

Article

Molecular Modeling of Allosteric Site of Isoform-Specific Inhibition of the Peroxisome Proliferator-Activated Receptor PPAR γ

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Abstract: The peroxisome proliferator-activated receptor gamma (PPAR γ) is a nuclear receptor and controls a number of gene expressions. The ligand binding domain (LBD) of PPAR γ is large and involves two binding sites: orthosteric and allosteric binding sites. Increased evidence has shown that PPAR γ is an oncogene and thus the PPAR γ antagonists have potential as anticancer agents. In this paper, we use Glide Dock approach to determine which binding site, orthosteric or allosteric, would be a preferred pocket for PPAR γ antagonist binding, though antidiabetic drugs such as thiazolidinediones (TZDs) bind to the orthosteric site. The Glide Dock results show that the binding of PPAR γ antagonists at the allosteric site yielded results that were much closer to the experimental data than at the orthosteric site. The PPAR γ antagonists seem to selectively bind to residues Lys265, Ser342 and Arg288 at the allosteric binding site, whereas PPAR γ agonists would selectively bind to residues Leu228, Phe363, and His449, though Phe282 and Lys367 may also play a role for agonist binding at the orthosteric binding pocket. This finding will provide new perspectives in the design and optimization of selective and potent PPAR γ antagonists or agonists.

Keywords: PPAR gamma; anticancer; prostate cancer; docking; allosteric; orthosteric; and selectivity



Citation: Almahmoud, S.; Zhong, H.A. Molecular Modeling of Allosteric Site of Isoform-Specific Inhibition of the Peroxisome Proliferator-Activated Receptor PPAR γ . *Biomolecules* **2022**, *12*, 1614. <https://doi.org/10.3390/biom12111614>

Academic Editor: Jörg Gsponer

Received: 3 October 2022

Accepted: 28 October 2022

Published: 1 November 2022

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1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that function as transcription factors upon activation, regulating as lipid and glucose metabolism and cellular differentiation [1–3]. PPAR family is consisted of three isoforms, PPAR α , PPAR γ , and PPAR δ ; each isoform has different tissue distribution, selectivity, and responsiveness to ligands [1,4]. PPAR γ is an interesting target because of its association with disorders such as atherosclerosis, diabetes, obesity, and cancer [5]. The thiazolidinedione (TZD) drugs such as rosiglitazone and pioglitazone are classical PPAR γ agonists and have been used as antidiabetic drugs to treat type 2 diabetes [6]. However, increased PPAR γ expression has been found to control other pathways that could induce cancer development and progression. Extended pioglitazone use increased the risk of bladder cancer in a dose- and time-dependent manner [7]. However, a meta-analysis of randomized clinical trials with rosiglitazone and control did not confirm a significant risk of cancer in patients with rosiglitazone treatment. The effects of rosiglitazone on incident malignancies were 0.88 in gastro-intestinal (GI), 0.67 in pulmonary, 1.19 in mammary, and 1.02 in prostate, which suggests some protective effect of rosiglitazone use in GI and lung cancer patients but negative effect in breast and prostate cancer patients [8]. Nevertheless, rosiglitazone was associated with a significantly increased risk of myocardial infarction and death from cardiovascular causes [9].

PPAR γ was once considered to be a tumor suppressor, and thus PPAR γ agonists such as troglitazone were used to treat prostate cancer in clinical trials [10–13]. However, further analysis showed that the observed inhibition of prostate cancer growth by these compounds was mediated by PPAR γ -independent mechanisms such as the inhibition of the CXCR4/CXCL12 axis, or the Bcl-xL/Bcl-2 functions or the suppression of the androgen receptor expression [14–16]. In fact, an increased level of PPAR γ expression was observed in prostate cancer and was positively correlated with stage and grade of prostate cancer, and thus PPAR γ has been considered as an oncogene [17–20]. Overexpression of PPAR γ in androgen receptor-negative prostate cancer cell lines such as DU-145, PC3, and PC3M, promoted cell proliferation, migration levels and metastases to the lungs and lymph nodes [17]. siRNA knockdown of PPAR γ and treatment of PPAR γ antagonist (warfarin) led to reduced prostate cancer tumor size and thus inhibited prostate cancer cell growth [17,21]. Via virtual screening and biological screening, we have identified seventeen hit molecules with single digit micromolar concentration in terms of growth inhibition of PPAR γ activity in the LNCaP cells [22]. Therefore, PPAR γ antagonists could be used to treat prostate cancer.

In addition, PPAR γ antagonists were shown to inhibit the growth of bladder, breast, pancreatic, and prostate cancer cells [23–26]. Several PPAR γ antagonists (such as SR1664) have also yielded therapeutic effects in the treatment of obesity with better therapeutic index comparing with PPAR γ agonists by overcoming thiazolidinedione drugs' undesirable side effects, such as heart failure, and fluid retention with increased risk of weight gain, loss of bone mineral density [27,28]. Therefore, it is highly desirable to design PPAR γ antagonists. They have potentials to be anticancer or antidiabetic drugs.

The PPAR γ structure constitutes the N-terminal domain co-regulator-binding activation function 1 (AF1), a central DNA-binding zinc-finger domain, and a C-terminal domain, which includes ligand-binding domain and activation function 2 (AF-2) for a second co-regulator. There is a hinge region that connects the ligand-binding domain and DNA-binding domain [29,30]. The C-terminal domain plays a vital role in ligand binding, dimerization, and transactivation functions [29]. The crystal structure (PDB ID, 2HFP, a human PPAR γ /ligand complex) of ligand binding domain of PPAR γ consists of 13 α -helices and four β -sheet strands. The PPAR γ shares similar ligand binding domain with other nuclear-receptor structures from helix 3 to carboxy-terminal domain, but it has a longer helix H2b [29]. The PPAR γ ligand binding domain has a large, hydrophobic, and T-shaped cavity which allows it to accommodate two ligands. One is for orthosteric binding, the other for allosteric binding (Figure 1). The orthosteric binding domains that thiazolidinedione drugs such as rosiglitazone and pioglitazone bind lie in the pocket defined by helices 5, 7, and 11, whereas the alternate binding site is consisted of helices H2b, H3 and the β -sheets (an allosteric site) [31].

TZDs are PPAR γ agonists that initiate transcription through binding to a canonical orthosteric pocket (Figure 1). Residues for agonist-binding include Cys285, Ser289, His323, Tyr327, Lys367, His449, and Tyr473. In addition to orthosteric binding, several PPAR γ ligands have alternate binding site located between helices H2b, H3 and the β -sheets (Figure 1). PPAR γ ligands that bind to this alternate site do not compete with endogenous ligands for their established orthosteric binding pocket, so this binding site can be identifies as an allosteric site. Allosteric binding residues include Glu259, Lys265, His266, Arg288, Ser289, Glu295, Ser342, Glu343, and Lys367. Several molecules have been reported to bind to the allosteric site [32,33]. It is found that the presence of an orthosteric ligand promotes the binding of the allosteric ligand [33].

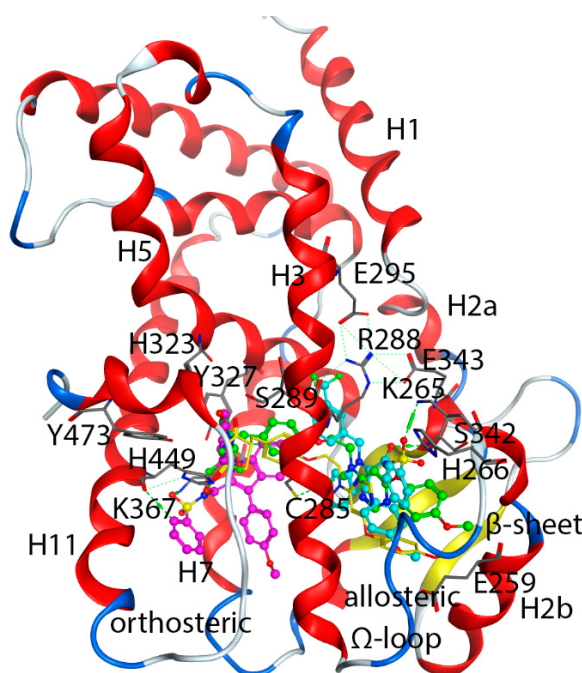


Figure 1. Co-crystal structure of NSI-bound PPAR γ (PDB id: 2HFP). Among two bound ligands (PDB ligand ID: NSI), one binds to the orthosteric pocket (magenta NSI) and the other to the allosteric site (cyan NSI). Lobeglitazone of native bound structure (PDB id: 5Y2T), yellow; Lobeglitazone from the docked pose of this study, green. Some helices and interacting residues for the orthosteric and allosteric bindings were labeled. Residues were presented as one letter code for clarity.

Since PPAR γ antagonists have potentials as anticancer agents, it is important to identify which binding site, orthosteric or allosteric, is preferred for PPAR γ antagonists. The answer to this question and the structural basis for PPAR γ antagonist binding will help future PPAR γ antagonist drug discovery. To achieve this goal, we ran a series of docking studies of verified PPAR γ antagonists with the crystal structures of PPAR γ and analyzed the protein-ligand interactions to identify which residues are responsible for antagonist binding.

2. Computational Methods

2.1. Preparation of Protein Structures

We downloaded the wild type X-ray crystal structures of PPAR γ complexed with *N*-sulfonyl-2-indole carboxamides (NSI) ligand (PDB ID: 2HFP) (Figure 1) from the RCSB Protein Data Bank (<https://www.rcsb.org/structure/2hfp>, accessed on 1 July 2021 [31]). There are no missing residues in the crystal structure of 2HFP. Structure of 2HFP was imported to Maestro program in the Schrödinger software suite. The Protein Preparation module was used for protein structure preparation. The pKas of protein side chains were calculated using PROPKA under pH 7.0. The side-chain structures of Gln, Asn and His were permitted to flip to maximize H-bond interaction. All lysines and arginines were protonated to +1 charge and all glutamic acid and aspartic acid were deprotonated to −1 charge. Histidine 425 were assigned as protonated HIP and histidines at 217, 266 and 466 were assigned as HIE while His323, Gln294 and Gln430 were flipped to maximized H-bond interactions. In addition, water molecules in the crystal structure were deleted before energy minimization. During the minimization, the backbone atoms were first restrained and then allowed to move freely using the OPLS force field in the MacroModel module in the Schrödinger software suite [34].

2.2. Preparation of Ligands

We collected forty-seven PPAR γ antagonists (Supplementary Tables S1 and S2) from different sources [27,30,31]. These antagonists were built and minimized using MOE program [35] based on the bound ligand, *N*-sulfonyl-2-indole carboxamides (NSI), in 2HFP as a template. In addition, we also built nine agonists (Figure S1) for analyzing the protein-ligand interactions for agonists. All PPAR γ ligands were imported to the Maestro, subjected to the EPik calculation and the subsequent optimization by the MacroModel program using the OPLS2005 force field. In addition, we downloaded a database of 3D molecules from the National Cancer Institute (NCI) [36], and then four hundred and twenty-three drug-like molecules (obtained after filtration based on the Lipinski's rule of five, calculated in the MOE program) were randomly selected from this database to assess the enrichment factor and to validate our docking method. The molecules were then subjected to EPik calculation and energy minimization using the MacroModel program. The EPik program predicted the pKa for all aromatic amines at 1.27–2.59, the pKa for oxazoline ring nitrogen at 1.22, and the pKa for imidazoline ring nitrogen at 2.29, suggesting neutral, non-protonated nitrogen atoms for these moieties. The pKa for pyridine was predicted to be 6.01–7.56, suggesting a neutral aromatic amine.

2.3. Molecular Dockin

Docking of the antagonists into the orthosteric and allosteric binding sites of PPAR γ was completed using the Schrödinger software suite. We generated two grid files for the crystal structure of 2HFP using the Glide Grid Generation protocol with the two bound ligands as respective centroids with box size of 25 Å from the centroid, one for orthosteric binding site and the other for allosteric binding site of PPAR γ . All 47 PPAR γ antagonists were docked to each of the two grid files, and we also ran docking for the 423 National Cancer Institute (NCI) drug-like molecules to these two grid files to calculate the enrichment factor. During the docking process, the scaling factor for receptor van der Waals for the nonpolar atoms was set to 0.8 to allow for some flexibility of the receptor, and the precision was set as extra precision. All other parameters were used as defaults. The output docking scores were given as extra precision glide scores (GScore). The GScore is considered as a predicated binding affinity as well as a predicated free energy of binding (ΔG_{PRED}). The output ΔG_{PRED} was then related to the experimental ΔG_{EXP} , calculated from the experimental IC₅₀ (nM) using the following equation [37].

$$\Delta G_{\text{EXP}}(\text{kcal/mol}) = RT \ln \left(\text{IC}_{50} (\text{nM}) \times 10^{-9} \right) / 1000$$

The Pearson's R (Pearson's correlation coefficient) was estimated using Microsoft Excel and the root-mean-square error and mean-absolute-error were measured from MatLab.

To evaluate the effect of flexibility of binding pockets on ligand binding, we ran induced fit docking of nine agonists to the orthosteric site and the allosteric site using the Induced Fit Docking (IFD) program in the Schrödinger software suite. During the IFD, the bound ligands at the orthosteric and allosteric sites were set as the centroid for respective docking. Maximum number of poses was set to 20 for the first round of Glide Docking and to 5 for the Glide Redocking. The best docking score and the average score of the top five reported poses were tabulated and were compared to the results from the regular Glide Dock protocol.

2.4. Protein-Ligand Interactions

From the docking output, each docked pose was analyzed with its bound protein and the interacting residues were tabulated to show which residues have high frequency in ligand binding, suggesting their essential roles for ligand binding.

3. Results and Discussion

3.1. Glide Docking

We ran docking experiments of 47 PPAR γ antagonists against the two PPAR γ binding sites to evaluate which is more predictable for the PPAR γ antagonists, the orthosteric or the allosteric binding site. PPAR γ antagonist docking scores are listed in Table 1. The glide docking shows that the predicted glide dock scores (GScore) at the allosteric binding site were in good agreement with the experimentally obtained values (ΔG_{EXP}), which was calculated from the IC_{50} s using the equation as described in the Section 2. Table 2 shows that PPAR γ antagonists bind preferably to the allosteric site rather than the orthosteric site in that the binding to the allosteric site produced binding affinity that was closer to the experimental values.

Table 1. The glide score (kcal/mol) for the 47 PPAR γ antagonists against the allosteric and orthosteric binding site.

Compound	IC_{50} (nM)	ΔG_{EXP} (kcal/mol)	Allosteric GScore	Orthosteric GScore	Compound	IC_{50} (nM)	ΔG_{EXP} (kcal/mol)	Allosteric GScore	Orthosteric GScore
NSI	3	−11.63	−12.59	−14.28	22	380	−8.76	−9.74	−11.45
SR1664	80	−9.68	−10.31	−10.96	23	330	−8.84	−9.37	−11.19
SR11023	108	−9.50	−10.27	−11.85	24	330	−8.84	−9.57	−11.84
1	7	−11.12	−12.64	−14.48	25	770	−8.34	−10.89	−10.06
2	1	−12.28	−12.75	−11.42	26	540	−8.55	−9.74	−10.15
3	7	−11.12	−12.69	−14.14	27	6	−11.22	−11.19	−11.68
4	2	−11.87	−11.46	−11.42	28	32	−10.22	−11.42	−11.08
5	290	−8.92	−10.33	−10.97	29	24	−10.39	−11.04	−11.17
6	720	−8.38	−10.07	−11.63	30	30	−10.26	−12.32	−12.48
7	280	−8.94	−10.25	−10.92	31	7	−11.12	−11.54	−11.63
8	80	−9.68	−10.78	−10.85	32	5	−11.32	−12.43	−12.25
9	290	−8.92	−10.19	−11.07	33	8	−11.05	−11.47	−10.93
10	180	−9.20	−10.21	−11.45	34	5	−11.32	−11.84	−11.83
11	90	−9.61	−11.14	−11.96	35	17	−10.60	−11.91	−12.73
12	80	−9.68	−10.50	−11.02	36	40	−10.09	−11.66	−11.95
13	80	−9.68	−10.20	−11.19	37	7	−11.12	−11.07	−11.06
14	680	−8.41	−8.93	−9.34	38	22	−10.45	−11.13	−11.32
15	700	−8.40	−9.28	−9.90	39	77	−9.70	−12.26	−12.17
16	140	−9.35	−9.40	−11.51	40	62	−9.83	−10.68	−11.27
17	90	−9.61	−10.74	−10.80	41	148	−9.32	−10.96	−10.66
18	2440	−7.66	−7.74	−8.39	42	17	−10.60	−13.23	−12.17
19	400	−8.73	−9.94	−10.27	43	1100	−8.13	−10.98	−6.61
20	50	−9.96	−11.55	−10.55	44	80	−9.68	−11.45	−12.28
21	280	−8.94	−9.93	−11.24					

The Pearson's correlation coefficient at allosteric binding site was 0.80 and the correlation R^2 was 0.64 (Figure 2), better than the respective numbers at the orthosteric binding site (0.62, and 0.39, respectively). In addition, the average of the difference (or error) between ΔG_{EXP} and ΔG_{PRED} ($\Delta\Delta G$), the mean of absolute error (MAE), and the root-mean-square (RMS) error for PPAR γ antagonists docked at allosteric binding site were 1.08, 1.10, and 1.29, respectively whereas the respective numbers at the orthosteric binding site were $\Delta\Delta G$ (1.50), MAE (1.63), and RMSE (1.83) (Table 2). Taken together, our docking results indicate that glide docking is capable of predicting ligands binding affinity in different pocket sites of PPAR γ . The glide docking reveals that the ΔG_{PRED} of PPAR γ antagonists is

more correlated with the ΔG_{EXP} of PPAR γ antagonists at allosteric binding site, and the statistical results such as Pearson's correlation coefficient, correlation R^2 , $\Delta\Delta G$, MAE, and RMSE further confirm the preference of the allosteric binding sites for PPAR γ antagonists. Therefore, for future structure-based PPAR γ antagonist design, it is more reasonable to use the allosteric site, rather than the orthosteric site.

Table 2. Statistical results, the correlation and residual errors between the experimentally obtained ΔG_{EXP} and the glide scores for the 47 PPAR γ antagonists against the allosteric and orthosteric binding site.

	Allosteric Binding Site	Orthosteric Binding Site
Pearson's R	0.80	0.62
Correlation R^2	0.64	0.39
$\Delta\Delta G$	1.08	1.50
MAE	1.10	1.63
RMSE	1.29	1.83

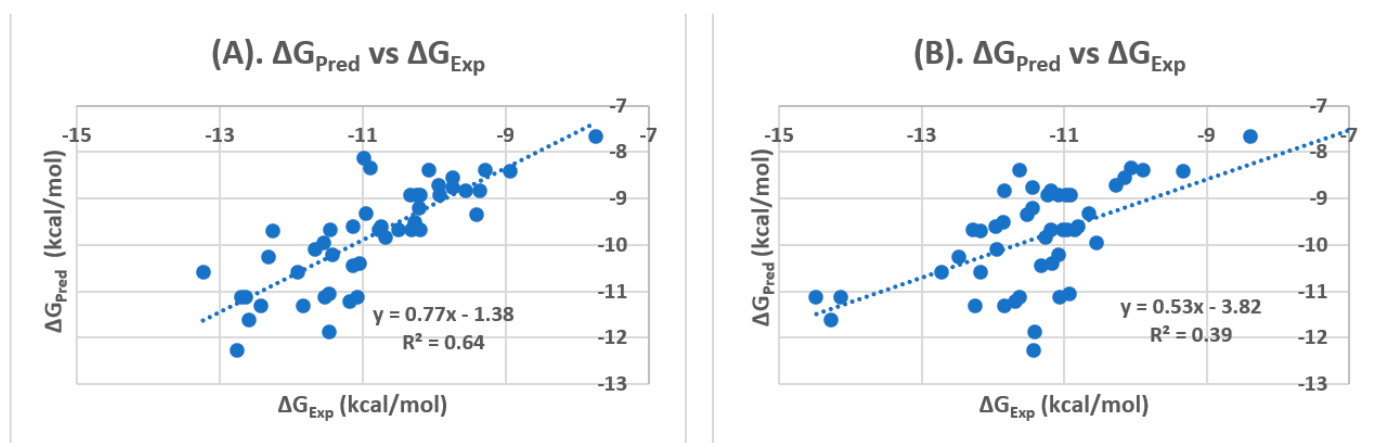


Figure 2. Correlations of glide docking scores for PPAR γ antagonists: (A) Allosteric Binding site. (B) Orthosteric Binding Site.

Other than the correlation between the predicted binding affinity (GScore) and the experimentally obtained data (ΔG_{exp}), we also used other methods to validate our docking programs and scoring functions. One commonly used method is whether the docking program can reproduce the native binding conformation. Docking methods are considered trustworthy when they can generate poses very close to the native conformation, i.e., with low Root Mean Square Deviation (RMSD) value from the known conformation (usually 1.5 or 2 Å depending on ligand size) [38]. The superposition of the Glide-generated docked pose for NSI and the native conformation in 2HFP revealed that the RMSD between these two poses is 0.22 Å, so the glide docking can successfully generate the native conformation.

In addition, the enrichment factor (EF) is often used to evaluate the validity of a docking program. The EF is a general measurement of the efficiency of a docking program: the higher the EF, the more accurate the docking program [39,40]. The EF and the true hit% measure the concentration of active inhibitors in a specific subset divided (%) by the concentration of active inhibitors in the database [41]. The EF can validate if a docking method can identify active compounds from the drug-like molecules. To evaluate the EF, in addition to the docking of 47 PPAR γ antagonists to the binding sites, we also docked 423 drug-like compounds to the same binding sites. The Glide docking scores for the drug-like molecules at the allosteric site were listed in Supplementary Table S3. Among a total of 470 compounds in the database, only 47 were verified active compounds. Among the top 10% compounds in the total database (47), 33 were reported PPAR γ antagonists,

resulting in the EF score of 7.0 and hit rate (or true hit%) of 70% (Table 3), which suggested that the docking program was able to identify the active compounds. The docking scores of the 423 drug-like compounds against the 2HFP crystal structure are listed (Table S1).

Table 3. Enrichment factor of the glide docking against PPAR γ at allosteric site.

Number of active PPAR γ antagonists	47
Number of total compounds in the database	470
Number of active PPAR γ antagonists in the top 10% subset	33
Enrichment factor (EF)	7.0
Hit rate (HR)	70%

3.2. Binding Mode of PPAR γ Ligands at the Allosteric Binding Site

Forty-seven PPAR γ antagonists and nine agonists were docked to the allosteric binding site and the orthosteric site to identify the binding difference between antagonists and agonists. After docking was completed, we first analyzed the protein-ligand interactions of antagonists and agonists at these two sites. Residues interacting with the antagonists at the allosteric site are listed in Table 4 and residues for the orthosteric antagonist binding are provided in Supplementary Table S4. We also tabulated the number of residues interacting with antagonists at the two sites and the frequency of interacting residues was calculated (Figure 3).

Table 4. Interacting residues of PPAR γ antagonists at the allosteric site.

Compound	Interacting Residues	Compound	Interacting Residues
NSI	Lys265, Ser342	22	Lys265, Ser342
SR1664	Lys265, Ser289, Ser342, Lys367	23	Lys265, Arg288, Ser342, Lys367
SR11023	Lys265, Ser289	24	Lys265, Arg288, Ser342
1	Lys265, Ser342	25	Lys265, Arg288, Ser342
2	Lys265, Ser342	26	Lys265, Arg288, Ser342
3	Lys265, Arg288, Ser342	27	Lys265, Ser289, Ser342
4	Lys265, Ser342	28	Lys265, Ser289, Ser342
5	Lys265, Ser342	29	Lys265, Ser289
6	Lys265, Ser342	30	Lys265, Phe282, Ser342, Lys367
7	Lys265, Ser342	31	Lys265, Ser289
8	Lys265, Arg288, Ser342	32	Lys265, Phe282, Ser342, Lys367
9	Lys265, Ser342	33	Lys265, Ser289
10	Lys265, Arg288, Ser342	34	Lys265, Ser289, Ser342, His449
11	Lys265, Arg288, Ser342	35	Lys265, Phe282, Ser342, Lys367, His449
12	Lys265, Ser342	36	Lys265, Phe282, Lys367
13	Lys265, Ser342	37	Lys265, Ser342
14	Lys265, Arg288, Ser342	38	Phe282, Lys367
15	Lys265, Ser342	39	Lys265, Phe282, Lys367
16	Lys265, Arg288, Ser342	40	Lys265, Gly284
17	Lys265, Ser342	41	Phe282
18	Ser342	42	Lys265, Phe282, Ser342, Lys367
19	Lys265, Arg288, Ser342	43	Arg288
20	Lys265, Ser342	44	Lys265, Phe282, Ser342, Lys367
21	Arg288, Ser342, Glu343		

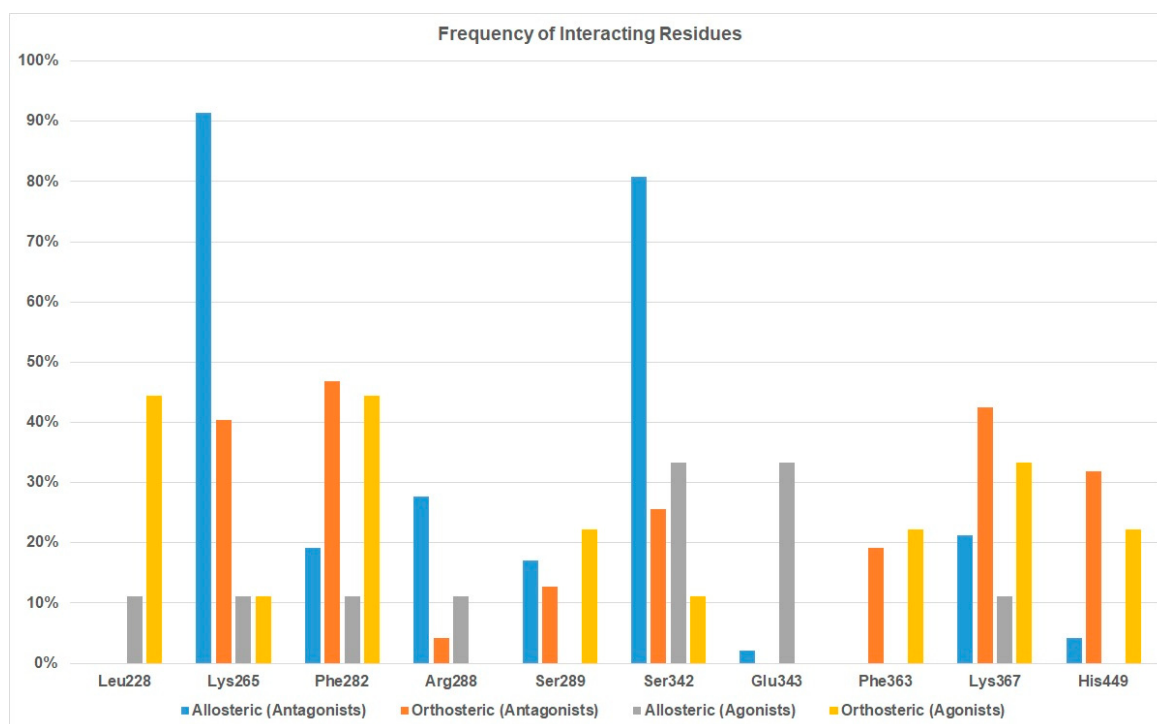


Figure 3. Frequency of interacting residues of PPAR γ antagonists and agonists at the orthosteric and allosteric binding sites.

Both Table 4 and Figure 3 show that residues Lys265, Arg288, and Ser342 are the preferred residues for antagonist binding at the allosteric site. The crystal structure of the PPAR γ /NSI complex at allosteric binding site reveals that residues Lys265 and Ser342 of PPAR γ form H-bonds to the NSI ligand. In addition, Lys367 and Ser289 form H-bonds with SR1664, an PPAR γ antagonist (Figure 4). In our docking study, the NSI ligand was docked to PPAR γ and formed two H-bonds with Lys265 and Ser342, which agreed with what was observed in the native ligand in the X-ray crystal structure (Figure 4A). Our previous work identified seventeen PPAR γ antagonists that showed single digit micromolar concentration in growth inhibition of the LNCaP cells and the residues responsible for PPAR γ binding were Arg288 and Ser342 [22].

Other than the non-covalent PPAR γ antagonists, some covalent PPAR γ antagonists have been discovered. MMT-160 is a PPAR γ antagonist with an alkyne function group that binds covalently to Cys285, a residue on helix 3 of the allosteric site [42].

Among all residues within the allosteric binding pocket, Lys265 and Ser342 appear to be the most important. In terms of agonist binding to the allosteric site, Ser342 interacted with 33% of agonists. Comparing to the 80% when it binds with antagonists, it is still reasonable to conclude that residues Lys265 and Ser342 are not only important for antagonist binding, but also can be utilized to design antagonists with selectivity over agonists. Residue Glu343 at the allosteric site appears to selectively bind to agonist (Figure 3).

In addition to Lys265, Ser342, and Arg288, residues Lys367, Phe282 and Ser289 have been observed as binding residues to some PPAR γ antagonists. However, they were not selective toward PPAR γ antagonist binding. On the contrary, Lys367, Phe282 and Ser289 were more selective toward PPAR γ agonist binding (Figure 3).

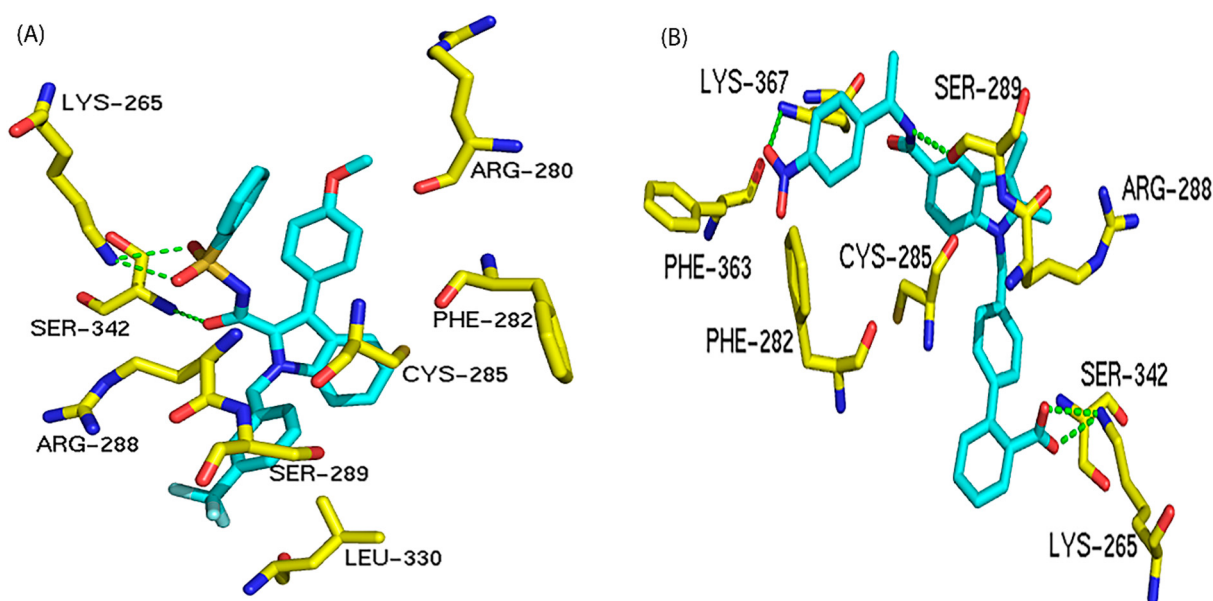


Figure 4. Interactions between PPAR γ and different antagonists at the allosteric binding site. The H-bond interactions are depicted as green dotted lines. **(A)** Interactions between PPAR γ and NSI (an antagonist). **(B)** Interactions between PPAR γ and compound SR1664 (an antagonist).

3.3. Binding Mode of PPAR γ Ligands at the Orthosteric Binding Site

To identify which residues are important for agonist binding at the orthosteric binding site, we docked nine reported agonists to both the orthosteric and allosteric binding pockets. The structures of nine PPAR γ agonists were given in Supplementary Figure S1 and were taken from published papers [43–49].

Table 5 shows that the docking scores for agonists at the orthosteric binding site in general were more negative (i.e., a stronger binding) than the same agonist at the allosteric site, which agreed with the fact that an agonist would prefer to bind to its target protein at the orthosteric site rather than the allosteric site. Residues responsible for orthosteric binding for an agonist are Leu228, Phe282, Lys367, His449 and Ser289 (Table 5 and Figure 3). It is interesting to observe that some of the agonists were able to bind to Ser342 and Glu343 of the allosteric site (Figure 3).

Table 5. The glide scores (kcal/mol) and interacting residues for the nine PPAR γ agonists against the allosteric and orthosteric binding site.

Names	Orthosteric GScore	Allosteric GScore	Orthosteric	Allosteric
Rosiglitazone	−8.45	−6.82	Leu228	NA
Lobeglitazone	−9.70	−8.56	Leu228, Phe282	Leu228
Pioglitazone	−8.96	−2.72	Ser289, Tyr327, Leu228	Ser342, Leu340
GW1929	−12.00	−9.93	Phe282, Phe363, Lys367	Lys265, Arg288
Farglitazar	−11.85	−12.58	Phe282, Lys367, Phe363	Lys367, Phe282
Edaglitazone	−9.98	−9.68	Leu228, His449	Glu343
Amorfrutin 1	−8.74	−8.42	Ser289 (phenol)	Glu343, Leu340
Amorfrutin 2	−7.89	−7.91	Phe282 (pp), Lys367	Glu343, Ser342
MRL20	−7.90	−9.72	Ser342, His449	Lys265, Ser342

Figure 5 showed that Leu228, a PPAR γ selective residue, as suggested in Figure 3, can provide main chain H-bond interactions with agonists and His449 provided an π - π interactions with aromatic rings on the ligand.

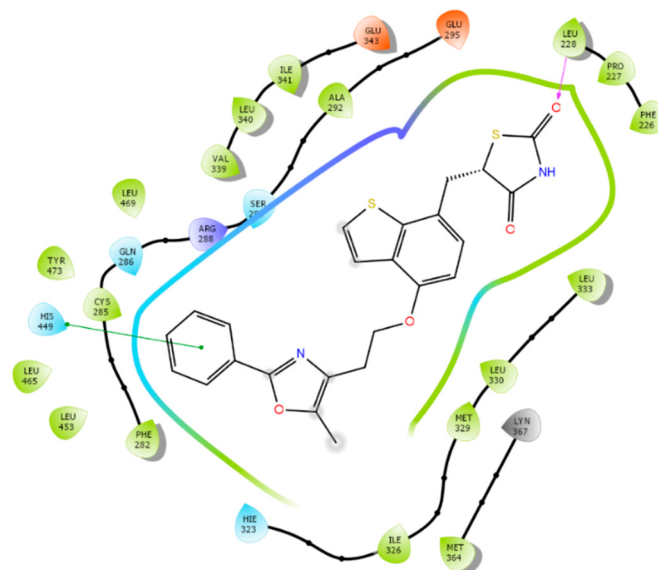


Figure 5. Interactions between PPAR γ and edaglitazone (an agonist) at the orthosteric site. Pink arrow: H-bond interactions with Leu228 main chain; green line: π - π interactions between phenyl group of edaglitazone and the histidine aromatic ring.

As we discussed in the Section 1, the large PPAR γ binding pocket allows the binding of two ligands, one at the orthosteric and the other at the allosteric binding site. Thus, it is not uncommon to find out that there are two molecules that occupy these two sites. The two molecules can be the same or different. When two of the same molecules bind to PPAR γ , the ratio between ligand and PPAR γ becomes 2:1, as what was observed in the crystal structure of 2HFP and 5UGM [31,49]. Brust et al. observed that an antagonist can bind to the orthosteric binding site and was still able to significantly reduce the cellular activation. However, dual-site binding can make an antagonist almost shut down the cellular activation [33]. Thus, it is not uncommon to observe that an antagonist can bind to the orthosteric site, which is what we observed in Table 1 and Table S4. By inspecting at Table 1, one may find that some antagonists show stronger binding at the orthosteric site than at the allosteric site. However, as what we discussed in Section 3.1, antagonists should bind to the allosteric site as the binding at the allosteric site is more agreeable to what was observed from experiments.

To design antagonists that can effectively compete with endogenous agonists, it is often true to introduce extra functional groups to the antagonists. Thus, antagonists normally have larger molecule weights, and more interacting surface. The average molecular weight (MW) of the nine agonists was 430.16 Dalton, whereas the mean MW of the 47 antagonists was 531.00 Dalton. To assess the effect of flexibility of binding pockets on ligand binding, we ran induced fit docking of nine agonists to the orthosteric site and the allosteric site using the Induced Fit Docking program in the Schrödinger software suite.

By comparing the IFD-based docking scores to those from the Glide Dock, one can notice that these two sets of scores were overall very comparable with mean absolute error of 1.12 kcal/mol for the orthosteric site and 1.47 kcal/mol for the allosteric site (Table 6), although some large differences (greater than 2.0 kcal/mol) can be found in some ligands. This suggested that the way the Glide docking to implicitly incorporate the induced-fit effect by using the scaling factor for receptor van der Waals for the nonpolar atoms of 0.8 to allow for some flexibility of the receptor was sufficient in most cases.

Table 6. The glide scores (kcal/mol) (the best docked pose and the average of the top five poses) and the absolute errors (AE) between the average top 5 IFD-glide scores and the respective Glide Dock glide scores for the nine PPAR γ agonists against the allosteric and orthosteric binding site.

Names	Ortho. Best	Ortho. Mean	Ortho. AE	Allo. Best	Allo. Mean	Allo. AE
Rosiglitazone	−11.74	−8.71	0.26	−9.80	−9.05	2.22
Lobeglitazone	−12.14	−10.39	0.69	−11.65	−10.95	2.39
Pioglitazone	−8.62	−6.34	2.61	−9.00	−6.83	4.11
GW1929	−11.01	−9.65	2.35	−11.73	−8.88	1.05
Farglitazar	−11.61	−10.79	1.05	−13.48	−12.54	0.04
Edaglitazone	−9.30	−8.59	1.39	−11.06	−9.86	0.18
Amorfrutin 1	−8.97	−8.44	0.30	−8.94	−8.60	0.18
Amorfrutin 2	−7.96	−7.66	0.23	−8.14	−7.67	0.24
MRL20	−9.57	−6.72	1.18	−9.91	−6.89	2.82
MAE			1.12			1.47

Note: Abbreviation: Ortho. orthosteric; Allo. allosteric.

Table 6 also showed that the average docking scores of the top five poses between the agonists at the orthosteric site and the allosteric site were very close, with mean of the absolute errors of 0.61 kcal/mol. To find out what caused such a small difference for the same agonist that was docked from different allosteric and orthosteric site. We found out that the Induced-Fit Dock program was able to generate similar poses for the agonists, no matter they were docked from the orthosteric site or the allosteric site. The reason might be stemmed from the following two reasons, first, the large box size for docking (set at 25 Å from the centroid) covered both the orthosteric site and the allosteric site, which allowed the program to find their best binding conformation, though starting from a different centroid; second, all the agonists under study were able to adopt an extended conformation which allowed it to occupy both the orthosteric and allosteric sites. Figure 6 showed that the docking poses of the rosiglitazone and lobeglitazone, generated from two different centroids, occupied very similar conformational space, thus having very similar interactions with binding residues Ser289, Tyr327, His323, Lys367 and Tyr473 of the orthosteric site and residues Cys285, Lys265, Glu343, and Ser342 of the allosteric site. This is also in accordance with our Glide Dock observation that agonists were able to bind to Ser342 and Glu343 (Figure 3). The interacting residues of PPAR γ agonists at the orthosteric and allosteric site based on the Induced-Fit Docking were listed in Table S5. The crystal structure of the PPAR γ / lobeglitazone (PDB id: 5Y2T) [50] also showed that lobeglitazone can occupy both sites (Figure 1). Our docking pose of lobeglitazone agreed well with what was observed from the experiments. The antagonists from Hopkins' laboratory [31], on the other hand, were able to adopt a curled conformation and thus preferentially bind to the allosteric site.

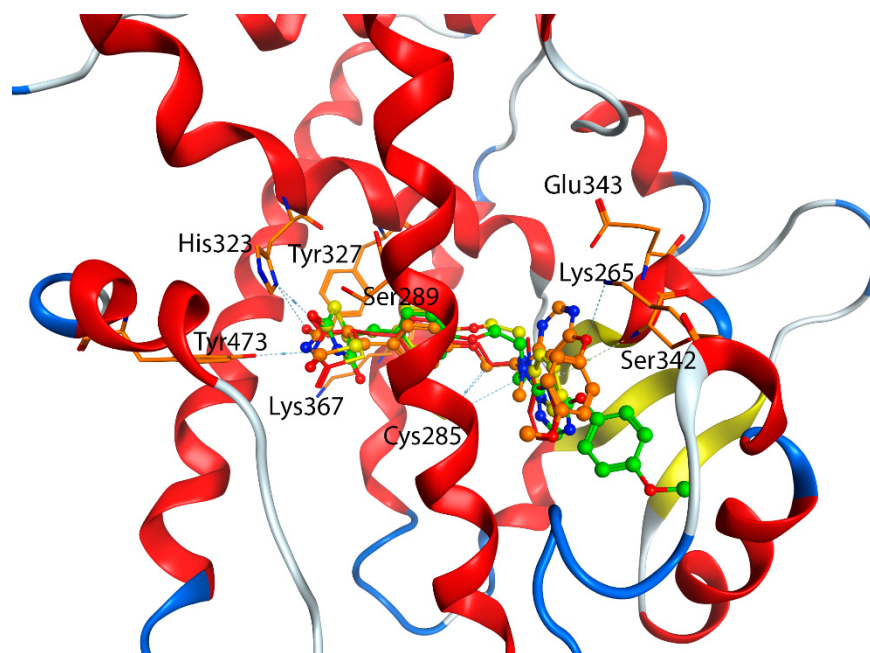


Figure 6. Interactions between PPAR γ agonists at the orthosteric site and allosteric site based on the Induced-Fit Docking. Yellow: Rosiglitazone from the orthosteric site; Red: Rosiglitazone from the allosteric site; Green: Lobeglitazone from the orthosteric site; Orange: Lobeglitazone from the allosteric site.

4. Conclusions

PPAR γ is an attractive target for drug discovery and development. PPAR γ antagonists have showed antitumor activity against different tumors. The crystal structures of PPAR γ complexed with antagonists revealed that antagonists can occupy two binding sites, orthosteric pocket, and allosteric pocket. Docking of PPAR γ antagonists resulted in predicted binding affinity that was in good agreement with the experimentally observed binding affinity. Docking studies show that the experimental binding affinity of PPAR γ antagonists is more correlated to the allosteric binding site than the orthosteric binding site. In addition, the statistics numbers of docking scores further confirmed the validity of the docking approach and favored the allosteric site. Therefore, the allosteric site is the most favorable binding site for PPAR γ antagonists. Residues important for selective allosteric antagonism appeared to be Lys265, Ser342 and Arg288. Our data also suggested that the results from the Glide Dock in most cases were in good agreement with those of the Induced-Fit Docking.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/biom12111614/s1>. The supporting information include the chemical structures of PPAR γ antagonists (Tables S1 and S2), the chemical structures of PPAR γ agonists (Figure S1), the Glide docking scores (GScore, kcal/mol) of drug-like molecules extracted from the NCI database against the PPAR γ (2HFP) at allosteric site (Table S3), interacting residues of PPAR γ antagonists at the orthosteric site (Table S4), and Interacting residues of PPAR γ agonists at the orthosteric site and the allosteric site based on the Induced-Fit Docking (Table S5).

Author Contributions: Conceptualization, S.A. and H.A.Z.; methodology, S.A. and H.A.Z.; formal analysis, S.A. and H.A.Z.; writing—original draft preparation, S.A.; writing—review and editing, H.A.Z.; supervision, H.A.Z.; project administration, H.A.Z., funding acquisition, S.A. and H.A.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the University of Nebraska at Omaha (HAZ for software support). SA was supported by Qassim University (Buraydah, Saudi Arabia).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The researchers would like to thank the Deanship of Scientific Research, Qassim University for funding the publication of this project.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rosen, E.D.; Spiegelman, B.M. Molecular Regulation of Adipogenesis. *Annu. Rev. Cell Dev. Biol.* **2000**, *16*, 145–171. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Maglich, J.M.; Sluder, A.; Guan, X.; Shi, Y.; McKee, D.D.; Carrick, K.; Kamdar, K.; Willson, T.M.; Moore, J.T. Comparison of complete nuclear receptor sets from the human, *Caenorhabditis elegans* and *Drosophila* genomes. *Genome Biol.* **2001**, *2*, 1–7. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Evans, R.M. The steroid and thyroid hormone receptor superfamily. *Science* **1988**, *240*, 889–895. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Willson, 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. [\[CrossRef\]](#)
5. Rosen, E.D.; Spiegelman, B.M. PPAR γ : A Nuclear Regulator of Metabolism, Differentiation, and Cell Growth. *J. Biol. Chem.* **2001**, *276*, 37731–37734. [\[CrossRef\]](#)
6. Wagstaff, A.J.; Goa, K.L. Rosiglitazone: A review of its use in the management of type 2 diabetes mellitus. *Drugs* **2002**, *62*, 1805–1837. [\[CrossRef\]](#)
7. Tang, H.; Shi, W.; Fu, S.; Wang, T.; Zhai, S.; Song, Y.; Han, J. Pioglitazone and bladder cancer risk: A systematic review and meta-analysis. *Cancer Med.* **2018**, *7*, 1070–1080. [\[CrossRef\]](#)
8. Monami, M.; Lamanna, C.; Marchionni, N.; Mannucci, E. Rosiglitazone and risk of cancer: A meta-analysis of randomized clinical trials. *Diabetes Care* **2008**, *31*, 1455–1460. [\[CrossRef\]](#)
9. Nissen, S.E.; Wolski, K. Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes. *New Engl. J. Med.* **2007**, *356*, 2457–2471. [\[CrossRef\]](#)
10. Butler, R.; Mitchell, S.H.; Tindall, D.J.; Young, C.Y. Nonapoptotic cell death associated with S-phase arrest of prostate cancer cells via the peroxisome proliferator-activated receptor gamma ligand, 15-deoxy-delta12,14-prostaglandin J2. *Cell Growth Differ. Mol. Biol. J. Am. Assoc. Cancer Res.* **2000**, *11*.
11. Hisatake, J.I.; Ikezoe, T.; Carey, M.; Holden, S.; Tomoyasu, S.; Koeffler, H.P. Down-Regulation of prostate-specific antigen expression by ligands for peroxisome proliferator-activated receptor gamma in human prostate cancer. *Cancer Res.* **2000**, *60*, 5494–5498. [\[PubMed\]](#)
12. Kubota, T.; Koshizuka, K.; Williamson, E.A.; Asou, H.; Said, J.W.; Holden, S.; Miyoshi, I.; Koeffler, H.P. Ligand for peroxisome proliferator-activated receptor gamma (troglitazone) has potent antitumor effect against human prostate cancer both in vitro and in vivo. *Cancer Res.* **1998**, *58*, 3344–3352.
13. Mueller, E.; Smith, M.; Sarraf, P.; Kroll, T.; Aiyer, A.; Kaufman, D.S.; Oh, W.; Demetri, G.; Figg, W.D.; Zhou, X.-P.; et al. Effects of ligand activation of peroxisome proliferator-activated receptor γ in human prostate cancer. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 10990–10995. [\[CrossRef\]](#)
14. Qin, L.; Gong, C.; Chen, A.-M.; Guo, F.-J.; Xu, F.; Ren, Y.; Liao, H. Peroxisome proliferator-activated receptor γ agonist rosiglitazone inhibits migration and invasion of prostate cancer cells through inhibition of the CXCR4/CXCL12 axis. *Mol. Med. Rep.* **2014**, *10*, 695–700. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Shiau, C.-W.; Yang, C.-C.; Kulp, S.K.; Chen, K.-F.; Chen, C.-S.; Huang, J.-W.; Chen, C.-S. Thiazolidinediones Mediate Apoptosis in Prostate Cancer Cells in Part through Inhibition of Bcl-xL/Bcl-2 Functions Independently of PPAR γ . *Cancer Res.* **2005**, *65*, 1561–1569. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Yang, C.-C.; Wang, Y.-C.; Wei, S.; Lin, L.-F.; Chen, C.-S.; Lee, C.-C.; Lin, C.-C.; Chen, C.-S. Peroxisome Proliferator-Activated Receptor γ -Independent Suppression of Androgen Receptor Expression by Troglitazone Mechanism and Pharmacologic Exploitation. *Cancer Res.* **2007**, *67*, 3229–3238. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Ahmad, I.; Mui, E.; Galbraith, L.; Patel, R.; Tan, E.H.; Salji, M.; Rust, A.G.; Repiscak, P.; Hedley, A.; Markert, E.; et al. *Sleeping Beauty* screen reveals *Pparg* activation in metastatic prostate cancer. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 8290–8295. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Salgia, M.M.; Elix, C.C.; Pal, S.K.; Jones, J.O. Different roles of peroxisome proliferator-activated receptor gamma isoforms in prostate cancer. *Am. J. Clin. Exp. Urol.* **2019**, *7*, 98–109.
19. Rogenhofer, S.; Ellinger, J.; Kahl, P.; Stoeck, C.; Hartmann, A.; Engehausen, D.; Wieland, W.-F.; Müller, S.C.; Hofstädter, F.; Walter, B. Enhanced expression of peroxisome proliferator-activated receptor gamma (PPAR- γ) in advanced prostate cancer. *Anticancer Res.* **2012**, *32*, 3479–3483.
20. Segawa, Y.; Yoshimura, R.; Hase, T.; Nakatani, T.; Wada, S.; Kawahito, Y.; Kishimoto, T.; Sano, H. Expression of peroxisome proliferator-activated receptor (PPAR) in human prostate cancer. *Prostate* **2002**, *51*, 108–116. [\[CrossRef\]](#)

21. Tew, B.Y.; Hong, T.B.; Otto-Duessel, M.; Elix, C.; Castro, E.; He, M.; Wu, X.; Pal, S.K.; Kalkum, M.; Jones, J.O. Vitamin K epoxide reductase regulation of androgen receptor activity. *Oncotarget* **2017**, *8*, 13818–13831. [CrossRef] [PubMed]
22. Almahmoud, S.; Elix, C.C.; Jones, J.O.; Hopkins, C.R.; Vennerstrom, J.L.; Zhong, H.A. Virtual screening and biological evaluation of PPAR γ antagonists as potential anti-prostate cancer agents. *Bioorg. Med. Chem.* **2021**, *46*, 116368. [CrossRef] [PubMed]
23. Goldstein, J.T.; Berger, A.C.; Shih, J.; Duke, F.F.; Furst, L.; Kwiatkowski, D.J.; Cherniack, A.D.; Meyerson, M.; Strathdee, C.A. Genomic Activation of *PPARG* Reveals a Candidate Therapeutic Axis in Bladder Cancer. *Cancer Res.* **2017**, *77*, 6987–6998. [CrossRef] [PubMed]
24. Zaytseva, Y.Y.; Wallis, N.K.; Southard, R.C.; Kilgore, M.W. The PPAR γ antagonist T0070907 suppresses breast cancer cell proliferation and motility via both PPAR γ -dependent and -independent mechanisms. *Anticancer Res.* **2011**, *31*, 813–823. [PubMed]
25. Nakajima, A.; Tomimoto, A.; Fujita, K.; Sugiyama, M.; Takahashi, H.; Ikeda, I.; Hosono, K.; Endo, H.; Yoneda, K.; Iida, H.; et al. Inhibition of peroxisome proliferator-activated receptor γ activity suppresses pancreatic cancer cell motility. *Cancer Sci.* **2008**, *99*, 1892–1900. [CrossRef] [PubMed]
26. Burton, J.D.; Goldenberg, D.M.; Blumenthal, R.D. Potential of Peroxisome Proliferator-Activated Receptor Gamma Antagonist Compounds as Therapeutic Agents for a Wide Range of Cancer Types. *PPAR Res.* **2008**, *2008*, 1–7. [CrossRef]
27. Asteian, A.; Blayo, A.-L.; He, Y.; Koenig, M.; Shin, Y.; Kuruvilla, D.S.; Corzo, C.A.; Cameron, M.D.; Lin, L.; Ruiz, C.; et al. Design, Synthesis, and Biological Evaluation of Indole Biphenylcarboxylic Acids as PPAR γ Antagonists. *ACS Med. Chem. Lett.* **2015**, *6*, 998–1003. [CrossRef]
28. Choi, J.H.; Banks, A.S.; Kamenecka, T.M.; Busby, S.A.; Chalmers, M.J.; Kumar, N.; Kuruvilla, D.S.; Shin, Y.; He, Y.; Bruning, J.B.; et al. Antidiabetic actions of a non-agonist PPAR γ ligand blocking Cdk5-mediated phosphorylation. *Nature* **2011**, *477*, 477–481. [CrossRef]
29. Nolte, R.T.; Wisely, G.B.; Westin, S.; Cobb, J.E.; Lambert, M.H.; Kurokawa, R.; Rosenfeld, M.G.; Willson, T.M.; Glass, C.K.; Milburn, M.V. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor- γ . *Nature* **1998**, *395*, 137–143. [CrossRef]
30. Frkic, R.L.; Marshall, A.; Blayo, A.-L.; Pukala, T.; Kamenecka, T.M.; Griffin, P.R.; Bruning, J.B. PPAR γ in Complex with an Antagonist and Inverse Agonist: A Tumble and Trap Mechanism of the Activation Helix. *iScience* **2018**, *5*, 69–79. [CrossRef]
31. Hopkins, C.R.; O’Neil, S.V.; Laufersweiler, M.C.; Wang, Y.; Pokross, M.; Mekel, M.; Evdokimov, A.; Walter, R.; Kontoyianni, M.; Petrey, M.E.; et al. Design and synthesis of novel N-sulfonyl-2-indole carboxamides as potent PPAR- γ binding agents with potential application to the treatment of osteoporosis. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5659–5663. [CrossRef] [PubMed]
32. Hughes, T.S.; Giri, P.K.; de Vera, I.M.S.; Kuruvilla, D.S.; Shin, Y.; Blayo, A.-L.; Kamenecka, T.M.; Burris, T.P.; Griffin, P.R.; Kojetin, D.J. An alternate binding site for PPAR γ ligands. *Nat. Commun.* **2014**, *5*, 3571. [CrossRef] [PubMed]
33. Brust, R.; Lin, H.; Fuhrmann, J.; Asteian, A.; Kamenecka, T.M.; Kojetin, D.J. Modification of the Orthosteric PPAR γ Covalent Antagonist Scaffold Yields an Improved Dual-Site Allosteric Inhibitor. *ACS Chem. Biol.* **2017**, *12*, 969–978. [CrossRef] [PubMed]
34. Maestro, MacroModel, Protein Preparation Wizard, Glide Dock, and Epik; Schrödinger, LLC.: New York, NY, USA, 2019.
35. The Molecular Operating Environment (MOE); Chemical Computing Group Inc.: Montreal, QC, Canada, 2019.
36. NCI Open Database Compounds, Release 3; National Cancer Institute, National Institutes of Health: Bethesda, MD, USA, 2003. Available online: <http://Cactus.nci.nih.gov/download/nci> (accessed on 1 June 2019).
37. Zhong, H.A.; Santos, E.M.; Vasileiou, C.; Zheng, Z.; Geiger, J.H.; Borhan, B.; Merz, K.M. Free-Energy-Based Protein Design: Re-Engineering Cellular Retinoic Acid Binding Protein II Assisted by the Moveable-Type Approach. *J. Am. Chem. Soc.* **2018**, *140*, 3483–3486. [CrossRef] [PubMed]
38. Hevener, K.E.; Zhao, W.; Ball, D.M.; Babaoglu, K.; Qi, J.; White, S.W.; Lee, R.E. Validation of Molecular Docking Programs for Virtual Screening against Dihydropteroate Synthase. *J. Chem. Inf. Model.* **2009**, *49*, 444–460. [CrossRef]
39. Bender, A.; Glen, R.C. A Discussion of Measures of Enrichment in Virtual Screening: Comparing the Information Content of Descriptors with Increasing Levels of Sophistication. *J. Chem. Inf. Model.* **2005**, *45*, 1369–1375. [CrossRef]
40. Almahmoud, S.; Zhong, H.A. Molecular Modeling Studies on the Binding Mode of the PD-1/PD-L1 Complex Inhibitors. *Int. J. Mol. Sci.* **2019**, *20*, 4654. [CrossRef]
41. Bissantz, C.; Folkers, G.; Rognan, D. Protein-Based Virtual Screening of Chemical Databases. 1. Evaluation of Different Docking/Scoring Combinations. *J. Med. Chem.* **2000**, *43*, 4759–4767. [CrossRef]
42. Yoshizawa, M.; Aoyama, T.; Itoh, T.; Miyachi, H. Arylalkynyl amide-type peroxisome proliferator-activated receptor γ (PPAR γ)-selective antagonists covalently bind to the PPAR γ ligand binding domain with a unique binding mode. *Bioorganic Med. Chem. Lett.* **2022**, *64*, 128676. [CrossRef]
43. Fuhr, L.; Rousseau, M.; Plauth, A.; Schroeder, F.C.; Sauer, S. Amorfrutins Are Natural PPAR γ Agonists with Potent Anti-inflammatory Properties. *J. Nat. Prod.* **2015**, *78*, 1160–1164. [CrossRef]
44. de Groot, J.C.; Weidner, C.; Krausze, J.; Kawamoto, K.; Schroeder, F.C.; Sauer, S.; Büsow, K. Structural Characterization of Amorfrutins Bound to the Peroxisome Proliferator-Activated Receptor γ . *J. Med. Chem.* **2013**, *56*, 1535–1543. [CrossRef] [PubMed]
45. Bruning, J.B.; Chalmers, M.J.; Prasad, S.; Busby, S.A.; Kamenecka, T.M.; He, Y.; Nettles, K.W.; Griffin, P.R. Partial Agonists Activate PPAR γ Using a Helix 12 Independent Mechanism. *Structure* **2007**, *15*, 1258–1271. [CrossRef] [PubMed]
46. Shang, J.; Kojetin, D.J. Structural mechanism underlying ligand binding and activation of PPAR γ . *Structure* **2021**, *29*, 940–950.e4. [CrossRef] [PubMed]

47. Jang, J.Y.; Bae, H.; Lee, Y.J.; Choi, Y.I.; Kim, H.-J.; Park, S.B.; Suh, S.W.; Kim, S.W.; Han, B.W. Structural Basis for the Enhanced Anti-Diabetic Efficacy of Lobeglitazone on PPAR γ . *Sci. Rep.* **2018**, *8*, 1–11. [[CrossRef](#)] [[PubMed](#)]
48. Haffner, C.D.; Lenhard, J.M.; Miller, A.B.; McDougald, D.L.; Dwornik, K.; Ittoop, O.R.; Gampe, J.R.T.; Xu, H.E.; Blanchard, S.; Montana, V.G.; et al. Structure-Based Design of Potent Retinoid X Receptor α Agonists. *J. Med. Chem.* **2004**, *47*, 2010–2029. [[CrossRef](#)]
49. Shang, J.; Brust, R.; Mosure, S.A.; Bass, J.; Munoz-Tello, P.; Lin, H.; Hughes, T.S.; Tang, M.; Ge, Q.; Kamenekca, T.M.; et al. Cooperative cobinding of synthetic and natural ligands to the nuclear receptor PPAR γ . *eLife* **2018**, *7*, e43320. [[CrossRef](#)]
50. Lee, M.A.; Tan, L.; Yang, H.; Im, Y.-G.; Im, Y.J. Structures of PPAR γ complexed with lobeglitazone and pioglitazone reveal key determinants for the recognition of antidiabetic drugs. *Sci. Rep.* **2017**, *7*, 1–11. [[CrossRef](#)]