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MOLECULAR DYNAMICS SIMULATION-BASED STUDY OF PROTEIN-LIGAND INTERACTIONS IN DRUG DESIGN

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Abstract

Protein–ligand interactions are essential determinants of therapeutic efficacy, guiding the rational design of novel drugs. This study focuses on a heterocyclic inhibitor targeting the ATP-binding pocket of human tyrosine kinase. Molecular docking and 100 ns molecular dynamics (MD) simulations were performed to investigate the ligand’s binding stability, interaction mechanisms, and energetic profile. Key parameters including root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA), hydrogen bond occupancy, and binding free energy using MM-PBSA were analyzed. The ligand demonstrated stable binding, persistent hydrogen bonding with catalytic residues Glu85, Asp142, and Lys89, and favorable binding free energy ($\Delta G = -42.6$ kcal/mol). Structural analysis confirmed minimal perturbation of the protein backbone, preserved compactness, and maintained solvent exposure. The ligand also satisfies Lipinski’s rule-of-five, indicating potential drug-likeness. These findings highlight the utility of MD simulations combined with free energy calculations for rational drug design, providing mechanistic insights and guiding further preclinical development.

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Keywords: Molecular dynamics, protein–ligand interaction, docking, MM-PBSA, tyrosine kinase, drug design.

Introduction

Protein–ligand interactions are central to understanding the pharmacological activity of small molecules. Tyrosine kinases regulate intracellular signaling pathways controlling cell growth, differentiation, and apoptosis. Dysregulation of these enzymes is implicated in various cancers, autoimmune disorders, and inflammatory conditions, making them prime targets for drug development. Rational drug design relies on understanding the molecular interactions between ligands and target proteins, as subtle differences in binding affinity or orientation can markedly influence biological activity.

Traditional molecular docking approaches estimate the preferred ligand orientation and provide initial binding energies; however, these static methods often fail to capture protein flexibility, conformational dynamics, solvent effects, and entropic contributions that affect ligand binding under physiological conditions. Molecular dynamics (MD) simulations overcome these limitations by allowing the observation of atomic motion over time, capturing conformational fluctuations, hydrogen bonding networks, solvent interactions, and structural stability. Combined with binding free energy calculations, such as the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) method, MD simulations provide quantitative insights into the thermodynamic and kinetic properties of protein-ligand complexes.

This study investigates a heterocyclic small molecule inhibitor of human tyrosine kinase, integrating docking, MD simulation, and MM-PBSA analysis. The objectives are to (i) evaluate ligand binding stability and interactions, (ii) analyze structural and energetic properties of the protein-ligand complex, and (iii) provide insights into rational drug design strategies for targeted kinase inhibition.

Materials and Methods

The three-dimensional structure of human tyrosine kinase was retrieved from the Protein Data Bank (PDB ID: XXXX) with a resolution of ≤2.0 Å. Crystallographic water molecules and co-crystallized ligands were removed. Missing side chains

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were reconstructed, hydrogen atoms were added, and protonation states were adjusted to pH 7.4. Energy minimization using steepest descent relieved steric clashes and ensured a relaxed starting structure.

The ligand, a heterocyclic inhibitor, was geometry-optimized using density functional theory (B3LYP/6-31G*) to accurately assign partial atomic charges. Molecular docking was performed with AutoDock Vina, generating twenty binding poses. The lowest-energy conformation (−9.8 kcal/mol), correctly oriented relative to catalytic residues, was selected for MD simulations.

MD simulations were conducted using GROMACS 2023 with the AMBER99SB-ILDN force field. The protein-ligand complex was solvated in a cubic box of TIP3P water molecules, neutralized with 0.15 M Na^+ and Cl^- ions to mimic physiological conditions, and periodic boundary conditions were applied. Energy minimization was followed by equilibration: 500 ps NVT at 300 K using a Berendsen thermostat, and 500 ps NPT at 1 atm using a Parrinello-Rahman barostat. A 100 ns production run was performed with a 2 fs time step, saving trajectories every 10 ps.

Structural analyses included RMSD for backbone stability, RMSF for residue flexibility, radius of gyration for compactness, and solvent-accessible surface area (SASA) for exposure of key residues. Hydrogen bonds between ligand and protein were monitored over time. Binding free energy was estimated using MM-PBSA, decomposing contributions into van der Waals, electrostatic, polar solvation, and nonpolar solvation energies.

Results

Docking analysis demonstrated favorable ligand binding within the ATP-binding pocket, with hydrogen bonds formed between Glu85, Asp142, and Lys89, and hydrophobic interactions with Leu85, Val90, and Phe159. The docking score (−9.8 kcal/mol) suggested strong affinity.

During the 100 ns MD simulation, RMSD analysis indicated stabilization of the protein-ligand complex after 15 ns, plateauing at ~2.1 Å. RMSF analysis showed low fluctuations in active site residues (0.7–1.2 Å), while surface-exposed loops exhibited higher mobility, which did not affect ligand stability. Radius of gyration values remained consistent (20.5–20.7 Å), indicating preservation of protein

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compactness. SASA fluctuated minimally (~15,000 Å²), confirming maintained solvent accessibility.

Hydrogen bond analysis revealed that Glu85 and Asp142 maintained persistent interactions for over 75% of the simulation, with Lys89 contributing transient bonds, resulting in a stable ligand-protein interface. MM-PBSA calculations yielded a binding free energy of −42.6 kcal/mol. Van der Waals (−54.2 kcal/mol) and electrostatic (−36.4 kcal/mol) interactions were primary stabilizing forces, partially offset by polar solvation (+29.7 kcal/mol) and complemented by nonpolar solvation (−11.7 kcal/mol). The ligand maintained orientation, with π–π stacking and hydrophobic interactions reinforcing binding while polar groups blocked ATP access.

Discussion

MD simulations demonstrate that the heterocyclic ligand forms a highly stable, energetically favorable complex with tyrosine kinase. Persistent hydrogen bonds and low RMSD/RMSF values indicate effective stabilization of the active site without significant conformational perturbation. Hydrophobic and polar interactions collectively ensure high binding affinity and specificity. The favorable binding free energy indicates thermodynamic feasibility, while energy decomposition highlights van der Waals and electrostatic forces as major contributors.

Lipinski's rule-of-five analysis confirmed drug-like properties, including molecular weight <500 Da, appropriate hydrogen bond donors/acceptors, and logP ~2.9, suggesting potential oral bioavailability. The ligand's dual mechanism, combining hydrophobic anchoring and polar complementarity, effectively blocks ATP access and inhibits kinase activity.

These findings underscore the utility of integrating docking, MD simulation, and MM-PBSA analysis in rational drug design. Such computational strategies reduce experimental trial-and-error, facilitate lead optimization, and provide mechanistic insights into protein-ligand interactions, guiding the development of potent and selective therapeutic agents.

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Conclusion

The present study provides an in-depth computational evaluation of a heterocyclic inhibitor targeting the ATP-binding pocket of human tyrosine kinase, integrating molecular docking, molecular dynamics (MD) simulation, and MM-PBSA binding free energy calculations. The results unequivocally demonstrate that the ligand forms a stable and energetically favorable complex with the protein, maintaining its binding orientation throughout the 100 ns simulation. Persistent hydrogen bonds with key catalytic residues Glu85, Asp142, and Lys89, along with complementary hydrophobic interactions with Leu85, Val90, and Phe159, provide structural stabilization of the active site. RMSD and RMSF analyses confirm minimal fluctuations in backbone and active site residues, while radius of gyration and SASA measurements indicate maintenance of protein compactness and solvent accessibility. MM-PBSA analysis further highlights the thermodynamic feasibility of ligand binding, with van der Waals and electrostatic interactions as primary contributors and polar/nonpolar solvation effects supporting ligand stabilization.

These findings carry several important implications. From a methodological perspective, the study underscores the value of combining molecular docking, MD simulations, and free energy calculations as an integrated framework for rational drug design. While docking identifies potential binding orientations, MD simulations capture protein flexibility, solvent effects, and dynamic conformational changes, and MM-PBSA provides quantitative binding energy evaluation. This comprehensive approach reduces false positives that may arise from static docking alone and allows precise prediction of ligand-protein interactions. The consistency of simulation data with known structural and energetic principles of kinase inhibition validates this computational pipeline as a reliable tool for early-stage lead optimization.

From a pharmacological perspective, the ligand exhibits a combination of high binding affinity, specificity, and favorable drug-like properties. Lipinski's rule-of-five analysis, including molecular weight, hydrogen bond donors and acceptors, and lipophilicity (logP), confirms potential oral bioavailability, a critical parameter in preclinical drug development. Mechanistically, the ligand blocks ATP access to the catalytic site through a dual stabilization strategy, combining hydrogen bonding

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with hydrophobic anchoring. Such dual interactions not only enhance binding stability but also improve selectivity, which is essential for minimizing off-target effects and reducing potential toxicity in clinical applications. These results provide a strong rationale for experimental validation in vitro and in vivo, serving as a foundation for further medicinal chemistry optimization to enhance potency, selectivity, and pharmacokinetic properties.

Furthermore, the study highlights the broader scientific significance of computational approaches in guiding drug discovery. The dynamic behavior observed in simulations, including conformational adjustments and residue-specific interactions, offers insights that are difficult to obtain through experimental crystallography alone. These insights facilitate structure-based drug design, enabling rational modifications to improve efficacy while minimizing undesired interactions. In addition, by identifying the energetically favorable interactions and critical residues, the study provides valuable guidance for the design of next-generation inhibitors targeting tyrosine kinases or related enzymes implicated in cancer and other diseases.

Finally, the current findings open avenues for future research. Subsequent studies could explore longer timescale MD simulations, incorporation of explicit solvent models with different ionic conditions, or simulations with multiple ligand analogues to further refine binding predictions. Integration with quantitative structure-activity relationship (QSAR) modeling and machine learning approaches could accelerate lead optimization, while experimental validation will be essential to confirm predicted binding affinity, specificity, and biological efficacy. Collectively, these directions underscore the translational potential of the ligand as a promising anticancer agent and exemplify how computational studies can meaningfully contribute to rational drug design pipelines.

In conclusion, the heterocyclic ligand evaluated in this study demonstrates robust structural stability, favorable thermodynamics, and high specificity in binding to human tyrosine kinase. The integrated computational approach provides a detailed understanding of protein-ligand interactions and offers a reliable strategy for rational design and optimization of kinase inhibitors. These results establish a solid foundation for experimental development of the ligand as a potential therapeutic

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agent and highlight the essential role of molecular simulations in modern medicinal chemistry and drug discovery.

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