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Evaluating Molecular Docking Accuracy: A Comparative Study with In Vitro EGFR Inhibition Data

Farah Haidar Abdulredha
(Department of Pharmacy,
Faculty of Pharmacy, The University of Mashreq, Baghdad 10023, Iraq) farah.haidar@uom.edu.iq

Abstract
Cancer, particularly non-small cell lung cancer (NSCLC), remains a major global health challenge, necessitating the development of novel and more effective therapeutic agents. Epidermal growth factor receptor (EGFR) inhibitors, such as erlotinib, have shown clinical efficacy in inhibiting EGFR-mediated tumor growth. However, the emergence of resistance mechanisms and concerns regarding bioavailability highlight the need for more potent and selective EGFR inhibitors. Molecular docking has emerged as a valuable tool in the early stages of drug discovery, providing insights into ligand-receptor interactions and predicting binding affinities. This review evaluates the accuracy of molecular docking by comparing in silico binding predictions with in vitro cytotoxicity data. Erlotinib, a well-established EGFR inhibitor, serves as a reference for assessing the performance of docking simulations. We further explore the structure-activity relationship (SAR) of novel 1,3-diazetidin-2-one derivatives as potential EGFR inhibitors, highlighting key pharmacophoric features that contribute to enhanced binding and specificity. Despite the utility of docking studies, limitations such as neglecting pharmacokinetic factors and metabolic stability are discussed. The review concludes by proposing strategies to integrate docking with in vitro and in vivo testing to improve the accuracy of predictive models and optimize lead compounds for clinical development.
Keyword: Epidermal Growth Factor Receptor (EGFR), Erlotinib, 1,3-Diazetidin-2-one derivatives, Molecular docking, Structure-activity relationship (SAR).

INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide, driving continuous research into novel therapeutic agents[1].Epidermal growth factor receptor (EGFR) is a crucial target in cancer treatment, particularly in non-small cell lung cancer (NSCLC), due to its role in tumor growth and progression[2]. Tyrosine kinase inhibitors (TKIs), such as erlotinib, have demonstrated clinical efficacy by selectively inhibiting EGFR activation. However, resistance mechanisms and limited bioavailability necessitate the discovery of new, more effective EGFR inhibitors.[3]

Molecular docking has emerged as an essential tool in drug discovery, allowing researchers to predict ligand-receptor interactions based on binding affinity calculations (Abdulredha et al., 2023). It provides valuable insights into potential drug candidates by evaluating binding energy, hydrogen bonding, and hydrophobic interactions. Despite its utility, docking alone is insufficient to determine actual biological activity, as it does not consider pharmacokinetics, metabolic stability, and in vivo efficacy. Therefore, integrating docking studies with in vitro and in vivo experiments is critical for validating computational predictions (Abdulredha et al., 2023).[4]

This review aims to assess the accuracy of molecular docking by comparing in silico binding affinities with in vitro cytotoxicity data. Using erlotinib as a reference EGFR inhibitor, we analyze the docking performance and structure-activity relationships (SAR) of novel 1,3-diazetidin-2-one derivatives. We investigate why

compounds with higher docking scores exhibit superior in vitro activity and discuss key pharmacophoric features that contribute to enhanced EGFR inhibition. Additionally, we highlight the limitations of docking methodologies and propose strategies to improve predictive accuracy for drug development.

MATERIALS AND METHODS

Molecular Docking Studies Molecular docking was performed to evaluate the binding affinities of novel 1,3-diazetidin-2-one derivatives against EGFR, following the methodology previously described by Abdulredha et al. (2023). The docking studies were conducted using the Genetic Optimization for Ligand Docking (GOLD) software[5]. The crystal structure of EGFR was obtained from the Protein Data Bank (PDB), and ligand structures were optimized using Chem3D software.[6] The docking simulations were performed using flexible ligand docking, and scoring functions such as PLP Fitness were applied to assess binding affinities. Key molecular interactions, including hydrogen bonding and hydrophobic contacts, were analyzed.[7] In Vitro Cytotoxicity Assays To validate docking predictions, in vitro cytotoxicity assays were conducted on the A549 lung cancer cell line, as previously reported in Abdulredha et al. (2023). The compounds were tested at varying concentrations, and their half-maximal inhibitory concentration (IC50) values were determined using the MTT assay. Erlotinib was used as a reference inhibitor for comparison. The results were analyzed to assess the correlation between docking scores and experimental anticancer activity.[8] Correlation Analysis The docking scores and in vitro IC50 values were statistically compared to evaluate the predictive accuracy of molecular docking. Pearson correlation coefficients were calculated to determine the relationship between binding affinities and cytotoxic activity[9]. Additionally, structure-activity relationship (SAR) analyses were performed to identify key molecular features contributing to enhanced EGFR inhibition.[10]Structure-Activity Relationship (SAR) Analysis Erlotinib's Pharmacophore and Binding Interactions Erlotinib, a clinically effective EGFR inhibitor, contains several key structural elements that contribute to its high binding affinity and specificity for EGFR. The quinazoline core serves as the central scaffold, which engages with the ATP-binding site of EGFR through hydrogen bonds with critical residues, including Met769 and Glu738. The aromaticity of the quinazoline ring provides additional stability to the ligand-receptor complex, enhancing the binding efficiency.[11]The anilino group attached to the quinazoline ring is crucial for hydrophobic interactions with hydrophobic residues such as Val702, Leu764, and Ala719 in EGFR, significantly contributing to both the potency and specificity of erlotinib. This interaction helps distinguish EGFR from other kinases, thereby improving selective inhibition.[12]A hydrophobic tail, characterized by the bulky ethynyl group (–C≡C–), extends from the quinazoline core, contributing to further hydrophobic interactions with residues like Leu694 and Ile759 in the receptor[13]. Although these interactions stabilize the ligand-receptor complex, the steric bulk of the ethynyl group can limit binding efficiency, as observed in some variants of erlotinib.[14]The 3-ethyl aniline group in erlotinib enhances binding affinity and specificity by establishing additional hydrophobic interactions with Leu820 and Met769. This modification not only improves binding but also aids in reducing off-target interactions, thereby increasing the selectivity of erlotinib for EGFR.[15] The combination of these structural features results in the high potency of erlotinib as an EGFR inhibitor, making it a valuable therapeutic agent for treating non-small cell lung cancer (NSCLC).[16]Comparison with 1,3-Diazetidin-2-One DerivativesThe novel 1,3-diazetidin-2-one derivatives, which are the subject of this study, introduce a four-membered diazetidin-2-one ring structure that shows promise in interacting with key EGFR residues. This core structure enhances binding potential by facilitating additional interactions with crucial residues such as Lys721, Leu820, and Asp831 in the EGFR ATP-binding pocket. Similar to erlotinib, these derivatives could form hydrogen bonds and hydrophobic contacts, which are crucial for their EGFR inhibitory activity.[17]

Docking studies have suggested that certain derivatives, such as M4c and M4e, exhibit stronger binding affinities due to improved hydrogen bonding and hydrophobic interactions. Specifically, the introduction of functional groups that mimic the hydrophobic tail of erlotinib, along with modifications to the diazetidin ring, further optimizes the binding profile of these derivatives[18]. These improvements correlate with the observed IC50 values, which are comparable to or even exceed the potency of erlotinib in in vitro assays, supporting the hypothesis that structural modifications can enhance both potency and specificity.[19]

Limitations of Docking Accuracy While docking studies provided valuable insights into the relative binding affinities of the diazetidin-2-one derivatives, they also highlighted several limitations.[17] Notably, docking does not account for key pharmacokinetic properties such as solubility, bioavailability, and metabolic stability. For instance, derivatives like M4c and M4e, despite exhibiting strong binding to EGFR, showed lower bioavailability scores due to excessive lipophilicity. Additionally, the rapid metabolism of highly hydrophobic compounds could reduce in vivo efficacy.[20]

Moreover, docking does not consider important aspects of drug absorption, distribution, metabolism, and excretion (ADME), such as drug permeability, plasma protein binding, or efflux mechanisms (e.g., P-glycoprotein). These factors must be taken into account in the development of EGFR inhibitors to ensure their clinical success. [21&22] Conclusion and Future Directions Molecular docking has proven to be a valuable tool for predicting ligand-receptor interactions, but its limitations must be acknowledged. The results of this study confirm that compounds with higher docking scores generally exhibit stronger in vitro efficacy, supporting the utility of docking in early-stage drug design. However, modifications to enhance solubility, bioavailability, and metabolic stability are essential to improve clinical outcomes. Future research should focus on integrating docking studies with molecular dynamics simulations and ADMET profiling to refine predictions and optimize lead compounds for clinical development.

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