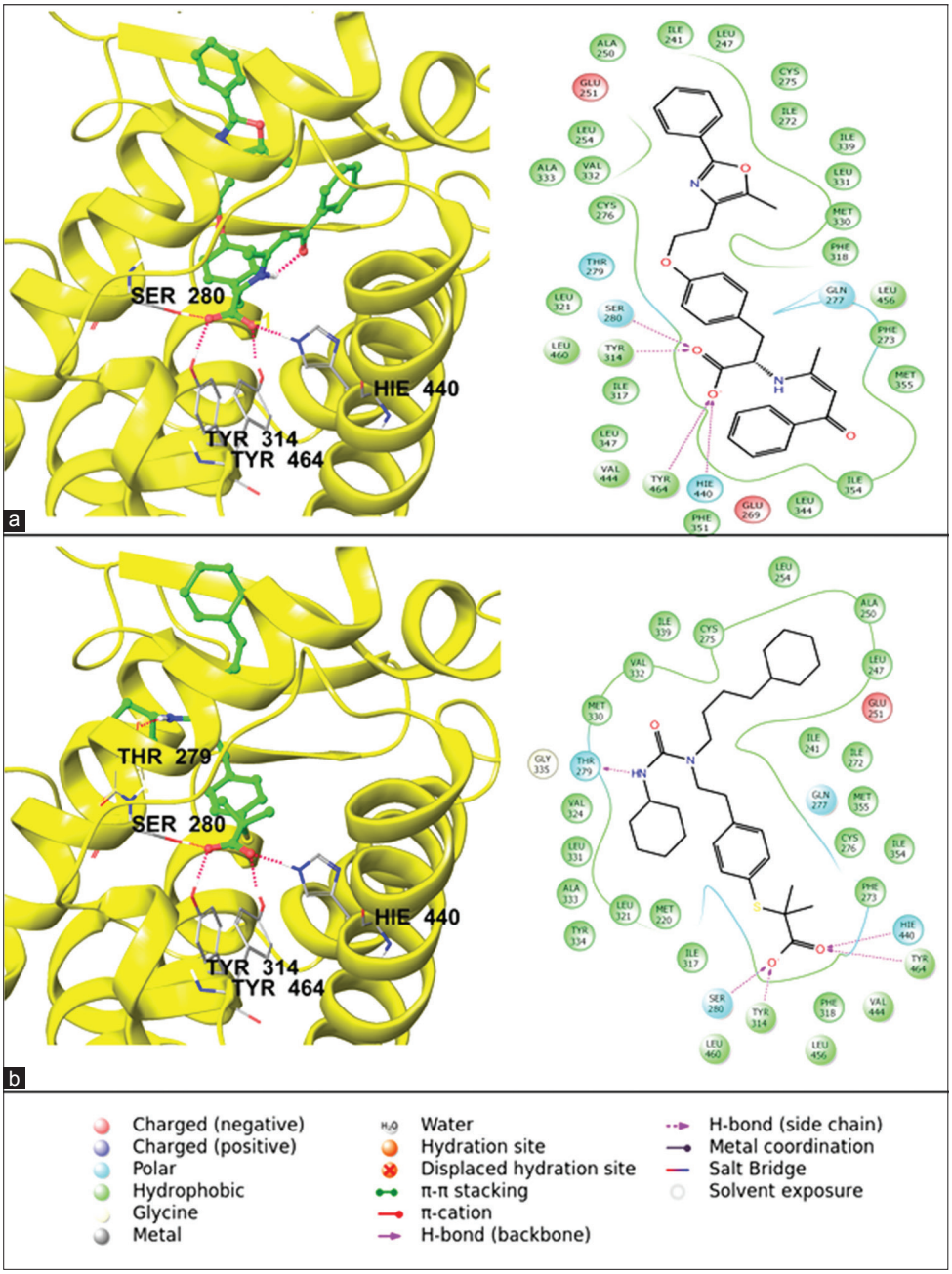

Fig. 2: Molecular interactions after docking studies (a) binding mode of crystal ligand GW409544 with the residues of active site (b) binding mode of known inhibitor GW7647 with the residues of active site

−2 means the molecule does not show any activity over the CNS and Lipinski's rule of five as zero means no violations. The Lipinski's rules are molecular weight <500, donor HB ≤5, QPlogPo/w <5, accept HB ≤10. The molecules, which satisfy the above rules, were considered as drug-like molecules. Finally, the screened 19 molecules were preceded to the next level of lead molecules identification process (Table 1).

Glide-based docking studies

Obtained 19 molecules from the above screening protocol were further analyzed for their binding modes within the receptor active site. GW7647 (2-[[4-[2-[[(cyclohexylamino) carbonyl](4-cyclohexylbutyl) amino]ethyl]phenyl]thio]-2-methylpropanoic acid) a highly potent and selective agonist of PPAR alpha was also incorporated in the docking studies along with the screened molecules. In the initial studies, a grid file was generated for the redocking of crystal ligand with the receptor that grid was reutilized in the present docking studies. Receptor grid generation procedure from the glide module was used to lock the receptors active site and into that the crystal molecule was docked using the ligand docking (XP) protocol from the same module. The crystal ligand pose was cross checked with pose generated after the docking studies, produced pose after docking studies were replica of the crystallized pose. Hence, thereby confirming that the grid file and the docking protocol are preferable for continuing further docking studies with the molecules.

The redocked crystal ligand inside the active site of the receptor produced four hydrogen bonds. Both Ser 280 (OH group) and Tyr 314 (OH group) residues of the active pocket formed a single hydrogen bond with the =O atom of the ligand. Other two Hie 440 (NH group) and Tyr 464 (OH group) were also formed a single hydrogen bond with the O^- atom of the crystal molecule. These = O and O^- are present in the form of branch adjacent to the NH group of the molecule (Fig. 2a). The complex produced a G-score of −12.7 with glide energy of −70.92 kcal/mol. Hie represents histidine with hydrogen present on the epsilon nitrogen. The selective agonist GW7647 and the receptor complex produced five hydrogen bonds with a G-score of −9.8. The similar type of four hydrogen bindings displayed in the crystal ligand with receptor same residues but with small difference, i.e., Ser 280, Tyr 314 with O^- and Hie 440, Tyr 464 with the = O of the agonist in the reverse manner (Fig. 2b). The fifth hydrogen bonding, which was absent in the crystal ligand-receptor complex was with Thr 279 residue. The hydrogen bond was shared between the NH group of the agonist with the OH group of Thr 279 with glide energy of −61.52 kcal/mol.

The prepared screened 19 molecules were docked into the active site of the receptor using the extra precession docking protocol as mentioned in the above section. All the molecules were docked into the active site of the receptor, produced G-scores, and glide energies. Among 19 molecules, only three molecules satisfied the binding modes as produced in the above crystal ligand and selective agonist. The

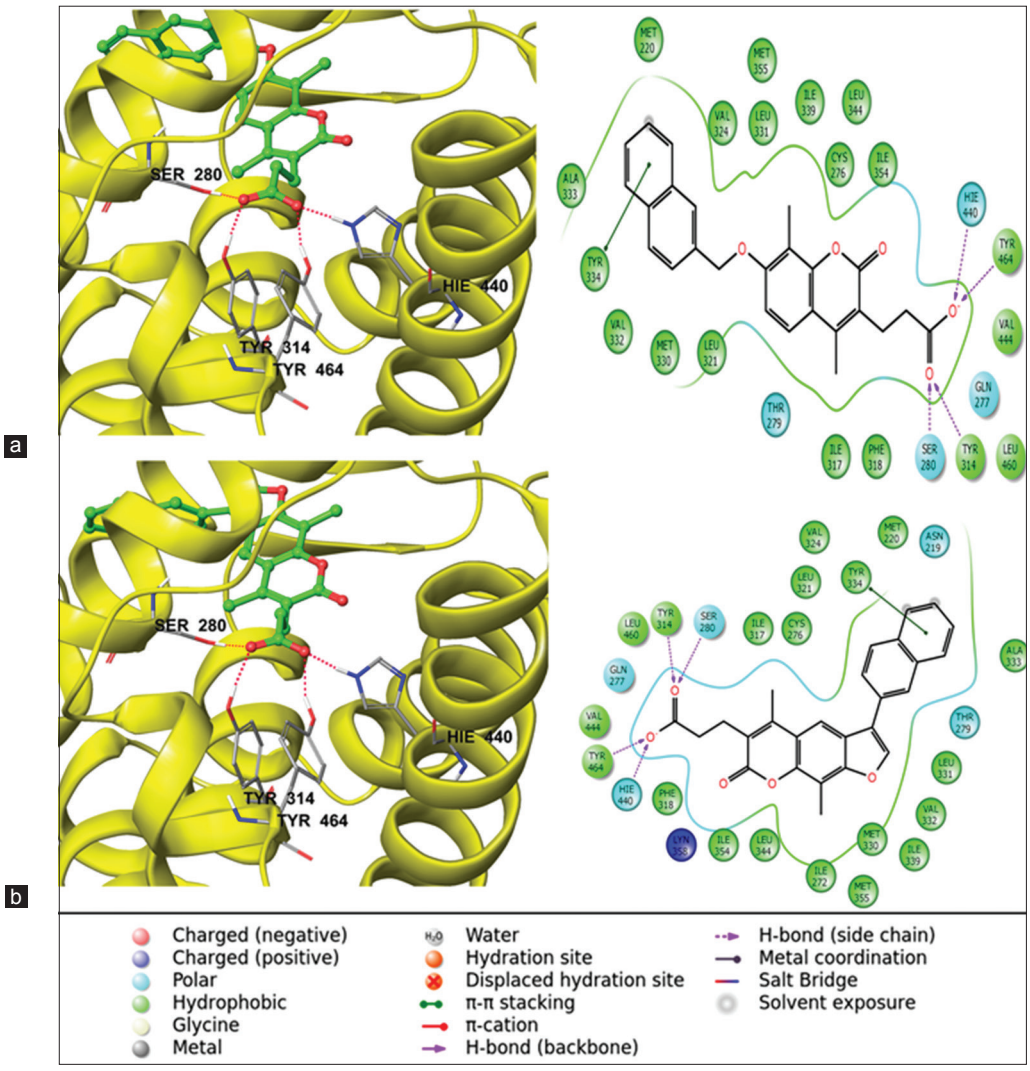

Fig. 3: Binding modes of the molecules (a) zinc02091671 (b) zinc02137525 with the active site of peroxisome proliferator-activated receptor-alpha

three molecules are zinc02137525, zinc02091671, and zinc02151654, respectively.

Zinc02137525 and zinc02091671 with receptor as individual complex produced four hydrogen bonds and one pi-pi interaction. The hydroxyl group presents in Ser 280and Tyr 314 residues formed a single hydrogen bond with the = O atom of the molecules. The NH group Hie 440 and OH group of Tyr 464 hydrogen bond with the O^- atom of the molecules. Both these two complexes produced the hydrogen bond network similar to that of the crystal ligand-receptor complex. Apart from that, one more interaction was observed in these two complexes, i.e. pi-pi interactions with Tyr 334 residue which was not seen in both crystal and agonist receptor complexes. The G-scores of the above two complexes are −8.9 and −8.6, respectively. Zinc02137525 and zinc02091671 with receptor produced Glide energy −51.43 and −59.15 kcal/mol, respectively. The final zinc02151654-receptor complex produced a similar type of four hydrogens with the ligand, and no other interactions were observed. The complex produced G-score of −8.2 and glide energy −56.74 kcal/mol. Structurally, both the zinc02091671 and zinc02151654 molecules are similar except a methyl group at the 8^{th} position was absent in zinc02151654 molecule. Due to this based on the G-scores molecule, zinc02091671 was taken into consideration omitting zinc02151654. Binding interactions of zinc02091671 and zinc02137525 with PPAR alpha were represented in the Fig. 3a and b.

Using the above methods, we have screened out two molecules as final leads targeting PPAR. These two molecules made the similar type of binding modes exhibited by both crystal and agonist molecules. The major four interactions Ser 280, Tyr 314, His 440, and Tyr 464 are important and necessary in performing agonist activity. The majority part of the active pocket was hydrophobic in nature with small polar regions. The common feature what we have observed in all leads, crystal ligand and agonist was the formation of hydrogen bond. All these molecules formed a hydrogen bond with the branched = O and O^- groups present in them. Further, the bond lengths between the molecules and the active residues are nearly same. The remaining 16 molecules, out of them, three molecules were found with no interactions and others displayed one or two interactions with the residues present in the active site. Hence, these molecules were not taken into consideration for the further studies.

CONCLUSION

This study is mainly focused on the molecular interactions between the molecules and the residues present in the receptors active pocket. Using techniques such as e-pharmacophore modeling continuing with screening protocol from Phase module, QikProp, and finally screened in molecules were docked using the Glide XP module of the Schrodinger suite. Based on the present investigation; using the above drug designing tools, it was predicted that to perform agonist activity against PPAR alpha the molecules must contribute hydrogen bonding with the four important resides Ser 280, Tyr 314, His 440, and Tyr 464. Two molecules were proposed as final leads to fulfilling all the ADME properties and importantly the binding interactions with the four important residues. In concluding, we predicted that molecules zinc02091671 and zinc02137525 as good agonists with G-score nearer to the specific agonist taken in this study satisfying all the criteria and hence this can be further evaluated using the *in vitro* methods.

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