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Computational Approaches in Drug Design Molecular Docking Studies of Ace Inhibitors on Angiotensine Converting Enzyme

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Abstract:- A molecular docking is a computational technique essential in drug design, particularly in predicting how small molecules (ligands) bind to target proteins. It begins with the significance of Computer-Aided Drug Design (CADD) in modern drug discovery and elaborates on its types—Structure-Based Drug Design (SBDD) and Ligand-Based Drug Design (LBDD). Molecular docking, a key aspect of SBDD, involves predicting how ligands interact with target proteins, crucial in developing treatments for diseases like HIV, where protease inhibitors were created using this approach. The document outlines methodologies like Monte Carlo and fragment-based approaches, along with docking types (rigid, semi-flexible, and flexible). It also highlights tool like AutoDock used for molecular docking studies. A case study involving docking of ACE inhibitors (Captopril, Enalapril, Ramipril) for antihypertensive activity is presented. Results showed promising binding energies, with Captopril exhibiting the highest binding energy at the ACE active site, indicating its effectiveness. The document concludes by discussing the relevance of molecular docking in drug design and its broader applications in industrial and environmental studies.

Keywords:- *Docking ACE Inhibitors, Molecular Docking, Molecular Modelling, Ligand-Protein Interaction, Captopril, Enalapril, Ramipril Docking, CADD.*

I. INTRODUCTION

A. Computer Aided Drug Design (CADD)

Across the ages, diseases like cholera, smallpox, chicken pox, and the plague have frequently brought entire civilizations or at least significant portions of them perilously close to extinction. A persistent phenomenon that is controlled by evolution itself, this threat to human civilization has given rise to diseases like AIDS caused by HIV that were unknown only a few years ago. After being believed to have been contained, some illnesses, such as dengue, malaria, and others, reappear and force people to use their creativity to find new defenses against these evil forces[1]. It is becoming increasingly popular to explore, use, and admire computational approaches in the drug design, discovery, and development process. The process of releasing a new medication onto the market is labor-intensive, expensive, and time-consuming. Research typically indicates that the process of finding and developing new drugs requires between 10 and 14 years, as well as over $1 billion in total funding that leads to discovery of CADD[2]. An Overview of CADD's past but there were restrictions: 2. Size, Reversible Analysis: The 1980s saw the advent of CADD Molecular Biology, X-ray Crystallography, a versatile model of NMR Molecular, and computer Graphics. Modern methods like as combinatorial chemistry, high-throughput screening, and human genome bioinformatics were brought to the field of innovative medical science in the 1990s [3]

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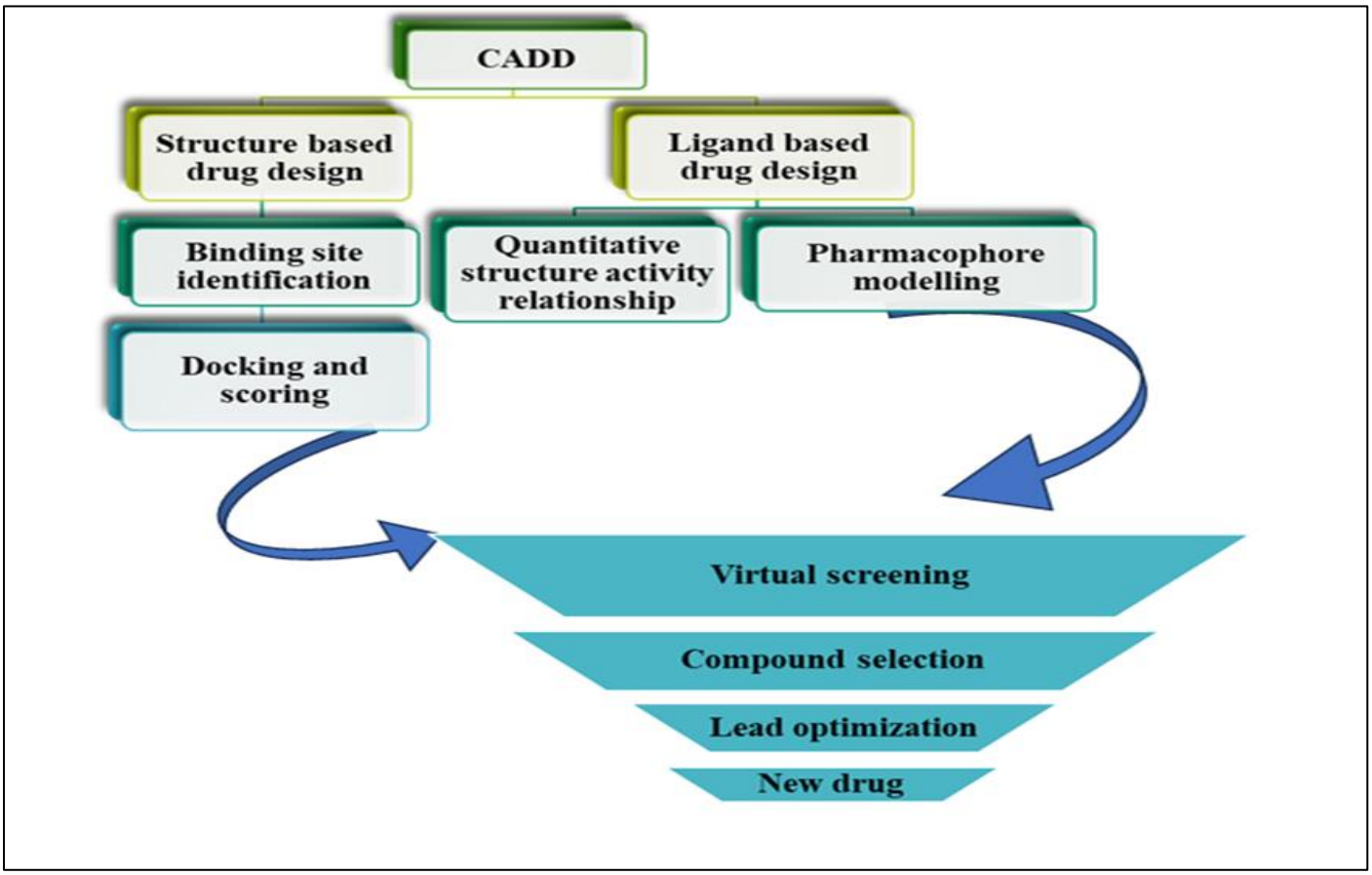

Fig 1: Outflow of CADD

B. Significance of CADD[3].

Relevance of CADD in Drug Design: CADD searches increased the number of chemicals filtered in a short amount of time by focusing on new compounds by outperforming trials and chemical compounds compared to conventional methods. Forecasts on cellular therapeutic auctions and possible discoveries for career advancement are provided by CADD. It serves the following functions:

- Predictive activity filtering of larger libraries of compound in tiny chemical groups. Testing is one way to test these. CADD has now supplanted traditional research, which involves an animal and human model, saving time and money.
- It is envisaged that computational drug design will be crucial in lowering the likelihood of drug resistance in the event of specific infections, such as influenza and thus leads to discovery of new drug.
- The strongest beats can be identified in a matter of weeks by using computerized approaches.
- CADD has also resulted in the development of well-known stocks and libraries with significant molecular diversity or similarity.

C. Types of CADD

- Structure based drug design
- Ligand based drug design
- Structure based drug design

D. Principle of SBDD[4]

The foundation of Structure based drug design is an idea of lock-and-key modeling, in which a drug attaches itself to a particular site's on the target protein in a manner similar to how a key fits into a lock. Depending on nature of the interaction, the drug molecule's binding to the target protein can either enhances or inhibit the target protein's function.

Getting the three-dimensional structure of target protein is the initial stage in SBDD. X-ray crystallography NMR spectroscopy, and cryo-electron microscopy, are the few methods that can be used for this. Finding tiny compounds that can attach to the target molecules and alter its activity comes next, once the structure of the molecules is established.

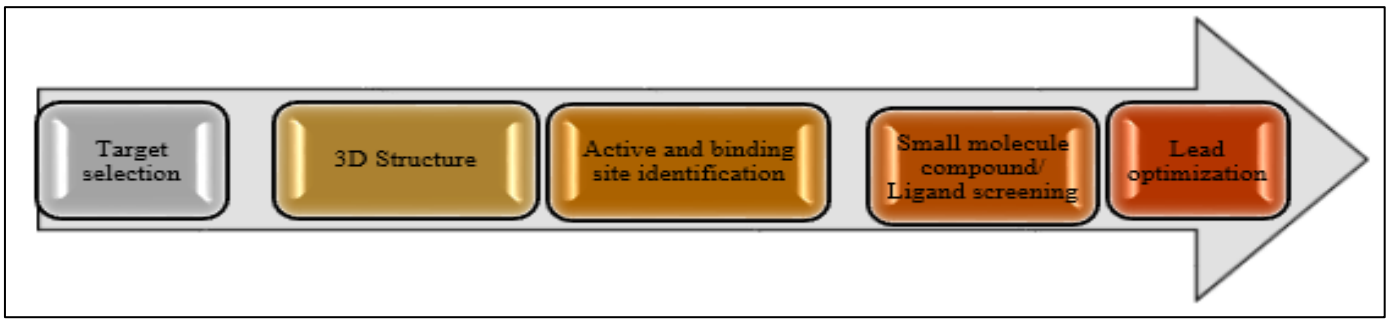

Fig 2: Process of SBDD

A computational method called "molecular docking" is used to forecast how tiny compounds will connect to their target proteins. A library of tiny molecules is screened for molecular docking's capacity to bind to the binding site of the target protein. The compounds chosen for additional testing are those that exhibit the best match. The goal of structure-activity relationship (SAR) study is to maximize the molecules' ability to bind to the target protein. In SAR analysis, the compounds' chemical structures are changed, and their ability to bind to the target protein is evaluated. SAR analysis can determine the essential characteristics of the molecule that are crucial for binding to the target protein by comparing the activity of the changed molecules to that of the original molecule. Predictive activity filtering of huge compound libraries in tiny chemical groups Testing is one way to test these. CADD has now supplanted traditional research, which involves an animal and human model, saving time and money. t is envisaged that computational drug design will be crucial in lowering the likelihood of drug resistance in the event of specific infections, such as influenza and thus leads to discovery of new drug. The strongest beats can be identified in a matter of weeks by using computerized approaches. CADD has also resulted in the development of well-known stocks and libraries with significant molecular diversity or similarity.

- **Example:** The creation of protease inhibitors for HIV treatment is one noteworthy instance. One enzyme that is essential to the virus's ability to replicate is called HIV protease. Small compounds that might bind to the protease's active site and limit its activity, so halting the virus's reproduction, were created using SBDD. Due to this strategy, medications like ritonavir and saquinavir, which are frequently used to treat HIV, have been developed.

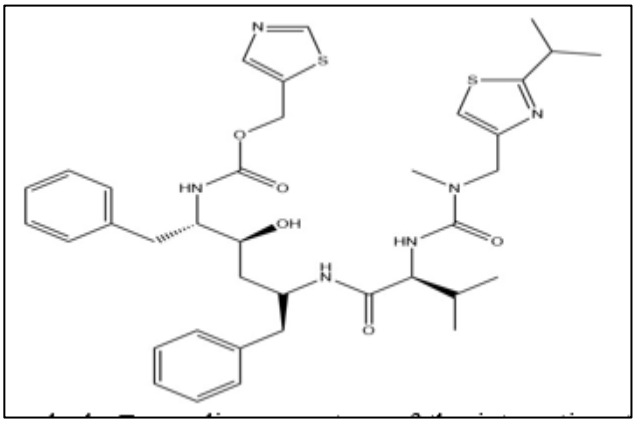

Fig 3: Ritonavir

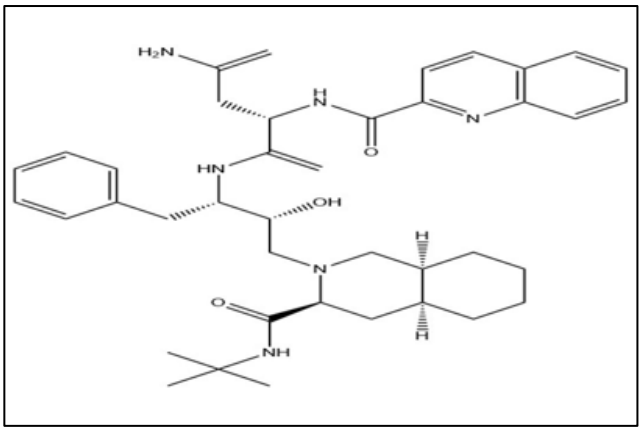

Fig 4: Saquinavir

II. LIGAND BASED DRUG DESIGN[5]

Indirect drug design, also known as ligand-based drug design (LBDD), aids in the identification of novel tiny molecules, or ligands, that bind to protein targets. The drug molecules supported by bioinformatics tools are recognized by the silico procedures used here, which are then investigated further on various platforms for the purpose of predicting putative active sites, generating similar structures, carrying out molecular docking with active ligands, catalyzing binding interactions, and optimizing the lead ones to improve their binding properties, efficacy, and safety.

Due to the lack of dependable 3D macromolecular structure, substances can now be recognized as targets extremely systematically utilizing specialized, cutting-edge computational methods. Based on experimental data sets, matched molecular pair (MMP) analysis, Free-Wilson models, and quantitative structure-activity relationship (QSAR) models are used to predict the potency of a lead compound in addition to its absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties. The flexibility and behavior of molecules for the bioactive conformation are determined in large part by conformational analysis, quantum mechanics, geometry, and optimization. The methods of scaffold hopping and fragment-based replacement are highly successful in resolving the metabolic instability of drugs and in directing the utilization of underutilized features in the model development process. In order to compare various sets of compound data, structure activity relationship (SAR) for virtual screening (VS) is linked using pharmacophoric methodologies and electrostatic similarity alignment methods based on ligand shape criteria.

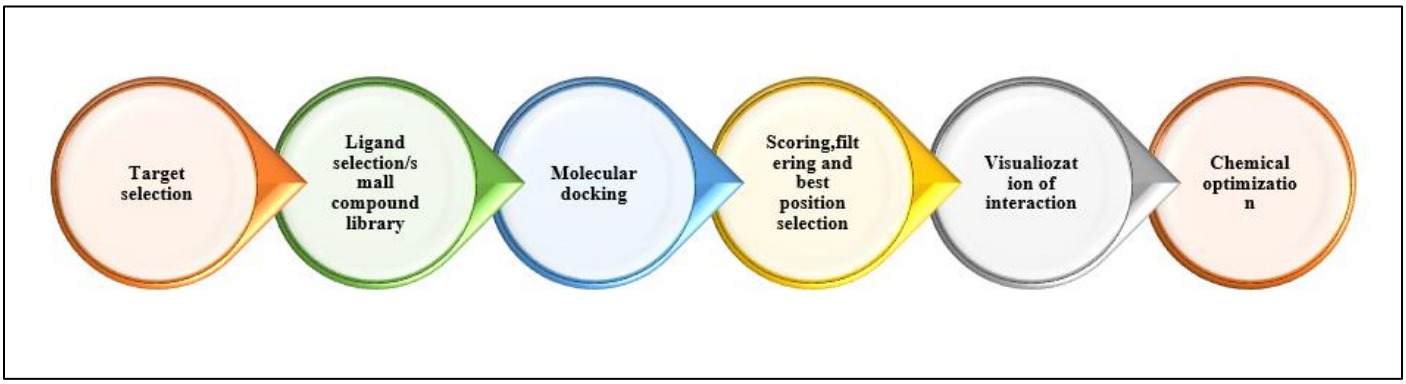

Fig 5: Process of LBDD

A. Molecular Docking

A fundamental challenge in structural biology is molecular recognition. Almost every biological event requires the interaction of molecules, whether they are tiny ligands or large macromolecules. Medicinal chemists have attempted to specifically modulate these interactions for more than a century. In order to obtain therapeutic drugs more quickly, we need to be able to anticipate the structural aspects of recognition events in addition to understanding them. For the sake of medication design, we could make predictions if we had a thorough grasp of the foundation for complementarity[6]. In recent times, Molecular Docking has become a crucial part of drug development done through computer simulations. This method entails foreseeing the atomic-level interaction between a protein and a small molecule. This allows researchers are examining the actions of tiny molecules, like nutraceuticals, inside the target's binding site comprehend protein and grasp the basic biochemical process involved in this interaction The method is efficient. The method relies on the structure of the target protein, which must be obtained in high-resolution 3D format using technology. Methods such as X-ray crystallography, NMR spectroscopy, and Cryo-EM are used for analysis[7].

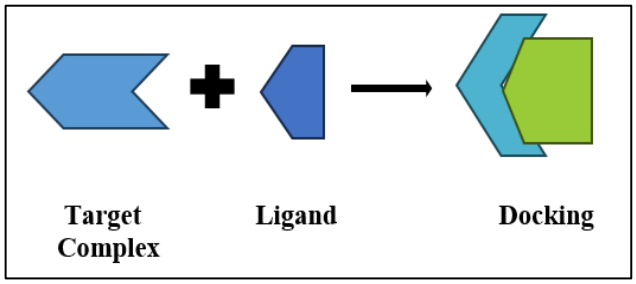

Fig 6: Molecular Docking Representation

B. Aim[8]

The primary objective of docking is to identify the optimal way for a ligand to bind to an active site, achieving the best conformation for both the ligand and receptor. Correct orientation minimizes energy usage, aids in locating the precise binding site, and helps estimate binding affinity, which is crucial for drug development.

C. The Molecular Docking Objective[8]

➢ *Goal of Optimization:*

The primary aim is to determine the ligand's most stable position in the protein's active site by minimizing binding energy and Root Mean Square Deviation (RMSD).

➢ *Methodological Comparisons:*

- Traditional single- and multi-objective approaches focus on reducing binding energy.
- A new multi-objective method integrates both binding energy and RMSD to achieve better docking results.

➢ *Assessment of Performance:*

The effectiveness of multi-objective methods is measured by evaluating convergence and diversity using specific quality indicators.

These approaches are compared to the single objective LGA method in AutoDock.

➢ *Advantages of the Multi-Objective Approach:*

This method shows great potential for discovering anticancer drugs targeting multidrug-resistant sites.

➢ *Broader Applications:*
Molecular docking is also applied in industrial and environmental studies to understand macromolecular interactions outside of medical research.

Computational methods enhance this understanding by incorporating data from co-crystallized ligands and refining scoring functions.

➢ *Progress in Docking Studies:*
Scoring functions can be customized for specific receptors or related targets. Studies on mutant T4 lysozyme structures demonstrate the utility of analyzing key features such as electrostatic forces, hydrophobic interactions, and solvation energies for improved docking accuracy.

D. Approaches of Molecular Docking [9]

➢ *Monte Carlo Method:*
Uses randomization techniques like rigid-body translation, bond rotation, or ligand rotation in the active site to predict molecular alignment.

Configurations are assessed based on energy criteria, and favorable ones are retained for further refinement.

➢ *Metropolis Criteria:*
A new configuration is retained if it outperforms the previous one, facilitating energy-efficient structural adjustments.

➢ *Fragment-Based Approach:*
Splits ligands into fragments for docking, then reconstructs possible conformations. Effectively reduces degrees of freedom (DOF) for improved efficiency.

➢ *Distance Geometry:*
Uses intra- or intermolecular distances to model molecular structures, enabling analysis of small molecules, peptides, and proteins.

➢ *Matching Approach:*
Focuses on the complementarity of protein-ligand surfaces for optimal shape matching, aiding in precise docking orientation.

➢ *Ligand Fit Approach:*
Identifies protein cavities as active sites and docks small molecule ligands based on shape complementarity.

➢ *Complimentary Points Approach:*
Evaluates the structural and chemical compatibility of interacting molecules to predict specific interactions.

➢ *Reverse Docking:*
Matches a small molecule to a library of receptor structures to identify potential biological targets or predict pharmacological profiles (one ligand–many targets).

➢ *Blind Docking:*
Docks a ligand onto a protein without prior knowledge of the binding pocket, exploring all possible surface interactions.

E. Mechanism of molecular docking [10]
The sequence of the particular protein is a prerequisite for performing a docking screen. A biophysical method like as x-ray crystallography or, less frequently, NMR spectroscopy is often used to find the structure. 7. This protein function and a database of chemicals are the inputs used by a docking tool. The performance of a docking program depends on three components: the scoring mechanism, the search algorithm, and the algorithm. All possible orientations and protein conformations are included in the conformational space that is searched when a protein is coupled to a ligand. It is challenging to fully explore the search space with the computational power available today, which would include listing every possible molecule distortion as well as every possible translational and rotational configuration of the ligand with respect to the It is challenging to fully explore the search space with the processing power available today. This would involve listing every possible molecular distortion as well as every possible translational and rotational configuration of the ligand reference to the protein at a moderate resolution criterion. 7. Most existing docking methods take into account flexible ligands, while some try to simulate a dynamic protein receptor.

F. Types of molecular docking [11]

➢ *Molecular Docking Methods are Classified Based on the Flexibility of the Ligand and Receptor:*

- **Rigid Docking (Lock and Key):** Both the ligand and receptor are treated as rigid, considering only translational and rotational movements. This method is commonly used for protein-protein docking and resembles the "lock and key" model.
- **Semi-Flexible Docking:** The receptor remains rigid while the ligand is flexible. Both translational/rotational movements and ligand flexibility are considered, enabling the ligand to adapt to the receptor.
- **Flexible Docking (Induced Fit):** Both the ligand and receptor are flexible, allowing mutual conformational adjustments for optimal binding. It involves conformational selection or induced fit modeling.

G. Procedure of Molecular Docking [12]
Important phases in the molecular docking mechanics The method used to investigate the in-silico intermolecular interaction between two molecules is called molecular docking. The protein receptor serves as the macromolecule in this process. The ligand molecule, act as an inhibitor, is the micromolecule. In the Docking process following steps are involved.

- **Step I: Protein preparation:** Get the protein's three-dimensional structure from the Protein Data Bank (PDB); pre process the structure once it has been obtained. According to the constraints at hand, this ought to permit the extraction of the water molecules from the cavity, stabilizing the charges, replacing the absent residues, producing side chains, etc.
- **Step II: Predicting the active site of the protein:** Following protein production, the protein's active site needs to be identified. The receptor may have many active sites; just the one that is of interest needs to be selected. Most of the water molecules and, if any, heteroatoms are eliminated.
- **Step III: Ligand preparation:** Ligands can be retrieved via a variety of databases, including ZINC and Pub Chem, or they can be drawn using the Chem sketch tool. Further preparation of ligand on openBableGUI and AUTODOCK tools.
- **Step IV: Docking:** The ligand is oriented against the protein and the resulting interactions are examined. A score based on the selection of the best docked ligand complex.

Table 1: Docking Tools[7]

Technique/Software	Key Features	Input Formats	Website
DOCK	Mimics enzyme-substrate transition states; uses amid hydrolase rigidity theory.	PDB, MOL2, SDF	http://dock.compbio.ucsf.edu/
AutoDock	Flexible/rigid docking; uses Lamarckian genetic algorithm; open-source.	PDB, MOL2, SDF	http://autodock.scripps.edu/
GOLD	Flexible docking; customizable scoring functions; handles metal atoms and water.	N/A	http://www.ccdc.cam.ac.uk/products/lifesciences/gold
MolDock	Uses FFT for rapid docking; scoring includes van der Waals, electrostatics, and shape complementarity.	PDB, MOL2, SDF	https://www.molsoft.com/about.html
Chimera	Visualization and docking prep tools; adds hydrogens, charges, and molecular surfaces.	N/A	https://www.cgl.ucsf.edu/chimera/

H. Docking study of ACE Inhibitors for Anti-Hypertensive Activity.

➢ *Hypertension*

Hypertension (HTN) is a significant health issue in South Asia, ranking as the third leading risk factor for disease burden in 2010. In India, hypertension heavily impacts healthcare systems and cardiovascular health, directly causing 57% of stroke-related deaths and 24% of deaths from coronary heart disease (CHD). The World Health Organization (WHO) identifies high blood pressure as one of the major global causes of premature death. In the Global and Regional Burden of Illness and Risk Factors study (2001), HTN ranked second only to child underweight for age as a contributor to illness burden in South Asia.[13]

Hypertension is defined as abnormally high diastolic and/or systolic blood pressure. Although mean arterial pressure is elevated in hypertension, it is not typically measured in humans. Historically, the diastolic pressure received greater attention, but studies have shown a strong correlation between elevated systolic pressure and an increased risk of coronary and cerebrovascular diseases such as stroke. Consequently, modern hypertension management emphasizes both systolic and diastolic readings.[14]

As the most common modifiable risk factor for mortality and disability, hypertension is associated with conditions such as stroke, heart failure, accelerated coronary and systemic atherosclerosis, and chronic kidney disease. Managing hypertension through antihypertensive medications significantly reduces the risk of cardiovascular events and organ damage. Lifestyle modifications, such as limiting sodium intake, achieving and maintaining a healthy weight, exercising regularly, moderating alcohol consumption, and increasing potassium-rich food intake, are also crucial in hypertension management.

The initial pharmacological treatment typically includes one of four medication classes proven effective in reducing cardiovascular risks: ACE inhibitors, angiotensin II receptor blockers (ARBs), calcium channel blockers, or thiazide diuretics. By combining these medications with recommended lifestyle changes, individuals can significantly reduce the adverse effects of hypertension on their health.[15]

➢ *ACE- Angiotensin Converting Enzyme*

The renin-angiotensin system primarily relies on ACE (angiotensin I-converting enzyme 1 [EC 3.4.15.1]), also known as peptidyl-dipeptidase A, peptidyl-dipeptidase I, dipeptidyl carboxypeptidase I (DCP1), kininase II, peptidase P, or carboxycathepsin. ACE plays a crucial role in converting angiotensin I into angiotensin II and degrading bradykinin. In mammals, ACE is a key component of the homeostatic mechanism responsible for regulating blood pressure and maintaining electrolyte balance in conjunction with renin. ACE activity is typically assessed by measuring the reduction in substrate cleavage in the presence of ACE inhibitors, such as captopril and lisinopril.[1]

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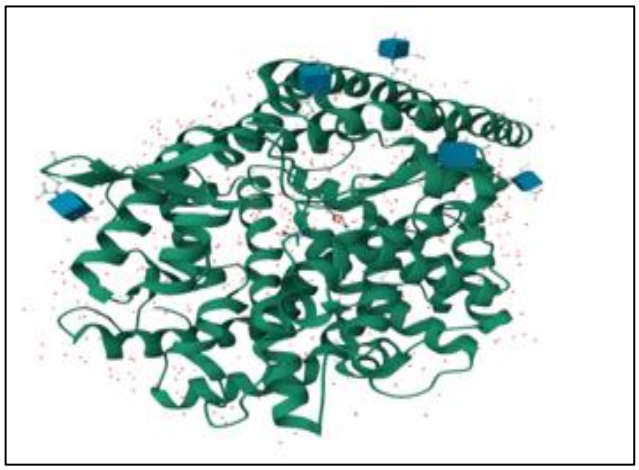

Fig 7: 3D Structure of Angiotensin Converting Enzyme

III. MATERIALS AND METHOD

➢ *In the Docking Process, Following Steps are Followed:*

- Protein Preparation: protein's three-dimensional structure is retrived from the Protein Data Bank (PDB). Once the structure is obtained, the removing water molecules from the cavity is done, stabilizing charges, replacing missing residues, and generating side chains as needed. PDB format is converted to PDBQT by using AutoDock tool
- Predicting the Active Site: After preparing the protein, Grid box containing the containing amino acids HIS383, GLU384, HIS387 and GLU411 was identified as the active site.[18]
- Ligand Preparation: Ligand was downloaded from PUBCHEM database in the SDF format then converted into PDB format by using Open Babel GUI then converted into PDBQT format in the AutoDock Tool.
- Docking: Finally, the docking is done by using the AutoDock tool (1.5.7).

IV. RESULTS

Molecular docking studies of ACE inhibitors such as Captopril, enalapril and Ramipril were carried out by using AutoDock (1.5.7) and results are tabulated in table no. 1, 2 and, 3 respectivly.

Table 2: Molecular Docking Studies of the Captopril

Rank	Run	Binding Energy
1	**3**	**-6.77**
1	10	-6.55
1	5	-6.20
1	2	-6.20
1	7	-6.18
1	8	-6.17
1	6	-6.04
1	9	-5.74
2	4	-4.99
2	1	-4.15

Table 3: Molecular Docking Studies of the Enalapril.

Rank	Run	Binding energy
1	**9**	**-6.39**
1	7	-6.13
1	2	-5.55
1	8	-4.28
1	3	-3.62
2	1	-5.18
3	10	-4.12
4	6	-3.72
5	4	-3.20
6	5	-2.76

Table 4: Molecular Docking Studies of the Ramipril

Rank	Run	Binding energy
1	**9**	**-3.35**
1	8	-0.36
2	10	-2.73
2	7	-0.59
3	1	-2.26
4	4	-1.53
5	2	-1.27
6	1	-0.93
7	6	+0.23
8	5	+0.24

Table 4: Comparative Docking Results of the Three Compounds

Sr. No.	Name of Compound	Binding Energy	Rank
1	Captopril	-6.77	1
2	Enalapril	-3.35	3
3	Ramipril	-6.39	2

V. DISCUSSION

All given compound showed promising binding energy. All compounds docked in their best conformation in the binding site of ACE enzyme are illustrated below.

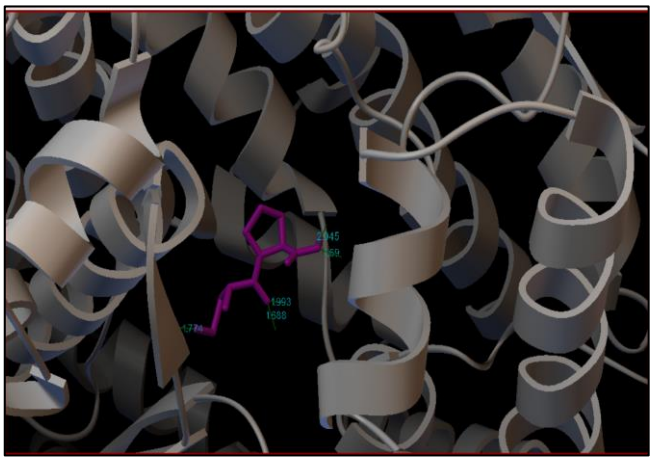

Fig 8: Captopril Docked in its Best Conformation at the Binding Site of ACE

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The oxygen of carbonyl group of Captopril forms two bond with polar hydrogen (HE) of HIS513 and another with HIS 353 of the ACE enzyme. The Hydroxyl group of Acid on Captopril forms one bond with polar hydrogen (HE21) of GLY 281 and one bond with hydrogen (HZ1) of LYS 511of the ACE enzyme and the -SH group forms hydrogen bond with unknown ligand (UNL1).

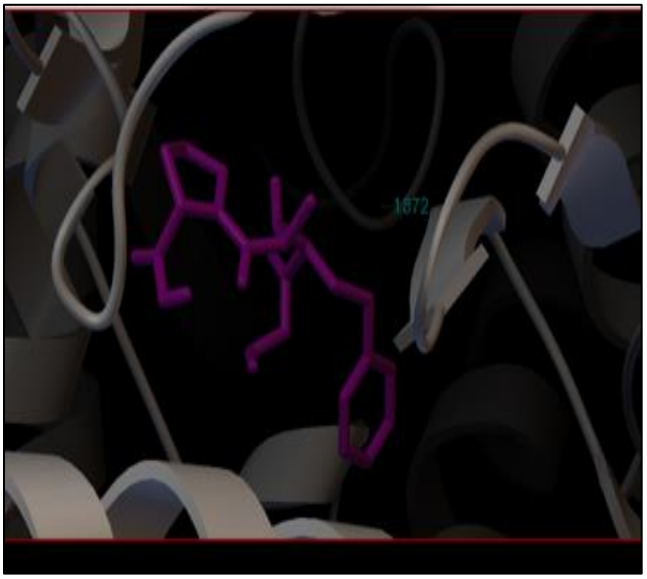

Fig 9: Enalapril Docked in its Best Conformation at the Binding Site of ACE The Oxygen of Carbonyl Group of Enalapril Forms One Bond with -NH of ALA356 of the ACE Enzyme

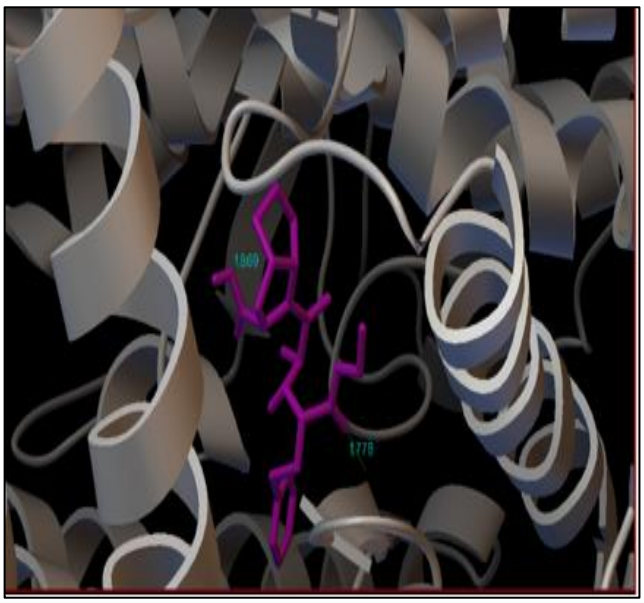

Fig 10: Ramipril Docked in its Best Conformation at the Binding Site of ACE.

The Hydroxyl group of Acid on Enalapril forms one bond with -NH of ALA356 and oxygen (=O) of Acetyl group on Ramipril forms another bond with HH11 of theARG522 of ACE enzyme.

VI. CONCLUSION

The molecular docking of the above three drugs i.e. Captopril, Enalapril, Ramipril, showed the promising binding energy. Out of these, Captopril showed the highest binding energy on the active site of ACE enzyme. Just like this, we can perform molecular docking studies of other compounds instead of directly synthesis and evaluation of thousands of new compounds. Instead, we perform docking of all of these compounds and synthesize only those compounds which having promising binding energy. Further synthesis and biological evaluation of these compounds when done, will reduce overall cost and time of new drug discovery by utilising computer aided drug design.

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