



Mechanistic exploration of paclitaxel as an anticancer against the OATP3A1 transporter a computational approach

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Abstract

Background: Breast cancer (BC) is second highest cause of cancer-related deaths all over the world, from uncontrolled cell growth that spreads to other organs in human body results metastasis. Paclitaxel is the popular drug used for treatment of breast cancer but it has demerit like poor membrane permeability and high side effects.

Methodology: In this study we performed structural homology modeling by I-Tasser, refine, validation of protein structure, molecular docking, molecular dynamics simulation and its interactions with PTX, and mechanism of Paclitaxel transportation by organic anion transporter in breast cancer cells by computational approaches.

Results: The protein constructed by using I-Tasser a computational tools shows excellence quality of protein structure when validated procheck. The topology of protein was demonstrated the 12 transmembrane helix which followed the MSF protein family. The molecular docking shows the hydrogen bond interaction with amino acid ARG⁵⁸². Molecular dynamics simulation with insertion of protein into the DPPC membrane shows the major alteration in protein backbone. Ligand also shows the shifting from its initial binding to another location which evidently that movement happen in dynamic system.

Conclusion: The model protein showed the excellent quality and molecular docking and dynamics results indicating the mechanism of PTX during the MD simulation.

Keyword: Breast cancer, Structure homology modelling, Molecular docking, SLCO, OATP, PTX, Molecular Dynamic Simulation, *In Silico* Analysis.

Abbreviation

BC Breast cancer ,SLC Solute carrier ,OATP Organic anion–transporting peptide ,2D- Two dimensional, 3D- Three dimensional ,RMSD, root mean square deviation; RMSF, root mean square fluctuation , MD, molecular dynamics, L-RMSF ligand root mean square fluctuation ,PTX Paclitaxel.

1. Introduction

In the year 2020, breast cancer became the most common type of cancer worldwide, surpassing lung cancer. It accounted for around 2.3 million new cases, which is 11.7% of all cancer cases. Additionally, breast cancer ranked as the fifth highest cause of death, with 685,000 fatalities, representing 6.9% of all deaths. The breast is a type of modified apocrine glands that have evolved to specifically provide sustenance to offspring^{1,2}. Although there are signs of notable advancement in the prevention and early identification of breast cancer, the achievement of effective treatment options still remains an unresolved objective. The heterogeneity of breast cancer is a significant obstacle to attaining treatment effectiveness^{3,4}. Breast cancer is an uncommon disease with significant variation, which can be noticed at many levels. Intertumor heterogeneity refers to variations between patients in how the disease presents itself, leading to different treatment approaches and outcomes. On the other hand, intratumoral heterogeneity refers to the presence of genetically and phenotypically diverse cell populations within a single tumor⁵. Drug targeting breast cancer shows chemo resistance due to improper transportation of drug from extracellular fluid to intra-cellular, other mechanism of drug resistance include fluidity of the membrane.^{6,7} Organic Anion Transporting Polypeptide 3A1 (OATP3A1) is a vital member of the solute carrier organic anion transporter family. It is responsible for facilitating the movement of several naturally occurring and externally introduced substances across cell membranes. One such transport protein OATP3A1 which is a Na⁺-independent transporters play a crucial role in transporting wide range of amphipathic chemical molecules, both endogenous and exogenous present in cell membrane of breast cancer which increased the transportation of drug molecule across the membrane. Paclitaxel is a well-known anticancer drug molecule which interfere the normal function of microtubule during cell division^{8,9}. OATP3A1 contains a way to define paclitaxel as a substance that can be transported, making it simple for the cells to take it in. This may raise the intracellular concentration of paclitaxel, therefore potentially enhancing its inhibitory effect on cancer cells. The expression level of OATP3A1 in cancer cells can impact the efficacy of paclitaxel. Elevated OATP3A1 expression may result in enhanced absorption of paclitaxel, hence increasing its therapeutic efficacy^{10–12}. The interaction between paclitaxel and OATP3A1 is a complex and crucial element in enhancing paclitaxel therapy for the treatment of cancer. Developing a comprehensive understanding of this interplay can result in the

development of enhanced therapeutic approaches¹³. The SLCO gene family encodes a group of membrane transport proteins known as organic anion transporter polypeptides (OATPs), which are known to be the most extensive family of such proteins. These OATPs may have a significant function in delivering medicines in breast cancer^{14,15}. OATP3A1, commonly referred to as SLCO3A1 is a part of the solute carrier organic anion transporter family. The expression of OATP3A1 in various tissues, including the liver, brain, and cancer cells, and plays a role in transporting a diverse array of naturally occurring and externally introduced organic anions¹⁶.

2. Material and methods

2.1 Sequence Database

Sequence for OATP3A1 protein was obtained from the UniProt KB database (<https://www.uniprot.org/uniprotkb/Q9UIG8/entry>) (UniProt id Q9UIG8). UniProt is the collective database for the primary knowledge.

2.2 Structural Homology Modeling

The tertiary structure (3D) of OATP3A1 protein has been modelled by using computation tools like Phyre2 and I-Tasser. In brief, the sequences submitted as a query to Phyre2 and I-Tasser respectively. The model constructed by I-TASSER showed the greater coverage, reliability, and overall quality. I-TASSER begins with the target protein's amino acid sequence and uses multiple threading alignments and iterative structural assembly simulations to create full-length atomic structural models. (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>), (<https://zhanggroup.org/I-TASSER/>).

2.3 Validation of protein structure

The constructed model refines by online computational tools like KoBaMIN and 3D refine for the improve the quality of protein structure. For improving protein structure by atomic-level energy minimization and hydrogen-bond network optimization with knowledge-based force fields, both tools provide an online platform. PROCHECK (<https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/>) tool was employing on the Ramachandran plot for assessed the secondary structural properties and stereochemistry of the protein structure¹⁷.

2.4 Prediction Binding Site and tunnel detection

We reviewed the literature and subjected the primary structure to computational ligand-binding site prediction tools, such as COACH and 3-D-LigandSite, to determine substrate binding pocket. The carrier route and membrane-lining sites where the residues engage in the ligand's transportation were discovered by the Pore Walker web online server (<https://www.ebi.ac.uk/thornton->

[srv/software/PoreWalker/](#)). It defines the optimal cavity, finds the centre of the pore axis, and evaluates pore properties such as pore size, geometrical frequency, atoms lining the cavity, and residues ¹⁸.

2.5 Molecular Docking

For the purpose of to conduct molecular docking analysis using AutoDock Vina package 1.5.7, the proteins were prepared by eliminating water molecules and introducing polar hydrogen atoms, Kollman charge, and Gasteiger charge to the ligands, respectively. The target proteins and ligands were transformed into the PDBQT format. In addition, we organized the grid box by utilizing the autogrid tool provided by the AutoDock wizard. The protein's grid box had x, y, and z coordinates of 58.53, 61.07, and 48.44, respectively. In order to enhance the binding affinity within the anticipated binding pocket, all residues that are involved in the substrate binding pocket were included within a grid box with a spacing of 4Å around the target proteins. Following the docking process, many ligand states were produced based on the interactions between amino acids and their binding affinities. These ligand states were then demonstrated using LigPlot ^{19,20}.

2.6 Molecular Dynamics (MD) Simulation

Desmond was employed to do the molecular dynamic (MD) simulation using the OPLS3e force field. A DPPC (dipalmitoylphosphatidylcholine) membrane, pre-equilibrated at 325.0 K, was utilized to construct the systems, in conjunction with the water salvation model. Sodium ions (Na⁺) and chloride ions (Cl⁻) were used as counter ions. The systems were built in an SPS environment, utilizing an orthorhombic box and a buffer distance of 10 Å. The membrane was aligned perpendicular to the central pore of the protein-ligand complex, in accordance with the shape of the helices. The simulation was run for duration of 300 nanoseconds on the prepared systems. The simulation was conducted in the NPγT ensemble at a temperature of 325 Kelvin. The simulations included a Nose-Hoover chain thermostat for temperature control and a Martyna-Tobias-Klein barostat for maintaining a constant pressure of 1.01 bar. We utilized the RESPA integrator to apply the default relaxation approach for bound atom pairs. We employed distinct approaches to handle the non-bonded interactions for atom pairings that were in close proximity (2 fs) and those that were far apart (6 fs). The threshold distance for short-range Columbic interactions was established at 9 units. We employed the Simulation Event Analysis pipeline in Maestro 2020.2 (Schrödinger LCC) to calculate atomic interactions and distances. We analyzed the molecular dynamics (MD) trajectories and generated visual representations using Pymol 5.7, software developed by Schrödinger LCC in New York, NY, USA. We utilized Maestro 2020.2 to scrutinize the

simulation data of Schrödinger LCC. Multiple trajectories were extracted at different time periods to calculate various complex parameters ^{21,22}.

3. Results

3.1 I-Tasser constructed the OATP3A1 model, which showed the highest quality among other modeling techniques.

It was demonstrated that there is 100% confidence in the amino acid sequence homology similarity between the Catspermasome transporter (PDB ID-7eeb) and OATP3B1 (S1). Proteins are thought to share a structure comparable to that of the Catspermasome transporter protein. I-Tasser model was selected for additional research because it outperformed the other web servers in terms of query coverage, confidence levels, overall models quality, and the innermost open model (Fig 1).

#	Template	Alignment Coverage	3D Model	Confidence	% Id.	Template Information
1	c7eeb_	Alignment		100.0	24	PDB header:protein transport Chain: I. PDB Molecule:succinyl-like domain-containing protein; PDBTitle: crystal structure of a bacterial homolog to human PDB Entry: 7eeb RCSB PDB
2	c7eeb_	Alignment		99.9	21	PDB header:transport protein Chain: A. PDB Molecule:major facilitator family transporter; PDBTitle: crystal structure of a bacterial homolog to human PDBEntry: 7eeb RCSB PDB
3	c7eeb_	Alignment		99.7	20	PDB header:transport protein Chain: A. PDB Molecule:multidrug resistance protein d; PDBTitle: crystal structure of a multidrug transporter from PDB Entry: 7eeb RCSB PDB
4	c7eeb_	Alignment		99.6	21	PDB header:transport protein Chain: A. PDB Molecule:sugar efflux transporter; PDBTitle: crystal structure of a multidrug transporter from PDB Entry: 7eeb RCSB PDB
5	c7eeb_	Alignment		99.6	21	PDB header:membrane protein Chain: A. PDB Molecule:multidrug resistance protein d; PDBTitle: crystal structure of a multidrug transporter from PDB Entry: 7eeb RCSB PDB
6	c7eeb_	Alignment		99.0	15	PDB header:membrane protein Chain: A. PDB Molecule:solute carrier family 15 member 1; PDBTitle: hisep1 bound to ala-phe in the outward facing occluded PDB Entry: 7eeb RCSB PDB
7	c7eeb_	Alignment		99.8	19	PDB header:membrane protein Chain: A. PDB Molecule:multidrug resistance protein d; PDBTitle: crystal structure of a multidrug transporter from PDB Entry: 7eeb RCSB PDB
8	c7eeb_	Alignment		99.8	15	PDB header:membrane protein Chain: A. PDB Molecule:multidrug resistance protein d; PDBTitle: crystal structure of a multidrug transporter from PDB Entry: 7eeb RCSB PDB
9	c7eeb_	Alignment		99.7	15	PDB header:transport protein Chain: A. PDB Molecule:multidrug resistance protein d; PDBTitle: crystal structure of a multidrug transporter from PDB Entry: 7eeb RCSB PDB
10	c7eeb_	Alignment		99.6	16	PDB header:transport protein, membrane protein Chain: B. PDB Molecule:glucose transporter gp; PDBTitle: the inward-facing structure of the glucose transporter PDB Entry: 7eeb RCSB PDB



Figure 1 The tertiary structure (3D) of the OATP3A1 protein has been modelled using computation tools like Phyre2 and I-Tasser.

3.2 Validation of protein structure

The Ramachandran plot illustrates the phi-psi torsion angles of all residues in the structure, except for those situated at the termini of the chain. Triangles unambiguously denote the existence of glycine residues, irrespective of their positioning within the anticipated sites for other types of side chains. The map utilises colouring and shading to visually depict the various regions. The darkest portions, shown

by the colour red, correlate to the core regions. These core regions represent the combinations of phi and psi values that are most advantageous. The regions that are most favoured are A, B, and L. The total number of residues is 523, which accounts for 86.7% of the whole. Moreover, there are additional permitted areas, namely [a, b, l, p]. The number of residues is 72, which accounts for 11.9%. The regions [$\sim$ a, $\sim$ b, $\sim$ l, $\sim$ p] have been granted with great generosity. The number of residues is 6, which accounts for 1.0% of the total. There are disallowed sections denoted by the symbol [XX]. There are 2 residues, which is equivalent to 0.3% (Fig 2).

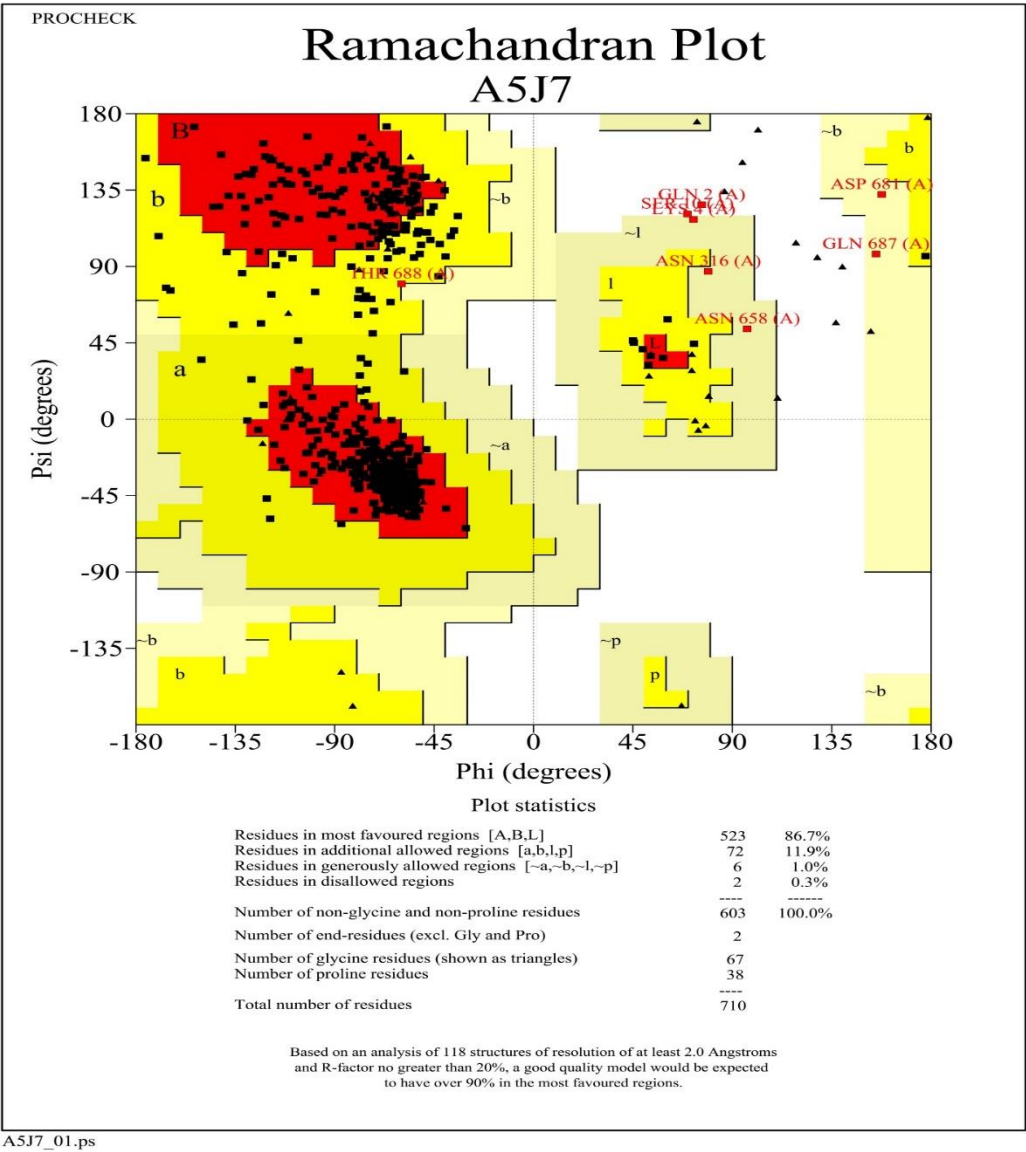
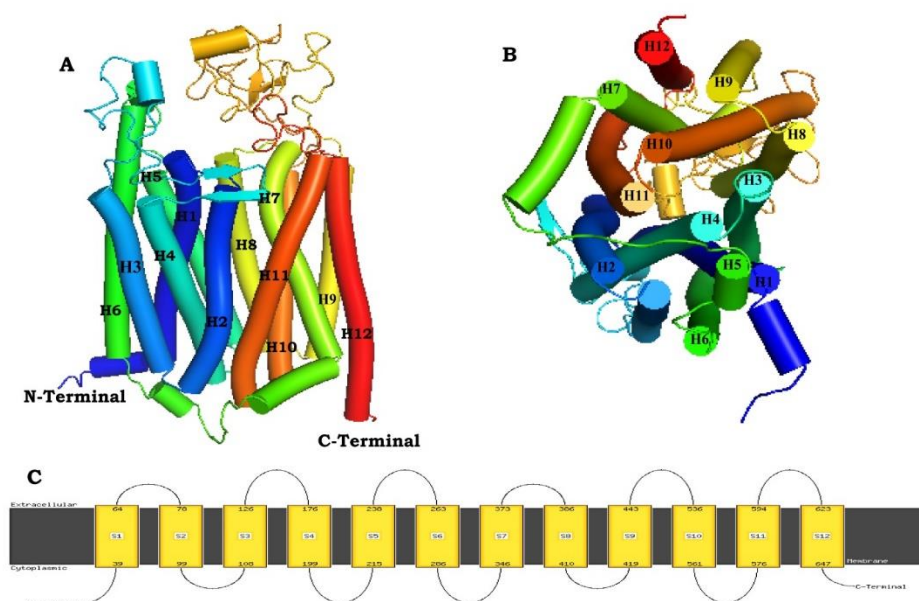


Figure 2 Ramachandran plot of OATP3A1 retrieved from PROCHECK (PDB A5J7).

3.4 The twelve-helix structure of the validated OATP3A1 model has a cytoplasmic terminus.

The protein's quality was evaluated using the Ramachandran plot. It revealed that the preferred region 86.7% included Number of the atomic residue. I-Tasser and phyre2 protein model was deemed suitable for more investigation, as evidenced by the 11.91% showing an additional favoured region and the 0.3% present being the two residues in the forbidden region. The protein's topology showed that it has twelve Tran's membrane helices. Five loops are intracellular, and the remaining six loops are extracellular (Fig



3).

Figure 3 (a) Three-dimensional structure model of the OATP3A1 protein generated by homology modelling in an inward-open conformation containing two N and C terminal domains consisting of 12 transmembrane helices. (b) Arrangement of helices on the periplasmic side (top view). (c) The topological arrangement of the OATP3A1 protein across the membrane from H1 to H12, with both terminal ends directed toward the cytoplasmic side.

3.5 Pore Walker identified a central tunnel with hydrophobic residues lining the extracellular to intracellular side of the protein.

For tunnel identification, the Pore Walker algorithm generated a single central tunnel that travelled from the extracellular to the intracellular side of the protein at the N-C domain contact. The majority of amino acids found in the pore residues included alanine, threonine, valine, leucine, serine, glycine, and others. The amino acid residues in the substrate-binding pocket overlapped with the transport channel that Pore Walker predicted Fig 4 (A-D).

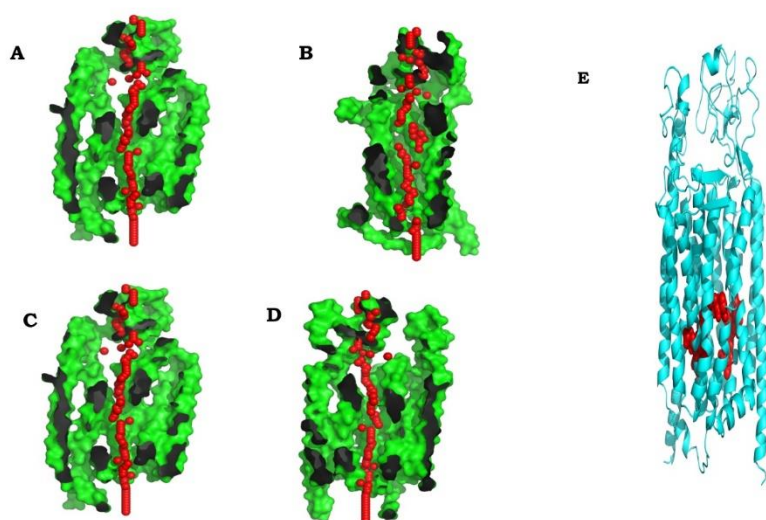


Figure 4. The path of ligand movement is predicted using the Porewalker programme for the structure model of the OATP3A1 protein (red spheres represent pore centres). The top left represents the XZ-plane section, with coordinates Y > 0 (B). Top right: XZ-plane section < 0 coordinates (C) Bottom left: XY-plane section, Z > 0 coordinates (D) Bottom right: XY-plane section, Z < 0 coordinates, and (E). The binding pocket of the protein showing amino acid residues that are actively participating in the binding with the ARG⁵⁸² amino acid residues were represented in the binding pocket.

3.6 Protein-ligand docking revealed a traditional MFS-like binding pocket.

A single substrate-binding pocket is typically observed in MFS proteins. PTX was docked utilising three different binding pockets: the one derived from pore walker and literature, the one identified from COACH, and 3-DLigandSite. ARG⁵⁸² amino acid residues were represented in the binding pocket showing the Fig 4(E).

3.7 Strong binding between PTX and OATP3A1 was demonstrated by hydrogen and hydrophobic interactions.

Docking studies were conducted to investigate the interaction between PTX and the OATP3A1 protein at a predicted binding site, after the identification of the potentially significant binding pocket. (Table 1) showed that PTX had a binding affinity of -11.4 kcal/mol towards the protein. Moreover, the ligand first established hydrogen bonds with ARG582. The remaining residues, including VAL558, GLN554, THR555, GLU361, GLY198, GLM195, PRO590, ALA370, and LEU222, exhibited hydrophobic interactions with the ligands. The OATP3A1-paclitaxel complex demonstrated ARG-582 hydrogen

bonding, electrostatic interactions, and hydrophobic interactions. The 2D depiction was created using the LigPlot+ tool; BIOVIA Discovery Studio Visualizer v21.1.020298 (Fig-5).Figure 7(A) illustrates the Schrodinger analysis of the 2D complex structure before and after molecular dynamics (MD) simulation. Figure 7(B) depicts the pre-MD simulation of the interaction between the protein and ligand.

Table 1

S.No	Target	PubChem Drug CID	Drug	Binding Energy (kcal/Mol)	No. of H-Bond	H-bond forming Residues	Average Distance of H-Bond (Å)
1	OATP3A1	36314	PTX	-11.4	1	ARG ⁵⁸²	~3.17

Table 1.The molecular docking scores show interaction between Paclitaxel and OATP3A1.

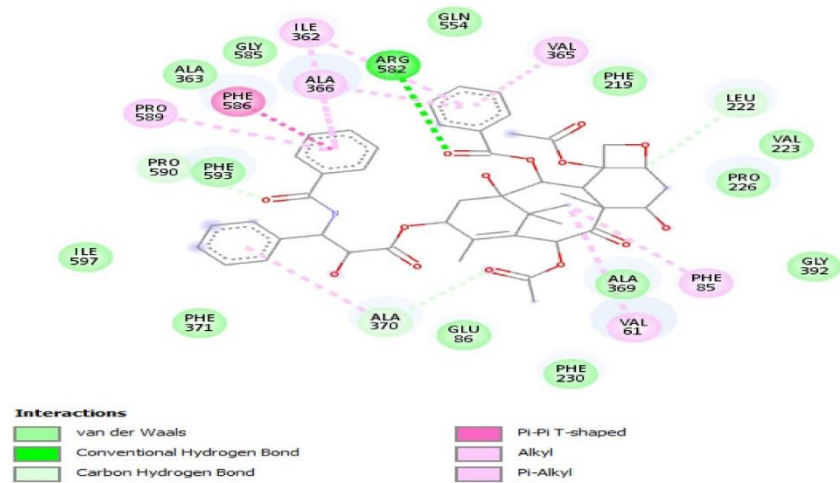


Figure 5. The OATP3A1-Pactitaxel complex displayed its 2D interaction pattern by utilising the LigPlot+ tool and BIOVIA Discovery Studio Visualizer v21.1.020298.

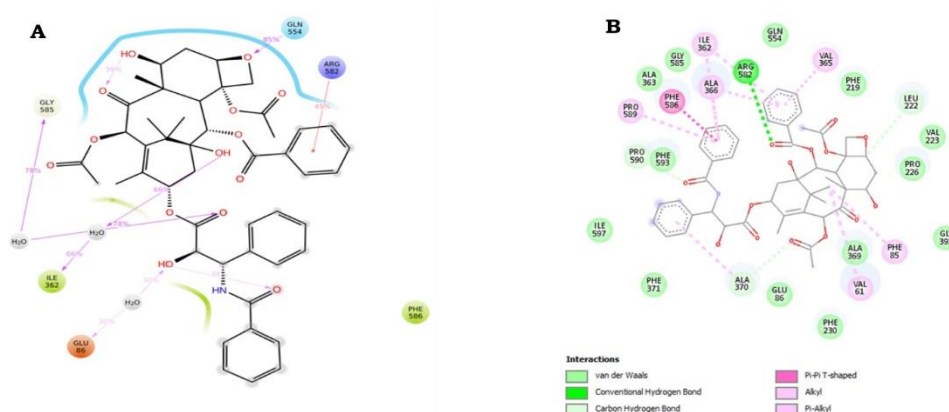


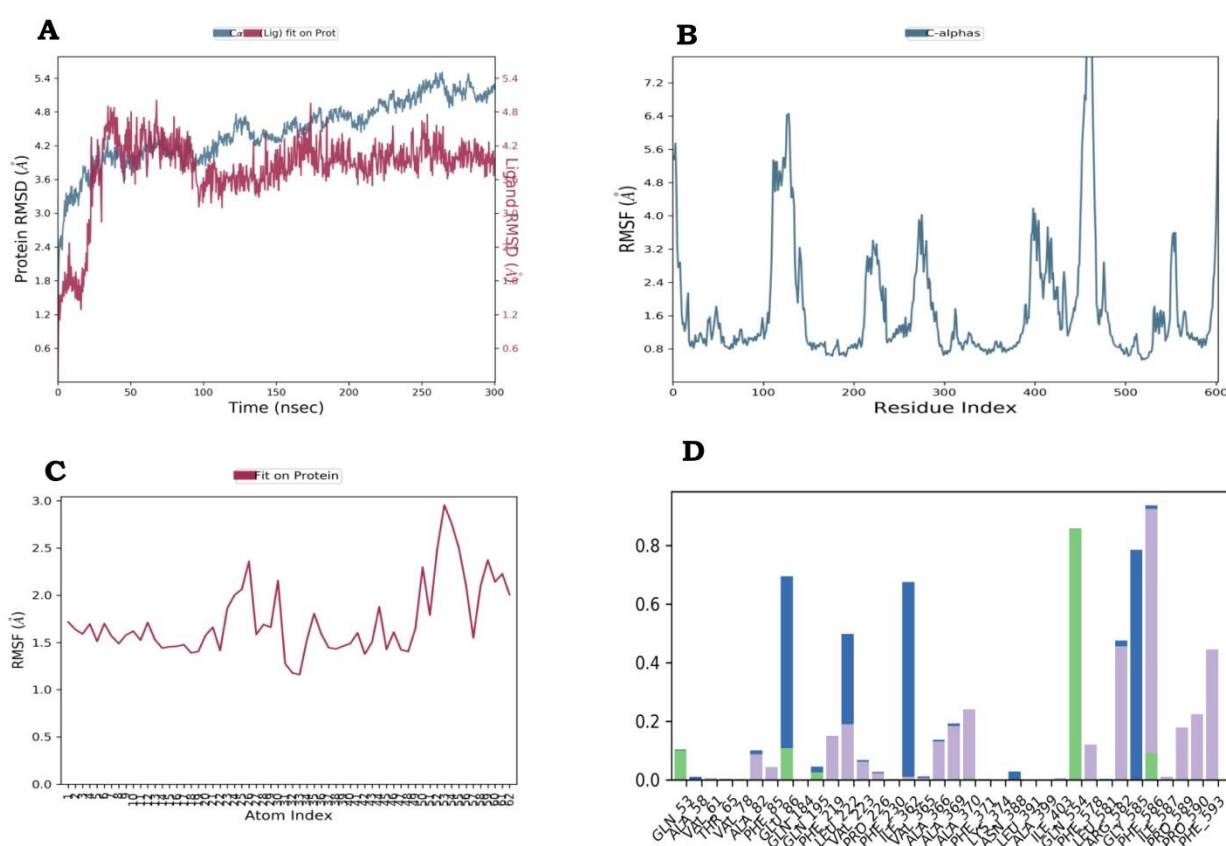
Figure 7. Binding energies and other interaction studies of the OATP3A1-paclitaxel complex. (A) The OATP3A1-paclitaxel complex exhibited ARG-582 hydrogen bonding, electrostatic interactions, and hydrophobic interactions. The 2D representation was generated using the ligand interactions module of Schrödinger. (B) The OATP3A1-Pactitaxel complex displayed its 2D interaction pattern by utilising the LigPlot+ tool and BIOVIA Discovery Studio Visualizer v21.1.020298.

3.8 MD- Simulation of OATP3A1 protein and ligand complex analysis

Molecular dynamics (MD) was employed to examine the physical movements of the molecule, as explained. We conducted a 300 ns molecular dynamics (MD) simulation to assess the stability of the docked complexes OATP3A1 and PTX, specifically focusing on any structural rearrangements. To understand the way systems (such as protein and ligand complexes) function as well as how they bind together, the Desmond software The Desmond platform accessed through Maestro 2020.2 (Schrödinger LCC) was used to evaluate their dynamics and stability. The system's dynamic stability was evaluated by analysing the root mean square deviation (RMSD) profile of the protein's backbone atoms at the 300 nanosecond mark (Figure 7A). During the whole MD simulation, the dock complex maintains a consistent RMSD value ranging from around 0.6 to 4.2 across a simulation length of 0 to 100 ns. This illustrates the process of protein stabilisation by counteracting the effects of PTX. The RMSF analysis confirmed the accuracy of the RMSD measurement by demonstrating the variability of residues. We monitored the displacement of particular residues employing an RMSF graph (Fig 6B). The observed phenomenon can be attributed to the alteration of the larger structural conformations (ranging from 0.8 to 7.2 Å) of the amino acid residues located between positions 440 and 480. Notably, these residues exhibited more significant alterations in their C α atoms compared to other regions. Small structural alterations (0.8–3.2 Å) are detected in the areas consisting of amino acid residues 480–600. The Ligand

root mean square fluctuation (L-RMSF) measures the variations in the position of the ligand atoms and examine the hydrogen bonding in the OATP3A1-PTX complex. Fig 6 (A-D) depict the protein-ligand interaction of the complexes.

Figure 6. The OATP3A1-PTX complex root mean square deviation studies. The MD-Simulation study examined the conformational stability of the OATP3A1 protein over duration of 300 ns. (A) RMSD of the OATP3A1-PTX complex. (B) RMSF profile of the OATP3A1-PTX complex. (C) RMSF profile of the Ligand (L-RMSF). (D) The protein-ligand interaction in the OATP3A1-paclitaxel complex during the 300 ns simulation is represented using a stacked bar chart.



4. Discussion

PTX is an anti-cancer drug widely used for breast, prostate, and lung cancer. It displayed low membrane permeability and resistance. Because of its huge structure and high molecular weight, it might happen²³. Our theory validates that the OATP3A membrane protein transporter, which is highly expressed in breast cancer cells, our hypothesis is the mechanism by which PTX is transported inside membrane cells. While previous studies were reported the mechanism underlying the OATP3A1 that OATP3A1

operates via a rocker-switch mechanism ²⁴. The protein's 3D structure was not available at the PDB database. The structure of the protein was modelled in three dimensions using the fold recognition approach. With a confidence level of 100% and query coverage of 89%, the constructed model demonstrated the homology of the query and template sequences. It was thought that I-Tasser used the sequence similarities between the three-dimensional structure and the crystal structure. The Catspermasome transporter (PDB ID-7eebL) was most used as a template when creating the three-dimensional protein structure Fig.1 ^{25,26}. The 3D structural validity of the OATP3A1 protein was confirmed prior to energy minimization. Energy minimization is an essential procedure that seeks to maximise structural and conformational stability by lowering the system's total potential energy. It works by correcting atypical protein geometries in order to relieve internal constraints and build stronger structures with precise stereochemistry. As a result, this phase was critical to producing a superior, enhanced the quality of 3D structure ^{27,28}. Ramachandran plot was used to validate the 3D structural by PROCHECK online server. The findings show, the energy-minimized of protein structure is very stable, with a higher percentage of residues situated in the approved and favoured regions Fig 2. Using COACH and 3-D-LigandSite for proteins, we also predicted ligand-binding sites; the outcomes were the same as those reported Fig (4) ^{29,30}. Strong initial binding of PTX to OATP3A1 with a binding affinity of -11.4 kcal/mol and hydrogen bond interactions with ARG⁵⁸² was shown by our computational modelling and molecular docking experiments. These results showed that OATP3A1 might be involved in the transfer of PTX, shows in Fig-5 ³¹. To confirm the changes in structural stability of protein-ligand complex, we also performed the MD simulation. The RMSD shows the major fluctuation of more than 5.4 Å, and we also observed the highest fluctuation during 50-100ns in MD simulation Fig 6 (A). The RMSF of backbone protein also demonstrated the major changes in fluctuation spectra as usually recorded with global proteins and we recorded the maximum fluctuation in RMSF between the 400 to 500 amino residues of protein, it indicating the these amino acids showed major change in protein configuration Fig 6 (B,D). We also examine the types of interaction with ligand with protein during MD simulation and we recorded the hydrogen bond gradually decreases during the 300ns MD simulation Fig 6 (C). Hence the 2- Dimensional structure of ligand interaction also depicted the removing the hydrogen bond during MD simulation. It was indicated the shifting the ligand from initial location to another location and break the bond in revealed Fig 7 ^{32,33}.

5. Conclusion

In overall, our results confirm the idea that OATP3A1 plays a role in the interaction with PTX. The findings indicate that PTX has the ability to facilitate the transportation of the OATPs protein, which is highly expressed in breast cancer cells. Ultimately, these investigations provide valuable insights into

the temporal dynamics of the OAP3A1-PTX complex. The simulation emphasizes the intricate interplay between movements, adaptation, and structural changes. These findings can help understand the structural alterations of the protein-ligand complex and their functional importance.

6. Conflict of interest

The authors declare no conflicts of interest

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