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Homology modeling and docking studies of Peroxisomal carnitine O-octanoyltransferase

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ABSTRACT

Computational docking is used for optimizing known drugs and for defining new binders by simulating their binding mode and affinity. AutoDock tools have been widely cited as necessary tools in structure-based drug design. These methods are rapid enough to declare virtual screening of ligand libraries. We selected a target involved in transferases enzyme class and provided a fully reproducible docking protocol. This paper will show how docking techniques would be an important asset to identify new ligands interactions with transferases. We used Non-3D structures of chosen transferases and built models for the proteins in trying to find putative compounds against them. Five proteins have structural data available in uniprot with varying degrees of structural coverage. Using homology-based methods; structural coverage of these proteins and built models for them through Swiss model. Designed ligands are tested by Autodock vina to study the interacting sites with the proteins. We have predicted putative drug like molecules using molecular docking that could bind to transferases. The stability of a few of our top docked protein-inhibitor complexes was evaluated based on molecular docking simulations. Our proposed inhibitors should potentially bind to enzyme proteins and hinder their function.

Keywords: Autodock; Ligands; PyMol; Swiss model; Transferases; Uniprot

1. Introduction

Transferases are a class of enzymes that transfer specific functional groups (e.g. a methyl or glycosyl group) from one molecule (called the donor) to another (called the acceptor).They are involved in hundreds of different biochemical pathways throughout biology, and are integral to some of life's most important processes (McDonald, Boyce et al. 2009).

Based on the type of biochemical group transferred, transferases can be divided into ten categories (based on the Enzyme Commission numbers (EC) Number classification). These categories comprise over 450 different unique enzymes (McDonald and Tipton 2022).

Molecular docking simulates the behavior of small molecules in the binding site of a target protein. As more protein structures are detected experimentally using X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy, molecular docking is increasingly used as a tool in drug discovery. Docking in contradiction of homology-modeled targets also becomes promising for proteins whose structures are not known. Molecular docking programs achieve a search algorithm in which the conformation of the ligand is valued until the convergence to the lowest energy is gotten. Finally, an affinity scoring function, ΔG [U total in kcal/mol], is employed to rank the candidate poses as the sum of the electrostatic and van der Waals energies. The driving forces

for these specific interactions in biological systems aim on the way to complementarities between the shape and electrostatics of the binding site surfaces and the ligand or substrate (Pagadala, Syed et al. 2017).

In this study, we have used unknown-3D structures of chosen transferases and built models for the proteins in trying to find putative compounds against them. OCTC_HUMAN Q9UKG9 Peroxisomal carnitine O-octanoyltransferase (PCOT) with ID Q9UKG9 have structural data available in uniprot (2017) with varying degrees of structural coverage. Using homology-based methods; we have extended the structural coverage of these proteins and built models for them through Swiss model (Waterhouse, Bertoni et al. 2018). Designed ligands are tested by Autodock vina to study the interacting sites with the proteins. We have predicted putative drug like molecules using molecular docking that could bind to transferases. The stability of a few of our top docked protein-inhibitor complexes was evaluated based on molecular docking simulations. Our proposed inhibitors should potentially bind to enzyme proteins and hinder their function.

2. Material and methods

2.1. Protein sequence analysis

The amino acid sequences of our enzyme were retrieved from UniProt (https://www.uniprot.org) with the accession number of Q9UKG9. Subsequently, the protein sequences were subjected into protein sequence analysis using Expert Protein Analysis System (ExPASy) Proteomics web server (www.expasy.org/tools).

2.2 Model evaluation and Prediction of putative small molecules that can bind to NiV proteins

The optimized 3D model obtained from the previous step was used to determine the overall structural quality assessment (stereochemistry) based on different type of internal angles, main chain ionic forces and interatomic interaction distances through the Ramachandran plot.

Docking was used to identify putative small molecules that can potentially bind and inhibit the activities of the transferases proteins. In this project, transferases proteins that had models built from templates with high identity (> 50%) and high coverage (> 45%) were used as targets for ligand screening. The screening library consisted of 89 ligands constructed from drug like molecules of the ZINC database (Irwin, Sterling et al. 2012). The 89 ligands were chosen as a practical measure to ensure wide coverage. Further, we visualize that during experimental trials all structurally similar small molecules to our predicted hits would be tested. Docking was performed using Autodock vina (Trott and Olson 2010). The target proteins were prepared for docking by Autodock vina, by adding missing polar hydrogen atoms. The ligand docking site, marked by affinity grids were generated using the Autogrid module of Autodock. The centre of the grid, number of grid points in X, Y, and Z directions and separation of grid points were chosen based on the predicted binding pockets using the ADT viewer from MGL tools (Fakhar, Naiker et al. 2016). For Peroxisomal carnitine O-octanoyltransferase (PCOT) we choose the first five ligands with higher score then make visualization on PyMOL (Morris, Huey et al. 2009) program for understanding the structural similarity between template and predicted model. PyMOL is often used to generate images of biomolecular structures. Hundreds of parameters in PyMOL provide precise control over the appearance of structures. The help function prints the function's documentation to the command history window. This documentation includes the PyMOL commands that the user can reuse by copying and pasting onto the command line or into a script file.

3. Results

Our results include OCTC_HUMAN Q9UKG9 Peroxisomal carnitine O-octanoyltransferase (PCOT): The homology modelling project "OCTC_HUMAN Q9UKG9 Peroxisomal carnitine O-octanoyltransferase" submitted to SWISS-MODEL workspace on Dec. 13, 2019, 9:59 a.m.

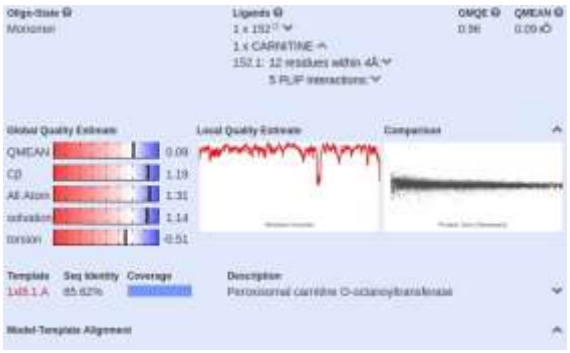


Figure 1: The model result of Peroxisomal carnitine O-octanoyl transferase (PCOT) with full coverage, 85.62% sequence identity, GMQE= .96, Qmean =0.09, oligo state is monomer and carnitine as ligand.

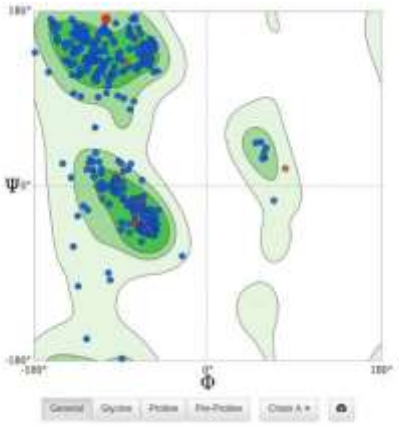


Figure 2: Ramachandran plot of Peroxisomal carnitine O-octanoyl transferase (PCOT); Ramachandran Favored was 96.33%, Ramachandran Outliers was 0.33% and Clash Score was 2.79

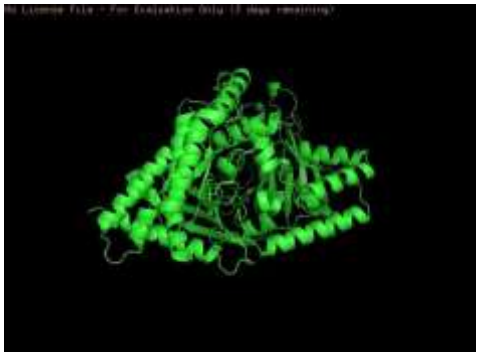
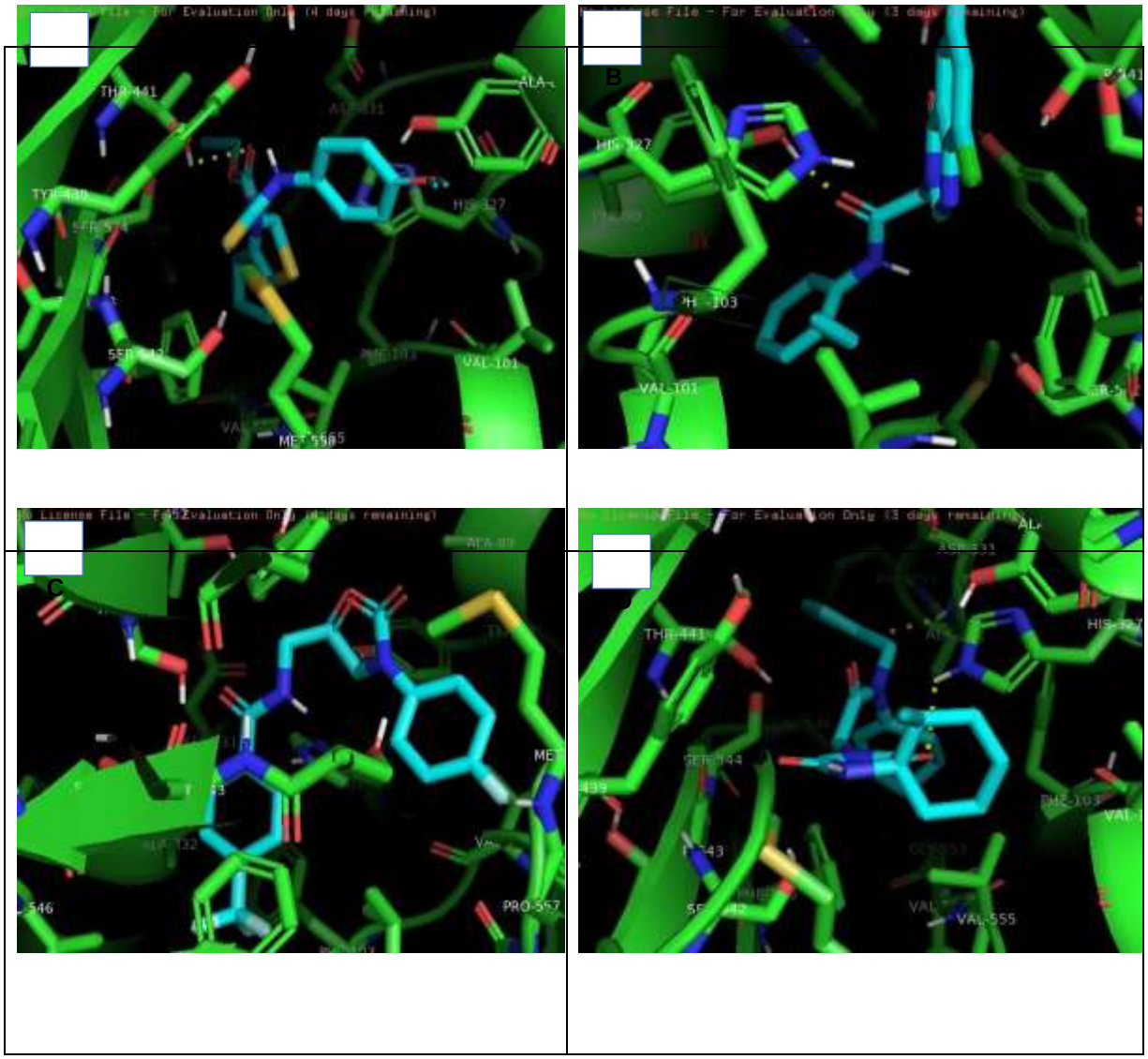


Figure 3: The molecular visualization by PyMOL of Peroxisomal carnitine O-octanoyl transferase (PCOT)

The 5 best hits from the library simulated for PCOT were: 84, 51, 48, 57 and 55 (Figures from 4.A-F)

Table (1): Q9UKG9 OCTC_HUMAN Peroxisomal carnitine O-octanoyltransferase (PCOT)

Ligand ID	Score
Cpds-mini84	-10.5
Cpds-mini51	-10.1
Cpds-mini48	-10
Cpds-mini57	-9.9
Cpds-mini55	-9.9



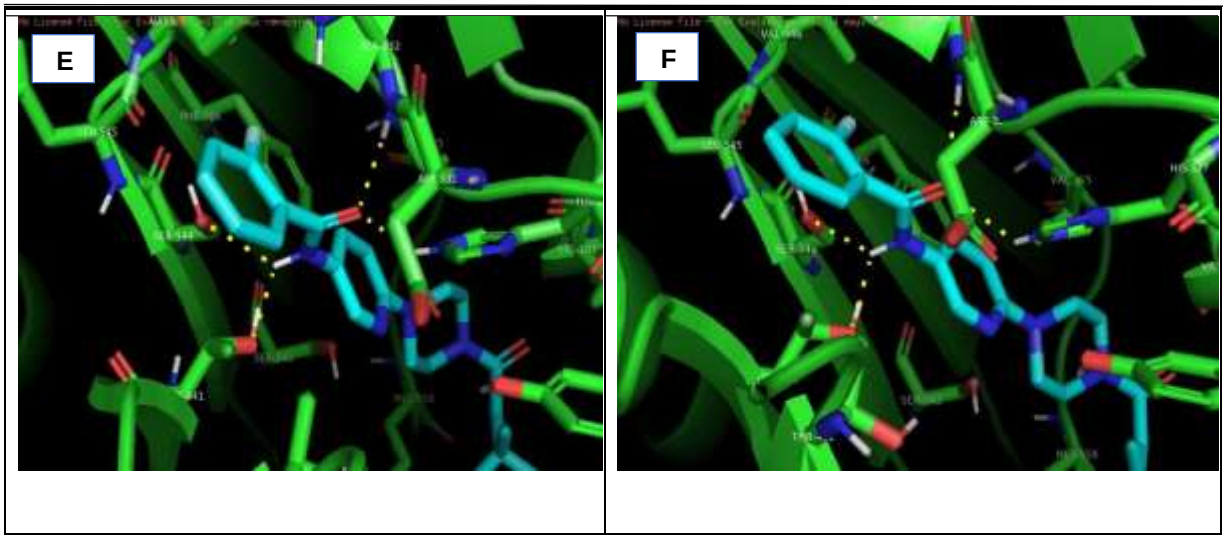


Figure 4: The 5 best hits from the library simulated for PCOT were: 84, 51, 48, 57 and 55 (from A-F), A: Ligand-84 includes only one H-bond with THR-441 in PCOT, B: Ligand-51 includes also only one H-bond with TYR-90 in PCOT, C: Ligand-48 does not include H-bond but actually it includes Van Der Waels interaction with Met-558 in PCOT, D: Ligand-55 attached by two H- bonds with HIS-327 and ALA-332 in PCOT, E: Ligand-57 is the most promising one in which it is attached to 4 H-bonds with: SER-544 and ALA-332, F:, Ligand-57 THR-441 and HIS-327 in PCOT.

The receptor-ligand interactions			
Q9UKG9 OCTC_HUMAN Peroxisomal carnitine O-octanoyltransferase (PCOT)			
Ligand ID	No. of H-bonds	H-bonds interacting residues	Residues involved in Hydrophobic interactions
Cpds-mini84	1	THR-441	-
Cpds-mini51	2	TYR-90	-
Cpds-mini48	-	-	Met-558
Cpds-mini57	4	SER-544 ALA-332 THR-441 HIS-327	-
Cpds-mini55	2	HIS-327 ALA-332	-

By drawing 2D structure by Chem draw for the most promising ligand (57), we found that the hydrogen bonds are included with 2 carbonyl groups (C=O), NH group and N of pyridine ring. Otherwise, there are electrostatic aromatic –aromatic interaction and 2 Vander Waels interaction with fluorine and tertiary butyl group.

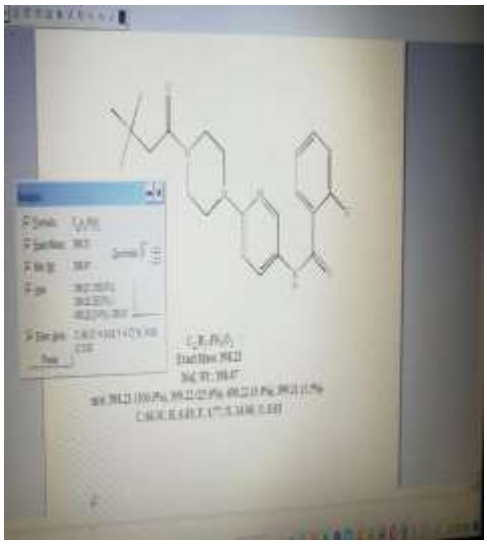


Figure 5: 2D structure for ligand-57 by Chem Draw

4. Discussion

Peroxisomal Carnitine octanoyltransferase (PCOT) facilitates the transport of medium- chain fatty acids through the peroxisomal membrane, that is physiologically inhibited by malonyl-CoA. Using an "in silico" macromolecular docking approach, (Morillas, Gómez-Puertas et al. 2002) built a model in which malonyl-CoA could be attached near the catalytic core. This disrupts the positioning of the acyl-CoA substrate in the channel. Putative malonyl-CoA domain contained **His 340**, implicated together with **His 131** in COT malonyl-CoA sensitivity. Morillas et al., 2002 mutated COT His 131 the malonyl-CoA competed with the substrate decanoyl-CoA. Mutation of COT **Ala 332**, present in the domain 8 amino acids away from His 340, decreased the malonyl-CoA sensitivity of COT. We conclude that this malonyl-CoA domain is present in COT protein and might be the site at which malonyl-CoA interacts with the substrate acyl-CoA.

Identification of residues responsible for the inhibition of COT by malonyl-CoA is an important step in elucidating the key to the control of β-oxidation. These open the way to study whether specific mutations in carnitine acyltransferases alter the metabolism of fatty acids. Understanding of malonyl-CoA interaction sites in COT is important in the design of drugs to control an excess of fatty acid oxidation in the pancreatic β -cell, and also to prevent the development of diabetes mellitus (Kim, He et al. 2014).

A few pharmacological analogues of the physiological inhibitor malonyl-CoA have been studied for their ability to regulate CPT I and PCOT, including the glycidic acids such as TDGA, clomoxir and etomoxir. They are functionally active only after metabolic conversion into their CoA esters and presumably inhibit CPT I via covalent binding to the protein through the reaction of their epoxide moiety (Morillas, Clotet et al. 2000). Taken together, the results of the present study, demonstrate that the 4 H-bonds with: SER-544 and ALA-332 (Fig. 4A), THR-441 and HIS-327 (Fig 4B) of PCOT are responsible for the action of ligand-57 on the protein.

Conclusion

In conclusion, these results can serve as a model to study the effect of ligand -57 on malonyl- CoA binding sites in carnitine octanyltransferase. The exact understanding of how ligand-57 interacts with those enzymes to which it binds may facilitate the development of other drugs that interfere with the metabolism of fatty acids in several tissues like liver, heart and muscle and in pancreatic β-cells. Studies on these topics could be confirmed experimentally.

Disclosure

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