Optimized Structure-based Methodology for Studying PPARγ Partial Agonists

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Abstract: *The peroxisome proliferator-activated receptor (PPAR) γ is a master regulator of the lipid and glucose metabolism, and thus is a valuable drug target. Since its full activation is accompanied by a number of adverse effects, researchers focus on discovery of novel compounds with ligand-receptor interaction patterns of PPARγ partial agonists. Molecular modelling is an appropriate way to achieve this goal.*
In this study we aimed at optimization of the docking algorithm for structure-based investigation of PPARγ partial agonists.
A dataset with structures and activities of PPARγ partial agonists was constructed. A comparative study of different scoring functions' performance was conducted by redocking the partial agonists' structures selected from experimentally resolved 3D structures of PPARγ protein-ligand complexes. The docking protocols' performance regarding pose scoring, reproducibility and interpretability in the context of the collected activity data was estimated.
An optimized docking protocol was developed to successfully correlate the docking scores of the studied compounds with their experimentally derived activity values and to provide the best matching degree with their experimental binding modes.
Overall, these results could be useful for further molecular modelling studies of novel PPARγ partial agonists by selection of reliable docking poses to predict their binding mode and for ranking them in respect to their agonistic activity using the calculated docking scores.

Keywords: *PPARγ, Partial agonists, Docking, Optimization.*

Introduction

PPARγ is a ligand-activated transcriptional regulator from the steroid-thyroid super-family of nuclear receptors. It has a wide tissue distribution and is an attractive target for treatment of cancer, metabolic disorders, cardiovascular diseases, inflammatory processes, Alzheimer's disease, skin disorders and addictions (to substances of abuse or as addictive behaviors) [2, 13].

PPARγ-mediated transaction involves several steps as described in Fig. 1: heterodimerization with the retinoid X receptor α (RXRα) at the specific promoter regions of the target genes (I), ligand binding (II), activation of PPARγ by ligand-induced conformational changes (including stabilization of helix H12 in active conformation), leading to release of the corepressor and attraction of the coactivator (III), necessary to initiate the gene transcription (IV). Full and partial agonists differ in their capacity to stabilize H12 and in the array of genes whose expression they trigger [12, 23]. The undesirable effects reported for the PPARγ activators are typical for the full agonists [15], while partial agonists possess improved safety profiles [4].

The reason relates to the different binding modes in the large PPARγ pocket. Molecular docking is a valuable tool to get a structural insight and to predict the probable binding modes [9, 11]. In fact it is a key approach in the virtual screening projects for drug discovery/development of novel PPARγ ligands [14, 19, 20], including those from natural origin [5-8, 12, 18, 22].

Therefore we aimed at optimization of a docking algorithm for structure-based study of PPARγ partial agonists.

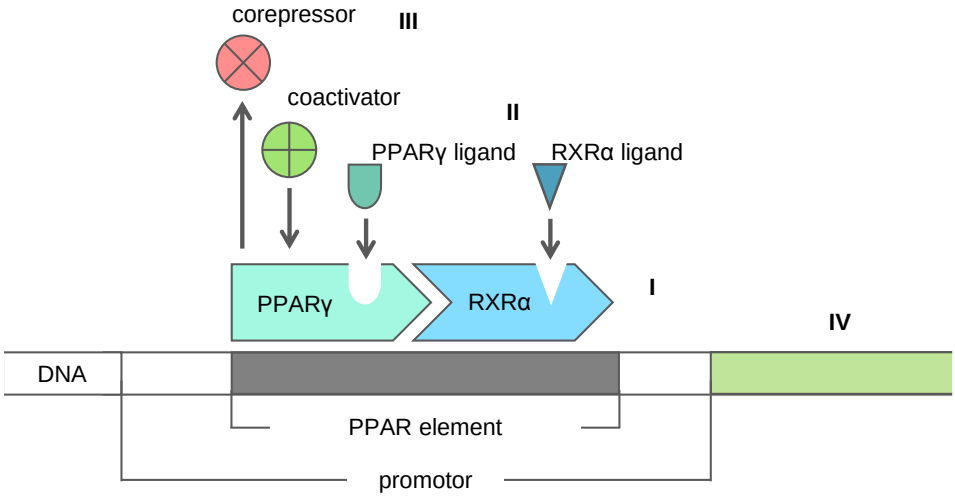

Fig. 1 Mechanism of the PPARγ-mediated transactivation

Materials and methods

Data selection and refinement

A Protein Data Bank (PDB) search resulted in 152 entries for PPARγ X-ray complexes of human origin [17]. The transactivation activity (EC_{50}, μM) and relative maximal activation (relative efficacy, E_{max}, %) data of PPARγ partial agonists were collected. For the needs of the analysis we set a 65% threshold for the reported relative efficacy of the ligands below which they are considered as partial agonists and thus restricted our initial dataset to 37 PDB entries [1, 3, 10]. Additional data processing was applied to reduce the inter- and intra-laboratory variations in the experimental settings reported in the corresponding literature: the activity and efficacy data measured using the HepG2 cell line, the chimeric Gal4-PPARγ construct and the referent PPARγ full agonist rosiglitazone were selected.

Molecular docking studies

The ligands were redocked in the protein structures of their own complexes using MOE software [16]. The docking site was defined by ligands' atoms. The default placement method "Triangle Matcher" was used. The scoring of the generated poses was performed by applying 5 different scoring functions implemented in MOE as follows: ASE, Affinity dG, Alpha HB, London dG, GBVI/WSA dG. The number of poses in the docking output database was set to 30. The docking scores approximate the binding energy of the complexes and are usually correlated to the ligand's binding affinity.

Protein-ligand interaction fingerprint (PLIF) analysis

The PLIF tool was used as a method for recording the interactions between ligands and proteins. Interactions such as hydrogen bonds, ionic interactions and surface contacts are

classified according to the participating residue, and built into a fingerprint scheme which is constructed for a given database of protein-ligand complexes.

Pose selection and statistical analysis

The best docking poses from the different docking simulations were selected based on a successful reproduction of the X-ray poses. For this purpose the root-mean-square deviations (RMSDs) of the docking poses from the original ones were used and their PLIFs were compared.

For the selected best poses the following data were recorded and used for further statistical analysis: RMSD and score, as well as their minimal and maximal values among the 30 docking poses of each compound.

Results and discussion

Clustering of the partial agonists in two activity subclasses

Within the selected set of 10 PPARγ-partial agonist complexes a good correlation (R = 0.8) was observed between the EC_{50} and the E_{max} values as shown on Fig. 2A. However, some clustering is observed on the graphic. In order to investigate it further and taking into account that the free energy of binding is linearly related to the negative logarithm of the effective concentrations, we built the graphical relationship between pEC_{50} and E_{max}. As seen from Fig. 2B the clustering is better identified. The area outlined in orange represents the subclass of partial agonists with lower maximal activation (9.4%-27%), and the one in blue includes partial agonists with higher E_{max} (33%-50.4%).

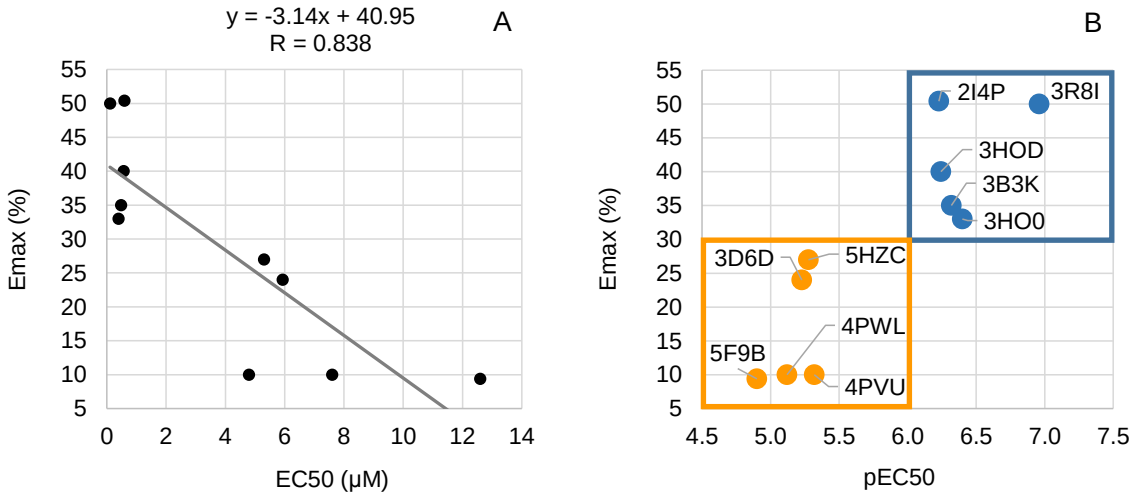

Fig. 2 Correlation of E_{max} to EC_{50} (A) and to pEC_{50} (B) values of the 10 PPARγ partial agonists

A visual inspection of the complexes allowed for further interpretation of this biological data-based clustering in the context of a preferred occupation of particular subregions in the large receptor's pocket. As shown in Fig. 3 the partial agonists from the subclass with higher E_{max} values are either located entirely in Arm I or occupy Arms I and III, while the representatives of the lower E_{max} subclass occupy Arms II and III. The possible suboptimal stabilisation of the activation helix H12 for the higher E_{max} subclass' partial agonists, compared to partial agonists in the lower E_{max} subclass suggests differences in the mechanisms of action between strong and weak partial agonists. In order to investigate the possibility for differentiation

between the two subclasses, based on the estimations of their binding energies, the 10 selected complexes were subjected to redocking.

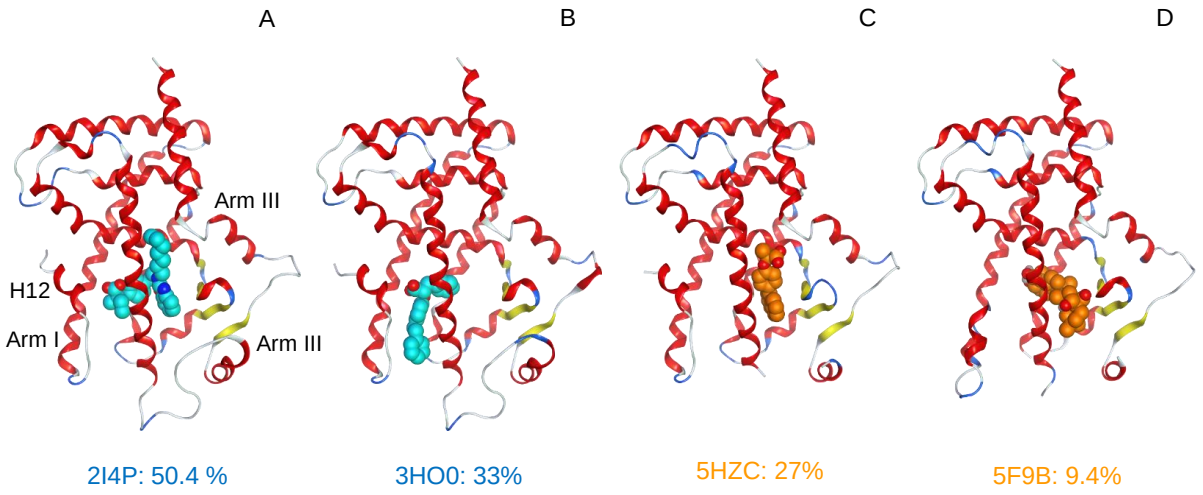

Fig. 3 Ligands' binding modes and relative efficacy data of representatives of the strong (A, B) and weak (C, D) subclasses PPARγ partial agonists

Redocking simulations with the selected set of PPARγ-partial agonist complexes

In total 50 docking runs were performed applying the 5 scoring functions, implemented in MOE, to the 10 receptor-ligand complexes. After selection of the best poses from the 50 molecular docking output sets, an analysis of the relationships between their docking scores and the experimentally measured pEC_{50} values was performed. This comparative analysis of the performance of the different docking protocols outlined the potential of two scoring functions (ASE and London dG) to reproduce the pre-established E_{max}-based discrimination of the partial agonists (Fig. 4). The selection of these particular scoring functions for further docking protocol optimization was additionally supported by the better correlation of the corresponding scores to the E_{max} values of the docked ligands (ASE, R = 0.6; London dG, R = 0.9). The docking scores relate to the binding energy of the complexes and are associated with the ligand's binding affinity. However, our analyses reveal also a relation between the gradually changing receptor activation and the score ranking of the PPARγ-partial agonist complexes. Stephenson had stated that the agonist's potency was determined both by its efficacy and its affinity for the receptors [21]. In this context, the established correlation between the ligands' potency (pEC_{50}) and the docking scores seemed reasonable. As illustrated in Fig. 4, the London dG gave better results. In Fig. 4B the energetically less favourable scoring range for the London dG function (between -9 and -12) is associated with the lower-efficacy partial agonists, while the energy estimation between -12 and -15 (suggesting a higher affinity of the ligands) is characteristic for the ligands with higher E_{max} values.

In order to compare the performance of the redocking simulations using the ASE and London dG scoring functions, we applied a Min-Max scaling to the scores of each output set of 30 poses. Comparing the scoring ranges of the 10 selected best poses for each scoring function, the London dG-based redocking produced a lower boundary (0) compared to the ASE-based redocking (0.3). This means that the London dG scoring function ranks the best poses better compared to the ASE scoring one. A detailed analysis of the scoring functions by complexes and a selection of the scoring function which gives the lower scaled value for each complex, confirmed the superiority of London dG to ASE scoring in the redocking of the

PPARγ partial agonists (Table 1). The comparison of the scaled RMSDs for these scoring functions resulted in very close ranges from 0 to 0.18 (ASE) or from 0 to 0.2 (London dG). The total ranking by complexes gives a precedence to the London dG function (Table 1).

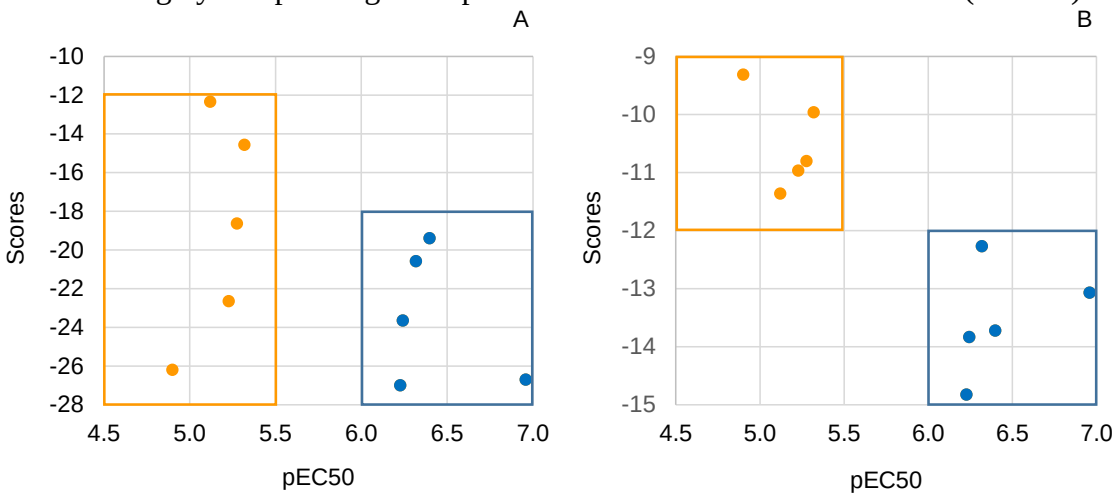

Fig. 4 Ligands' clustering based on the pEC_{50} values and the docking scores of the ASE- (A) and London dG-based scoring (B)

Table 1. Comparative analysis of the ASE and London dG (LdG) scoring functions regarding the scaled scores and RMSD values of the best poses of partial agonists

PDB ID (E_{max}, %)	Scoring		RMSD	
	ASE	LdG	ASE	LdG
2I4P (50.4)		*		*
3R8I (50)		*	*	
3HOD (40)		*	*	
3B3K (35)	*			*
3HO0 (33)		*		*
5HZC (27)	*			*
3D6D (24)		*		*
4PVU (10)	*			*
4PWL (10)		*	*	
5F9B (9.4)		*	*	
Ranking	3	7	4	6

* indicates that the given function produced a better scaled score or RMSD for the complex

Conclusion

We recorded a significant correlation between the binding energy determined by the scoring function and the relative maximal efficacy of the partial agonists. The docking protocol based on the London dG scoring function permits reproduction of the experimental data and is suitable for docking of new compounds to assess their receptor interactions and predict their potential to act as PPARγ partial agonists. Overall, these results could be useful for further molecular modelling studies of novel PPARγ partial agonists by selection of reliable docking poses to predict their binding mode and for ranking them in respect to their agonistic activity using the calculated docking scores.

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