Rational Discovery and Identification of New Lead Molecules Active on PPAR gamma using Flexible Docking Methodologies

Valerie Jean Smart, Giorgio Carta, Valeria Onnis, Dr. Darren Fayne, Dr. Daniela M. Zisterer, Dr. David G. Lloyd
Molecular Design Group – School of Biochemistry and Immunology – Trinity College Dublin

Introduction

In this project, we have validated and developed a Virtual Screening protocol employing a flexible docking program, Surflex to 'fish' for novel ligands for PPAR gamma that can potentially be useful therapeutically.
The Virtual Screening protocol includes the use of a flexible docking program Surflex which takes into account the 3D shape of the receptor's active site by calculating the conformations of the ligand inside the binding pocket.

Significance of PPAR-gamma:

PPARγ is a critical transcription factor in the regulation of adipocyte differentiation as illustrated in the picture here[1]. Role of PPARγ in glucose metabolism/insulin sensitivity and antidiabetic action. PPARγ agonists are involved in controlling the metabolism of tryglycerides, and cholesterol in adipose tissue. PPARγ agonists have been shown to decrease blood pressure in animal models suggesting their ability to prevent hypertension, suggesting potential use for treatment of cardiovascular disease. PPARγ is a potential candidate for use in the treatment of Cancer. PPARγ. Protective in models of Inflammation and has an immunomodulatory role in the innate immune system.

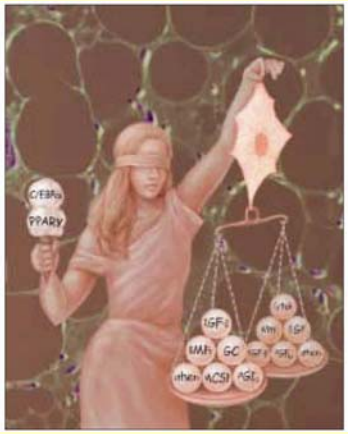
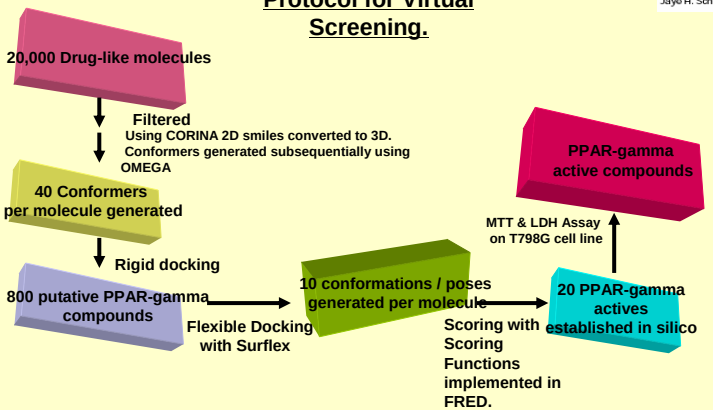


Fig. 2. The weighty decision of the preadipocyte. The imagery of this figure is that of the Goddess of Justice, who blindly weighs the balance of evidence and if necessary, uses the sword to exact justice. In this case, the Goddess of Adiposity weighs the positive and negative forces experienced by the preadipocyte. If the scales fall towards adipogenesis, she uses C/EBPα and PPARγ (here represented as a high-fat ice cream cone) to stimulate preadipocyte differentiation. Abbreviations: BMPs, bone morphogenetic proteins; C/EBP, CCAAT/enhancer binding protein; Cytok, inflammatory cytokines; EGF, epidermal growth factor; GC, glucocorticoids; IGF-I, insulin-like growth factor-I; MCSF, macrophage colony-stimulating factor; $PGF_{2\alpha}$, prostaglandin F2α; PGI_2, prostacyclin; PPAR, peroxisome proliferator-activated receptor; TGF-β, transforming growth factor β. Artwork by Jaye H. Schlesinger.

Protocol for Virtual Screening.

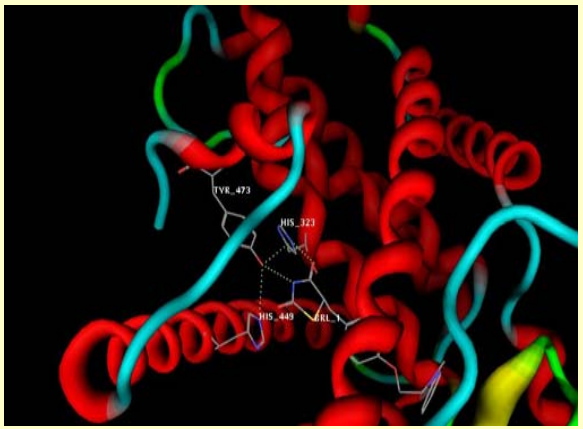


Virtual Screening Protocol:

The validation part was carried out by using a small database of PPARγ actives (needles) added to the validation database (haystack). We then tested Surflex's ability to retrieve the needles from the haystack, i.e. identify the PPARγ actives from the database. This ability is evaluated by calculating the enrichment of the results.
It is also important to ensure that the poses generated by Surflex for the compounds are docked and orientated in the correct position. We evaluate this by taking the pose that Surflex has scored as the best conformation of the active compound, and superimposed it onto the pose of the ligand in the protomol. We calculate RMSD (Root Mean Square Deviation) values to evaluate the similarity of the poses.

The scoring functions implemented in the Surflex docking tool did not rank the compounds in the correct order so we used the scoring functions implemented in a program called FRED instead to score and rank the compounds.

Application of virtual screen

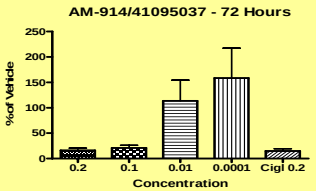


Surflex employs an idealized active site ligand (in our case AZ242) (called a protomol) as a target to gene generate putative poses of molecules or molecular fragments. This protomol takes into account residues essential for ligand binding and agonist activity in the ligand binding site.
At least four residues essential for ligand binding and agonist activity have been identified in the PPAR family of nuclear receptors. Common interactions seen in several PPAR agonists are the H bond interactions with His449, His323 and Tyr473. Tyr473 lies on helix AF-2. The ligand-binding site (1300 A) is Y shaped as can be seen in Fig.15 (a) above. The LBD extends from C-terminal α-helix (AF-2). Most known PPAR agonists share features such as a hydrophilic head group, a central hydrophobic part and a flexible linker tail as shown.

Results & Biological Activity

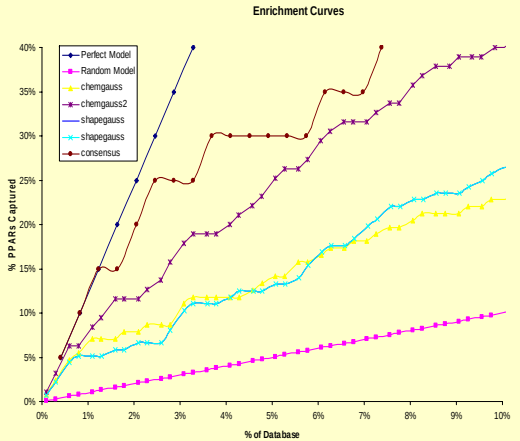
Hits retrieve through this protocol were then tested for antiproliferative and toxicity effects (MTT assay and LDH assay) on cell line T98G, which growth is known to be sensitive to PPARγ agonists.

To determine the activity in vitro of the compounds found to be actives in silico the standard MTT assay was used. Then the compounds were tested using an LDH assay to test for cytotoxicity. Four Compounds were tested. Two exhibited inhibition of proliferation of a human T798G cell line at micromolar concentrations. Activity was correlated to that of ciglitazone through the use of a control ciglitazone reference standard. Unfortunately all compounds were found to be cytotoxic when tested with the LDH assay.



Compound 1 after treatment for 72 hours, Absorbance (595nm)

Applying consensus scoring functions as implemented in FRED in our protocol gave the best enrichment values as shown below:



Enrichment rate using several protocols.

Conclusion

Blab blalb ablabla

References:

1. MacDougald O.A. & Mandrup S.(2002) Adipogenesis:forces that tip the scales. *TRENDS Endocrinol. Metab*, **13**, 5-11