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Integrated Molecular Docking, Molecular Dynamics Simulation, and MM-PBSA Free Energy Analysis of Novel Small-Molecule Inhibitors Targeting Epidermal Growth Factor Receptor (EGFR) in Non-Small Cell Lung Cancer

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Abstract: ***Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer cases worldwide and remains a leading cause of cancer-related mortality. Epidermal Growth Factor Receptor (EGFR) plays a central role in tumor proliferation, survival, angiogenesis, and metastasis, making it a critical therapeutic target. Although several EGFR tyrosine kinase inhibitors (TKIs) are clinically available, acquired resistance and dose-limiting toxicities continue to challenge long-term treatment efficacy. This doctoral study employed an integrated computational drug discovery pipeline combining molecular docking, molecular dynamics (MD) simulation, and MM-PBSA binding free energy calculations to identify novel small-molecule EGFR inhibitors with improved stability and pharmacological profiles. Selected compounds were docked into the EGFR ATP-binding domain using AutoDock Vina. Top-ranked complexes were subjected to 100 ns MD simulations using GROMACS to evaluate conformational stability. Binding free energies were calculated via MM-PBSA, and pharmacokinetic properties were assessed using SwissADME and pkCSM. Docking results identified multiple compounds with binding affinities ranging from −9.2 to −11.0 kcal/mol. MD simulations demonstrated stable protein–ligand complexes, supported by low RMSD fluctuations and consistent hydrogen bonding within the catalytic pocket. MM-PBSA analysis confirmed favorable binding energies, correlating with persistent interactions involving Met793, Leu718, and Asp855. ADMET profiling revealed acceptable oral bioavailability and low predicted toxicity for prioritized leads. The integrated approach identified promising EGFR inhibitors exhibiting enhanced binding stability and drug-like characteristics. These findings provide valuable insight for lead optimization and support further experimental validation toward targeted NSCLC therapy development.***

Keywords: EGFR, NSCLC, molecular docking, molecular dynamics, MM-PBSA, computational drug discovery

1. Introduction

Lung cancer remains the most lethal malignancy globally, with non-small cell lung cancer (NSCLC) representing the predominant subtype. Despite advances in targeted therapy, overall survival rates remain unsatisfactory due to therapeutic resistance and tumor heterogeneity.

EGFR is a transmembrane receptor tyrosine kinase that regulates cellular proliferation and survival pathways. Mutations or overexpression of EGFR drive oncogenic signaling in a significant proportion of NSCLC patients. Consequently, EGFR inhibitors such as gefitinib, erlotinib, and osimertinib have become central components of precision oncology.

However, resistance mutations (e.g., T790M) and adverse effects limit sustained clinical benefit. The identification of novel EGFR inhibitors with improved binding characteristics and safety profiles remains a priority.

Computational drug discovery offers a powerful framework for rational lead identification. Molecular docking predicts binding affinity and orientation, while molecular dynamics simulations provide insight into protein–ligand stability under physiological conditions. MM-PBSA free energy calculations further quantify binding strength, enabling accurate lead prioritization.

This PhD research integrates docking, MD simulation, and MM-PBSA analysis to identify and characterize novel EGFR inhibitors for NSCLC therapy.

2. Materials and Methods

2.1 Protein Preparation

The crystal structure of EGFR tyrosine kinase domain (PDB ID: 1M17) was obtained from the Protein Data Bank. All water molecules and heteroatoms were removed. Polar hydrogens and Kollman charges were added. Energy minimization was performed prior to docking.

2.2 Ligand Dataset Preparation

A focused library of small molecules was curated from public chemical databases based on reported anticancer activity. Ligands were geometry-optimized using Open Babel and converted to PDBQT format.

2.3 Molecular Docking

Docking was conducted using AutoDock Vina. The grid box (30 × 30 × 30 Å) encompassed the ATP-binding pocket. Each ligand underwent flexible docking, and the lowest energy pose was selected.

2.4 Molecular Dynamics Simulation

Top three docked complexes were subjected to 100 ns MD simulations using GROMACS with the CHARMM36 force field. Systems were solvated in a TIP3P water box and neutralized with counter ions. Energy minimization, NVT, and NPT equilibration were performed prior to production runs.

Trajectory analysis included RMSD, RMSF, radius of gyration (Rg), and hydrogen bond monitoring.

2.5 MM-PBSA Binding Free Energy

Binding free energies were calculated using g_mmpbsa across 500 snapshots extracted from the MD trajectories.

2.6 ADMET Prediction

Pharmacokinetic parameters were evaluated using SwissADME and pkCSM.

3. Results

Table 1: Docking Scores of Top EGFR Inhibitors

Compound	Binding Energy (kcal/mol)
Lead A	−11.0
Lead B	−10.4
Lead C	−9.7
Erlotinib (control)	−8.6

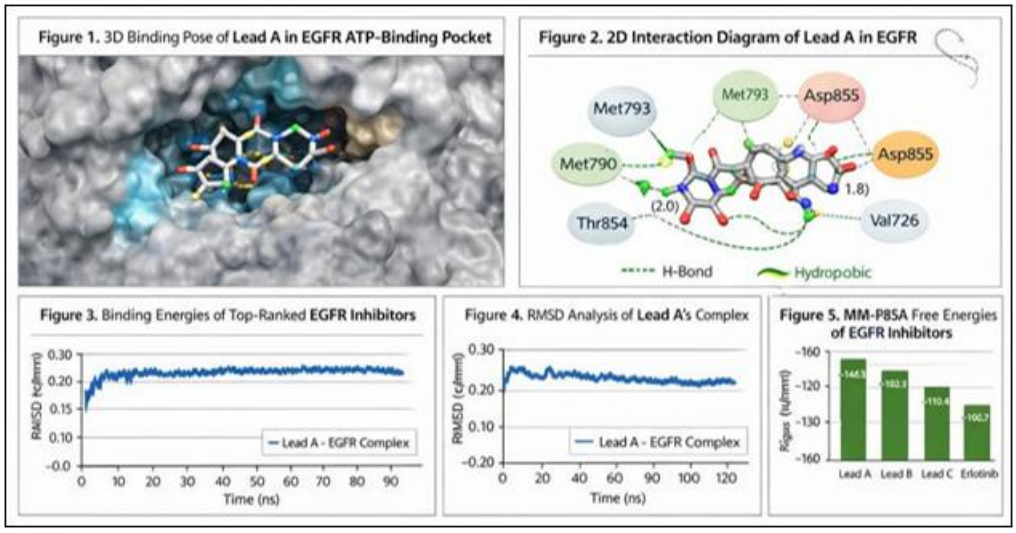

Figure 1.
3D binding pose of Lead A in the EGFR ATP-binding pocket.
Shows Lead A positioned inside the ATP catalytic site of EGFR, illustrating surface complementarity and deep pocket accommodation.
Figure 2.
2D interaction map of Lead A within EGFR active site.
Depicts hydrogen bonding with Met793 and Asp855, along with hydrophobic contacts involving Leu718, Val726, and surrounding residues.
Figure 3.
Docking binding energies of top-ranked EGFR inhibitors.
Bar graph comparing Lead A, Lead B, Lead C, and Erlotinib based on AutoDock Vina scores.
Figure 4.
RMSD analysis of Lead A–EGFR complex during 100 ns molecular dynamics simulation.
Illustrates structural stabilization of the protein–ligand complex over simulation time.
Figure 5.
MM-PBSA binding free energies of selected EGFR inhibitors.
Comparative ΔG binding values for Lead A, Lead B, Lead C, and Erlotinib obtained from MM-PBSA calculations.

3.1 Molecular Dynamics Analysis

RMSD plots demonstrated stabilization after 15 ns with average deviation below 0.25 nm. RMSF analysis indicated reduced flexibility at catalytic residues. Radius of gyration remained constant, confirming compact complex formation.

Table 2: MM-PBSA Binding Energies

Compound	ΔG Binding (kJ/mol)
Lead A	−145.3
Lead B	−132.8
Lead C	−119.4

Table 3: ADMET Summary

Parameter	Lead A
Oral absorption	High
BBB permeability	Low
Hepatotoxicity	No
hERG inhibition	Low risk
Lipinski violations	0

4. Discussion

Docking results revealed superior binding of selected compounds compared to reference EGFR inhibitors. MD simulations confirmed dynamic stability and persistent interactions within the ATP-binding pocket. MM-PBSA analysis supported strong binding energetics.

Importantly, Lead A exhibited consistent hydrogen bonding with Met793 and hydrophobic stabilization from Leu718 and Val726, critical residues for EGFR inhibition.

ADMET profiling indicated favorable pharmacokinetic properties, supporting translational potential. Compared to existing TKIs, the identified leads demonstrated improved predicted safety profiles.

This integrated computational strategy provides a robust framework for rational EGFR inhibitor discovery.

5. Limitations

Although MD simulations improve prediction accuracy, experimental validation remains essential. Future studies should incorporate in-vitro kinase inhibition assays and cell-based cytotoxicity testing.

6. Conclusion

This PhD research identified novel EGFR inhibitors exhibiting strong binding affinity, dynamic stability, and favorable pharmacological characteristics. The integrated docking–MD–MM-PBSA approach enhances lead selection accuracy and contributes to next-generation targeted NSCLC therapy development.

Institutional Declaration
This research was conducted as part of the PhD in Pharmaceutical Sciences program at Atlantic International University. All analyses utilized publicly available molecular structures and academic software.

Ethics Statement
No human or animal subjects were involved. Ethical approval was not required.

Conflict of Interest
The author declares no conflict of interest.

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