Repurposing of FDA approved drugs for Hereditary Angioedema – An Insilico Molecular Docking and Dynamics study

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Running Title: Repurposing of Drugs for the treatment of Hereditary Angioedema – An *in silico* Approach

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Abstract

Hereditary angioedema is characterized by repeated episodes of extremely severe swelling (angioedema) of the hands, feet, limbs, face, digestive system and respiratory tract. An attack can be triggered by a minor trauma or stress, but oedema usually occurs without warning. The inflammation of the airways can lead to obstruction and difficulty breathing. It is a rare autosomal dominant disease caused by the absence or dysfunction of a C1 inhibitory protein. The current treatment for HAE includes C1 esterase inhibitors (cinryze and haegarda), Plasma kallikrein inhibitors (Lanadelumab, Berotralstat) and bradykinin B2 receptor antagonist. Patients with hereditary angioedema have benefited enormously from the boom in new drug development and approval. However, the financial consequences have made it difficult to obtain these potentially revolutionary and life-saving drugs. Thus, with the drug repurposing approach, a set of test drugs from the FDA databases was paired with the chosen target protein 7n7x. Molecular dynamics was also performed to explore the conformational space. The result showed that tropism (binding affinity of -9.1) and losartan (-8.6) showed a higher binding energy than other drugs suggesting their potential as novel therapies.

Keywords: Drug repurposing, Hereditary angioedema, Losartan, Molecular dynamics, Tropism

Introduction

Hereditary angioedema (HAE) is a rare condition that causes episodic oedema formation with potentially fatal consequences. It is a rare disorder that affects one in fifty thousand people. The body components that swell most frequently are the limbs, face, digestive system, and airway. Laryngeal oedema can cause asphyxiation; abdominal angioedema attacks can result in unnecessary surgery and diagnostic delays, as well as narcotic dependency due to severe pain; and coetaneous attacks can be disfiguring and disabling.

A specific protein named bradykinin in the body is imbalanced in this condition. This causes fluid to be pushed near the areas of body by blood vessels due to which the swelling occurs. HAE is caused by a problem with a gene that produces a blood protein called C1 inhibitor. In most cases people are deficient in this protein. The primary role of C1INH is to regulate the activation of the complement system, the contact system, and intrinsic coagulation system. There are 2 types of HAE have been described. Type 1 HAE, is caused by the decreased production of C1-INH, resulting in subnormal blood and tissue inhibitor activity which is estimated to occur in 80 to 85 % of patients and in type 2 HAE, normal or elevated quantities of functionally impaired C1-INH are produced which occurs in the remaining 15 to 20 % of patients. A variant, possibly X-linked, inherited angioedema has recently been described and has been named "type 3" HAE (Davis., 2005). The main symptom of hereditary angioedema is swelling without any itching effect. It can affect different body parts throat, genitals, face, stomach, feet. The most dangerous sign is neck swelling, while puffiness in the hands and feet makes daily life painful and difficult. Vomiting, severe pain, nausea, and diarrhoea can all be caused by abdominal swelling (Veronica *et al*., 2020). Stress, common infections like cold, hormonal changes, drugs like oral contraceptive and angiotensin-converting enzyme (ACE) inhibitors are common risk factor for the cause of HAE (Abdulkarim and Craig., 2022). Current treatment strategies involves medications like C1 esterase inhibitors (cinryze and haegarda), Plasma kallikrein inhibitors (Lanadelumab, Berotralstat), bradykinin B2 receptor antagonist, plasma-derived C1 inhibitor (C1-INH) concentrates etc (Bork., 2012) (Lunn., 2010) (Aleena *et al*., 2017) (Manning and Kashkin., 2021). De novo drug development (the discovery of completely new treatments) is expensive and risky procedure. The entire average cost is between $2 and $3 billion, with a development span of at least 13 to 15 years. Furthermore, it has a high attrition rate: only 10 % of medications that reach phase 1 clinical trials are authorised, with the rest failing owing to adverse drug responses or inefficacy. Drug repurposing has been recommended as a less risky, less expensive, and faster way to develop new therapeutics than inventing a wholly new drug. Repositioning, reprofiling, retasking, rediscovery, and rescue are all terms used to describe drug repurposing. In the field of rare diseases, repurposing medications could be an effective way to develop cures (Roessler *et al*., 2021) (Surabhi and Singh., 2018) (Sudeep Pushpakom *et al*., 2019).

Material and Methods

Ligand structure

From FDA data base the chemical structure of each ligand was taken. Databases are created primarily for virtual screening and is utilised by researchers at pharmaceutical companies, research institutions, and biotech companies.

Protein structure

The typical structure file of the target protein was acquired from the RCSB Protein Data Bank, a database for information on the three-dimensional structures of large biological molecules including proteins and nucleic acids (Surabhi and Singh., 2018). The typical structure file from the PDB is not suitable for use immediately in calculations for molecular modelling. A typical PDB structure files consists only of heavy atoms and may include water molecules, metal ions, a co-crystallized ligand and co-factors. In order to interactively build complex systems and prepare their inputs, purification was then done using the web-based tool CHARMM-GUI.

Receptor Grid Generation

Receptor Grid Generation needs a prepared structure and all atom structures with the proper bond ordering and formal charges. Favourable interaction, AUTODOCK looks for a connection between one or more ligand molecules and a receptor molecule, which is typically a protein. The shape and characteristics of the receptors are continued, and they are reflected on a grid by a number of distinct sets of fields that offer ever more accurate scoring of ligand poses. The options in each tab of the receptor grid generation panel allow user to define the receptor's structure by excluding any potential co-crystallized ligand, choose the location and size of the active site as it will be represented as receptor grids, and set up AUTODOCK constants. Around the binding site of the receptor a grid area was generated.

Ligand Docking

AUTODOCK VINA is used to carry out ligand docking. One or more ligand molecules and a receptor molecule may interact properly when AUTODOCK VINA searches for that interaction. The receptor may include more than one molecule while each ligand acts as a single molecule. A protein and a co-factor. AUTODOCK VINA was run in flexible or rigid docking mode; for each input ligand, the latter produced conformations automatically. A ligand's pose in flexible docking is the culmination of its position, orientation, and conformation with respect to the receptor. The ligand pose that was generated by AUTODOCK VINA pass through series of hierarchical filters that evaluate the interaction of ligand with the receptor. Initial filters evaluate the ligand's spatial fit to the identified active site, and a grid-based approach modelled after the human cell grid is used to investigate the ligand receptor interactions. Then the end scoring is then carried out.

Docking procedure

The target protein the typical structure file was obtained from the Protein Data Bank (RCSB) and purified using CHARM-GUI. http://www.rcsb.org/pdb.

Using the program AUTODOCK VINA experiment were performed. By using the protein preparation wizard, full length substrate complex dimmer coordinates were created for AUTODOCK VINA calculations. A new receptor file is created by the P-PreP script, and it contains all residues, except those that are relative close to ligand are neutralized. Finally the software was allowed to run in order to start the docking process, where in each ligand was made to bind with the active site present in the active pocket of the target protein under studies.

Visualization

The pdbqt file obtained from the docking is complexed with the protein structure for visualization. In Maestro software the 2D and 3D structure of the complexes are visualized for studying ligand interactions and enhanced presentation.

Molecular Dynamics

The dynamic behaviour of the protein-ligand complex is studied by MD simulations. Molecular dynamics simulation is a method that involves numerically integrating Newton's equation of motion for a given interatomic potential, initial condition, and boundary condition in order to produce the atomic trajectories of a system of N particles. Molecular simulation was done for 100.102 nanoseconds at 300 kelvin temperature for trospium and Olmesartan and analysed by measuring the root mean square deviation (RMSD), root mean square fluctuation (RMSF) (Kalimuthu *et al*., 2022).

Results and Discussion

Molecular Docking

The protein target selected under the study was a crystal structure of BCX7353 (ORLADEYO) in combination with the serine protease of human plasma kallikrein. The PDB ID for this particular protein is 7n7x. The target protein along with the drug candidates were uploaded and run on the software. Higher the negative value of binding affinity, more potent is the activity that indicate whether or not the identified drug candidate is feasible. The overall binding affinity for FDA approved drugs lies between -9.1 to -8.2. So the drugs Trospium and Olmesartan can be potent candidates as the binding affinity score was -9.1 and -8.6 respectively as shown in the table 1.

Table 1. Docking score of the top 10 compounds from FDA database

S. No	FDA drugs	Docking scores	Interaction Residue	Type of Interaction	Distance
1	Trospium	-9.1	A:TYR 242	Hydrophobic Interactions	3.23
			A:LYS 262	Hydrophobic Interactions	3.63
			A:LYS 262	Hydrophobic Interactions	3.88
			A:TRP 285	Hydrophobic Interactions	3.60
			A:TRP 285	Hydrophobic Interactions	3.74
			A:TRP 285	Hydrophobic Interactions	3.44
			A:GLU 285	Hydrogen bonds	1.94
			A:HIS 285	pi-stacking	4.61
2	Adapalene	-8.5	A:LYS 262	Hydrophobic Interactions	4.443
			A:LYS 262	Hydrophobic Interactions	3.82
			A:GLY 288	Hydrogen Bond	2.92
3	NADH	-8.5	A:TYR 242	Hydrophobic Interactions	3.65
			A:TRP 285	Hydrophobic Interactions	3.80
			A:TRP 285	Hydrophobic Interactions	3.43
			A:ASP 47	Hydrogen Bonds	3.14
			A:GLU 89	Hydrogen Bonds	2.28
			A:TYR 242	Hydrogen Bonds	2.10
			A:ASP 259	Hydrogen Bonds	2.96
			A:LYS 262	Hydrogen Bonds	3.08
			A:SER 265	Hydrogen Bonds	2.37
			A:SER 284	Hydrogen Bonds	2.56
			A:GLY 286	Hydrogen Bonds	2.90
			A:GLY 288	Hydrogen Bonds	2.05
			A:BIS 44	Salt Bridges	5.11
			A:LYS 262	Salt Bridges	5.48
			A:LYS 262	Salt Bridges	4.24
4	Olmesartan	-8.6	A:HIS 44	Hydrophobic	3.71
			A:TYR 242	Hydrophobic	3.22
			A:TRP 285	Hydrophobic	3.75
			A:TRP 285	Hydrophobic	3.29
			A:HIS 44	Hydrogen Bonds	2.81
			A:GLY 286	Hydrogen Bonds	2.28
			A:GLY 288	Hydrogen Bonds	2.21
			A:HIS 44	pi-Stacking	4.55
			A:TYR 242	pi-Stacking	4.80

			A:TRP 285	pi-Stacking	4.07
			A:TRP 285	pi-Stacking	4.07
5	Indinavir	-8.5	A:PHE 211	Hydrophobic	3.93
			A:ALA 260	Hydrophobic	3.69
			A:LYS 262	Hydrophobic	3.57
			A:THR 283	Hydrophobic	3.24
			A:TRP 285	Hydrophobic	3.66
			A:TRP 285	Hydrophobic	3.63
			A:TRP 285	Hydrophobic	3.95
			A:TRP 285	Hydrophobic	3.69
			A:SER 265	Hydrophobic	3.32
			A:GLY 286	Hydrogen Bonds	2.82
			A:HIS 44	pi-Stacking	4.70
6	Cabergoline	-8.4	A:HIS 44	Hydrophobic	3.71
			A:TYR 85	Hydrophobic	3.89
			A:LYS 86	Hydrogen Bonds	3.00
			A:SER 265	Hydrogen Bonds	2.51
			A:ASP 259	Salt Bridges	4.52
			A:HIS 44	pi-Stacking	4.29
7	Octreotide	-8.4	A:GLU 214	Hydrophobic	3.69
			A:PYR 242	Hydrophobic	3.25
			A:ALA 260	Hydrophobic	3.98
			A:LYS 262	Hydrophobic	3.68
			A:THR 283	Hydrophobic	3.61
			A:TRP 285	Hydrophobic	3.53
			A:TRP 285	Hydrophobic	3.87
			A:SER 88	Hydrogen Bonds	2.06
			A:TYR 242	Hydrogen Bonds	2.24
			A:TYR 242	Hydrogen Bonds	2.00
			A:SER 265	Hydrogen Bonds	1.89
			A:GLU 287	Hydrogen Bonds	2.53
			A:GLY 282	Hydrogen Bonds	2.50
			A:ARG 291	Hydrogen Bonds	3.15
8	Folic-acid	-8.3	A:GLY 90	Hydrogen Bonds	3.16
			A:SER 265	Hydrogen Bonds	2.34
			A:SER 284	Hydrogen Bonds	2.96
			A:TRP 285	Hydrogen Bonds	3.86
			A:GLY 286	Hydrogen Bonds	3.58
			A:VAL 297	Hydrogen Bonds	3.38
			A:PYR 298	Hydrogen Bonds	2.69
			A:ASP 259	Salt Bridges	5.14
9	Amsacrine	-8.2	A:GLU 214	Hydrophobic	3.92
			A:LYS 262	Hydrophobic	3.71
			A:LYS 262	Hydrophobic	3.91
			A:THR 283	Hydrophobic	3.87
			A:GLY 286	Hydrogen Bonds	3.03
			A:GLY 286	Hydrogen Bonds	2.45
10	Argatroban	-8.2	A:TYR 242	Hydrophobic	3.74
			A:ALA 260	Hydrophobic	3.64
			A:TRP 285	Hydrophobic	3.66
			A:TRP 285	Hydrophobic	3.75

			A:HIS 44	Hydrogen Bonds	1.98
			A:VAL 87	Hydrogen Bonds	2.31
			A:VAL 87	Hydrogen Bonds	2.46
			A:LYS 262	Hydrogen Bonds	3.33
			A:GLY 263	Hydrogen Bonds	3.28
			A:SER 265	Hydrogen Bonds	3.23
			A:SER 265	Hydrogen Bonds	2.14
			A:ASP 47	Salt Bridges	4.14

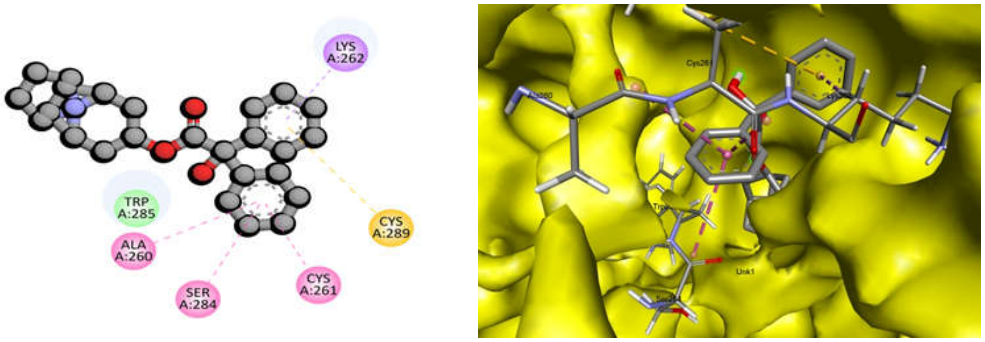

Figure 1. 2D and 3D interaction of Trospium with 7n7x

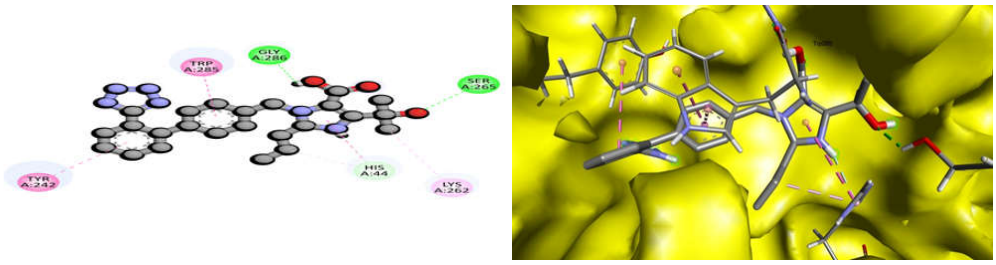

Figure 2. 2D and 3D Interaction of Olmesartan with 7n7x

Molecular Dynamics

MD is a technique for examining the physical movements of atoms and molecules when they are allowed to interact for a predetermined period of time through computer simulation. Newton's equation of motion is used in the analysis. For molecular dynamic simulations, the top two compounds, Trospium and olmesartan, were chosen because they showed highest binding affinity with the target 7n7x. A molecular dynamic simulation was run for 100.102 nanoseconds at 300 kelvin temperature. The following are the respective outcomes: MD simulations generate trajectories that depict atom motions by presenting atomic coordinates at predetermined time intervals. By computing metrics like root mean square deviation (RMSD) and root mean square fluctuation (RMSF), these trajectories are then used to analyse macromolecule dynamics.

Protein-Ligand RMSD

RMSD is a measurement of the average displacement of a group of atoms in a frame relative to a reference frame. It indicates the stability of the protein-ligand complex by describing the molecule's overall discrepancy with respect to a reference conformation. The RMSD is plotted against time to assess structural conformation variations.

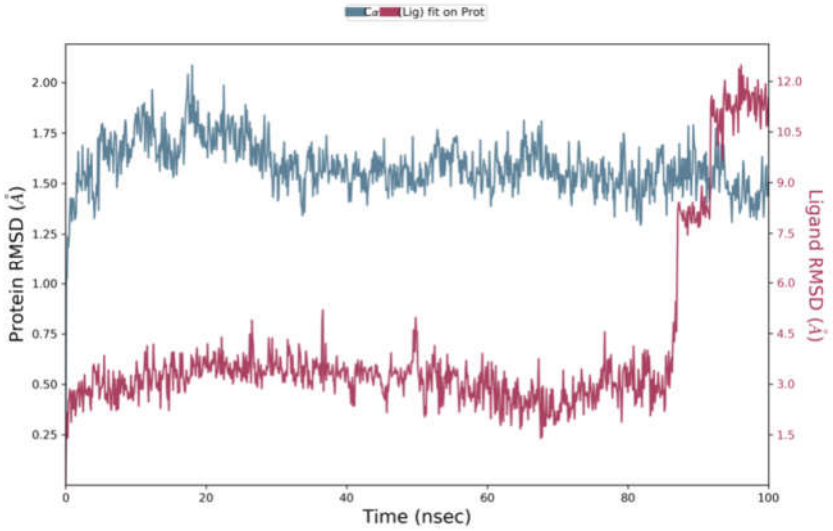

Figure 3. Protein – Ligand RMSD of 7n7x and Trospium complex

The complex of 7n7x and trospium initially showed fluctuations that were higher than RMSD of protein ranging 1.25 -1.85Aº from 2 to 25ns. The RMSD of the complex did not overlap with that of protein. At the end of the simulation i.e., 70 to 100ns, RMSD values of complex were lesser than that of the protein and stabilised around 2Aº and they also overlapped with each other from 82 to 85ns indicated that the complex system has equilibrated and the simulation may be long enough for rigorous analysis **(Fig. 3)**.

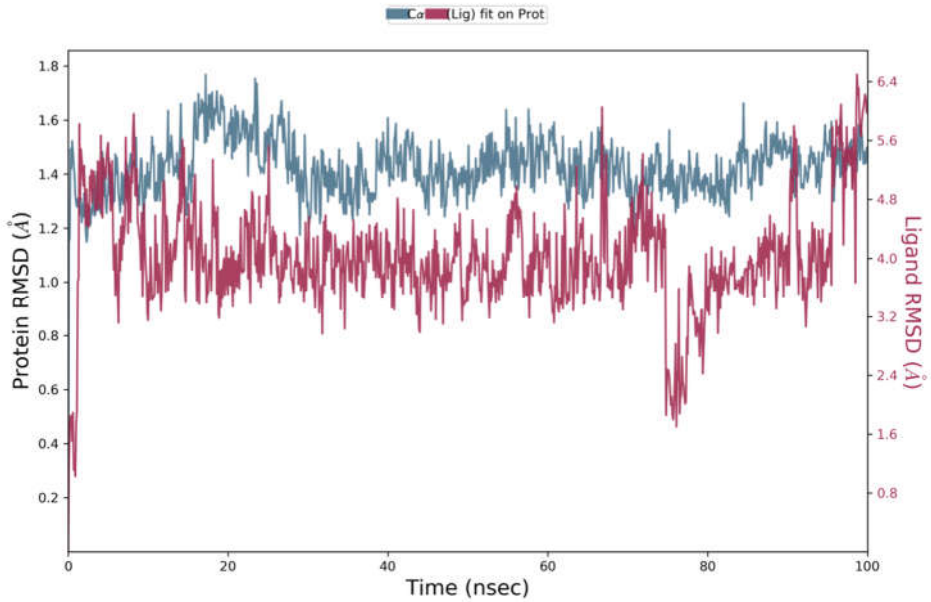

Figure 3: Protein – Ligand RMSD of 7n7x and Olmesartan complex

The complex of 7nx7 and olmesartan initially showed fluctuations ranging 0.4-1.6Aº till 10ns. The RMSD of the complex stabilised around 1.5Aº for the major portion of the simulation with a variation at 60ns. At the end of the simulation i.e., 90 to 100ns, RMSD values of complex slightly overlapped with that of the protein. This indicates that the complex system has equilibrated and the simulation may be time consuming for the analysis **(Fig. 4)**.

RMSF

Individual residue flexibility is measured by RMSF. During a simulation, it tracks the movement of a specific residue. The amino acids in the protein structure that contribute the most to molecular motion are indicated by a plot of RMSF vs. residue number. It aids in the analysis of changes in the behaviour of target protein amino acid residues following ligand binding.

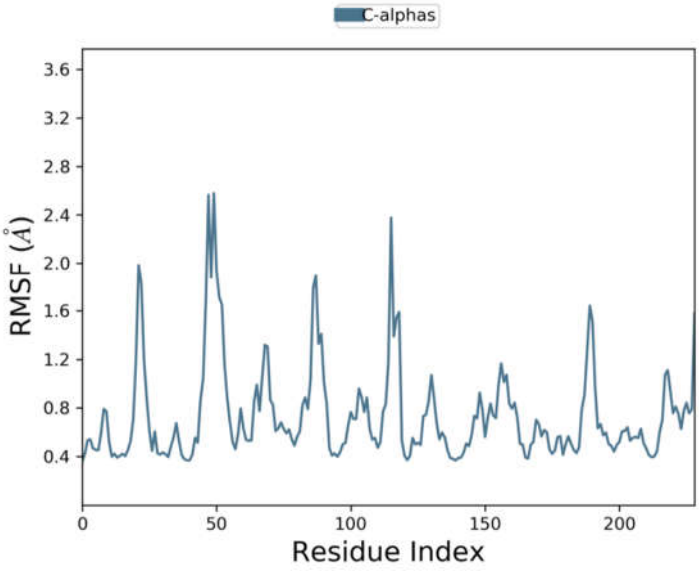

Figure 5. Protein RMSF with 7n7x and Trospium complex

Peaks in figure 5 represent regions of the protein where the simulation-induced fluctuations were greatest. In the examined complex, the amino acid residues showed major fluctuations at 25, 50, 80, and 185 for the entire simulation. However, the values of RMSF are below 5Aº. This shows that binding of the ligand did not stimulate any major effects on the flexibility of the protein.

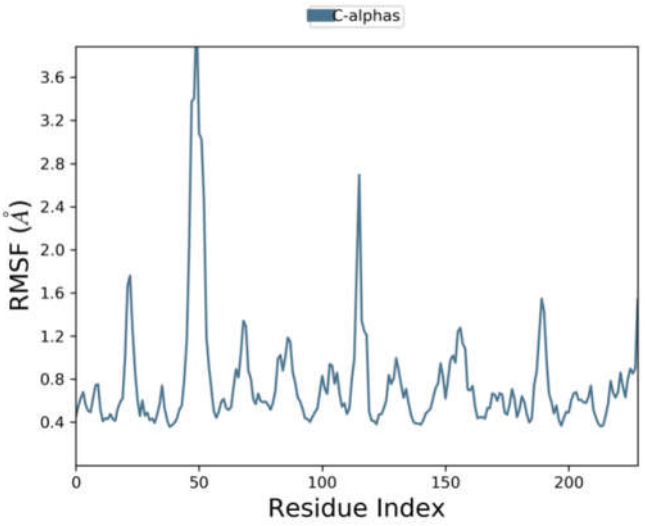

Figure 6. Protein RMSF with 7n7x and Olmesatran complex

In the examined olmesartan complex (Figure 6), the amino acid residues showed major fluctuations at 25, 50, 125, and 185 for the entire simulation. However, the values of RMSF are below 5Aº. This shows that binding of the ligand did not stimulate any major effects on the flexibility of the protein.

Protein-Ligand Contacts (PLCs)

Throughout the simulation, the protein interaction with the ligand was examined. The four different kinds of protein-ligand contacts or interactions are hydrogen bonds, hydrophobic, ionic,

and water bridges. There are numerous subtypes of each interaction type which were investigated using the simulation interactions diagram panel. Over the course of the trajectory, the stacked bar charts are normalised. The interactions between specific ligand atoms and protein residues are schematically shown in Figure 7 and 8.

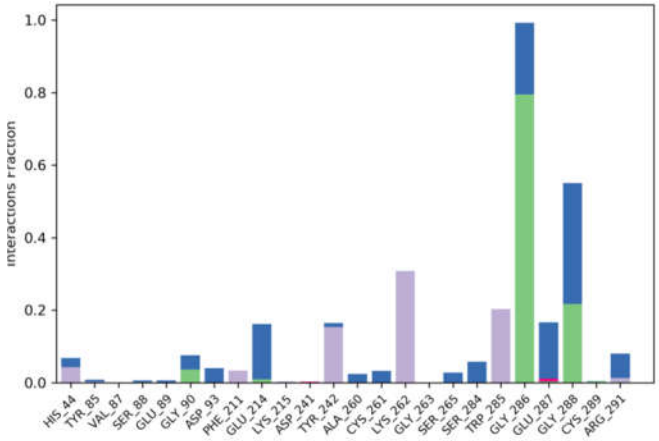

Figure 7. PLC of 7n7x and Trospium Complex

Hydrogen bonding interaction fractions of the ligand trospium with amino acid residues are as follows 0.002 with GLY_90, 0.001 with GLU_214, 0.8 with GLY_286, 0.22 with GLY_288. The percentage suggests the simulation time the interaction was maintained.

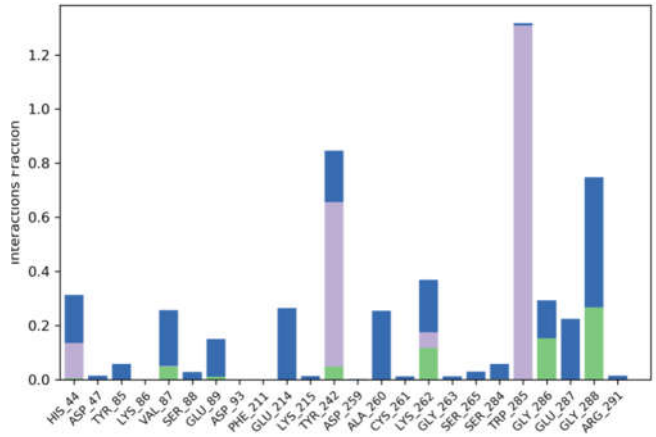

Figure 8. PLC of 7n7x and Olmesartan Complex

Hydrogen bonding interaction fractions of the ligand olmesartan with amino acid residues are as follows: 0.03 with VAL_87, 0.04 with TRY_242, 0.08 with LYS_262, 0.09 with GLY_286, 0.23 with GLY_288. The percentage suggests the simulation time the interaction was maintained.

Conclusion

The present study was conducted to determine the HAE activity of selected FDA drugs against the selected protein target 7n7x using AUTODOCK. The objective of the study was to select the biological target for HAE, identify the database for FDA approved drugs, and carry out molecular docking of FDA approved drugs against HAE disease target. The result showed that trospium (which showed a binding affinity of -9.1) and olmesartan (which showed a binding affinity of -8.6) than the other drugs with selected target. Molecular dynamics showed that the binding of these ligands did not stimulate any major effects on the flexibility of the protein. Hence we can

suggest that these studies can be used for future work. Further research can be undertaken to evaluate *in-vivo and in-vitro* studies of drugs to elucidate mechanism of action or pharmacological action.

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Disclosure Statement: *Conflict of Interest*

Authors have no conflict of interest

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