*In silico* Study to Recognize Novel Angiotensin-Converting-Enzyme-I Inhibitors by 2D-QSAR and Constraint-based Molecular Simulations

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1. **A platform for molecular docking**

The computational docking assessment of selected compounds with ACE as a target was executed via MOE 2019 software (Chemical Computing Group, Montreal, Canada), software.

1. **Protein preparation:**

The enzyme crystal structures were extracted from the protein databank with the PDB id: 1o86 (Crystal Structure of Human Angiotensin-Converting Enzyme in complex with lisinopril) and 3NXQ (Crystal structure of Angiotensin-Converting Enzyme N domain in complex with RXP407). The 1o86 (PDB ID: 1o86, having resolution ~2. Å, R-Value Free <0.25, R-Value Work<0.20) is a complex inhibitor with a monomer of ACE that plays a central key role in the management of hypertension [1, 2]. The 3NXQ (PDB ID: 3NXQ, having resolution ~2. Å, R-Value Free <0.25, R-Value Work < 0.20) is possible treatment for both cardiac and pulmonary fibrosis[3]. From both crystal structures, Chain A was used for macromolecule preparation, all other molecules like co-crystallized water molecules, unwanted chains, and nonstandard residues were deleted. The free target protein was then subjected to the QuickPrep procedure of MOE including corrections for missing atoms, alternate geometries, or other crystallographic artifacts, removing water molecules farther than 4.5 A⁰ from any receptor or ligand atom, and 3D protonation.

1. **Ligand preparations**

The three-dimensional structures of compounds were built using smiles notations in open babel. The energy of all ligands was minimized with an MMFF94 force field using openbabel.The chemical structures of all the ligands are depicted in Figure. S1.



**Figure S1. Selected chemical compounds for docking against C-domain ACE (PDB ID: 1o86) and N-domain ACE (PDB ID:3NXQ)**

1. **Molecular Docking Procedure**

A primary objective in molecular docking was the ability to estimate the scoring function and to evaluate interactions between a protein and small molecules based on the geometry to predict the binding affinity and activity of the ligand molecule [4, 5].

In this study to investigate the detailed intermolecular interactions, the molecular operating environment (MOE) software package (Molecular Operating Environment, 2015) was used to perform various steps involved in docking simulation. Pharmacophores constraints were generated using the Pharmacophore query editor containing metal chelation constraint and one positional constrain amide/amine Nitrogen with 1 A⁰ constraint sphere (Figure S2). This pharmacophore was used in the docking using the pharmacophore placement method at site centered on co-crystallized ligand atoms and top 1000 poses ranked by London dG scoring function. Although, the final results were analyzed and visualized based on docking scores using Discovery Studio 2020 Client[6], Ligplot v.4.5.3 [7], and PyMol software’s[8].



**Figure S2. An illustration of the metal chelation and positional constraints created and implemented for docking chemical compounds against C-domain ACE (PDB ID: 1o86) and N-domain ACE (PDB ID:3NXQ)**

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