ISSN 0976 – 9080

BIOMIRROR

An Open Access Journal

In Silico Identification Of A Potentially Novel Binding Modality For 1,3-Dicarbonyl Compounds Having 2(3H)-Benzazolonic Heterocycles Within The PPARγ Ligand Binding Pocket: A *De Novo* Design Study

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ARTICLE INFO

Received 22 November 2013
Revised 29 November 2013
Accepted 02 December 2013
Available Online 04 January 2014

Keywords:
T2DM,
PPAR,
SPPARγMs,
Thiazolidinediones,
S-26948.

ABSTRACT

Rosiglitazone withdrawal from the market has led to a renewed interest in the Peroxisome Proliferator Activated Receptor γ (PPARγ) as target for hypoglycaemic therapy – this time, via partial agonism. This may be achieved by using selective PPARγ modulators such as S-26948. A receptor-based drug design approach was adopted in this study, using the bound conformation of rosiglitazone within the PPARγ ligand binding pocket to identify S-26948 conformers, and consequently generate high affinity novel molecules. S-26948 conformer 17 was chosen, which exhibited an alternative binding modality with respect to rosiglitazone. Ligand binding pocket mapping of this orientation identified a larger pocket with respect to that delineated by the bound coordinates of rosiglitazone, and an additional theoretical novel pocket within PPARγ. Therefore, currently used PPARγ ligands may not occupy the entire breadth of the ligand binding pocket, warranting further investigation from a receptor modality point of view. Key words: T2DM, PPAR, SPPARγMs, Thiazolidinediones, S-26948.

Introduction:

Peroxisome Proliferator Activated Receptors (PPARs) represent a family of nuclear receptors (NRs) which are at the basis of various metabolic processes within the body – including glycaemic control[1]. Three subtypes of the PPAR receptor – the α, the γ, and the β/δ have been described[2,3,4], of which, the major target for glycaemic control in Type II Diabetes Mellitus (T2DM) is the γ subtype, as exemplified by the thiazolidinediones (TZDs)[3,5,6]. The 2010 withdrawal of rosiglitazone[7], a TZD, from the market, has left a lacuna in contemporary hypoglycaemic therapy – in turn spurring renewed rational design efforts at PPARγ as a design target.

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Citation: Stephanie Portelli * (In silico identification of a potentially novel binding modality for 1,3-dicarbonyl compounds having 2(3H)-benzazolonic heterocycles within the PPARγ Ligand Binding Pocket: a de novo design study)
BIOMIRROR: 1-7 / bm-2225010414

There is evidence[3] in the literature that different ligands bring about their action on the PPARγ receptor according to their structure – via the degree of Helix 12 (H12) stabilisation. Full agonists, such as the recently withdrawn rosiglitazone[7], stabilise H12 enough to recruit coactivators to a high extent, while partial agonists or selective PPARγ modulators (SPPARγMs), bring about improper H12 stabilisation – hence leading to a lesser recruitment of coactivators and/or lesser dissociation of corepressors.[3] This is the theoretical basis behind newer PPARγ-directed approaches – where the use of SPPARγMs is hypothesised to achieve the same therapeutic outcomes related to insulin sensitization, but with less adverse events related to body weight and dyslipideamia[3,6,8]. In the 2007 study led by Carmona[8], the experimental molecule S-26948 showed evidence of such traits, in that it exhibited a differential coactivator recruitment with respect to rosiglitazone, being unable to form neither PPARγ/DRIP205 nor PPARγ/PGC-1α complexes. The same study showed, however, that S-26948 was able to dose-dependently activate PPARγ, with a comparable EC_{50} and ligand binding affinity (LBA) to rosiglitazone, all the while not contributing to the increase in triglyceride concentration, hence not inducing weight gain in animal diabetic models, as opposed to rosiglitazone.[8]

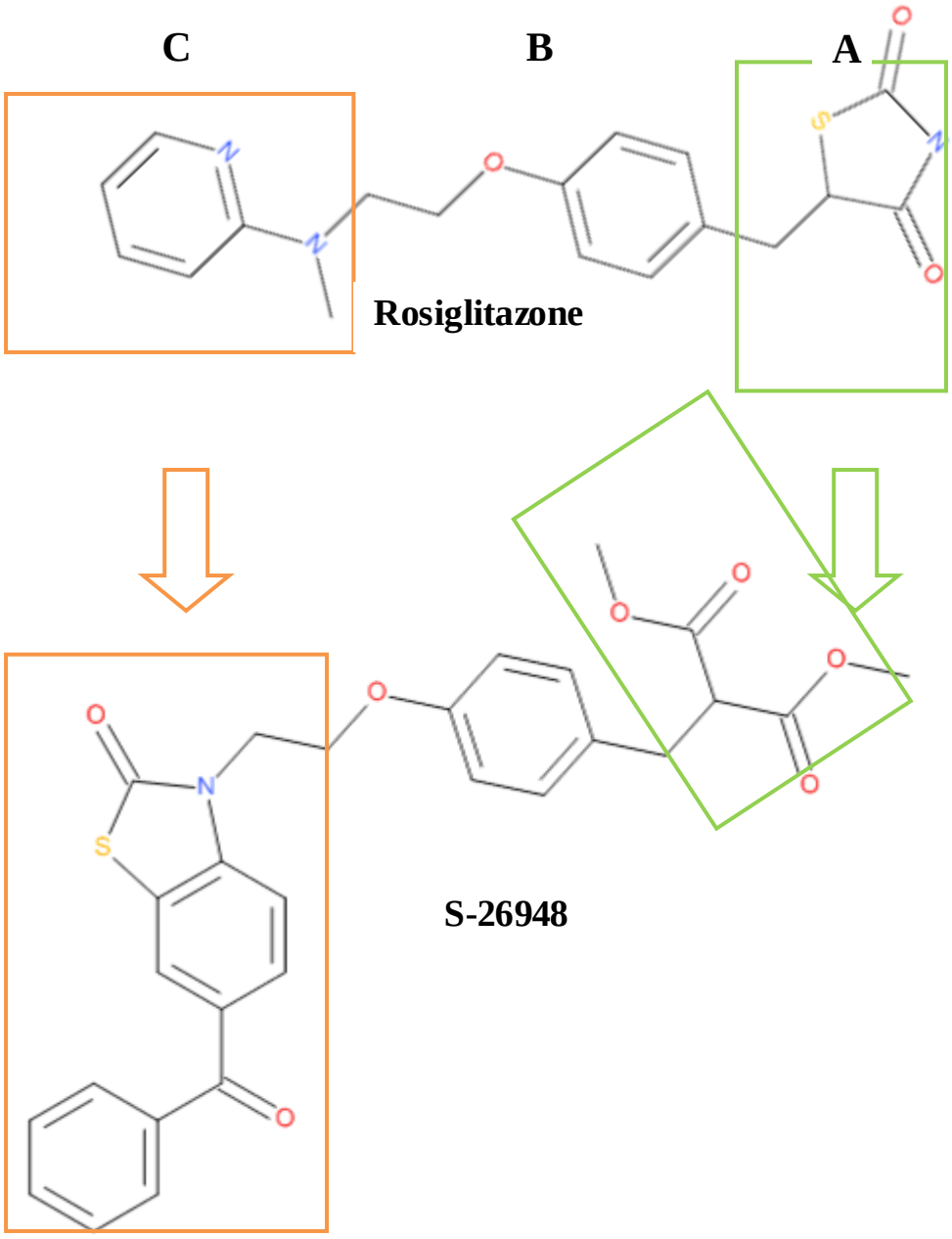
In a study led by Blanc-Delmas[9] in 2006, S-26948 was described as being a chemical derivative of the TZDs, where both structural backbones can be divided into three separate regions (Fig. 1): the acidic head (A), the linker region (B), and the hydrophobic tail (C). In synthesizing S-

26948, the thiazolidine-2,4-dione moiety (Fig. 1) was replaced with a non-cyclic 1,3-dicarbonyl derivative - the dimethyl malonate moiety (Fig.1).[9] The methylamino-pyridin-2-yl moiety in rosiglitazone, was in turn, replaced with a benzazolonic heterocycle – benzaldehyde-2(3H)-benzothiazolone (Fig. 1) while the linker region – 2-ethoxybenzyl, remained the same.[9]

Therefore, we have identified S-26948[8,9] as a suitable lead for this study, and as a template for novel structure generation. In choosing a PPARγ crystallographic structure, protein data bank (PDB) deposition 1FM6[10] was chosen for the purpose of this drug design exercise, since it is bound to rosiglitazone – to which, its derivative S-26948[9] was already compared during *in vivo* studies[8].

Therefore, it was decided to obtain and compare the conformations of S-26948 which bind with highest affinity to the three receptor conformations, and thence use these posologies to generate novel molecular cohorts in a *de novo* drug design approach.

Figure 1: A 2-Dimensional representation of the molecular similarities between rosiglitazone (above) and S -26948 (below); showing the changes carried out during the synthesis of S -26948. A denotes the acidic head, B the linker region, while C the hydrophobic tail.



Materials and Methods:

This study utilised a receptor based drug design approach[12] having a similar framework to that used by Ciantar[13] *et al* in 2012. The PPARγ ligand binding pocket (LBP) obtained from the rosiglitazone bound coordinates of PPARγ (PDB entry 1FM6)[10] was used as a template for the development of novel structures – all with the potential of obtaining glycaemic control *in vivo*, without, in this case, predisposing the patient to cardiac irregularities and side effects, as did the recently withdrawn rosiglitazone[7]. In order to facilitate the computational process, PDB entry 1FM6[10] was edited in a process that sought to remove all moieties co-crystallised with the protein during X-ray crystallography that were not considered critical to ligand binding. Therefore, prior to lead docking, the PDB entry 1FM6[10] was modelled in Sybyl-X® v1.1 with the initial removal of one of its dimers, followed by removal of crystallised water molecules at a distance ≥ 5 Å from the LBP, and the extraction of its cognate ligand rosiglitazone from its LBP. The two dimers in PDB entry 1FM6 were found to be similar, with the only differences being those pertaining to the steroid receptor coactivator-1 (SRC-1) peptides, which are not involved in ligand binding.[10] Therefore, one of the two dimers (composed of chains U, V, X and Y of PDB entry 1FM6)[10] was arbitrarily chosen in this preliminary step on the premise that either dimer would yield identical results.

Next, the score algorithm was used in order to calculate the predicted *in silico* LBA (p*K*d) of rosiglitazone for its cognate LBP. Sybyl-X® v1.1 was then used in order to construct *de novo* and minimise S-26948, which was subsequently guided, using the similarity suite algorithm of Sybyl-X® v1.1 into the rosiglitazone bound conformation of the PPARγ LBP (PDB entry 1FM6)[10] with conformational freedom being allowed within the confines of the respective PPARγ LBP. This algorithm subjected S-26948 to bond rotation, torsion and bending into a number of alternative positions, or conformations, all based on the bioactive conformation of rosiglitazone, thereby resulting in the identification of the 20 highest affinity S-26948 conformers for the rosiglitazone bound PPARγ LBP (PDB entry 1FM6).[10] This was followed by binding energy calculations for each conformer, expressed in kcalmol^{-1} and computed using Sybyl-X® v1.1. The *in silico* predicted LBA (p*K*d) of each conformer was obtained following scoring using X-SCORE® v1.3,[14] and a graph of LBA (p*K*d) and binding energies (kcalmol^{-1}) (*y*-axis) versus conformer number (*x*-axis) were plotted for the rosiglitazone bound PPARγ LBP (PDB entry 1FM6)[10] (Fig. 2). From this conformer cohort, the best conformer was chosen on the basis of the highest LBA (p*K*d) and lowest binding energy – using binding energy and binding affinity graphs, and also using visualisation tools such as VMD® v1.9[15] in order to observe the conformers visually (Figs. 2 and 3).

Figure 2: Graph showing the predicted *in silico* average ligand binding affinity (p*K*d) and binding energy (kcalmol^{-1}) of the 20 different S-26948 conformers for the rosiglitazone bound conformation of PPARγ LBP (PDB entry 1FM6). Chosen conformer 17 is marked in green

Graph of predicted *in silico* average ligand binding affinity (p*K*d) and binding energy (kcalmol^{-1}) of the 20 conformers of S-26948 generated within the rosiglitazone bound conformation of the PPARγ_LBP (PDB entry 1FM6)

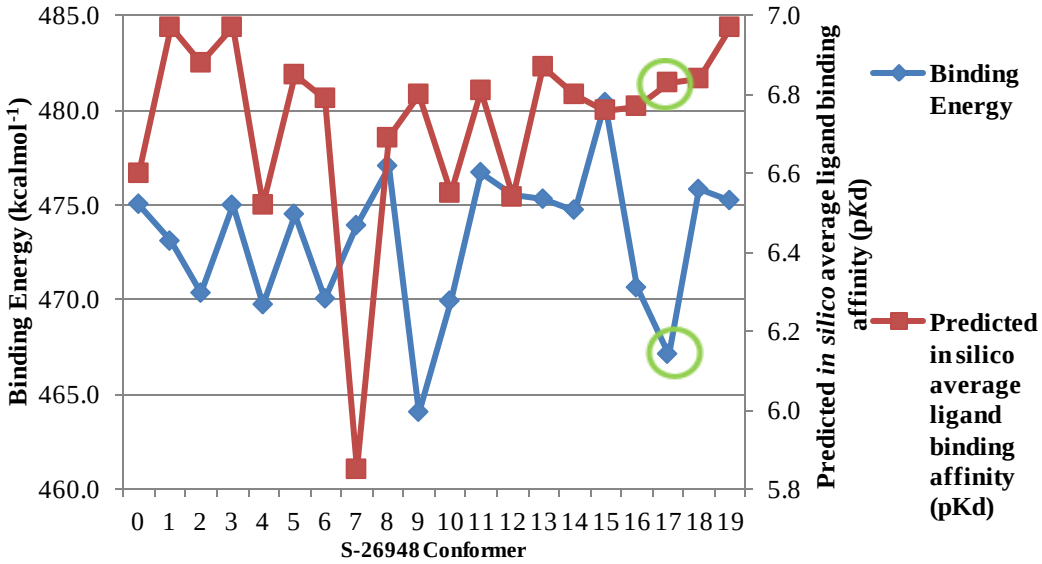
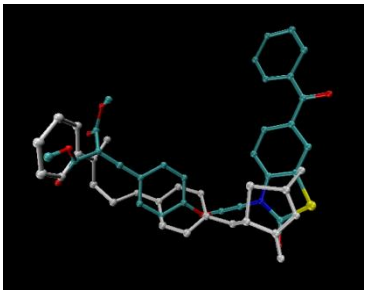


Figure 3: A 3-dimensional representation of rosiglitazone (white) and S-26948 conformer 17 (cyan) showing alternative binding modalities, rendered using VMD® v1.9



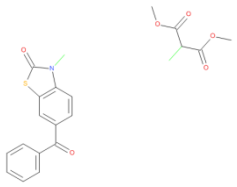
Analysis of LBA, corresponding binding energy and of visual representations, led to the identification of one particular conformer (conformer 17) within the rosiglitazone bound conformation of the PPARγ LBP (PDB entry 1FM6)[10]. Conformer 17 was considered significant from a drug design perspective owing to the fact that it exhibited an alternative orientation with respect to rosiglitazone, when the positioning of the functional moieties was compared (Fig. 3). More specifically, the dimethyl malonate moiety, representing the acidic head of S-26948, was superimposed on the methylamino-pyridin-2-yl moiety of rosiglitazone, as opposed to thiazolidine-2,4-dione moiety, which is the acidic head of rosiglitazone (Fig. 3). Moreover, the benzaldehyde-2(3H)-benzothiazolone moiety, representing the hydrophobic tail of S-26948, was superimposed on the acidic head of rosiglitazone, that is, the thiazolidine-2,4-dione moiety (Fig. 3). For this reason, it was decided to explore the possibility of alternative binding modalities within the PPARγ LBP, via a drug design exercise, aiming at the generation of novel molecules with high affinity to the same LBP, while also having an alternative binding modality with respect to known PPARγ agonists as is rosiglitazone.

Sybyl-X® v1.1 was used in order to model conformer 17, creating seed structures retaining, as evidenced by structure activity relationship (SAR) studies found in the literature[3,9], the moieties considered fundamental to partial agonism at the PPARγ LBP. These moieties were found to be the acidic head, as exemplified via the dimethyl malonate moiety within S-26948, and the hydrophobic tail, exemplified via the benzaldehyde-2(3H)-benzothiazolone moiety.[9] Hence, 3 seed structures were created, Seed A comprising the acidic head, Seed B the hydrophobic tail, and Seed C having both these moieties without the atoms linking the extremities (Fig. 4). These seeds were assigned growing sites, (through the designation of *H.spc* atoms) at which molecular growth was permitted, within the confines of the PPARγ LBP.

The 'pocket' algorithm within LigBuilder® v1.2[16] was used in order to map and analyse the 3-Dimensional structure of the rosiglitazone bound PPARγ LBP, also taking into account the nature of the amino acids lining the LBP. However this LBP map was only capable of accommodating Seed A (Fig. 4) which comprised the acidic head analogous to that of rosiglitazone. Seed B and consequently Seed C, (Fig 4) could not be rationally accommodated within the LBP space circumscribed by rosiglitazone when this was complexed with PPARγ. For this reason, a new LBP map was created, again using the 'pocket' module of LigBuilder® v1.2[16], this time based on the bound coordinates of S-26948 conformer 17. The 3 seeds were consequently docked within this novel pharmacophoric space which represented the newly identified binding modality of S-26948 conformer 17 at the interior of the PPARγ receptor. Following docking, the 'grow' (Seeds A and B) and 'link' (Seed C) algorithms of LigBuilder® v1.2[16] were invoked such that unidirectional growth could be sustained in the case of Seeds A and B, and that the separate moieties of Seed C could be joined together by a novel linker region.

In the analysis phase, where *de novo* generation created a large number of molecules, sample molecules were chosen from each family, on the notion that members of the same family present with similar structural moieties. The two molecules with the highest p*K*d and the two with the lowest p*K*d of each family of molecules were arbitrarily chosen, while an additional 2 molecules with intermediate p*K*d were chosen where families had a larger number of molecules. This was possible since members of the same family had similar structural characteristics, and were all listed in order of decreasing p*K*d – as computed via LigBuilder® v1.2.[16] The sample molecules were then rendered in Molsoft® ICM-Browser in both 2-Dimension and 3-Dimension, and analysed according to Lipinski Rule[17] compliance, the presence and locus of Hydrogen bond (H-bond) forming moieties, the sterric nature of the side chains – i.e. whether elongated aliphatic, or rigid and cyclic; and according to the presence or absence of intra-molecular H-bonding – owing to the fact that this could potentially hinder favourable interactions with the LBP. Binding energy ($kcalmol^{-1}$) calculations for the sample molecules were then computed using Sybyl-X® v1.1 and binding energy – binding affinity graphs were plotted in order to identify the *de novo* molecules with the highest p*K*d and lowest binding energy ($kcalmol^{-1}$) on the basis of highest LBA and greatest stability respectively.

Figure 4: A 2-dimensional representation of the moieties of S-26948 chosen to be used as seeds: the benzaldehyde-2(3H)-benzothiazolone moiety (left) was used as Seed B; the dimethyl malonate moiety (right) was used as Seed A, while both concomitantly were used as Seed C. The green bonds represent the growing sites assigned. The figure was rendered using Symyx® Draw v 4.0.



Results:

Three Novel structures were generated from Seed A. These structures were not compliant with the stipulations of Lipinski *et al*,[17] in predicting *in vivo* bioavailability – since all 3 had values for LogP over 5; with values ranging from 5.11 – 5.94, and a molecular mass over 500; with values ranging from 576 – 590. On the other hand, *de novo* design

using Seed B gave rise to 200 novel structures all of which were Lipinski Rule[17] compliant.

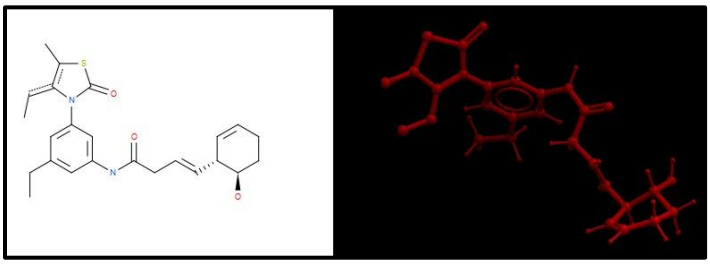
Furthermore, *de novo* design using Seed C yielded only 3 novel molecules all of which Lipinski Rule[17] compliant.

Therefore, a total of 206 molecules were generated in the *de novo* design phase of this study, while, of these, 203 (98.5%) were found to be Lipinski Rule[17] compliant. A total sample of 31 molecules were chosen for analysis.

The p*K*d for the 206 *de novo* molecules generated ranged from 5.08 to 7.94, while binding energy ranged from 112.063 kcalmol^{-1} to 453.377 kcalmol^{-1}. Moreover, molecular weight ranged from 305 to 590, and LogP ranged from 3.01 to 5.94.

The highest p*K*d recorded of all 206 molecules, having a value of 7.94 – which was considerably higher than that recorded for the cognate ligand rosiglitazone at 6.62, belonged to the Seed B cohort (Figs. 5 and 6)

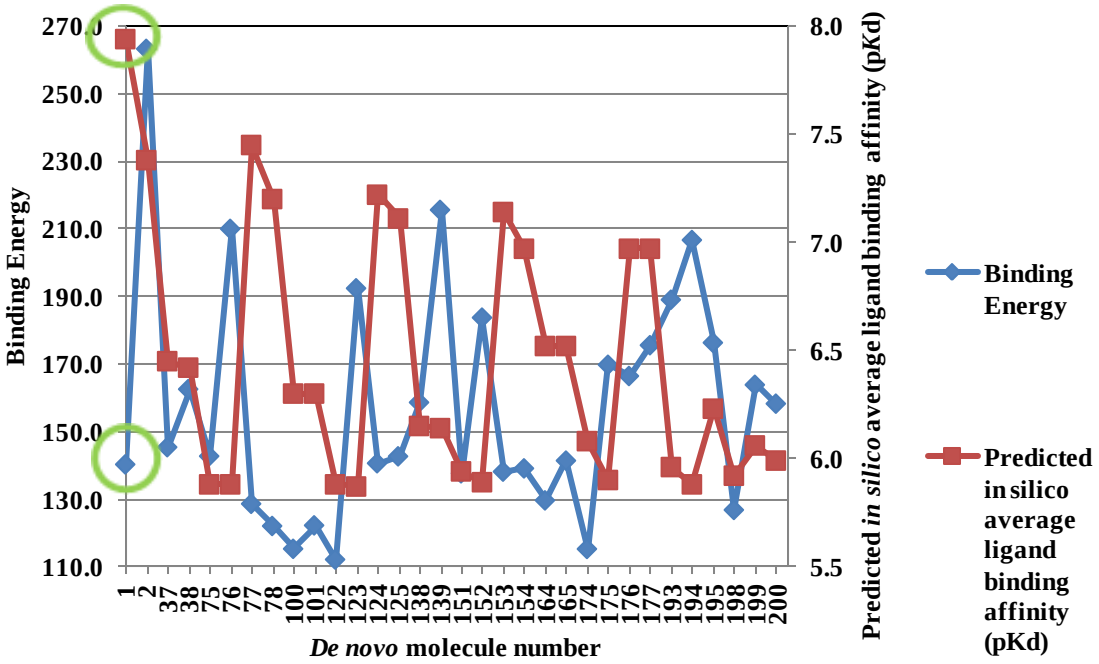
Figure 5: A 2-dimensional and 3-dimensional representation of the *de novo* generated molecule with the highest p*K*d – Molecule 1 of the Seed B cohort, as rendered in Molsoft® ICM Browser



When compared to the values of S-26948 conformer 17, molecules pertaining to the Seed C cohort, were seen to have a lower p*K*d, at values of 5.60, 5.50 and 5.43; and a lower binding energy, at values of 453.377 kcalmol^{-1}, 360.756 kcalmol^{-1}, and 338.870 kcalmol^{-1} respectively.

Figure 6: Graph showing the predicted *in silico* average ligand binding affinity (p*K*d) and binding energy (kcalmol^{-1}) of the sample *de novo* generated structures chosen from the Seed B cohort of S-26948 conformer 17, as generated within the S-26948 conformer 17 bound conformation of PPARγ_LBP (PDB entry 1FM6). Highest p*K*d value (molecule 1; 7.94) with corresponding binding energy value (140.234 kcalmol^{-1}) are marked in green

Graph of predicted *in silico* average ligand binding affinity (p*K*d) and binding energy (kcalmol^{-1}) of the sample *de novo* molecules of Seed B of conformer 17, generated within the S-26948 conformer 17 bound conformation of the PPARγ_LBP (PDB entry 1FM6)



Discussion:

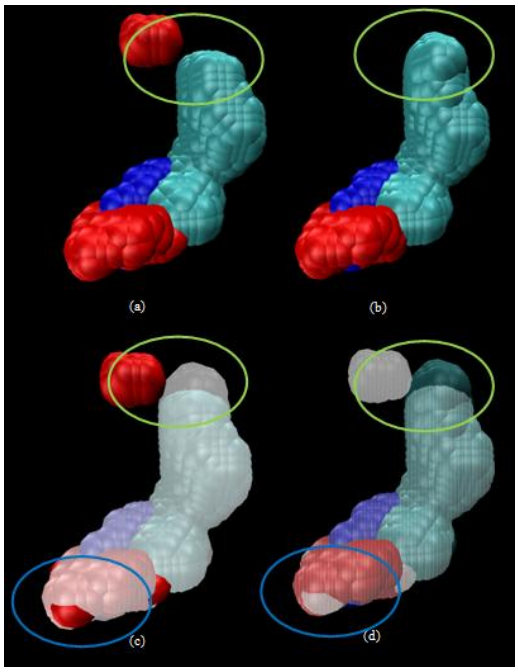
Analysis of the *de novo* generated molecular cohort is indicative of which structural moieties predispose to increased LBA (p*K*d). Specifically, it was apparent that an aliphatic chain terminating in a cyclic moiety within the hydrophobic locus was favorable to binding. Moreover, within the linker region, a cyclic substitution presented with a higher p*K*d, while a short aliphatic side chain terminating in a lateral H-bond donor was also favorable to binding. At this same region, a H-bond acceptor close to the acidic locus was found to increase p*K*d. Within the acidic region, a rigidified aliphatic side chain yielded molecules with a higher p*K*d, and was found more favorable to binding with respect to a cyclic moiety. Terminal H-bond forging moieties within the acidic region were also found to increase p*K*d.

Moreover, molecules within the Seed C cohort were all generated having a longer linker region when compared to S-26948, and a bulkier moiety close to the hydrophobic region – which could have caused sterric hindrance, leading to a lower p*K*d.

In exploring an alternative binding modality of ligands at the PPARγ LBP, as delineated via the orientation of S-26948 conformer 17, we have identified a hitherto unexplored channel within PPARγ, which is capable of being stabilised through small molecule binding. This new channel was able to accommodate a series of novel generated structures – having the structural prerequisites for partial agonism at the PPARγ LBP, while also abiding to the stipulations made by Lipinski *et al*[17] – which are the gold standard predictors of *in vivo* bioavailability. Further development of these *in silico* designed molecules could lead to a different coactivator recruitment – via an alternative H12 stabilisation; hence potentially reducing the possibility of inducing cardiac side effects which caused the marketing authorisation withdrawal of the TZD rosiglitazone[7].

Visual analysis of the binding pockets delineated by rosiglitazone and S-26948 conformer 17 within the PPARγ LBP, confirmed that a deeper, hydrophobic pocket was penetrated by the acidic head of S-26948 conformer 17, which is thought to be a similar locus to that penetrated by farglitazar[18]. More specifically, the hydrophobic area penetrated by rosiglitazone via its hydrophobic tail, and known to bind the bulkier hydrophobic tail of farglitazar[18] was also confirmed to be penetrated by the acidic head of S-26948 conformer 17 (Fig.7). Moreover, a theoretical novel binding pocket was penetrated via the benzaldehyde moiety of S-26948 conformer 17, since despite the superimposition of the latter moiety on the coordinates of the acidic thiazolidine-2,4-dione head of rosiglitazone, a high affinity S-26948 conformation (pKd: 6.83) comparable to that of rosiglitazone (6.62) was obtained.

Figure 7: A 3-dimensional representation of the rosiglitazone bound (a) and the S-26948 conformer 17 bound (b) configuration of the PPARγ LBP. Superimposition of the pockets (c) and (d) reveals a deeper pocket penetrated via the S-26948 conformer 17 delineated LBP. Changes are highlighted via the green circle, within which, in pocket (a), the hydrophobic tail of rosiglitazone is docked, while in pocket (b) the dimethyl malonate acidic head of S-26948 is docked. At the opposite terminal lies the theoretical novel binding pocket, penetrated via the benzaldehyde-2(3H)-benzothiazolone moiety at the opposing locus (blue circle)



Furthermore, the novel binding modality of S-26948 conformer 17 was identified within the rosiglitazone bound PPARγ LBP (PDB entry 1FM6)[10] according to a model that assumed a static LBP, and a small molecule that was allowed conformational rotation within a consequently confined space – which did not have the ability to move in tandem with the ligand. Nonetheless, it is probable, that the area within the PPARγ receptor currently assumed to form the LBP is actually larger than previously thought, and that the extended area to which this new molecular cohort binds, must be further exploited in the context of a rational drug design process. An in depth molecular dynamic study is essential in identifying the tandem motions of novel ligand and receptor – such that positive interactions could be further exploited in iterative optimizations.

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