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Development and Application of a Novel Protocol for Predicting Protein-Ligand Interactions: Comprehensive Docking Studies of Imidazole Derivatives as Anti-Bacterial Agents

A. Lazha[1], Ketki Rani[2], Priya Yadav[3], Monika Ghosh[4], Anil Kumar[5], Gopi Reddy Alugubelli[6], Satyabrat Sarma[7], Sunil Kumar Pandey[8], Dr. Imran A Sheikh[*9]

[1]Professor, Department of Physiology, Sri Sairam Siddha Medical College and Research Centre, Chennai, India
[2]Assistant Professor, Department of Pharmaceutical Sciences, SGT College of Pharmacy, SGT University, Gurugram, Haryana, India
[3]Associate Professor, Department of Pharmaceutical Chemistry, Orlean College of Pharmacy, Knowledge Park 3, Greater Noida, Uttar Pradesh, India
[4]M.Pharm Scholar, School of Pharmacy, Seacom Skills University, Kendradangal, Bolpur, Birbhum, West Bengal, India
[5]Assistant Professor & Head, Department of Chemistry (PG), Sahibganj College Sahibganj, Jharkhand, India
[6]Principal & Professor, Sana College of Pharmacy, Kodad , Suryapet , Telangana, India
[7]Assistant Professor, Faculty of Pharmaceutical Science, Assam downtown University , Gandhinagar, Panikhaiti, Guwahati, India
[8]Assistant Professor, Department of Pharmacognosy, B.R.Nahata College of Pharmacy,Mandsaur, Madhya Pradesh, India
*[*9]Associate Professor, Anjuman I Islam's Kalsekar Technical Campus, School Of Pharmacy, New Panvel Navi Mumbai , Maharashtra, India*
** Corresponding Author: Dr. Imran A Sheik, Associate Professor, Anjuman I Islam'S Kalsekar Technical Campus, School of Pharmacy, New Panvel Navi Mumbai, Maharashtra, India*

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Abstract:
Advancements in bioinformatics have greatly enhanced our ability to predict molecular interactions between proteins and ligands, crucial for drug discovery and development. This study focuses on the implementation and refinement of a computational protocol using AutoDock 4.2 for predicting protein-ligand interactions. The protocol begins with acquiring and preparing PDB and PDBQT format files, followed by grid and docking parameter file preparation using AutoDock tools. Molecular docking simulations were conducted using Cygwin, and the results were comprehensively analyzed. The study emphasizes AutoDock's capability to distinguish between compounds with varying binding affinities, ranging from millimolar to nanomolar levels, and its effectiveness in ranking molecules based on subtle affinity differences. Detailed directions provided in this research enable researchers, regardless of prior bioinformatics experience, to proficiently utilize AutoDock for molecular docking experiments. This approach holds promise for accelerating drug discovery processes by facilitating the screening of large chemical libraries and identifying potential drug candidates with specific binding properties.

Keywords: Bioinformatics, molecular docking, AutoDock 4.2, protein-ligand interactions, drug discovery, binding affinity, computational protocol

Introduction

The rising prevalence of antibiotic-resistant bacterial strains presents a significant challenge to public health, necessitating the urgent development of new antimicrobial agents[1]. Traditional drug discovery methods are often time-consuming and costly, prompting the scientific community to increasingly rely on computational approaches for the rapid identification of potential drug candidates[2]. This research focuses on the development and application of a novel protocol for predicting protein-ligand interactions, specifically targeting imidazole derivatives as anti-bacterial agents. Through comprehensive docking studies, this protocol aims to streamline the identification of promising compounds, offering a potential solution to the global antibiotic resistance crisis[3,4].

Protein-ligand interactions are fundamental to numerous biological processes, including enzyme activity, signal transduction, and cellular regulation. Understanding these interactions is critical in the context of drug design, as the binding affinity and specificity of a ligand to its target protein largely determine its therapeutic efficacy[5]. Traditional methods for studying these interactions, such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy, are indispensable but can be labor-intensive and costly[6]. Consequently, computational techniques like molecular docking have become invaluable tools in modern drug discovery. Molecular docking involves predicting the preferred orientation of a ligand when bound to a target protein to form a stable complex. This technique not only estimates the binding affinity but also provides insights into the molecular interactions at the binding site[7]. Despite its potential, the accuracy of molecular docking can be limited by the scoring functions used to predict binding affinities and the flexibility of both the ligand and the protein. Therefore, developing an advanced and accurate computational protocol is essential for reliable predictions[8,9]. Imidazole derivatives have long been recognized for their diverse biological activities, including antifungal, antiviral, and anti-inflammatory properties. Their potential as antibacterial agents has also been explored, given their ability to inhibit key bacterial enzymes and disrupt vital cellular processes[10,11]. However, the structural diversity and complex mechanisms of action of imidazole derivatives necessitate comprehensive studies to fully understand their interaction with bacterial targets. The primary objective of this research is to develop a novel computational protocol for predicting protein-ligand interactions, with a specific focus on imidazole derivatives as antibacterial agents[12]. This protocol aims to enhance the accuracy and efficiency of molecular docking studies, providing a robust tool for identifying promising drug candidates. The secondary objective is to apply this protocol to a diverse library of imidazole derivatives, conducting comprehensive docking studies to evaluate their potential as antibacterial agents[13,14]. By achieving these objectives, this research seeks to contribute to the development of new antibiotics and address the pressing issue of antibiotic resistance. The development of the computational protocol involves several key components, including the selection of appropriate docking software, optimization of scoring functions, and validation against known protein-ligand complexes[15]. Advanced docking software such as AutoDock Vina and Glide will be utilized for their proven efficacy in predicting binding affinities and poses. To enhance accuracy, multiple scoring functions will be integrated, allowing for a more comprehensive evaluation of binding interactions. Validation is a crucial step in protocol development[16,17]. By using known protein-ligand complexes with

experimentally determined binding affinities, the accuracy of the docking predictions can be assessed. This benchmarking process ensures that the protocol can reliably predict binding interactions for new ligands[18].

Material and method

Material

Softwares and tools: The computational protocol for predicting protein-ligand interactions was developed using several software and tools: AutoDock Vina, employed for molecular docking simulations due to its high accuracy and speed; Glide (Schrödinger), used for docking studies to compare and validate results from AutoDock Vina; Gaussian, utilized for energy minimization of ligands; AMBER, applied for molecular dynamics simulations and energy minimization of proteins; PyMOL and Chimera, used for protein structure preparation and visualization; and the Protein Data Bank (PDB), which served as the source for acquiring 3D structures of target proteins.

Method:

Ligand preparation: For ligand preparation, a diverse library of imidazole derivatives was compiled from chemical databases like PubChem and ChemSpider, as well as relevant literature, with selection criteria including structural diversity and reported antibacterial activity. The ligands were subjected to energy minimization using Gaussian, where each ligand was optimized to its lowest energy conformation and parameterized using AMBER force fields to ensure accurate molecular mechanics simulations. For protein preparation, bacterial protein targets essential for survival and pathogenicity, such as DNA Gyrase and Penicillin-Binding Proteins (PBPs), were selected. The 3D structures of these proteins were retrieved from the PDB and prepared for docking by removing water molecules and heteroatoms not part of the protein-ligand complex, assigning proper protonation states using Chimera based on physiological pH, and performing energy minimization using AMBER to refine the structures[19].

Docking Protocol: The developed protocol for docking studies was applied using AutoDock Vina and Glide. The process involved defining the grid box to encompass the protein's active site, conducting docking simulations to generate multiple binding poses for each ligand, and scoring and ranking the ligands based on their binding affinities. The docking results were analyzed to identify key interactions and binding poses by examining hydrogen bonds, hydrophobic interactions, and π-π stacking using PyMOL, and comparing predicted binding affinities to experimental values for validation. A Structure-Activity Relationship (SAR) analysis was conducted to correlate the structural features of imidazole derivatives with their binding affinities and antibacterial potential, identifying common structural features among high-affinity ligands and analyzing the relationship between specific structural features and binding affinity[20].

Compounds formulations: For Compound 1, 1-(1H-imidazol-1-yl)-2-phenylethanone, the preparation of the grid box and output was followed by removing the B chain and analyzing the interaction of the ligand with the receptor. Similarly, for Compound 2, 1-(2-methyl-1H-

imidazol-1-yl)-2-phenylethanone, the grid box was prepared, and after removing the B chain, the ligand-receptor interactions were studied. Compound 3, 1-(2-methyl-5-nitro-1H-imidazol-1-yl)-2-phenylethanone, underwent grid box preparation, output generation, and interaction analysis post-B chain removal. Compound 4, 2-(4-chlorophenyl)-1-(1H-imidazol-1-yl) ethanone, followed the same steps: grid box preparation, output, B chain removal, and interaction analysis. For Compound 5, 2-(4-chlorophenyl)-1-(2-methyl-1H-imidazol-1-yl) ethanone, the grid box was prepared, output generated, B chain removed, and interactions analyzed. Compound 6, 2-(4-chlorophenyl)-1-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanone, was processed by removing the B chain, output generation, and ligand-receptor interaction analysis. Compound 7, 1-(1H-imidazol-1-yl)-2-(o-tolyl) ethanone, included grid box preparation, output generation, and interaction analysis. For Compound 8, 1-(2-methyl-1H-imidazol-1-yl)-2-(o-tolyl) ethanone, grid box preparation, output generation, and post-B chain removal interaction analysis were performed. Compound 9, 1-(2-methyl-5-nitro-1H-imidazol-1-yl)-2-(p-tolyl) ethanone, was analyzed after B chain removal and output generation, focusing on ligand-receptor interactions. Compound 10, 1-(1H-imidazol-1-yl)-2-(p-tolyl) ethanone, underwent grid box preparation, output generation, and interaction analysis. Among these, Compounds 7 and 8 exhibited the most negative docking values, which are thermodynamically favorable, indicating stronger binding affinity due to favorable intermolecular interactions and better ligand fit within the protein's binding pocket. These negative scores suggest a higher likelihood of these ligands exhibiting the desired biological activity. Consequently, Compounds 7 and 8 are concluded to have the best drug-receptor binding interactions. The binding mode of Compound 8 at the binding pocket of 1RT2 Ligand is shown with amino acid residues interacting with the ligands, and hydrogen bond interactions are highlighted with yellow dotted lines[21].

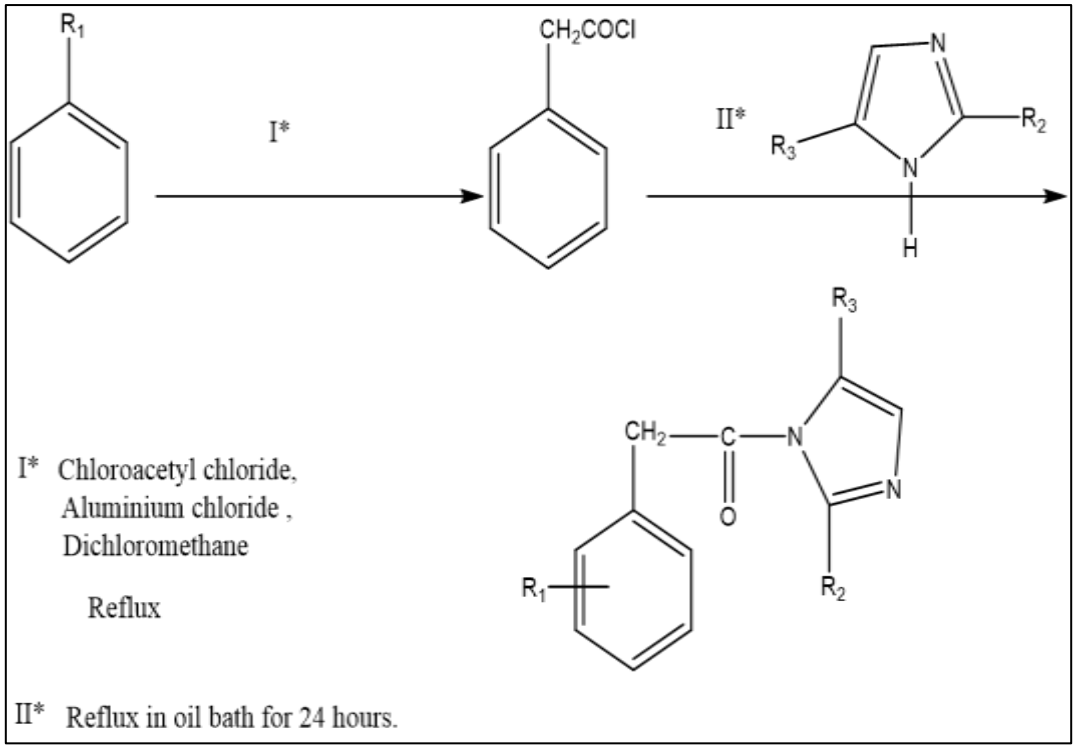
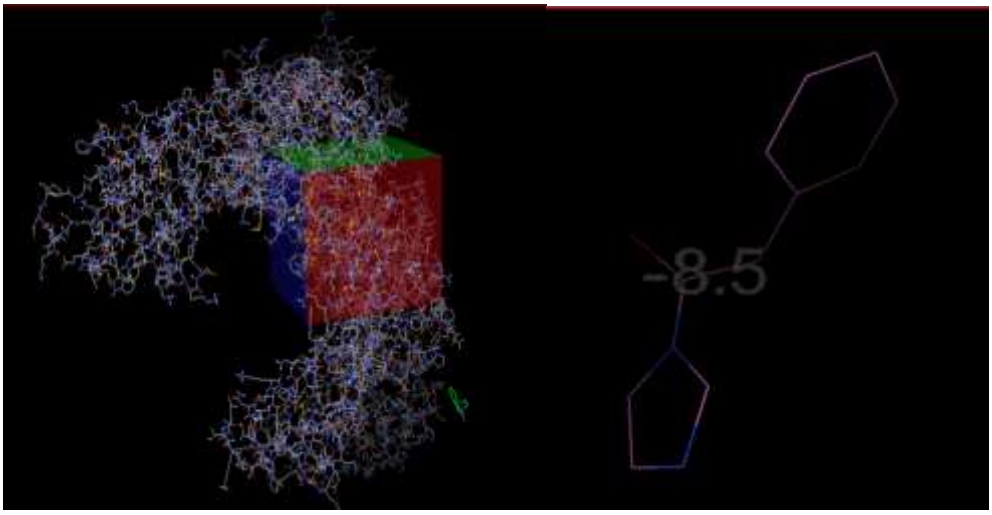
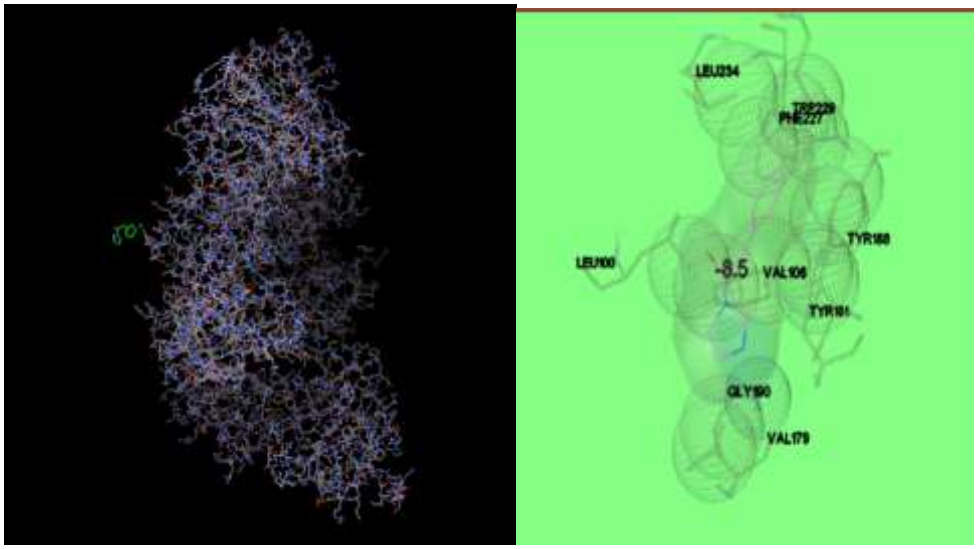


Figure 1: Schametic diagram of the synthesis

Development of compound 1: 1-(1H-imidazol-1-yl)-2-phenylethanone

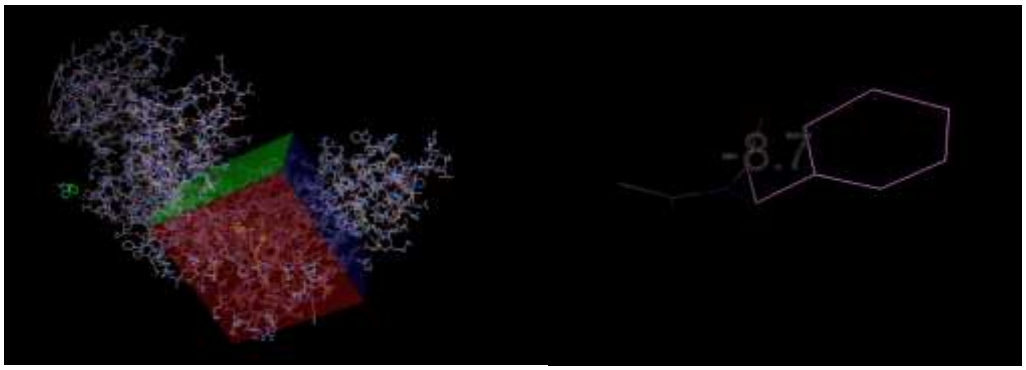


Preparation of Grid Box **Output**

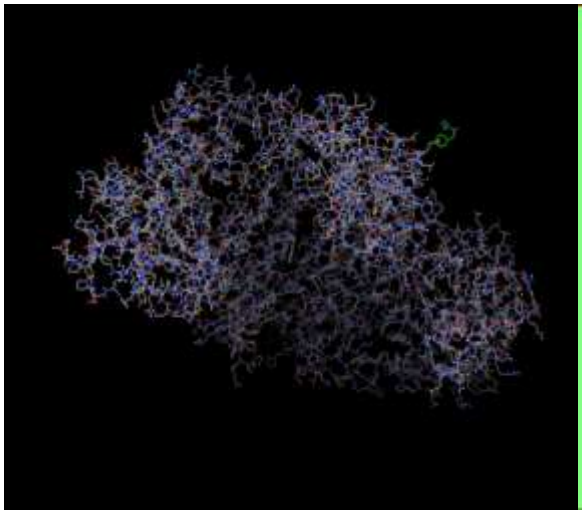


After removing b chain **Intraction of ligand with receptor**

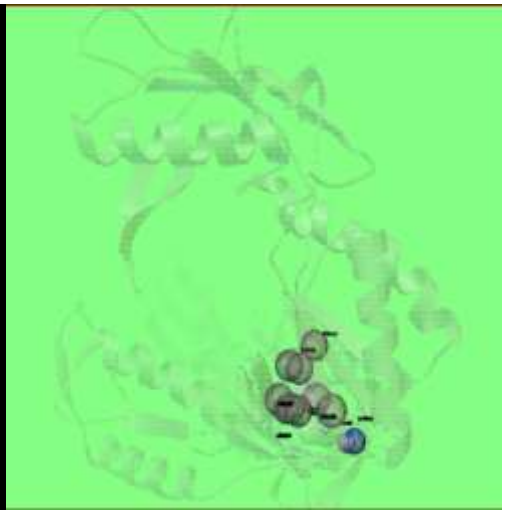
Development of compound 2: 1-(2-methyl-1H-imidazol-1-yl)-2-phenylethanone



Preparation of grid box **Output**

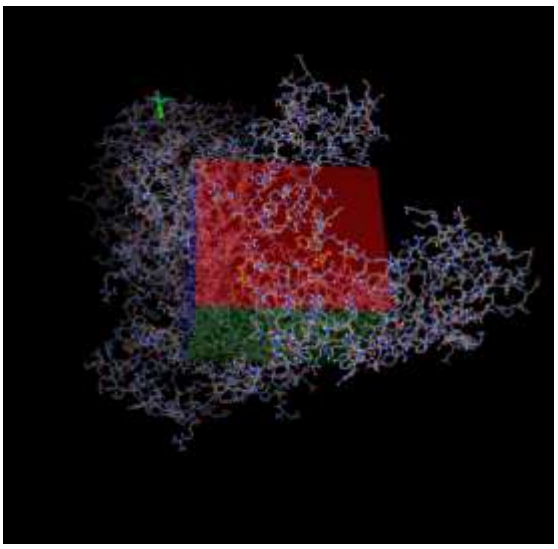


After removing b chain

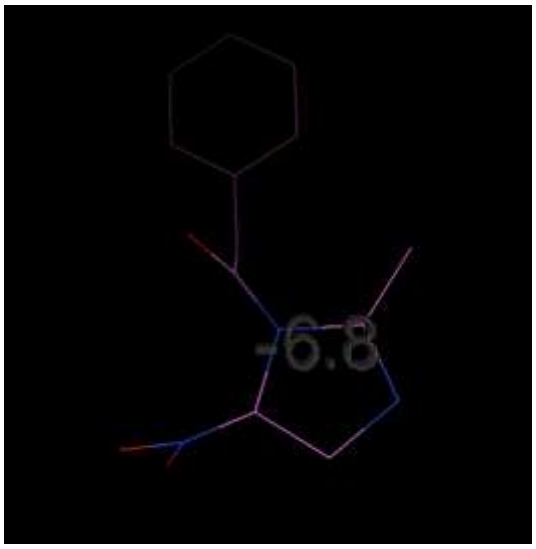


Intraction of ligand with receptor

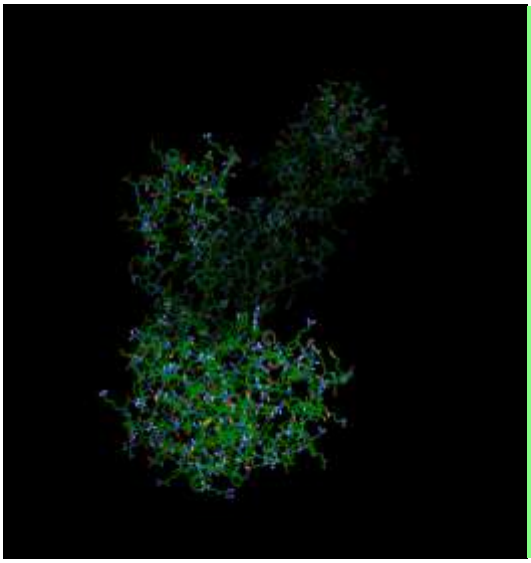
Development of compound 3: 1-(2-methyl-5-nitro-1H-imidazol-1-yl)-2-phenylethanone



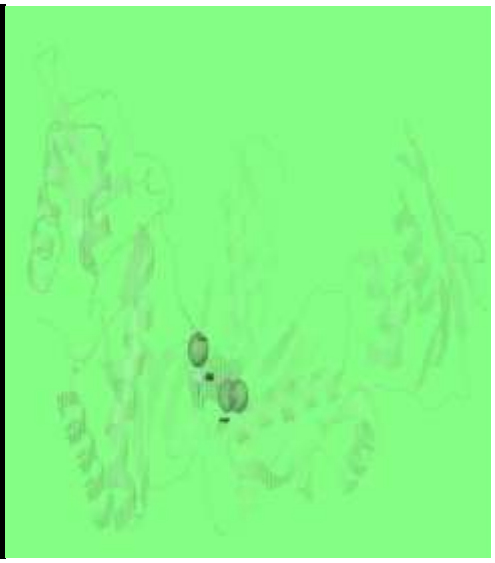
Preparation of grid box



Output

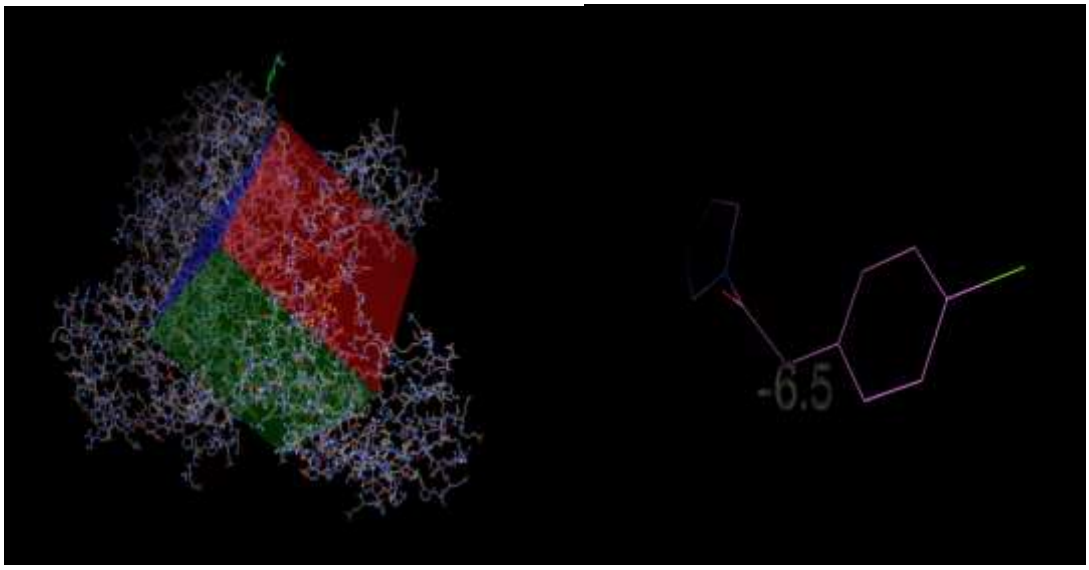


After removing b chain

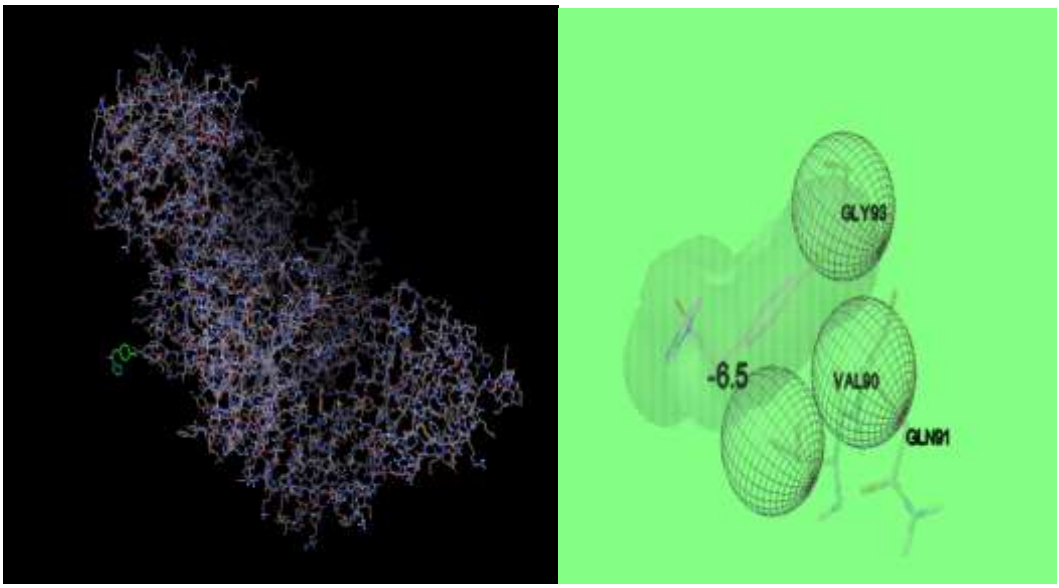


Interaction of ligand with receptor

Development of compound 4: 2-(4-chlorophenyl)-1-(1H-imidazol-1-yl) ethanone

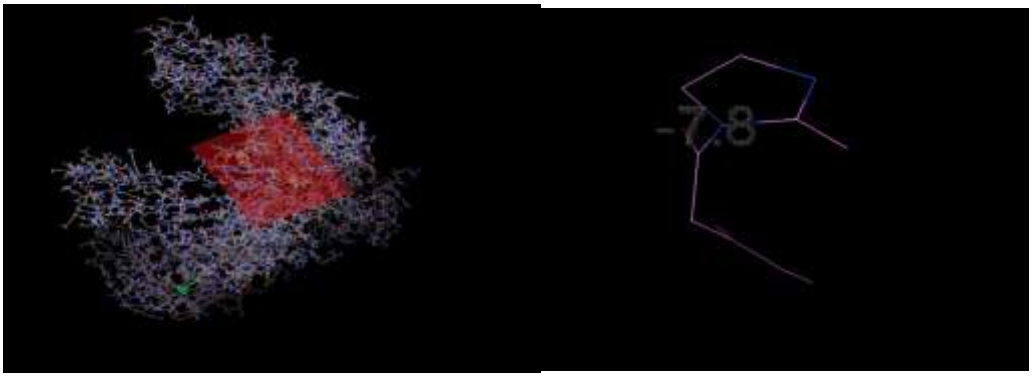


Preparation of grid box **Output**

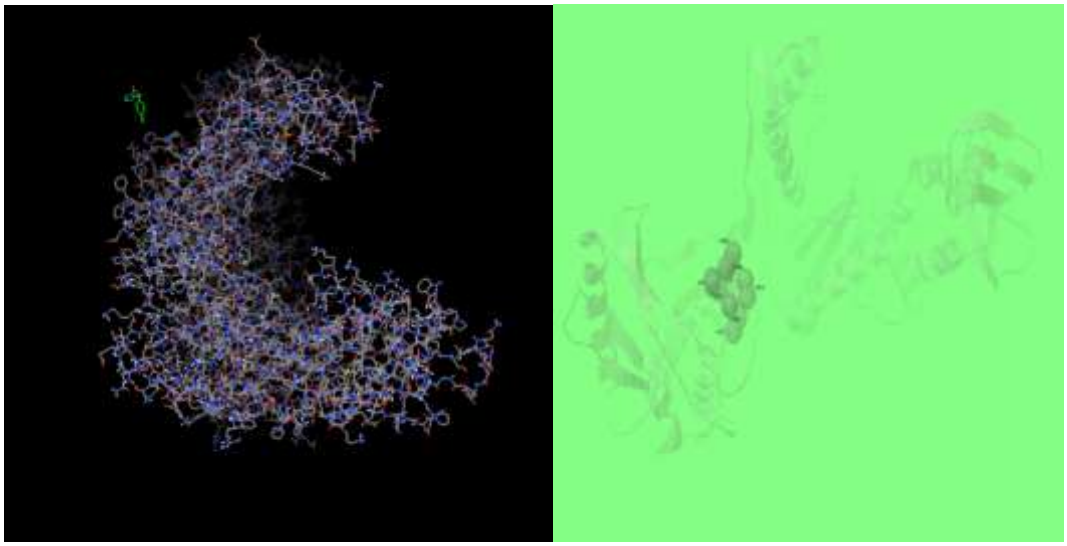


After removing b chain **Intraction of ligand with receptor**

Development of compound 5: 2-(4-chlorophenyl)-1-(2-methyl-1H-imidazol-1-yl) ethanone

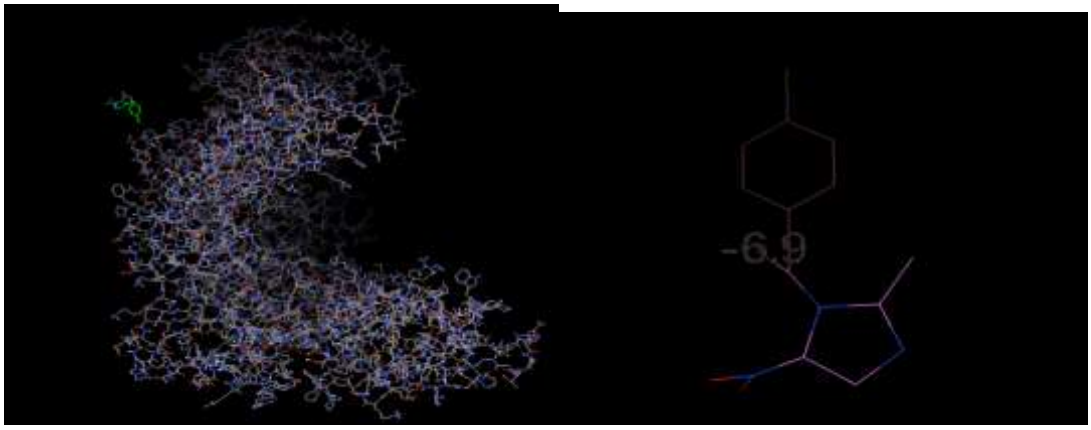


Preparation of grid box **Output**



After removing b chain **Iintraction of ligand with receptor**

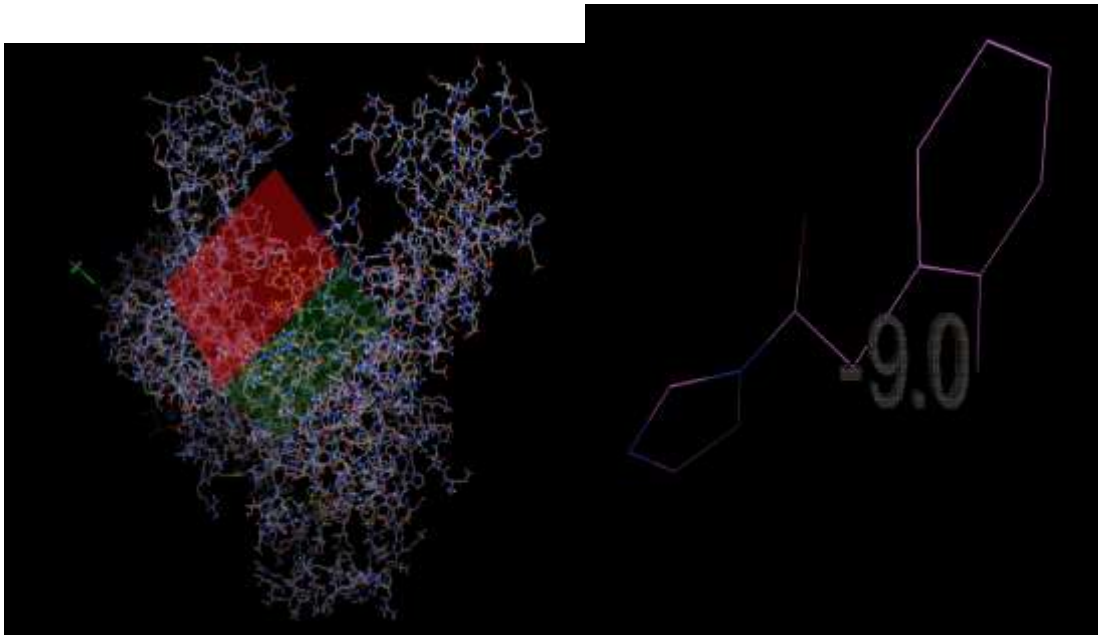
Development of compound 6: 2-(4-chlorophenyl)-1-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanone



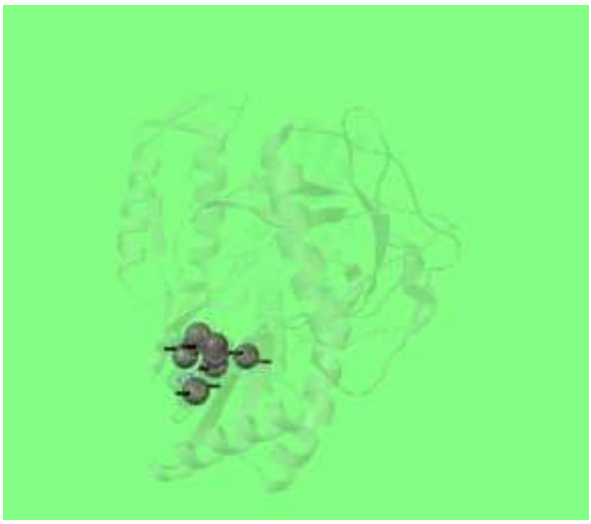
After removing b chain **Output**

Intraction of ligand with receptor

Development of compound 7: 1-(1H-imidazol-1-yl)-2-(o-tolyl) ethanone

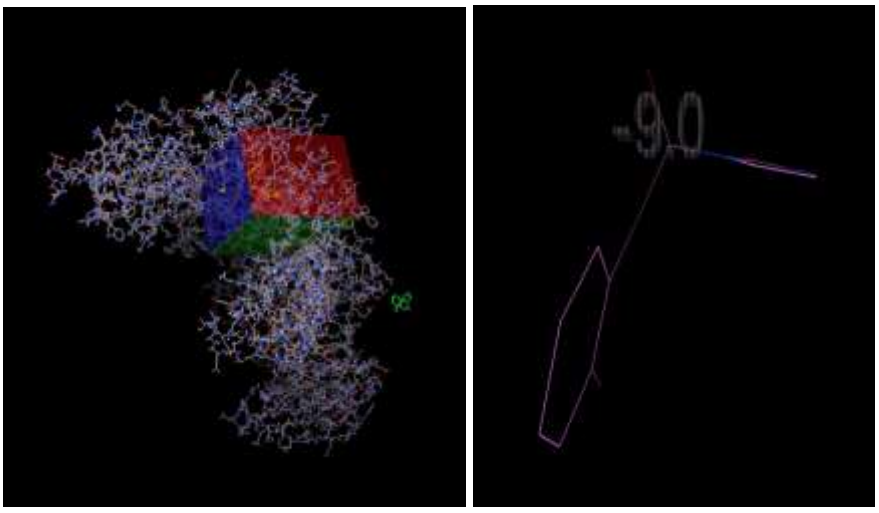


Preparation of grid box **Output**

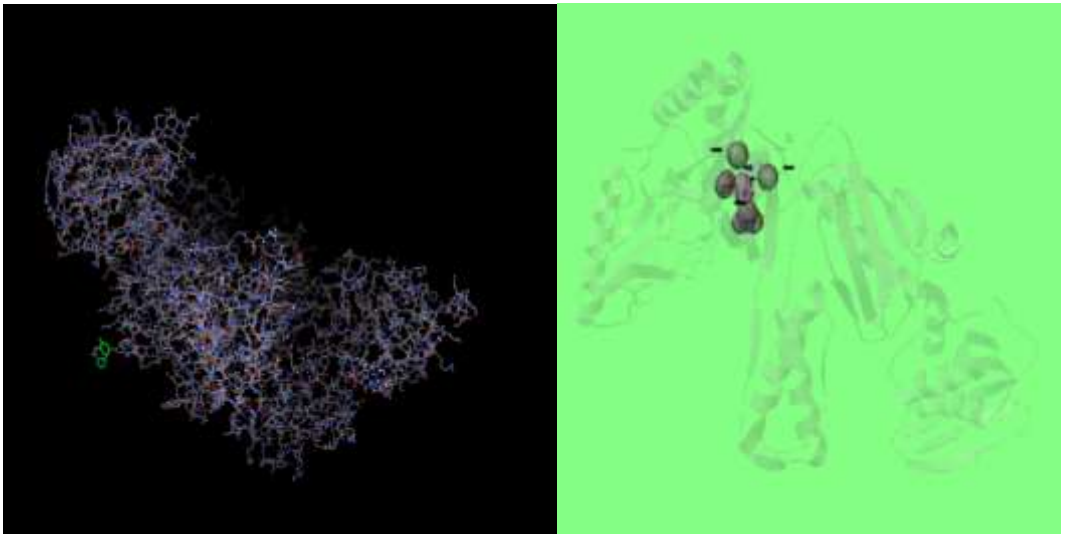


Intraction of ligand with receptor

Development of compound 8: 1-(2-methyl-1H-imidazol-1-yl)-2-(o-tolyl) ethanone

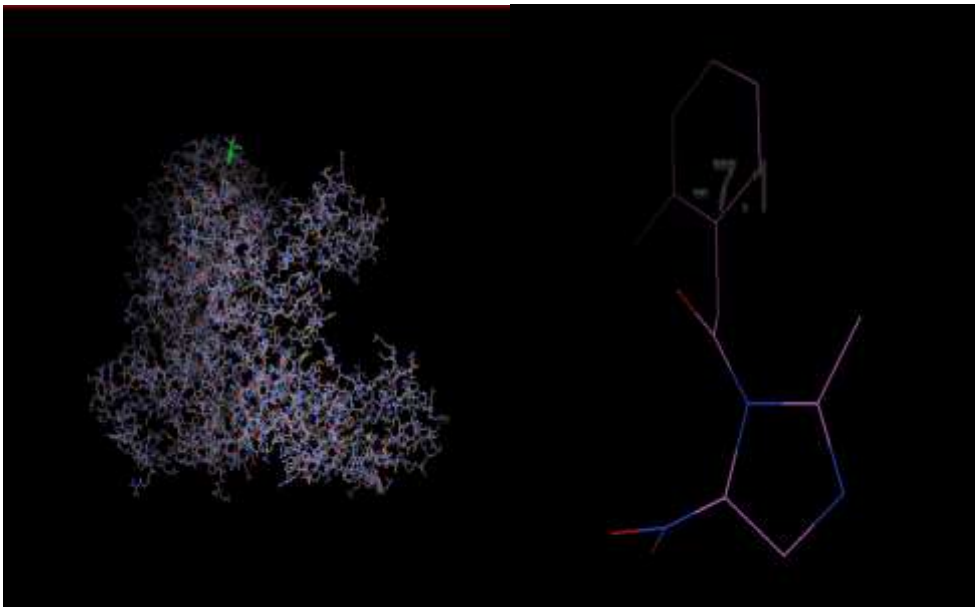


Preparation of grid box **Output**



After removing b chain **Intraction of ligand with receptor**

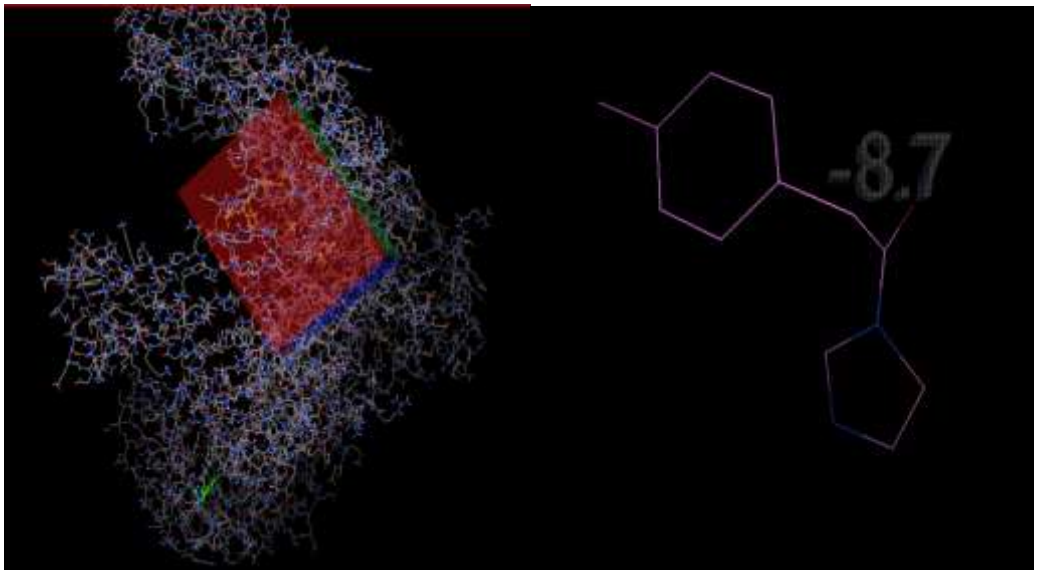
Development of compound 9: 1-(2-methyl-5-nitro-1H-imidazol-1-yl)-2-(p-tolyl) ethanone



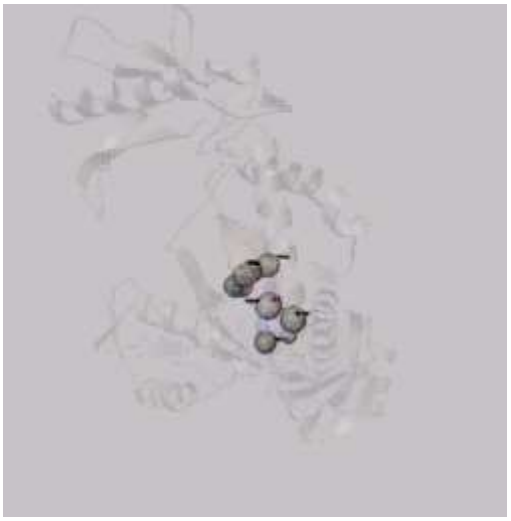
After removing b chain **Output**

Intraction of ligand with receptor

Development of compound 10: 1-(1H-imidazol-1-yl)-2-(p-tolyl) ethanone



Preparation of grid box **Output**



Intraction of ligand with receptor

Discussion

Compounds 7 [1-(1H-imidazol-1-yl)-2-(o-tolyl) ethanone] and 8 [1-(2-methyl-1H-imidazol-1-yl)-2-(o-tolyl) ethanone] exhibit the highest negative docking values, which are highly desirable in molecular docking for several key reasons. Firstly, a negative docking score (ΔG) indicates a thermodynamically favorable interaction, suggesting spontaneous binding and implying a stronger binding affinity. Secondly, such scores typically denote the presence of favorable intermolecular interactions between the ligand and the receptor, crucial for establishing robust binding. Thirdly, a more negative score often signifies a superior fit of the ligand within the protein's binding pocket, akin to a puzzle piece perfectly aligning with its counterpart. Lastly, while docking scores serve as estimates, a more negative value suggests a heightened likelihood that the ligand will exhibit the desired biological activity by effectively interacting with its target protein. Based on these considerations, both Compounds 7 and 8 are identified as having the best drug-receptor binding interactions due to their notably large negative docking values compared to other compounds examined.

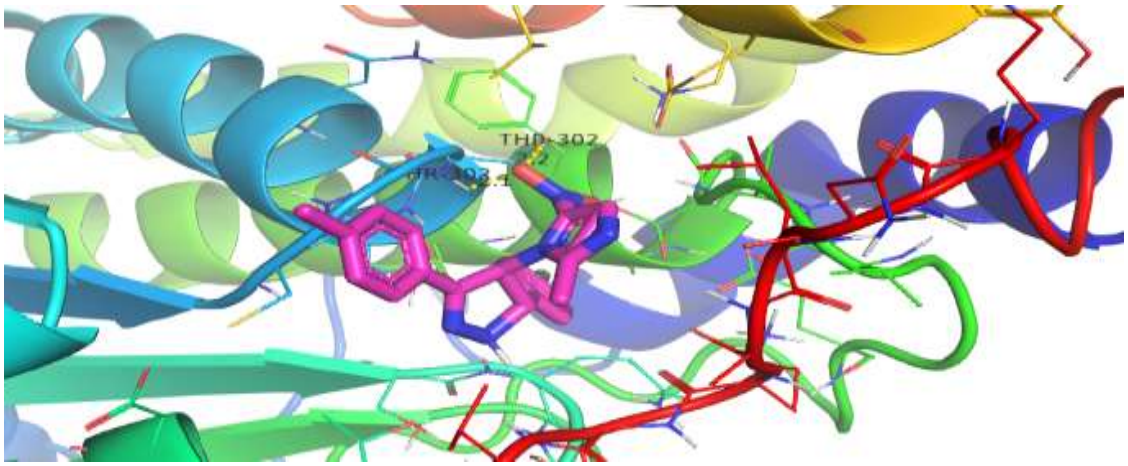


Figure 2: Binding Configuration of Compound 8 in the Binding Pocket of 1RT2Ligand: Amino Acid Interactions and Hydrogen Bonding

The binding configuration of compound 8 within the binding pocket of 1RT2Ligand is depicted, illustrating the specific amino acid residues that interact with the ligand. Hydrogen bonds are indicated by yellow dotted lines.

Conclusion

Recent advancements in bioinformatics have significantly enhanced our ability to predict molecular interactions between proteins and ligands in their bound states with remarkable accuracy. Tools like AutoDock have revolutionized this field by enabling precise prediction of binding affinities, distinguishing between compounds with varying binding constants ranging from millimolar to nanomolar levels, and effectively ranking molecules based on subtle affinity differences. By facilitating the screening of large chemical libraries and facilitating the exploration of chemical space for new compounds with specific binding properties, AutoDock offers a robust platform for drug discovery and molecular design. The protocol outlined in this study provides a user-friendly approach to utilizing AutoDock, starting from acquiring and preparing PDB and PDBQT format files to performing molecular docking and analyzing results. This streamlined approach ensures that even researchers without prior bioinformatics

experience can proficiently conduct molecular docking experiments using AutoDock 4.2, leveraging the detailed guidance provided in this article.

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