In silico Molecular Docking Analysis of Afatinib Targeting the Marker Proteins of Lung Cancer

Sheeja Rekha A G[1], *Manoj Dilip Patil[2], Lal Prasanth[3], Subash Chandran M P[4], K. Anitha[5], Ajay Kumar T, V[6].

[1]Associate Professor, The Dale view College of Pharmacy & Research Centre, Punalal, Kerala
[2]Principal, Shinde Institute of Pharmacy and Research, Jalgaon, Maharashtra
[3]Principal, DM WIMS College of Pharmacy, Wayanad, Kerala
[4]Professor, Immanuel Arasar College of Pharmacy, Nattalam, Tamilnadu
[5]Associate Professor, A. P. A. College for Women, Palani, Tamilnadu
[6]Senior Research Associate, Accent Pharma, Puducherry

***Corresponding Author:**

Manoj Dilip Patil, Principal, Shinde Institute of Pharmacy and Research, Jalgaon, Maharashtra

Abstract

Cancer encompasses a group of diseases marked by the uncontrolled proliferation of cells and their tendency to infiltrate or metastasize to various organs. This progression stems from genetic mutations that interfere with normal cellular regulations. Among the types, Lung cancer ranks as a leading global cause of cancer mortality, claiming more lives than breast, prostate and colorectal cancers combined. Tobacco use remains the most significant risk factor. Common treatments include chemotherapy (cisplatin, carboplatin, and etoposide), targeted therapies (afatinib, erlotinib, and osimertinib) and immunotherapies (pembrolizumab and nivolumab). This investigation focuses on the binding specificity of Afatinib against critical molecular drivers of lungs cancer namely Anaplastic Lymphoma Kinase (ALK), Epidermal growth factor receptor (EGFR), Kirsten rat sarcoma viral oncogene (KRAS), Protein Phosphatase 2A (PP2A), ROS proto-oncogene 1 (ROS1) and Vascular Endothelial Growth Factor (VEGF). Using Schrodinger's molecular docking tools and computational simulations, the study assessed Afatinib's interaction potential and pharmacokinetic characteristics. Findings indicate Afatinib exhibits stronger affinity for EGFR and ALK, suggesting its therapeutic promise could be optimized based on target expression within the tumor microenvironment.

Keywords: Cancer; lung cancer; molecular docking; Schrodinger; In Silico; Afatinib; binding affinities; ADME.

Introduction

Cancer arises from disrupted cellular signals and metabolism, leading to unchecked cell growth and survival. Various biological and environmental triggers contribute to its onset and progression [1]. These illnesses represent over a hundred genetically varied disorders that share similar molecular and metabolic traits [2 & 3]. The influence of tissue microenvironment and inflammation on tumor growth and persistence is widely acknowledged [4 & 5]. Despite ongoing research, the precise triggers of cancer remain unclear. Numerous genetic mutations have been linked to the transformation of healthy cells into tumor forming cancer cells [6 & 7]. Overtime, many scientific and technological strategies have been used to study and combat these complex diseases. Oncological research has drawn from fields like genetics, molecular biology, immunology, proteomics, and computational biology [8]. Treating cancer with a single drug is challenging due to the wide diversity in cancer types and patient-specific variations [9, 10, and 11].

Lung cancer therapies target key proteins such as VEGF, EGFR, KRAS, ALK, ROS1, BRAF, RET, MET, NTRK, HER2, HER3, and pathways like PI3K/AKT/mTORC, as well as immune check points like PD1 and CTLA-4 [12]. Lung cancer treatment involves various drug categories:

Chemotherapy

Used for both NSCLC and SCLC to target rapidly dividing cells:

- Cisplatin, Carboplatin, Paclitaxel, Docetaxel
- Gemcitabine, Pemetrexed, Vinorelbine
- Etoposide, Topotecan and Doxorubicin

Targeted Therapy:

Focus on specific genetic mutations (mainly in NSCLC):

- EGFR inhibitors: Erlotinib, Gefitinib, Osimertinib
- ALK inhibitors: Crizotinib, Alectinib, Brigatinib, Ceritinib, Lorlatinib
- Others: Bevacizumab, Ramucirumab, Selpercatinib, Entrectinib, Capmatinib and Sotorasib

Immunotherapy

Enhances immune response against cancer cells:

- PD-1/PD-L1 inhibitors: Nivolumab, Pembrolizumab, Atezolizumab, Durvalumab
- CTLA-4 inhibitors: Ipilimumab and Tremelimumab

Other Notable Agents

Advances or specified treatments:

- Lurbinectedin (SCLC), Amivantamab (NSCLC)
- Fam-trastuzumab deruxtecan for NSCLC [13 and 14].

Researchers worldwide are working to develop effective lung cancer therapies using diverse methods. Molecular docking, a key technique in drug discovery, helps evaluate how well a drug binds to its target. In this study, *in silico* analysis was used to assess Afatinib's specificity towards the target proteins Anaplastic Lymphoma Kinase (ALK) [15], Epidermal growth factor receptor (EGFR) [16], Kirsten rat sarcoma viral oncogene (KRAS) [17], Protein Phosphatase 2A (PP2A) [18], ROS proto-oncogene 1 (ROS1) [19] and Vascular Endothelial Growth Factor (VEGF) [20].

ALK, a receptor tyrosine kinase from the insulin receptor superfamily, shares structural features with LTK and is encoded by a gene on chromosome 2p23. It matures into a 200-220 kDa protein with three main components: an extracellular ligand-binding domain, a short transmembrane segment, and an intracellular tyrosine kinase domain involved in cellular signaling [21]. EGFR, or erbB1, is a receptor tyrosine kinase within the erbB family that shares structural domains with HER2, erB3, and erB4 but differs in its kinase activity. Upon ligand binding, EGFR undergoes conformational changes and phosphorylation, triggering signaling cascades such as MAPK, PI3/Akt, and STAT that regulates cell proliferation and survival by suppressing apoptosis [16].

KRAS, a key member of the RAS GTPase family, acts as a molecular switch that cyles between inactive and active states to regulate cellular signaling, impacting processes such as cell growth, differentiation, and apoptosis. Structurally, it contains domains that anchor it to membranes and enable interaction with downstream effectors like RAF1 and PI3K, playing a pivotal role in cancer-related pathways including MAPK and PI3K-AKT [22]. PP2A is a Serine/Threonine phosphatase composed of catalytic, structural, and regulatory subunits that overseas major cellular signaling processes, including apoptosis, proliferation, and DNA repair. Its dysregulation plays a key role in cancer progression, making both its reactivation and inhibition

valuable strategies in therapeutic development alongside kinase inhibitors and conventional treatments [18].

ROS-1 encodes a transmembrane with tyrosine kinase activity and structural similarity to insulin receptors and ALK, playing a role in epithelial differentiation during embryonic development. Its oncogenic potential was uncovered through a fusion with the FIG gene, resulting in abnormal activation of signaling pathways and tumorigenesis, making it a targetable driver in certain [19].VEGF) is a critical driver of tumor angiogenesis, comprising several isoforms (VEGFA –E and placental growth factor) that stimulate new blood vessel formation and vascular permeability. Discovered and characterized between 1939 and the early 1990s, VEGF is now recognized for its central role in promoting tumor growth, metastasis, and malignant cell survival by enhancing oxygen and nutrient delivery through activated endothelial cells [23].

Afatinib is an irreversible ErbB family tyrosine kinases inhibitor approved for treating advanced NSCLC with EGFR mutations and as a second-line therapy for squamous cell NSCLC. It is also under investigation for HER2-positive breast cancer cases resistant to trastuzumab, though not yet FDA approved for that use. Alongside gefitinib and erlotinib, afatinib is one of the three TKIs used for advanced NSCLC treatment. While it shows comparable efficacy to erlotinib in EGFR mutant cases, afatinib demonstrates better outcomes in advanced squamous cell carcinoma [24].

Molecular docking is a vital technique in drug design that predicts how a ligand interacts with a protein’s 3D structure to identify potential binding modes. It enables virtual screening and ranking of compounds from chemical libraries, helping generate structural insights that guide lead optimization of a protein [25]. Docking techniques also support in vitro assays by predicting key properties like lipophilicity, solubility, and plasma stability during a ligand’s absorption, distribution, metabolism and excretion (ADME) process. These insights help assess a compound’s pharmacological profile before its progresses to in vivo studies and clinical trials. [26]. Computational protocol helps forecast potential ADME characteristics and toxicity concerns, significantly reducing the need for extensive animal based experiments. By refining early stage drug profiling, they support ethical research while streamlining candidate selection for further testing [27].

This study involves an in silico molecular docking assessment of Afatinib targeting lung cancer related proteins including ALK, EGFR, KRAS, PP2A, ROS1 and VEGF using Schrödinger software. The selected protein structures, retrieved from protein data bank, include PDBIDs such as 2XP2, 3AOX (ALK), 1M17, 4WKQ (EGFR), 6OIM (KRAS), 2IE4 (PP2A), 3ZBF (ROS1) and 3B8R (VEGF). Afatinib's drug-likeness and ADME properties were evaluated based on Lipinski's rule of five to support pharmacokinetic profiling.

Materials and Methods

Protein Preparation

The 3D crystal structures of key lung cancer related proteins were retrieved for docking studies, including ALK (PDB IDs: 2XP2 and 3AOX), EGFR (1M17 and 4WKQ), KRAS (6OIM), PP2A (2IE4), ROS1 (3ZBF) and VEGF (3B8R). The three-dimensional (3D) crystal structures of the following proteins were retrieved from the protein data bank (PDB) (https://www.rcsb.org).

- AKT (PDB IDS): 2XP2 – 1.90 Å; 3OAX – 1.75 Å) [28, 29]
- EGFR (1M17 – 2.60 Å; 4WKQ – 1.85 Å) [30, 31]
- KRAS (6OIM – 1.65 Å) [32]
- PP2A (2IE4 – 2.60 Å) [33]
- ROS1 (3ZBF – 2.20 Å) [34]
- VEGF (3B8R – 2.70 Å) [35]

These high resolution protein structures were imported into the "Protein preparation wizard" for preprocessing. Appropriate modeling calculations were performed using Schrodinger software to optimize the protein geometry and ensure suitability for further molecular studies [36].

The protein structures obtained from the protein data bank included heavy atoms, metal ions, missing hydrogen atoms, water molecules, co-crystallized ligands, and incomplete or terminal amide groups. Using the protein Preparation Wizard, corrections were applied to bond orders and formal charges, missing protons were added, metals were appropriately treated, and water molecules beyond 5Å from hetero atoms were removed. Ionization states of the ligand were generated using Epik [37]. Finally, the protein structures were subjected to controlled minimization using the OPLS-2005 force-filed, maintaining a root mean square deviation (RMSD) tolerance of 0.3 Å [38].

Receptor Grid Generation

The ligands present in all proteins structures were retained, and grids were generated using the receptor grid generation module of the Schrodinger software suite. Grid placement was centered on the ligand bound within the active site of the protein. The resulting cubical centroid formation delineates the active site region, facilitating accurate docking simulations [39, 40].

Ligand Preparation

The molecular structure and formula of Afatinib ($C_{24}H_{25}ClFN_5O_3$) was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/compound/Afatinib) and saved in .SDF format (Fig.1). The ligand was then imported into the LigPrep module of Schrodinger maestro 14.2, where its 2D structure was converted into a low energy 3D conformation [39]. Multiple ligand variants were generated, encompassing possible ionization states, tautomers, stereo-isomers, and ring conformations. Geometry optimization and energy minimization were performed to refine the structures. Using the Epik module, ionization and tautomeric states were predicted within a physiological pH range of 6.8 to 7.2. Final minimization was carried out using the OPLS- 2005 force-filed, achieving a root mean square deviation (RMSD) of 1.8 Å [41].

Molecular Docking

Molecular docking studies were carried out using the Glide module of Schrodinger, utilizing the previously generated receptor grid and prepared ligand structures. Ligand binding interactions were assessed and ranked using the Ligand docking program within Glide. Docking calculations employed the Extra Precision (XP) mode along with the OPLS- 2005 force-field to ensure reliable conformational sampling and scoring.

A flexible docking protocol was applied to explore multiple ligand-receptor interaction modes. The algorithm incorporated a series of hierarchical filters to identify favorable interactions such as hydrophobic contacts, hydrogen bonding and metal coordination while penalizing steric clashes and unfavorable binding geometries. In the final phase, the docked poses underwent energy minimization using OPLS-2005, and the optimized conformations were re-ranked using the Glide Scoring function [42].

Lipinski Rule and the Analysis of ADME Properties

In silico analysis plays a crucial role in the early preclinical evaluation of new chemical entities, helping to minimize late-stage failures in the drug discovery process. By streamlining development timelines and reducing resource expenditure, it offers a more efficient pathway for molecule assessment. Notably, nearly 40% of drug candidates fail due to poor adsorption, distribution, metabolism and excretion (ADME) characteristics. To counter this, high-throughput screening (HTS) techniques are employed to predict ADME profiles and eliminate unsuitable candidates early on. Remarkably, this approach also enables structural optimization of failed compounds, enhancing their drug-like properties to meet ADME criteria more effectively [43].

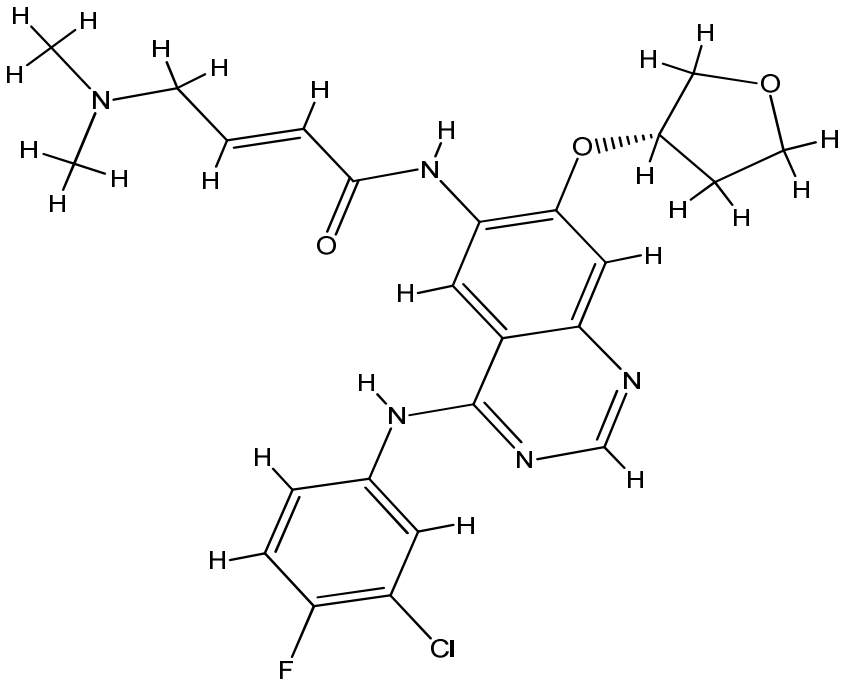

Figure 1: The structure of Afatinib

Bioavailability of a molecule is more effectively assesses using Lipinski's rule of five, a widely accepted filter in drug discovery. According to this rule, a compound is considered to have favorable drug-like properties if it meets the following criteria:

- Molecular weight < 500 daltons
- Hydrogen bond donors ≤ 5
- Hydrogen bond acceptors ≤ 10

- Octanol-water partition coefficient (log P) ≤ 5 [44]

These guidelines serve as a foundational checkpoint for predicting oral bioavailability and ensuring molecular suitability for further development. Additionally, pharmacokinetic parameters – absorption, distribution, metabolism, and excretion (ADME) – were computed using the QikProp tool (Schrodinger 2024.4), offering high-throughput in silico insights into a compound's drug-likeness [45, 46].

Results and Discussion

Molecular docking studies were carried out to investigate the interaction of the ligand Afatinib with the active sites of several lung cancer-related target proteins using Schrodinger maestro version 14.2, an advanced computational docking tool. Was initially processed and optimized using the LigPrep module, ensuring structural accuracy and readiness for docking analysis. The target proteins selected for the study included ALK, EGFR, KRAS, PP2A, ROS1 and VEGF, all of which ae implicated in lung cancer pathogenesis. These proteins were meticulously prepared through the protein preparation wizard tool to ensure proper geometry, protonation states, and minimized energy structures suitable for molecular docking.

To define the binding regions, a cube shaped grid was generated using the receptor grid generation module. This grid was strategically positioned to encompass the active site of each target protein, enabling precise docking simulations with Afatinib. The docking analysis assessed the binding affinity of Afatinib to each protein target. Based on the docking scores and interaction profiles, the protein were ranked to determine which targets exhibited the strongest affinity towards Afatinib. These results contribute valuable insights into Afatinib's potential mechanisms and efficacy in targeting multiple molecular pathways involved in lung cancer.

Molecular docking simulations were conducted for all six target proteins implicated in lung cancer such as ALK, EGFR, KRAS, PP2A, ROS1, and VEGF using the GLIDE module in Schrodinger. The ligand Afatinib was docked against each protein to evaluate in binding potential and interaction profile. Key parameters such as docking score, Glide evdw (Van Der Waals energy), ecoul (Coulomb energy), and Glide energy were analyzed in detail. Additionally, the nature of molecular interactions including Hydrogen bonds and π- π stacking interactions between Afatinib and the active site residues of each protein was meticulously examined.

The results revealed that Afatinib successfully docked into the binding sites of all six proteins, exhibiting favorable binding conformations and interaction energies. These findings suggest a strong affinity between Afatinib and the target proteins, highlighting its potential efficacy in disrupting multiple oncogenic pathways. For clarity, results corresponding to ALK, EGFR, KRAS, PP2A, ROS1 and VEGF are designated as A, B, C, D, E and F respectively (see Table 1).

The molecular docking study revealed that Afatinib displayed favorable interactions with all six lung cancer-associated target proteins. The binding affinities, as determined by docking scores, followed the order: EGFR > ALK > VEGF > ROS1 > KRAS > PP2A. These results suggest that Afatinib has the strongest binding interaction with EGFR, followed by ALK, indicating their potential as primary molecular targets. The docking scores of the EGFR protein are -10.23 (PDB ID: 1M17) & -11.14 (PDB ID: 4WKQ), ALK protein are -9.55 (PDB ID: 2XP2), & -7.63 (PDB ID: 3AOX), VEGF protein is -8.62 (3B8R), ROS1 protein is -8.62 (PDB ID: 3ZBF), KRAS protein is -7.01 (PDB ID: 6OIM) and PP2A protein is -4.77 (PDB ID: 2IE4) respectively.

In molecular docking analysis, a lower docking score typically corresponds to a stronger binding affinity between the ligand and receptor. Based on the principle, Afatinib demonstrates the most potent interaction with EGFR (particularly with PDB ID: 4WKQ), followed closely by ALK, while PP2A exhibited the weakest affinity. These insights highlight Afatinib's potential in targeting EGFR and ALK-driven oncogenic pathways in lung cancer.

A detailed molecular docking study was carried out to examine the energetic interactions between the ligand Afatinib and six lung cancer-associated target proteins ALK, EGFR, KRAS, PP2A, ROS1 and VEGF using the glide module of Schrodinger. The van der Waals (Glide evdw) values indicated significant hydrophobic interactions between Afatinib and the binding pockets of the target proteins. Among them EGFR (PDB ID: 4WKQ) exhibited the lowest evdw value at -51.15, suggesting a strong van der Waals interaction, followed by VEGF (-49.01) and EGFR (-48.40, PDB ID: 1M17). ALK showed moderate values (-41.89, -42.19), while KRAS (-34.82) and PP2A (-32.33) had the least evdw interaction energies.

The Coulomb energy (Glide ecoul) values provided insights into electrostatic contributions. EGFR proteins presented notable ecoul values (-13.17, -15.70), closely matched by ALK (16.04, -6.41) and ROS1 (-11.88), KRAS (-9.91), PP2A (-9.54), and VEGF (-6.64) exhibited comparatively lower electrostatic interactions. The total Glide energy combined van der Waals

and Coulomb contributions, reflecting the overall binding strength of Afatinib with each target. EGFR proteins displayed the strongest interactions (-61.57, -66.86), followed by ALK (-57.93, -48.60), VEGF (-55.66), ROS1 (-54.46), KRAS (-44.73), and PP2A (-41.87).

Furthermore, Afatinib exhibited notable interactions through hydrogen bonding, π-π stacking, and polar contacts with amino acids at the active sites of all target proteins. These molecular interactions are tabulated in Table 1, highlighting the specific residues involved in stabilizing the ligand-receptor complexes.

Table 1: The Table provides the docking scores, Glide van der Waals energy (Glide evdw), Coulomb energy (Glide ecoul), interacting residues, and the types of interactions between Afatinib and various lung cancer marker proteins. The docking scores were calculated using Glide module of Schrodinger (Version 2024.4). In the table: HB denotes hydrogen bonding; Pi-Pi denotes π-π stacking interaction.

Ligand Name	Target protein	PDB ID	Docking Score	Glide Ecoul	Glide energy	Glide evdw	Interacting residues/type (HB/Pi-Pi)
Afatinib	ALK	2XP2	**- 9.55**	- 16.04	- 57.93	- 41.89	GLU1210, SER1206, LEU1122, ASP1203, & MET1199
		3AOX	- 7.63	- 6.41	- 48.60	- 42.19	MET1199
	EGFR	1M17	**- 10.23**	- 13.17	- 61.57	- 48.40	ASP776, CYS773, MET769, & THR766
		4WKQ	**- 11.14**	- 15.70	- 66.86	- 51.15	LEU792, THR854, ASP800
	KRAS	6OIM	-7.01	-9.91	-44.73	-34.82	GLN61, ARG68 (Pi-cation) TYR96 (pi-pi)
	PP2A	2IE4	- 4.77	- 9.54	- 41.87	- 32.33	GLU192, GLY215, & ARG214
	ROS1	3ZBF	- 8.48	- 11.88	- 54.46	- 42.58	ASP2033 & LEU2028
	VEGF	3B8R	- 8.62	- 6.64	- 55.66	- 49.01	LEU840 & CYS919, LYS868 (pi-cation)

Figures 2 to 5 depict the 3D and 2D molecular interactions of Afatinib with the series of lung cancer marker proteins, based on the docking simulations. These include:

- ALK: Panels A1 (PDB ID: 2XP2) and A2 (PDB ID: 3AOX)
- EGFR: Panels B1 (1M17) and B2 (4WKQ)

- KRAS: Panel C (6OIM)
- PP2A: Panel D (2IE4)
- ROS1: Panel E (3ZBF)
- VEGF Panel F (38BR)

Each figure highlights Afatinib's binding orientation, along with hydrogen bonding, π–π stacking, and π–cation interactions observed within the active sites of these proteins.

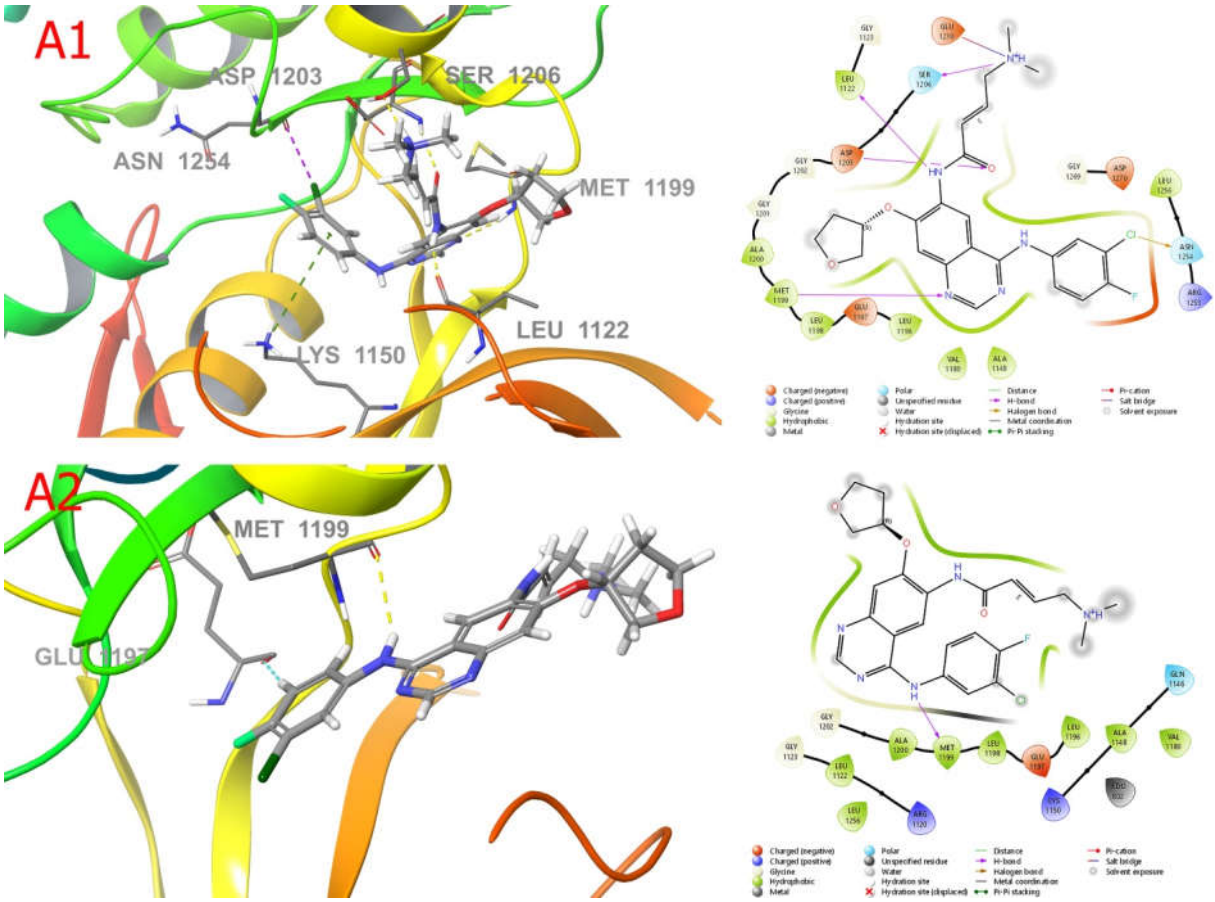

Figure 2: Binding orientations of Afatinib with the crystal structure of ALK (Panels A1 and A2), highlighting key bond interactions with amino acid residues in the active site.

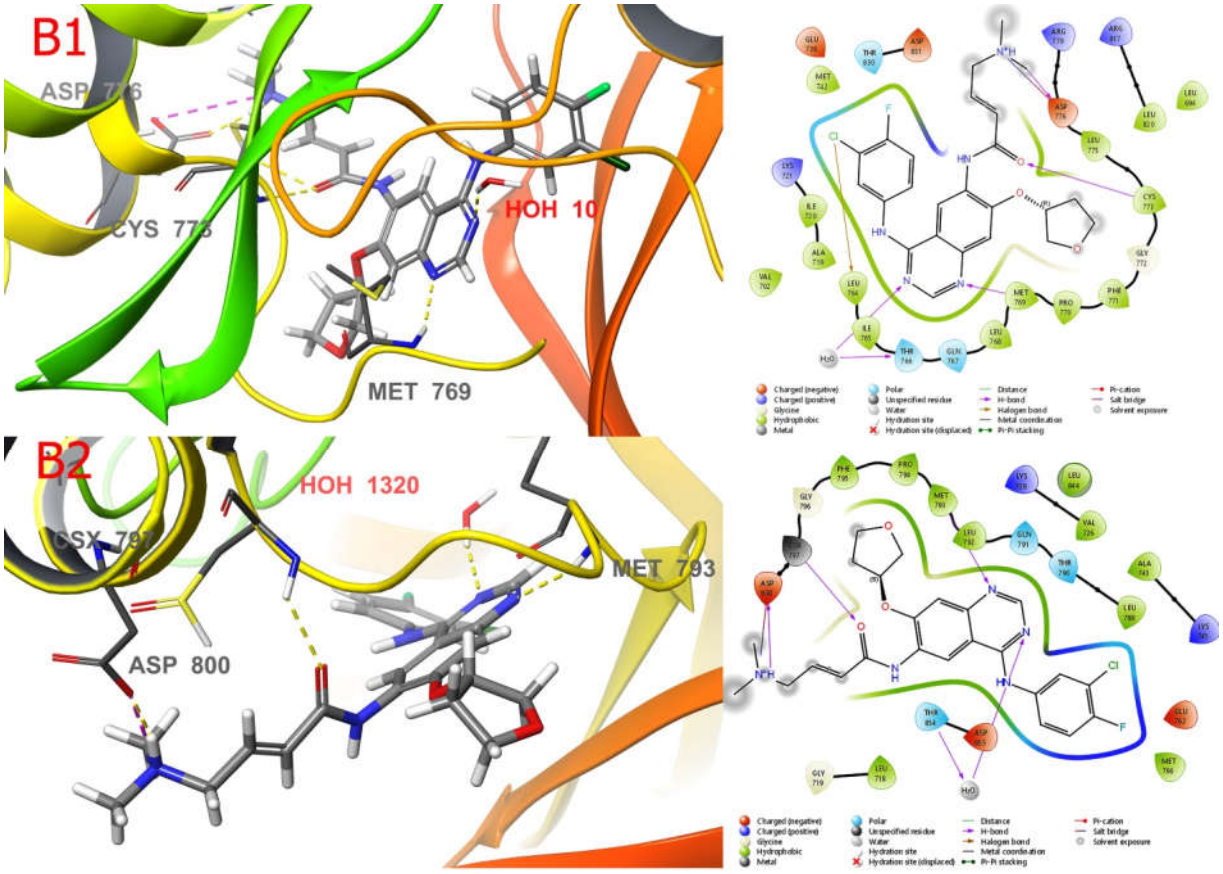

Figure 3: Binding orientations of Afatinib with the crystal structure of EGFR (Panels B1 and B2), highlighting key bond interactions with amino acid residues in the active site.

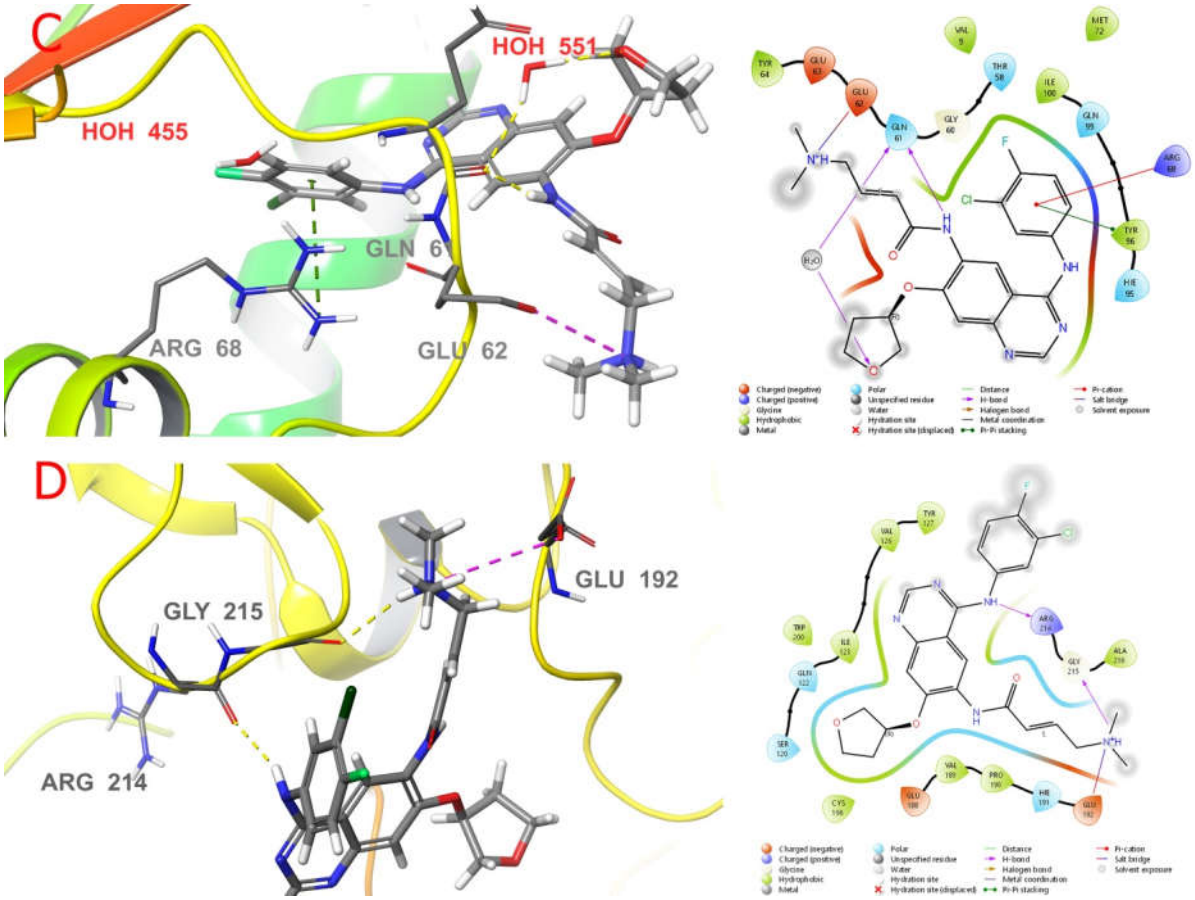

Figure 4: Binding orientations of Afatinib with the crystal structure of KRAS and PP2A (Panels C and D), highlighting key bond interactions with amino acid residues in the active site.

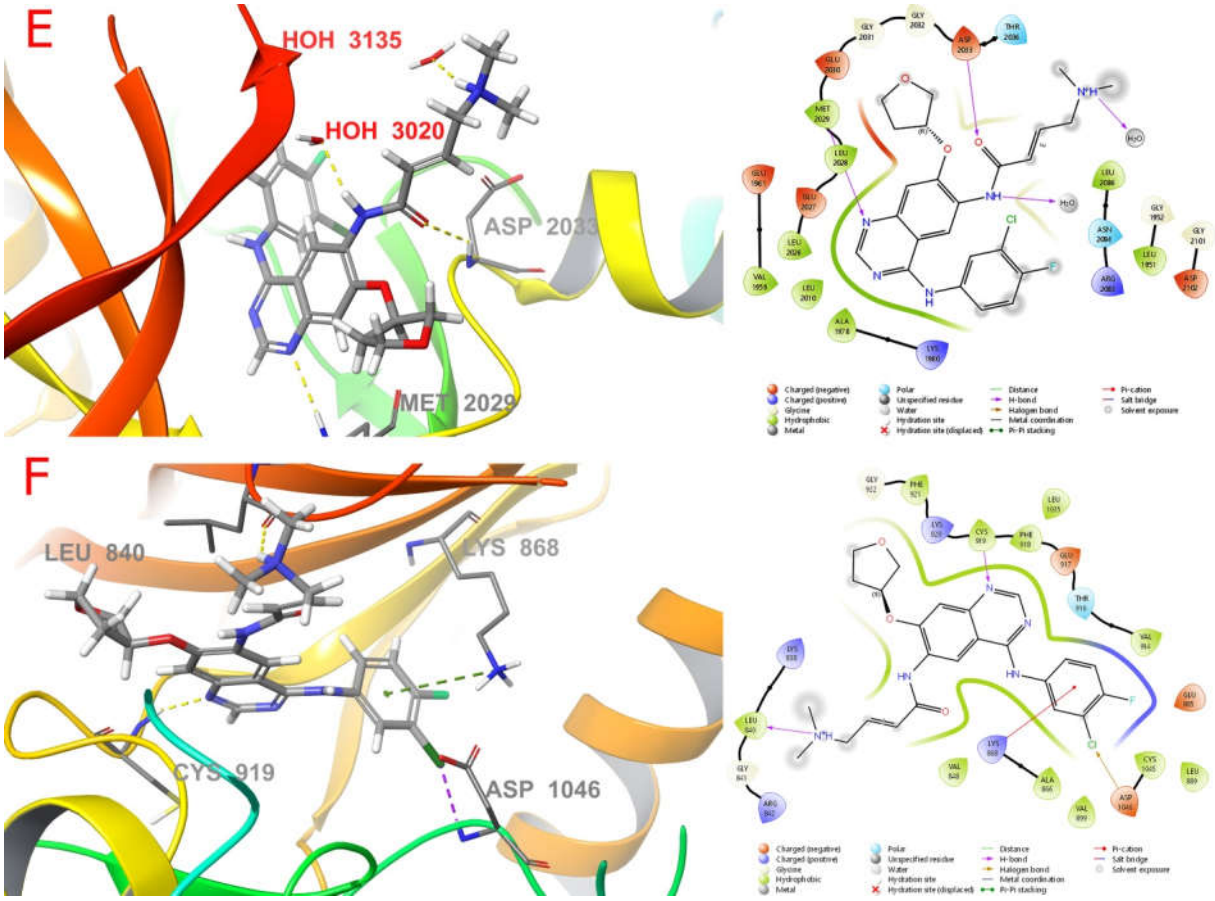

Figure 5: Binding orientations of Afatinib with the crystal structure of ROS1 and VEGF (Panels E and F), highlighting key bond interactions with amino acid residues in the active site.

Validation of the Docking Programme

The reliability of the molecular docking protocol was validated by examining the binding conformations of Afatinib with its respective target proteins, based on the lowest-energy poses generated through the Glide scoring function. The docking scores obtained were consistent with experimental binding profiles established via X-ray crystallography, reinforcing the accuracy of the computational predictions.

Key parameters used for validation included the analysis of hydrogen bonding interactions and the calculations of root mean square deviation (RMSD) between the predicted ligand poses and their experimentally resolved conformations. To further enhance accuracy, Extra Precision (XP) docking mode was employed by intentionally removing the native co-crystallized ligand from

the binding site and re-docking Afatinib, thereby assessing its ability to replicate experimentally observed interactions [33]. The docking scores for each target were subsequently compared. Notably, Afatinib demonstrated strong and consistent binding affinities across all eight lung cancer-associated proteins. The ranking of binding strength, based on Glide score analysis is as follows:

EGFR > ALK > VEGF > ROS1 > KRAS > PP2A

Lipinski Rule and ADME Properties

The ADME (Absorption, Distribution, Metabolism, and Excretion) characteristics of the investigational drug Afatinib were further evaluated through the QikProp module of Schrodinger software, focusing specifically on compliance with Lipinski's Rule of Five – a key set of criteria used to assess drug-likeness and oral bioavailability. The parameters analyzed included:

- Molecular weight (MW)
- Hydrogen bond donor count (HBD)
- Hydrogen bond acceptor count (HBA)
- Predicted octanol/water partition coefficient (QPlogP (O/W)
- Number of Rule of Five violations.

Afatinib's predicted values were MW: 485.945 (<500), HBD: 2 (<5), HBA: 9.45 (<10), QPlogP (O/W): 3.956 (<5) and Rule of Five violations: 0 respectively (Table 2). All measured parameters remained within the recommended thresholds, indicating that Afatinib adheres to Lipinski's criteria and exhibits favorable pharmacokinetic potential.

Table 2: Scores of Afatinib predicted by the QikProp module in Schrodinger software, based on Lipinski Rule of Five. MW denotes molecular weight, HB refers to hydrogen bonding characteristics, and QPLogP (O/W) indicates the predicted octanol/water partition co-efficient logP.

Name of the Drug	**Factors of Lipinski rule of 5**				
	MW (<500)	**HB-Donor (<5)**	**HB-Acceptor (<10)**	**QPlogP (O/W) (<5)**	**Rule of 5 (0)**
Afatinib	485.945	2	9.45	3.956	0

The pharmacokinetic properties of Afatinib were computed using the QikProp module in Schrodinger, with focus on parameters relevant to drug absorption and distribution. These included:

- Aqueous solubility (QPlogS)
- Predicted IC_{50} value for HERG K^+ channel blockade (QplogHERG)
- Brain/blood partition coefficient (QPlogBB)
- Predicted human oral absorption (PHOA)
- Cell permeability via Caco-2 monolayer (QPPCaco)

The corresponding predicted values were: QPlogS: -5.683 (range: -6.5 to 0.5), QplogHERG: -7.352 (< -5indicates potential HERG inhibition), QPlogBB: 0.309 (range: -3.0 to 1.2), PHOA: 95.357 (% absorption; >80 = high, < 25 = poor) and QPPCaco: 337.316 (nm/s; >500 = high permeability, <25 = poor) respectively (Table 3).

Table 3: ADME parameters of Afatinib, calculated using the QikProp module of Schrodinger software. The listed properties include: QPlogS: Aqueous solubility, QplogHERG: Predicted IC_{50} value for blockage of HERG K^+ channels, QPlogBB: Brain/blood partition coefficient, PHOA: Predicted human oral absorption, and QPPCaco: Gut-blood barrier/cell permeability in nm/s

Name of the Drug	**Pharmacokinetic properties**				
	QPlogS (-6.5 to 0.5)	**QplogHERG (< -5)**	**QPlogBB (-3 to 1.2)**	**PHOA (>80 high, < 25 poor)**	**QPPCaco (>500 high, <25 poor)**
Afatinib	-5.683	-7.352	-0.309	95.357	337.316

The pharmacokinetic assessment of Afatinib revealed that all predicted values fell within acceptable parameters, indicating no deviation from established limits. Additionally, the in silico analysis demonstrated strong binding affinity of Afatinib toward the target protein. The compound successfully compiled with Lipinski's Rule of Five and met the criteria for key ADME properties, reinforcing its potential as a drug candidate.

Conclusion

The present study systematically evaluated the molecular interactions and pharmacokinetic properties of Afatinib with selected target proteins namely ALK, EGFR, KRAS, PP2A, ROS1 and VEGF using the Schrodinger Maestro platform. Molecular docking and ADME analyses were conducted utilizing the GLIDE and QikProp modules respectively. The results revealed strong binding affinities of Afatinib to the target proteins, facilitated by hydrogen bonding, polar interactions, and π–π stacking.

The predicted ADME parameters and Lipinski's rule scores were found to be within acceptable limits, underscoring the drug's potential drug-likeness. Notably, Afatinib exhibited low interaction energies with the lung cancer-related proteins, suggesting effective inhibitory activity. Among the six proteins, EGFR and ALK demonstrated the highest binding affinities, followed by VEGF, ROS1, KRAS and PP2A respectively.

These findings highlight Afatinib's promise as a candidate for targeted anticancer therapy. Moreover, the data support the strategic use of Afatinib based on the expression levels of these target proteins within the tumor microenvironment, potentially aiding in personalized treatment approaches.

Disclaimer

The products utilized in this research are widely recognized and commonly used within our scientific community and country. The authors declare no conflict of interest with the manufacturers or suppliers of these products. This study is intended solely for academic and research purposes, aimed at advancing scientific knowledge. No part of this work is intended for litigation or commercial promotion. Furthermore, the research was entirely self-funded by the authors and received no financial support from any commercial entity.

Competing Interests

Authors have declared that no competing interests exist.

References

1. Upadhyay A. Cancer: An unknown territory; rethinking before going ahead. Genes Dis. 2020 Sep 18; 8(5):655-661. doi: 10.1016/j.gendis.2020.09.002. PMID: 34291136; PMCID: PMC8278524.
2. Vander Heiden MG, DeBerardinis RJ. Understanding the Intersections between Metabolism and Cancer Biology. Cell. 2017 Feb 9;168(4):657-669. doi: 10.1016/j.cell.2016.12.039. PMID: 28187287; PMCID: PMC5329766.
3. Lambert AW, Pattabiraman DR, Weinberg RA. Emerging Biological Principles of Metastasis. Cell. 2017 Feb 9;168(4):670-691. doi: 10.1016/j.cell.2016.11.037. PMID: 28187288; PMCID: PMC5308465.
4. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013 Nov;19(11):1423-37. doi: 10.1038/nm.3394. PMID: 24202395; PMCID: PMC3954707.
5. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008 Jul 24;454(7203):436-44. doi: 10.1038/nature07205. PMID: 18650914.
6. Ujvari B, Klaassen M, Raven N, Russell T, Vittecoq M, Hamede R, Thomas F, Madsen T. Genetic diversity, inbreeding and cancer. Proc Biol Sci. 2018 Mar 28;285(1875):20172589. doi: 10.1098/rspb.2017.2589. PMID: 29563261; PMCID: PMC5897632.
7. Mroz EA, Rocco JW. The challenges of tumor genetic diversity. Cancer. 2017 May 15;123(6):917-927. doi: 10.1002/cncr.30430. Epub 2016 Nov 8. PMID: 27861749; PMCID: PMC5370554.
8. Bishayee A, Block K. A broad-spectrum integrative design for cancer prevention and therapy: The challenge ahead. Semin Cancer Biol. 2015 Dec;35 Suppl:S1-S4. doi: 10.1016/j.semcancer.2015.08.002. Epub 2015 Aug 7. PMID: 26260004.
9. GCC Lim. Clinical oncology in Malaysia: 1914 to present. Biomedical Imaging and Intervention Journal. 2006;2(1):e18. DOI: 10.2349/biij.2.1.e18.
10. GCC Lim. Overview of cancer in Malaysia. Japan Journal of Clinical Oncology. 2002;32:37-42. DOI: https://doi.org/10.1093/jjco/hye132.
11. Balachandran P, Ajay Kumar TV, Parthasarathy V. Screening of potential anticancer compounds from Sargassum wightii to target breast cancer specific HER2 receptor using in-silico analysis. The Natural Products Journal. 2016;6(2):108- 115. DOI:10.2174/2210315506666160218224112.
12. Araghi M, Mannani R, Heidarnejad Maleki A, Hamidi A, Rostami S, Safa SH, Faramarzi F, Khorasani S, Alimohammadi M, Tahmasebi S, Akhavan-Sigari R. Recent advances in non-small cell lung cancer targeted therapy; an update review. Cancer Cell Int. 2023 Aug 11;23(1):162. doi: 10.1186/s12935-023-02990-y. PMID: 37568193; PMCID: PMC10416536.

13. Bao R, Chan P. Novel compounds in the treatment of lung cancer: current and developing therapeutic agents. J Exp Pharmacol. 2011 Mar 16;3:21-34. doi: 10.2147/JEP.S7804. PMID: 27186107; PMCID: PMC4863377.
14. National cancer institute, About cancer, cancer treatment, cancer drugs, https://www.cancer.gov/about-cancer/treatment/drugs/lung.
15. Gupta R, Amanam I, Rahmanuddin S, Mambetsariev I, Wang Y, Huang C, Reckamp K, Vora L, Salgia R. Anaplastic Lymphoma Kinase (ALK)-positive Tumors: Clinical, Radiographic and Molecular Profiles, and Uncommon Sites of Metastases in Patients With Lung Adenocarcinoma. Am J Clin Oncol. 2019 Apr;42(4):337-344. doi: 10.1097/COC.0000000000000508. PMID: 30741758; PMCID: PMC7597761.
16. Bethune G, Bethune D, Ridgway N, Xu Z. Epidermal growth factor receptor (EGFR) in lung cancer: an overview and update. J Thorac Dis. 2010 Mar;2(1):48-51. PMID: 22263017; PMCID: PMC3256436.
17. Li JX, Li RZ, Ma LR, Wang P, Xu DH, Huang J, Li LQ, Tang L, Xie Y, Leung EL, Yan PY. Targeting Mutant Kirsten Rat Sarcoma Viral Oncogene Homolog in Non-Small Cell Lung Cancer: Current Difficulties, Integrative Treatments and Future Perspectives. Front Pharmacol. 2022 Apr 20;13:875330. doi: 10.3389/fphar.2022.875330. PMID: 35517800; PMCID: PMC9065471.
18. Mirzapoiazova T, Xiao G, Mambetsariev B, Nasser MW, Miaou E, Singhal SS, Srivastava S, Mambetsariev I, Nelson MS, Nam A, Behal A, Arvanitis LD, Atri P, Muschen M, Tissot FLH, Miser J, Kovach JS, Sattler M, Batra SK, Kulkarni P, Salgia R. Protein Phosphatase 2A as a Therapeutic Target in Small Cell Lung Cancer. Mol Cancer Ther. 2021 Oct;20(10):1820-1835. doi: 10.1158/1535-7163.MCT-21-0013. Epub 2021 Jul 12. Erratum in: Mol Cancer Ther. 2022 Apr 1;21(4):700. doi: 10.1158/1535-7163.MCT-22-0104. PMID: 34253596; PMCID: PMC8722383.
19. Gendarme S, Bylicki O, Chouaid C, Guisier F. *ROS-1* Fusions in Non-Small-Cell Lung Cancer: Evidence to Date. Curr Oncol. 2022 Jan 28;29(2):641-658. doi: 10.3390/curroncol29020057. PMID: 35200557; PMCID: PMC8870726.
20. Frezzetti D, Gallo M, Maiello MR, D'Alessio A, Esposito C, Chicchinelli N, Normanno N, De Luca A. VEGF as a potential target in lung cancer. Expert Opin Ther Targets. 2017 Oct;21(10):959-966. doi: 10.1080/14728222.2017.1371137. Epub 2017 Aug 30. PMID: 28831824.
21. Della Corte, C.M., Viscardi, G., Di Liello, R. *et al.* Role and targeting of anaplastic lymphoma kinase in cancer. *Mol Cancer* **17**, 30 (2018). https://doi.org/10.1186/s12943-018-0776-2
22. O'Sullivan É, Keogh A, Henderson B, Finn SP, Gray SG, Gately K. Treatment Strategies for KRAS-Mutated Non-Small-Cell Lung Cancer. Cancers (Basel). 2023 Mar 7;15(6):1635. doi: 10.3390/cancers15061635. PMID: 36980522; PMCID: PMC10046549.

23. Chen Y, Mathy NW, Lu H. The role of VEGF in the diagnosis and treatment of malignant pleural effusion in patients with non-small cell lung cancer (Review). Mol Med Rep. 2018 Jun;17(6):8019-8030. doi: 10.3892/mmr.2018.8922. Epub 2018 Apr 23. PMID: 29693703; PMCID: PMC5983970.
24. Moosavi L, Preuss CV, Polineni R. Afatinib. 2024 Feb 28. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–. PMID: 31194405.
25. Morris GM, Lim-Wilby M. Molecular docking. Methods in Molecular Biology. 2008;443:365-82. DOI: 10.1007/978-1-59745-177-2_19.
26. Arne K, Vinicius GM, Carsten W, Thales K. ADME profiling in drug discovery and a new path paved on silica, drug discovery and development - new advances. Intechopen. 2019:1-31. DOI: 10.5772/intechopen.86174.
27. Fernando DPM, Edgar LL, Euridice JMK, Jose LMF. Chapter 2 - Computational drug design methods-Current and future perspectives. In silico drug design repurposing techniques and methodologies. 2019:19-44. DOI: https://doi.org/10.1016/b978-0-12- 816125-8.00002-x.
28. Cui JJ, Tran-Dubé M, Shen H, Nambu M, Kung PP, Pairish M, Jia L, Meng J, Funk L, Botrous I, McTigue M, Grodsky N, Ryan K, Padrique E, Alton G, Timofeevski S, Yamazaki S, Li Q, Zou H, Christensen J, Mroczkowski B, Bender S, Kania RS, Edwards MP. Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK). J Med Chem. 2011 Sep 22;54(18):6342-63. doi: 10.1021/jm2007613.
29. Sakamoto H, Tsukaguchi T, Hiroshima S, Kodama T, Kobayashi T, Fukami TA, Oikawa N, Tsukuda T, Ishii N, Aoki Y. CH5424802, a selective ALK inhibitor capable of blocking the resistant gatekeeper mutant. Cancer Cell. 2011 May 17;19(5):679-90. doi: 10.1016/j.ccr.2011.04.004. PMID: 21575866.
30. Stamos J, Sliwkowski MX, Eigenbrot C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. J Biol Chem. 2002 Nov 29;277(48):46265-72. doi: 10.1074/jbc.M207135200. Epub 2002 Aug 23. PMID: 12196540.
31. Yosaatmadja, Y., Squire, C.J., McKeage, M., Flanagan, J.U, 1.85 angstrom structure of EGFR kinase domain with gefitinib, to be published.
32. Canon J, Rex K, Saiki AY, Mohr C, Cooke K, Bagal D, Gaida K, Holt T, Knutson CG, Koppada N, Lanman BA, Werner J, Rapaport AS, San Miguel T, Ortiz R, Osgood T, Sun JR, Zhu X, McCarter JD, Volak LP, Houk BE, Fakih MG, O'Neil BH, Price TJ, Falchook GS, Desai J, Kuo J, Govindan R, Hong DS, Ouyang W, Henary H, Arvedson T, Cee VJ, Lipford JR. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. Nature. 2019 Nov;575(7781):217-223. doi: 10.1038/s41586-019-1694-1. Epub 2019 Oct 30. PMID: 31666701.
33. Xing Y, Xu Y, Chen Y, Jeffrey PD, Chao Y, Lin Z, Li Z, Strack S, Stock JB, Shi Y.

Structure of protein phosphatase 2A core enzyme bound to tumor-inducing toxins. Cell. 2006 Oct 20;127(2):341-53. doi: 10.1016/j.cell.2006.09.025. PMID: 17055435.

34. Awad MM, Katayama R, McTigue M, Liu W, Deng YL, Brooun A, Friboulet L, Huang D, Falk MD, Timofeevski S, Wilner KD, Lockerman EL, Khan TM, Mahmood S, Gainor JF, Digumarthy SR, Stone JR, Mino-Kenudson M, Christensen JG, Iafrate AJ, Engelman JA, Shaw AT. Acquired resistance to crizotinib from a mutation in CD74-ROS1. N Engl J Med. 2013 Jun 20;368(25):2395-401. doi: 10.1056/NEJMoa1215530. Epub 2013 Jun 1. PMID: 23724914; PMCID: PMC3878821.
35. Weiss MM, Harmange JC, Polverino AJ, Bauer D, Berry L, Berry V, Borg G, Bready J, Chen D, Choquette D, Coxon A, DeMelfi T, Doerr N, Estrada J, Flynn J, Graceffa RF, Harriman SP, Kaufman S, La DS, Long A, Neervannan S, Patel VF, Potashman M, Regal K, Roveto PM, Schrag ML, Starnes C, Tasker A, Teffera Y, Whittington DA, Zanon R. Evaluation of a series of naphthamides as potent, orally active vascular endothelial growth factor receptor-2 tyrosine kinase inhibitors. J Med Chem. 2008 Mar 27;51(6):1668-80. doi: 10.1021/jm701098w. Epub 2008 Mar 7. PMID: 18324759.
36. Nida Iqbal, Naveed Iqbal. Imatinib: A breakthrough of targeted therapy in cancer. Hindawi Publishing Corporation. Chemotherapy research and practice. 2014;57027:1-9. DOI: 10.1155/2014/357027.
37. Shelley JC, Cholleti A, Frye L, Greenwood JR, Timlin MR, Uchimaya M. Epik: a software program for pKa prediction and protonation state generation for drug-like molecules. Journal of Computer aided Molecular Design. 2007; 21:681-691. DOI: https://doi.org/10.1007/s10822-007- 9133-z.
38. Ajay Kumar TV, Athavan AAS, Loganathan C, Saravanan K, Kabilan S, Parthasarathy V. Design, 3D QSAR modeling and docking of TGF-β type I inhibitors to target cancer. Computational Biology and Chemistry. 2018; 76:232-244. DOI:10.1016/j.compbiolchem.2018.07.011.
39. Balachandran P, Ajay Kumar TV, Parthasarathy V. Screening of potential anticancer compounds from Sargassum wightii to target breast cancer specific HER2 receptor using in-silico analysis. The Natural Products Journal. 2016; 6(2):108- 115. DOI: 10.2174/2210315506666 1602182241 12.
40. Rajagopal K, Sundaram S, Selvaraj J, Byran G. Molecular docking studies and in- silico ADMET screening of some novel oxazine substituted 9-Anilinoacridines as topoisomerase II Inhibitors. Indian Journal of Pharmaceutical Education and Research. 2017;51(1):110-115. DOI:10.5530/ijper.51.1.15.
41. Banks JL, Beard HS, Cao Y, Cho AE, Damm W, Farid R, Felts AK, Halgren TA, Mainz DT, Maple JR, Murphy R, Philipp DM, et al. Integrated modeling program, applied chemical theory (IMPACT). Journal of ComputationalChemistry. 2005; 26:1752-80. DOI: 10.1002/jcc.20292.
42. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz DT. Extra precision glide: Docking and scoring incorporating a

model of hydrophobic enclosure for protein-ligand complexes. Journal of Medicinal Chemistry. 2006; 49:6177-6196. DOI: https://doi.org/10.1021/jm051256o

43. Mullard A. Re-assessing the rule of 5, two decades on. Nature Reviews Drug Discovery. 2018; 17(11):777. DOI: 10.1038/nrd.2018.197.
44. Lipinski CA. Lead and drug-like compounds: the rule-of-five revolution. Drug discovery today: Technologies. 2004; 1(4):337-341. DOI: 10.1016/j.ddtec.2004.11.007.
45. Anonymous. Schrodinger Release 2024-4: QikProp, Schrodinger, LLC, New York, NY, 2024.
46. Anonymous. Schrodinger Release 2024-4: Protein Preparation Wizard; Epik, Schrodinger, LLC, New York, NY, 2024; Impact, Schrodinger, LLC, New York, NY, 2024; Prime, Schrodinger, LLC, New York, NY, 2024.