Identification of Lead Molecules Capable of the Simultaneous Agonism of the PPARγ and PPARα Subtypes for the Dual Management of Diabetes Mellitus and Dyslipidaemia

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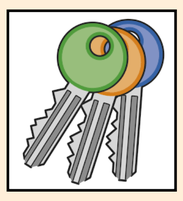
INTRODUCTION

This is a rational drug design study aiming to identify lead molecules capable of successfully interacting with more than one Peroxisome Proliferator Activated Receptor (PPAR) subtype in order to simultaneously manage more than one PPAR mediated condition. The PPARs are nuclear receptors involved in the regulation of cellular differentiation, development, and metabolism (carbohydrate, lipid, protein), and tumorigenesis of higher organisms.



This study stems from the loss of the glitazone hypoglycaemics (full PPARγ agonists) from the market due to their unacceptable side effect profile, and from the realisation that full PPARγ agonism could not be separated from this adverse effect spectrum. It uses the PPARγ partial agonist angiotensin receptor blocker telmisartan (pdb ID 3VN2[1]) and an experimental fibrate PPARα agonist GW590735 (pdb ID 2P54[5]) to probe these respective PPAR Ligand Binding Pockets (PPAR_LBPs).

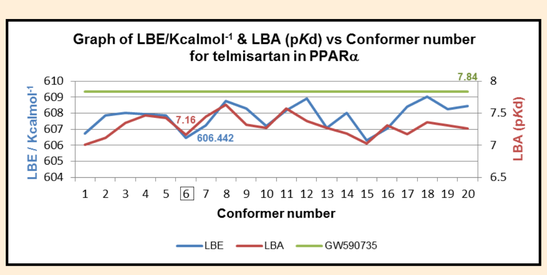
AIMS

- To understand the interactions forged between PPARγ and telmisartan and those arising between those of PPARα and the experimental fibrate GW590735.
- To compare the interactions observed between PPARγ and telmisartan to those forged by the endogenous ligand 13-hydroxyoctadecadienoic acid with PPARγ as described in pdb crystallographic deposition 2VST[2].
- To design *de novo*, analog series of PPAR modulators based on the scaffolds of the PPARγ partial agonist telmisartan and on that of the PPARα fibrate agonist GW590735.
- To identify, through virtual screening, using the online database ViCi[3], molecules which are spatially and electronically similar to the lead structures telmisartan and GW590735.
- To filter the molecular cohorts obtained from each approach on the bases of affinity to both PPARγ/α and of Lipinski Rule compliance.
- To analyse the structures, physicochemical parameters and critical interactions forged by the selected molecules and to compare these to known complete and partial dual PPARγ/α agonists.

METHOD

The small molecules telmisartan and GW590735 were extracted from the LBP of their cognate receptors such that *in silico* binding affinity (p*K*d) could be quantified in XScore®[8]. These values were established as baselines for comparison for each receptor subtype.

The extracted small molecules telmisartan and GW590735 were docked into their non-cognate counterparts, conformational analysis (Graph 1) performed in each case and affinity of the optimal conformation for its non-cognate LBP quantified in XScore®[8].



Graph 1: Graph of LBE/Kcalmol^{-1} and LBA (p*K*d) vs Conformer number for telmisartan docked in PPARα. The LBA (p*K*d) of the PPARα agonist GW590735 for its cognate receptor is included as a reference as a green horizontal line.

These optimal conformations were used in parallel processes:

The first was a virtual screening exercise, using the molecular database ViCi[3], in which they were used as query molecules for the identification of spatially and electronically analogous structures capable of forging similar or enhanced interactions within the non-cognate LBPs. This process yielded molecular cohorts for each query molecule ranked in order of similarity to the query.

The second was a fragment based *de novo* approach which was carried out using LigBuilder® v1.2[7]. Here, molecular moieties from the telmisartan and GW590735 scaffolds considered critical to binding were identified and modelled in Sybyl® v1.2[6], in order to produce a number of seed structures capable of sustaining molecular growth at user directed sites designated as *H.spc* atoms subsequent to their being docked within the non-cognate LBPs.

Molecular growth was carried out using the Link and Grow Algorithms of LigBuilder® v1.2[7].

The resultant structures were, for each seed structure segregated into families of pharmacophoric similarity and ranked within each family according to ligand binding affinity. Physicochemical data including molecular weight and logP were also included, as was an indicator of synthetic feasibility.

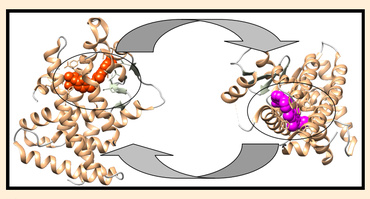


Figure 1:

Left Test molecule bound with non-cognate PPARα

Right Test molecule bound with cognate PPARγ

The molecular cohorts identified through both approaches were filtered for Lipinski Rule compliance. The molecules that survived filtering were then redocked into their original cognate receptor LBP (Figure 1), conformational analysis re-performed and the affinity of the optimal conformer measured for each using XScore®[8]. Comparison was made to the baseline and non-cognate receptor affinities initially established, and the molecules exhibiting dual affinities exceeding baseline values selected for further optimisation.

RESULTS

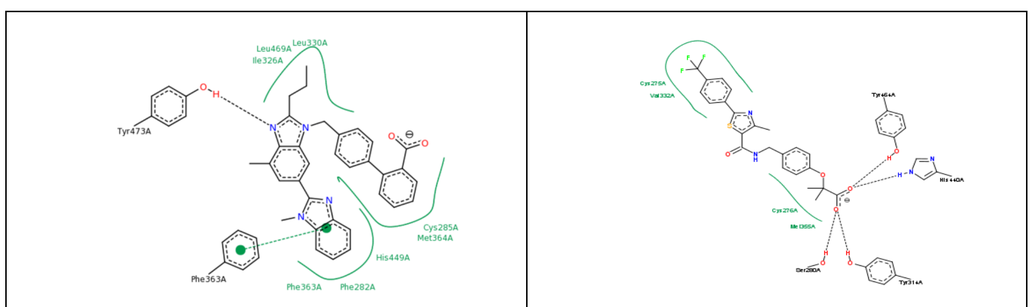
The Virtual Screening exercise yielded 1000 molecules for each submitted query (n=2). The *de novo* generated molecular cohorts (n=5) consisted of 200 molecules each.

Subsequent to filtering the generated cohorts from both approaches for Lipinski Rule compliance, molecules were ranked according to binding affinity (p*K*d) and logP values.

- 10 molecules from each molecular cohort (n=2) were chosen from the structures generated from the virtual screening exercise
- 5 molecules from each cohort (n=5) were chosen from the *de novo* generated structures

PoseView®[4] was used to generate 2D topology maps depicting the interactions of the template structures telmisartan and GW590735 in their cognate receptors and of the representative chosen molecules from both approaches as shown in figures 2, 3 and 4:

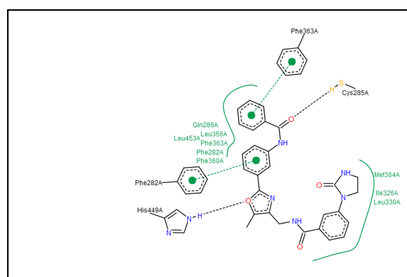
Figure 2 below shows the critical interactions forged by telmisartan and GW590735 with their cognate receptors



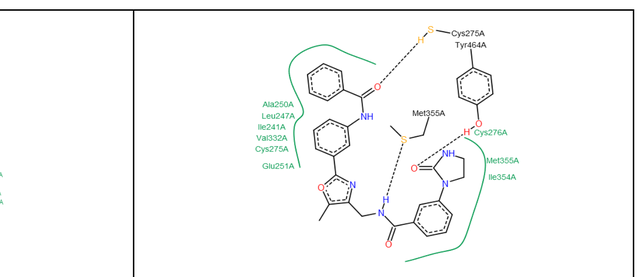
- Telmisartan has a LBA (p*K*d) of 9.26 for the cognate PPARγ LBP (pdb ID 3VN2)
- Hydrophobic interactions with residues Phe[282], Cys[285], Ile[326], Leu[330], Phe[363], Met[364], His[449], Leu[469], H-bond with Tyr[473] and π-π stacking with Phe[363]

- GW590735 has a LBA (p*K*d) of 7.84 for the cognate PPARα LBP (pdb ID 2P54)
- Hydrophobic interactions with residues Cys[275], Cys[276], Val[332] and Met[355],and H-bonds with Ser[280], Tyr[314], His[440] and Tyr[464]

Figure 3 below shows the optimal conformer of molecule 509 identified through virtual screening using the optimal conformer of telmisartan within its non-cognate PPARα_LBP as a query docked in the PPARγ LBP on the left and the PPARα LBP on the right

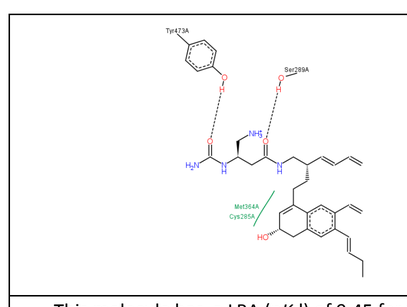


- This molecule has a LBA (p*K*d) of 8.44 for the PPARγ LBP
- Hydrophobic interactions with residues Phe[282], Ile[326], Leu[330], Phe[363] and Met[364] and π-π stacking with Phe[363] also seen in telmisartan with its cognate receptor

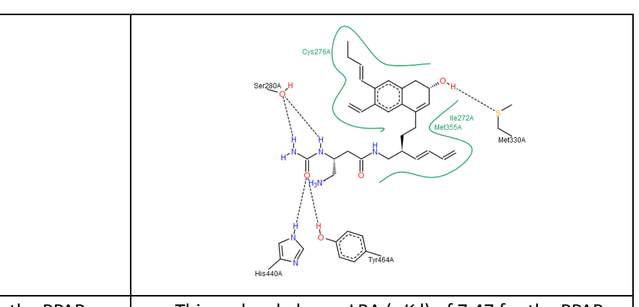


- This molecule has a LBA (p*K*d) of 7.73 for the PPARα LBP
- Hydrophobic interactions with residues Cys[275], Cys[276], Val[332] and Met[355],and H-bonds with Tyr[464] also seen in GW590735 with its cognate receptor

Figure 4 below shows molecule number 115 derived from the *de novo* generated GW5_seed001 molecular cohort docked in the PPARγ LBP on the left and the optimal conformer of molecule number 115 docked in the PPARα LBP on the right



- This molecule has a LBA (p*K*d) of 9.45 for the PPARγ LBP
- Hydrophobic interactions with residues, Cys[285], Met[364]and H-bond with Tyr[473] also seen in telmisartan with its cognate receptor



- This molecule has a LBA (p*K*d) of 7.47 for the PPARα LBP
- Hydrophobic interactions with residues, Cys[276], Met[355]and H-bonds with Ser[280], His[440] and Tyr[464] and also seen in GW90735 with its cognate receptor

CONCLUSION

A comparison of the physicochemical parameters and critical interactions of the molecules identified in this study to those of the lead molecules towards the PPARγ and PPARα LBPs indicates that the ligand-protein contacts that seem significant for dual agonism are:

- Hydrophobic contacts with Met[364] in the PPARγ receptor and with Cys[276] and Met[355] in the PPARα receptor
- Hydrophilic contact with Tyr[464] in the PPARα receptor

Preliminary results consequently suggest that further optimisation of these molecules could have significant clinical impact, with dual PPARγ/α agonists having the potential of simultaneously managing type 2 diabetes mellitus and hypertriglyceridaemia. The added antihypertensive effect of the telmisartan scaffold increases the breadth of the potential effect of derivatives of this molecule to the management of the highly prevalent metabolic syndrome.

References:

1. Amano Y, Yamaguchi T, Ohno K, Niimi T, Orita M, Sakashita H, Takeuchi M. Structural basis for telmisartan-mediated partial activation of PPAR gamma. Hypertens Res. 2012 Feb 23;35:715-719.
2. Itoh T, Fairall L, Amin K, Inaba Y, Szanto A, Balint BL *et al.* Structural basis for the activation of PPAR gamma by oxidized fatty acids. Nat Struct Mol Biol. 2008 Sep;15(9):924-31.
3. Lamzin V. ViCi - In-silico Ligand-based Drug Design. 2016 [cited 2016 Apr 5]. Available from: EMBL Hamburg, http://www.embl-hamburg.de/vici/index
4. Rarey M, Stierand PC. PoseView – Molecular interaction patterns at a glance. *J Cheminform.* 2010; 2: 50. Available from: URL: www.poseview.de
5. Sierra ML, Beneton V, Boullay AB, Boyer T, Brewster AG, Donche F *et al.* Substituted 2-[(4-aminomethyl)phenoxy]-2-methylpropionic acid PPAR alpha agonists. Discovery of a novel series of potent HDLc raising agents. J Med Chem. 2007 Feb 22;50(4):685-95.
6. SYBYL-X 1.2, Tripos International, 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA, 2010.
7. Wang R, Gao Y, Lai L. LigBuilder. A multi-purpose program for structure-based drug design. J Mol Model. 2000:6:498-516.
8. Wang R, Liu L, Lai L, Tang Y. SCORE: A New Empirical Method for Estimating the Binding Affinity of a Protein-Ligand Complex. J Mol Model. 1998:4:379-394.