Journal of Molecular Structure 1028 (2012) 22–30

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

A combined ligand and structure based approach to design potent PPAR-alpha agonists

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HIGHLIGHTS

- Ligand and structure based pharmacophore model was developed for PPAR-alpha agonists.
- The developed pharmacophore model was validated with multiple approaches.
- Four different databases were screened by means of validated pharmacophore model.
- Finally three novel scaffolds were identified as PPAR-alpha agonists.
- The novelty of virtual hits was confirmed by using PubChem and SciFinder search tool.

ARTICLE INFO

Article history:
Received 19 April 2012
Received in revised form 14 June 2012
Accepted 14 June 2012
Available online 19 June 2012

Keywords:
LigandScout
Molecular docking
PPAR-alpha
Pharmacophore
Virtual screening

ABSTRACT

A combined ligand and structure based pharmacophore modeling approach was employed to reveal structural and chemical features necessary for PPAR-alpha agonistic activity. The best HypoGen pharmacophore model Hypo1 for PPAR-alpha agonists contains two hydrogen-bond acceptor (HBA), two general hydrophobic (H), and one negative ionizable (NI) feature. In addition, one structure based pharmacophore model was developed using LigandScout3.0, which has identified additional three hydrophobic features. Further, molecular docking studies of all agonists showed hydrogen bond interactions with important amino acids (Ser280, Tyr314 and Tyr464) and these interactions were compared with Hypo1, which shows that the Hypo1 has a good predictive ability. The screened virtual hits from Hypo1 were subjected to the Lipinski's rule of five, structure based pharmacophore screening and molecular docking analysis. Finally, three novel compounds with diverse scaffolds were selected as possible candidates for the designing of potent PPAR-alpha agonists. Combination of these two approaches results in designing an ideal pharmacophore model, which provides a powerful tool for the discovery of novel PPAR-alpha agonists.

1. Introduction

Peroxisome proliferator activated receptors (PPARs) are the ligand activated transcription factors belonging to the nuclear hormone receptor (NHR) super family [1]. To date, three subtypes of PPAR receptors, have been identified namely PPAR-alpha, PPAR-beta and PPAR-gamma [2]. Each receptor displays distinct tissue-selective expression patterns, ligand selectivity, and biological actions. PPAR-alpha is predominantly expressed in the liver [3] and to a lesser extent in variety of cell types, including smooth muscle cells, endothelial cells, and macrophages, playing a pivotal role in atherosclerosis and inflammation process [4–7]. The role of PPAR-alpha in the regulation of hepatic lipid metabolism was revealed by its association with well known natural fatty acids and the fibrate class of hypolipidemic drugs (fenofibrate, gemfibrozil, and bezafibrate). Activated PPARs get heterodimerized with the 9-cis-retinoic acid receptor (RXR) [8,9]. The heterodimer receptor complex binds to peroxisome proliferator response elements (PPREs) located in the promoter regions of the target genes (acyl-CoA oxidase (AOX) [10], liver-fatty acid-binding protein (L-FABP) [11], apolipoprotein C-III (apo C-III) [12], and lipoprotein lipase (LPL)) [13], which controls lipid and glucose metabolism [14]. In addition, it has been reported that activated PPAR-alpha produce insulin sensitizing effects to improve glucose tolerance in type II diabetes [15].

In the current scenario, the clinical use of hypolipidemic drugs as PPARs agonist have very weak affinity towards PPAR-alpha, which results in the comparatively high doses (e.g., 200 mg of fenofibrate) to attain desired clinical efficacy, and leads to dose-limiting side effects [16]. The role of fibrates in protecting against cardiovascular disease was restricted because of these unwanted

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http://dx.doi.org/10.1016/j.molstruc.2012.06.032

side effects [17]. Since from the last few years, several potent selective PPAR-alpha agonists have been developed and passed through various stages of clinical development but none of them have been reached to the market because of their safety issues [18].

The main aim of this study is to identify the basic structural requirements for PPAR-alpha agonistic activity and thereby designing novel potent and selective agonists as hypolipidemic agents. Ligand and structure based pharmacophore modeling approaches have been employed to achieve this goal. The validated pharmacophore hypothesis has been employed in virtual screening to identify the potent lead. The screened virtual hits were subjected to several filters such as Max. Fit value, Lipinski's rule of five, and afterward subjected to the molecular docking studies using Glide5.5. We have reported three novel compounds with diverse scaffolds as possible candidates for the designing of potent PPAR-alpha agonists. Finally, potential lead compounds will be shifted to the subsequent *in vitro* and *in vivo* studies.

2. Materials and methods

2.1. Data sets

In last few decades, more than 380 compounds were reported as PPAR-alpha agonists. Out of these, 104 agonists were selected based on the biological assay method and used further for generation of pharmacophore model [19–27]. The agonist activity of these compounds was expressed as EC_{50} (i.e., concentration of a compound where 50% of its maximal effect is observed).

The EC_{50} values of dataset spanned across a wide range from 1 to 950,000 nM. The most important step in the pharmacophore modeling is the selection of suitable training set, responsible for the determining the quality of the generated pharmacophore. HypoGen pharmacophore model was built using 20 training set compounds (Fig. 1), which were selected based on the principles of structural diversity and activity range. The data set selected covered an activity range of at least 4 orders of magnitude and the most active compound was also included in the training set. The rests of the 84 compounds from the dataset were taken as a test set for pharmacophore model validation.

2.2. Pharmacophore modeling

The pharmacophore model is a combined representation of 3D (hydrophobic groups, hydrogen bond donors/acceptors, and ionizable groups), 2D includes substructures, and 1D (physical and biological) properties, which are responsible for the desired biological activity [28]. All the pharmacophore modeling calculations were carried out by using the 3D QSAR Pharmacophore Generation and Ligand Pharmacophore Mapping module within Accelrys Discovery Studio 2.5 (DS2.5) software package [29] and LigandScout3.0 [30] on IBM graphic workstation.

2.2.1. Ligand based pharmacophore

Multiple acceptable conformations for all training set compounds were generated by Cat-Conf program within DS2.5 software package. The BEST method was employed for generation of conformations, which provides complete and improved coverage of conformational space by performing a rigorous energy minimization and optimizing the conformations in both torsional and cartesian space using the poling algorithm [31]. During the conformation generation, maximum number of conformers was set to 255 with 4.0 kcal/mol as energy cut-off, and all other parameters were set to default. The quantitative pharmacophore models were then generated by using the HypoGen algorithm, which uses training set compounds to generate hypotheses with features common amongst active molecules and missing from the inactive molecules. The process of pharmacophore hypothesis generation accomplished in three steps namely a constructive step, a subtractive step and an optimization step [32]. In constructive step, hypotheses features common amongst the active compounds are identified. The determination of active compounds involved the simple calculation based on the activity and uncertainty values. Thus uncertainty value is a crucial parameter in constructive step. The hypotheses features common among inactive compounds are removed from the previous result in the subtractive step. The consequential hypotheses are then optimized using simulated annealing to further fine tune the model parameters, thereby improving model quality. The quality of the mapping between a compound and a hypothesis is indicated by the fit value. Features such as the hydrogen bond acceptor (HBA), hydrophobic feature (H), ring aromatic (RA) and negative ionizable (NI) features were included for the pharmacophore generation assuming common features present in the studied compounds. The uncertainty value was set to 3, which means that the biological activity of a particular agonist is assumed to be located somewhere in the range three times higher to three times lower of the true value of that agonist. Ten pharmacophore model hypotheses with significant statistical parameters were generated. The best model was selected on the basis of high correlation coefficient (r^2), lowest total cost, highest cost difference and low RMSD values.

2.2.2. Structure based pharmacophore models using LigandScout

Nine crystal structures of PPAR-alpha agonist (PDB ID: 1I7G, 1K7L, 2NPA, 2P54, 2REW, 2ZNN, 3ET1, 3KDT, 3KDU) are available in a protein data bank, out of these three were selected based on their EC_{50} value and crystal structure resolution for generation of the structure-based pharmacophore models. The interaction between ligand and amino acids present in the active site provide sufficient input to generate the structure based pharmacophore. LigandScout, an automated tool for pharmacophore generation, was used to study the interaction between the agonists and amino acid in the active site of PPAR-alpha. It identifies protein–ligand interactions such as hydrogen bond, charge transfer, hydrophobic regions. It defines excluded volume spheres based on the side chain atoms to characterize the inaccessible areas for any potential ligand. All the generated structure based pharmacophore hypotheses were exported in hypoedit format and then converted into chm format using hypoedit tool in the discovery studio, which was used as 3D query for the virtual screening process.

2.3. Validation of pharmacophore model

The validation of a developed pharmacophore model was done to determine whether it is capable of identifying active and inactive compounds, predicting their activities and further performing a virtual screening of databases [33]. To validate quantitative ligand based pharmacophore model three different methods were employed based on cost analysis, test set prediction and Fischer randomization test. A test set of 84 diverse PPAR-alpha agonists were selected to validate the best pharmacophore model. The cost functions calculated during the pharmacophore generation process were used as first validation method. The different cost values were generated during pharmacophore generation such as weight cost, configuration cost and error cost. Weight cost is a value that depends on the deviation of the feature weight in a model from an ideal value of 2. The configuration cost is $\log_2 P$, where P is the number of initial hypotheses created in the constructive phase, and that survived the subtractive phase. The standard configuration cost value should not be greater than 17.0. The deviation between the actual and predicted activities of the training set is signified by the error cost. The fixed cost represents the entropy