

Rational Discovery for identification of new lead PPAR γ agonists using rigid docking and a focused – consensus scoring function

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Introduction

R&D production of new drugs has remained constant over the last number of years with major pharmaceuticals each launching roughly one new drug per year(1) Traditionally these molecules are found either by: Serendipitous Random Screening, as Natural products as Leads in Drug Design, Combinatorial Approaches, Rapid Throughput Screening, Structure-Based Drug Design – 'in silico' screening (Virtual screening). The goal is to accelerate the overall drug discovery process, or to 'fail early' compounds that would fail further down the road in clinical or pre-clinical testing.

In this respect, we have developed a Virtual High Throughput Screening protocol a for the identification of new lead PPAR γ agonists based on a focused – consensus scoring function.

Significance of PPAR γ

- PPAR γ is a critical transcription factor in the regulation of adipocyte differentiation.
- PPAR γ is currently used as a target for the treatment of type 2 diabetes.
- PPAR γ agonist decreases the levels of tryglycerides, cholesterol, and fatty acids in dyslipidemia.
- PPAR γ agonists have shown to decrease blood pressure in animal models suggesting their ability to prevent hypertension.
- PPAR γ agonists have been show to trigger cell cycle arrest fibroblasts and malignantly transformed adipogenic cells.

Binding of PPAR γ Agonist with the receptor LBD⁽¹⁾

The structure of PPAR γ LBD has been determined by X-ray crystallography and the structure has been solved in the absence of ligand (apoPPAR) and bound with several agonists.

- The Crystal structures revealed a large (1300 Å) Y-shaped ligand-binding site.
- The LBD site extends from C-terminal α -helix (AF-2) to the β -sheet between helices 3 and 6.
- Common interaction among several ppar agonists are the H bond interaction with His⁴⁴⁹, His³²³ and Tyr⁴⁷³ the last one lies on helix AF-2.
- It's likely that the interaction with AF-2 locks the receptor in an activated state.



Figure 1: PPAR γ LBD bound with agonist TZD

Validation study

A haystack built from 1000 'drug-like' compounds from WDI collection (Daylight) was seeded with a set of known actives (19 PPARs agonists).

The PPAR agonists we selected belonged to the family of TZD and Dihydro cinnamate derivate (fig 2), we did not include the Tyrosine derivate because they show slightly different modality in binding the receptor.

Physiochemical properties such as cLogP, numbers of H-bond donors/acceptors, numbers of rotatable bonds calculated and 2D filters were applied to remove inorganics, and compounds that were not drug-like (Filter light by openeyes updated for our purpose). The filtered dataset was converted from 2-D to 3- D using CORINA and conformational ensembles generated subsequently using OMEGA, to allow sequential docking of each ligand in the dataset.

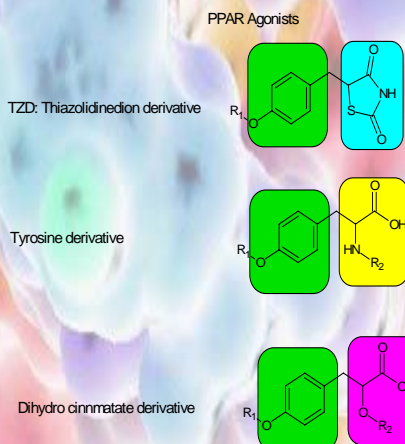


Figure 2: Main classes of PPAR agonists

Name	RMSD (min values)	
	MMFF	noMin
1FM9	1.43	1
1I7I	0.8	0.93
1K74	1.37	1.28
1KNU	0.91	0.71
1NYX	0.77	0.7
1WMO	0.51	0.76
2PRG	0.79	0.52

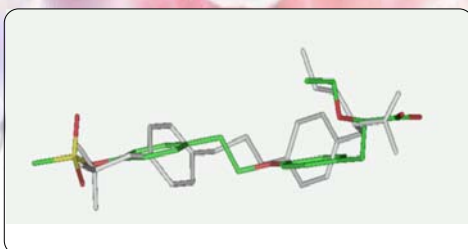
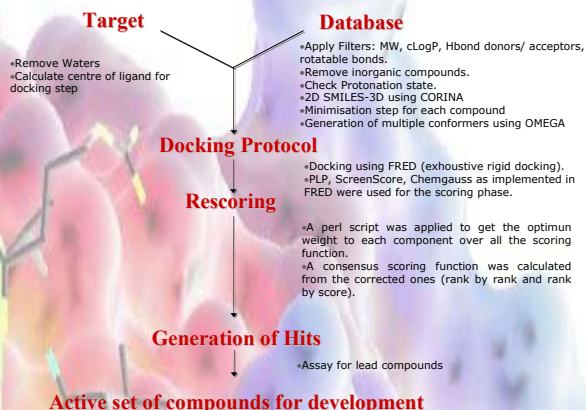


Figure 3: Left: RMSD table of Crystal Structure and conformers Generated with corina-omega (minimised and non). Right: Example of superposition with the conformer generated (rmsd 1=1.2)

Summary of Virtual Screening Protocol



Screening Result

The enrichment rate is the increase in the proportion of hits found in any given sample of compounds, compared with the proportion expected from a random sample. In figure 3 we compare our focused scoring function with the single scoring function taken individually. The focused scoring function is obtained using a perl script that applies different weights to some components of the scoring function (ex: Hydrogen Bond, Rotatable Bond). The same script then calculates the consensus scoring function (Rank by Rank). Figure 4 shows the improvement in applying the coefficients to the scoring function evaluating the first 5 positions on the database screened.

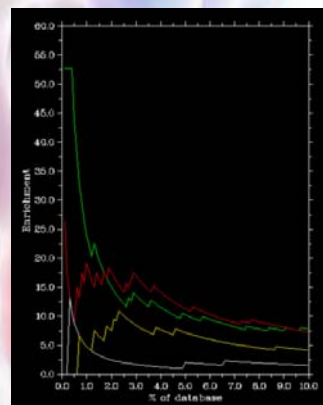
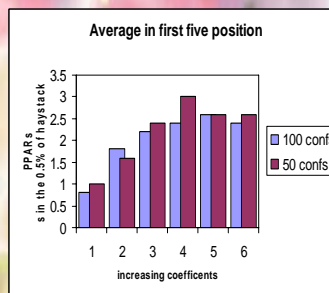


Figure 3 shows the enrichment using each scoring function and our focused one to re-score the database of ligands. Green: Our protocol, Red: Screenscore, White: PLP, Yellow: ChemoGauss.

Figure 4. The graph shows the increase of hits in the first 0.5% of the database when the coefficients of each scoring function are properly corrected. There is no improvement generating more conformers.



We then scanned the database Peakdale (about 8000 structures). We pre-filtered the database using a modified version of Filter (daylight) including some 2D descriptors specific for PPARs gamma. We generated the conformers using Omega (Openeye) and finally we docked and scored the structures using FRED (Openeye). As well as for the training set we applied our own focused-consensus scoring function. In figure 5 we show an example of one of the structures retrieved.



Fig 5. Docked structure of a hit retrieved scanning the database Peakdale (about 8000 structures).

REFERENCE

Wilson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. The PPARs: from orphan receptors to drug discovery. *J Med Chem* 2000, 43, 527-550.