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Molecular docking of Glyceroneogenesis pathway intermediates with Peroxisome Proliferator-Activated Receptor-Alpha (PPAR-α)

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Abstract:
Peroxisome proliferator-activated receptor alpha (PPAR-α) belongs to the nuclear receptor superfamily of proteins. It is one of the principle regulators of metabolism and lipid homeostasis whose malfunction leads to complications including obesity and type 2 diabetes. In the adipose tissue, glyceroneogenesis is a unique pathway through which pyruvate is converted into glycerol-3-phosphate (G3P) in a multistep process. Previous findings demonstrated that glyceroneogenesis regulates triacylglycerol synthesis and adipogenesis. This led us to hypothesize that one of the pathway intermediate is physiologically relevant PPAR-α ligand. In the present study using *in silico* docking, we proved that glycerate, dihydroxy acetone phosphate, glyceraldehyde-3-phosphate, and G3P are key glyceroneogenesis pathway intermediates which bind to PPAR-α. They bind PPAR-α with comparable binding energy and docking score to that of (2s)-2-ethoxy-3-[4-(2-{4-[(methylsulfonyl)oxy]phenyl}ethoxy)phenyl]propanoic acid(AZ-2), a synthetic high affinity ligand of PPAR-α. These intermediates could be studied further as potential physiologically relevant activators of PPAR-α *in vitro* and *in vivo*.

Key words: PPAR-α, *in silico* docking, endogenous ligands, glyceroneogenesis.

Background:
Peroxisome proliferator-activated receptors (PPARs) are a group of proteins that belong to nuclear receptor super family **[1].** There are three main isotypes of PPARs found in humans PPAR-α, PPAR-β/δ and PPAR-γ. PPAR-α, also known as NR1C1 has been shown to express in organs like eyes, kidney and nerves and currently it is a candidate target for anti-diabetic drugs **[2, 3].** Lipid lowering drugs like fenofibrate, ciprofibrate, GW9578 act through PPAR-α **[4].** Endogenous compounds like leukotriene B4 (LTB4) **[5],** eicosanoids **[6]** and a number of long chain fatty acids **[7]** have been shown to activate PPAR-α but physiological relevance is still ambiguous due to their low physiological concentrations. Glyceroneogenesis is a pathway in which pyruvate is converted to glycerol-3-phosphate via gluconeogenesis pathway **[8].** It is also the dominant source of glycerol to which fatty acids are attached to form triglycerides in adipocytes **[9].** It has been shown that organic extracts of media after adipogenic induction and before triglyceride production of various pre-adipose cell lines were capable of inducing adipogenesis of other pre-adipose cells **[10].** Also inhibition of glyceroneogenesis resulted in lipodystrophy and overexpression leading to obesity **[11].** This strongly provoked us to question the involvement of glyceroneogenesis in activating PPARs that plays an important role in adipogenesis. Glide extra precision (XP) docking introduced new binding energy and scoring function which is considered to be more advantageous than most other docking and scoring tools including for PPAR-α **[12].** A number of leads were proved to be PPAR-α agonists using glide **[13].** In this study we report the docking studies done on PPAR-α with glyceroneogenesis pathway intermediates.

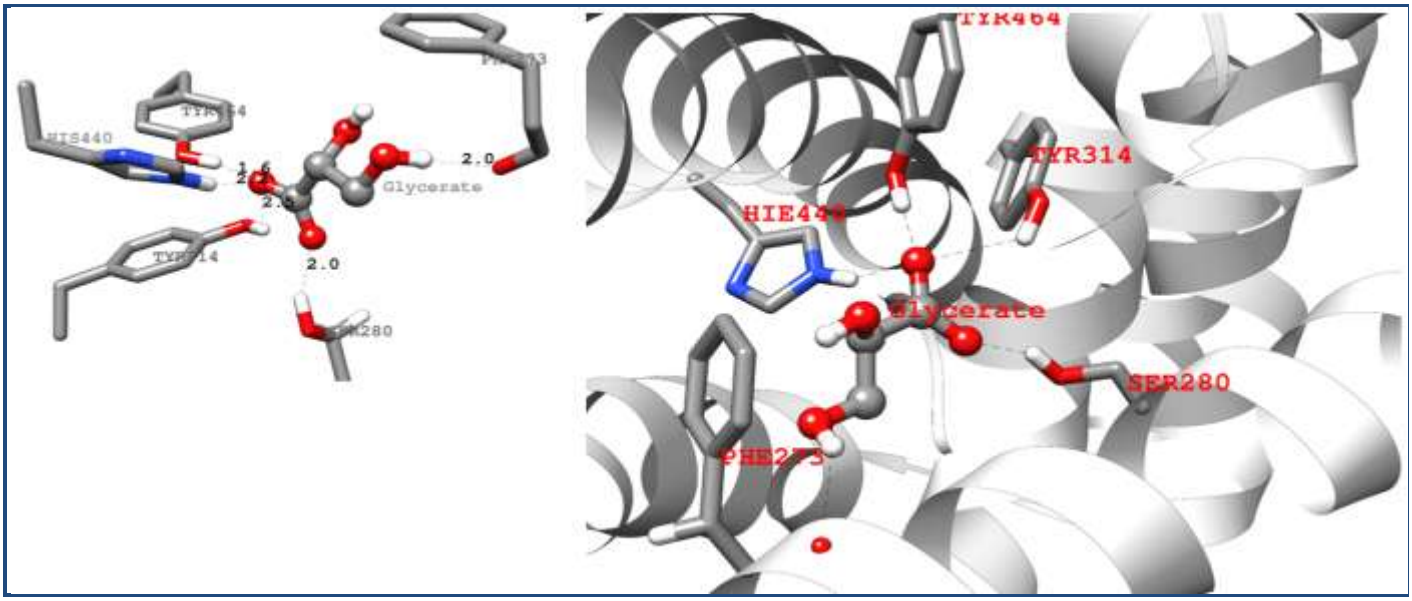

Figure 1: PPAR-α interaction with glycerate.

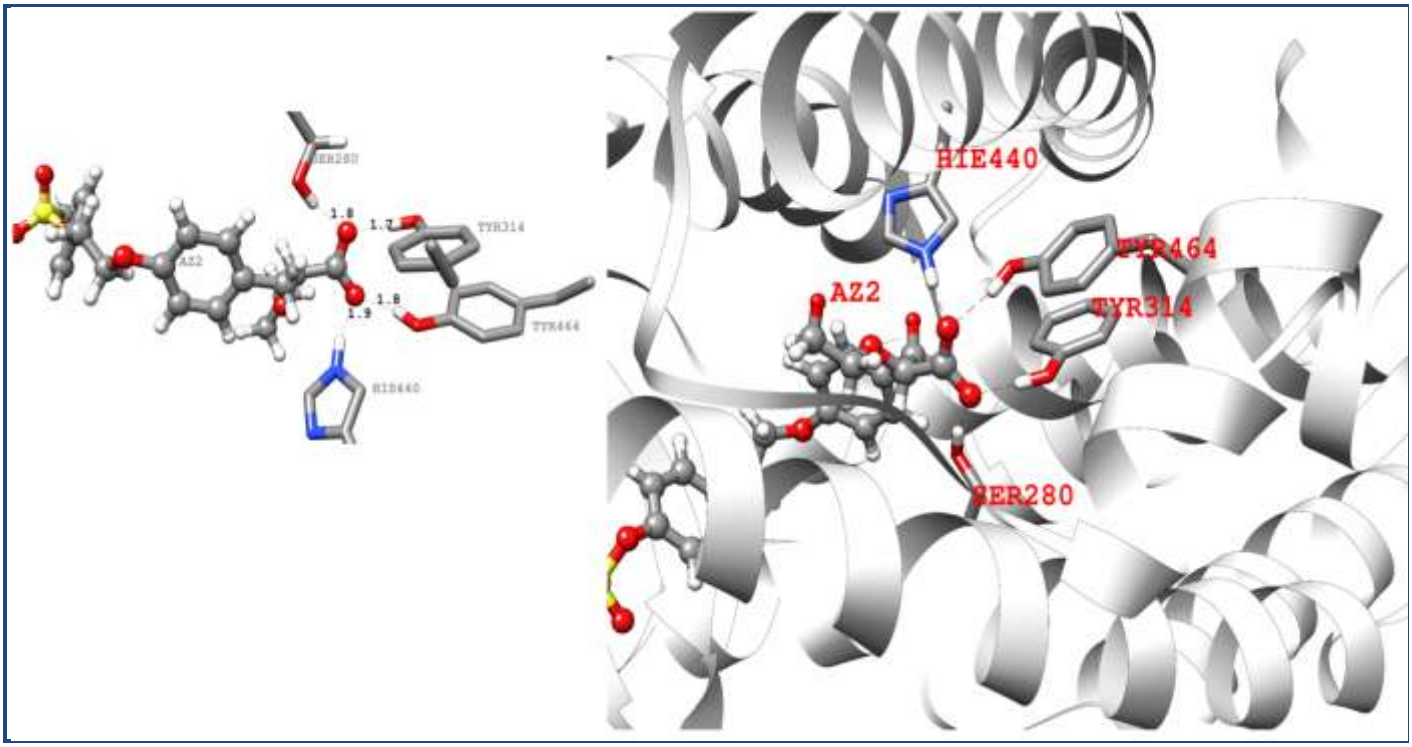

Figure 2: PPAR-α interaction with the co-crystallized compound AZ-2.

Methodology:

Target protein structure

The ligand binding domain (LBD) of human PPAR-α PDB: 1I7G receptor co-crystallized with agonist (2s)-2-ethoxy-3-[4-(2-{4-[(methylsulfonyl)oxy]phenyl}ethoxy)phenyl]propanoicacid (AZ-2), taken from the Protein Data Bank (www.rcsb.org).

Ligand compounds and structures

Ligands were downloaded in .mol format from pubchem (http://pubchem.ncbi.nlm.nih.gov/) website and prepared by LigPrep 2.5 (Schrödinger, Inc., NY, 2012).

Molecular energy minimization and preparation: Downloaded ligands were first converted from 2D to 3D structures; ionized using Epik 2.3 and energy minimized using Optimized Potentials for Liquid Simulations 2005 (OPLS_2005) force field. The protein was prepared automatically by prepwizard available in Maestro 9.3. Briefly, the preparation process included adding hydrogen, defining bond order of hydrogen, histidine protonation (Epik 2.3), minimizing the new structure in OPLS_2005 force field and adding missed side chains (Prime 2.3). Waters that are forming more than three hydrogen bonds and 5Å away from ligand binding pocket were deleted for clarity.

Molecular docking

The grid box was generated by clicking an atom of the co-crystallized ligand AZ-2. Extra precision (XP) Glide was used

for docking the prepared ligands into the grid (Glide 5.8, Schrödinger, Inc., 2012). All these processes were carried out in a PC ran on Windows7.

Binding energy calculation and scoring

The XP visualization tool (XPVT) provides a unique opportunity to visualize Glide results. Almost all the weak interactions were considered and more score will be awarded to the ligand that forms more hydrophobic contact with the active site of the protein. XPVT also provides XP scores which is more accurate than conventional glide and dock scores. High resolution images were obtained using Chimera 1.6.2 **[14].**

Results & Discussion:

The intermolecular hydrogen bonding interaction of the best-fit ligands were found to be associated with Tyr314, Ser280, His440, and Tyr464 amino acid residues at the receptor active site of PPAR-α. As shown in the **Table 1 (see supplementary material),** strong binding was observed for glycerate and found similar interactions **(Figure 1)** to that of co-crystallized AZ-2 **(Figure 2)** with a best glide score of -7.1. Previous studies have established that PPAR-α activators form hydrogen bonds to Tyr314, Ser280, His440, and Tyr464 of the receptor and these are necessary for receptor activation **[15, 16].** The docking structures of all the compounds showed that they bind in a very similar pattern with the active site of PPAR-α (data not shown). The best results obtained with docking scores are -7.1, -6.5, -6.4 and -6.0 of ligands glycerate, glyceraldehyde-3-phosphate (GAP), dihydroxy acetone phosphate (DHAP) and phosphoglycerate respectively. Guerre-Millo *et al.* **[4]** proved that lipid lowering drugs act through PPAR-α and improve insulin sensitivity besides reducing adiposity. Thus by finding a novel endogenous PPAR-α ligand with high physiological abundance is the need of the hour for diabetes researchers.

Docking study is particularly useful as a preparatory step for identifying ligands. It provides a faster, cheaper but highly efficient means of detecting the agonists. Previously Das *et al.* **[17]** have proved that Benzoxazinones as PPAR-γ ligand using Glide. In a recent study LTB4 has been reported as a PPAR-α ligand through docking and established its activity *in vitro* **[5].** A number of synthetic and semi-synthetic compounds were identified as PPAR agonists using molecular docking, especially through the recent advancement in tools like core hopping and high throughput virtual screening. But right from the beginning only certain class of drugs like fibrates were considered as potential ligands for PPAR-α. Here we show for the first time that some of the gluconeogenesis intermediates are physiologically relevant PPAR-α ligands by employing in silico docking.

Conclusion:

Availability of glycerol is the most critical for triacylglycerol synthesis. As mentioned earlier, adipogenesis is activated by triacylglycerol biosynthesis pathway intermediates. But most of the researchers till date ponder either the last few steps of the pathway i.e. after the formation of diacylglycerol or dietary triacylglycerols. This is based on the notion that PPAR-α, with a big ligand binding pocket and very large earlier reported ligands, can bind only huge molecules like fatty acids and triglycerides. Here for the first time we consider the first phase of triacylglycerol synthesis, the synthesis of glycerol itself, as a potent pathway for ligand identification. Studies conducted by Pastorius *et al.* **[18]** show that PPAR-α regulates the conversion of glycerol to glucose in the liver and PPAR-α ligands reduce free glycerol in serum. Moreover glycerate is the connection link between gluconeogenesis and glyceroneogenesis **[19].** This strongly coincides with our hypothesis and docking studies show that glyceroneogenesis pathway intermediate, glycerate can act as plausible PPAR-α ligand. We suggest that further investigation is necessary to confirm the biological activity of these compounds *in vitro* and *in vivo*. We conclude that small molecule precursors of the glyceroneogenesis pathway may activate PPAR-α and leads to adipogenesis.

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Supplementary material:

Table 1: Results of extra precision (XP) glide docking.

Compounds	Pubchem ID	Docking score	XP Score	Glide score
Glycerate	439194	-7.10821	-7.10821	-7.10821
Glyceraldehyde-3-phosphate	24794350	-6.51083	-6.60093	-6.60093
Dihydroxy acetone phosphate	668	-6.44694	-6.51694	-6.51694
3-phosphoglycerate	724	-6.06109	-6.20269	-6.20269
Glyceraldehyde	751	-3.79024	-3.79024	-3.79024
Dihydroxyacetone	670	-2.39398	-2.39398	-2.39398
AZ2 (co-crystallized ligand)	--	-10.0151	-10.0151	-10.0151