Optimizing Gefitinib for Toxicity Reduction and Dual-Route Administration: A Structure–Property–Toxicity Analysis

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1. Abstract

Gefitinib is a first-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) used to treat non-small cell lung cancer (NSCLC) with EGFR mutation. By binding competitively and reversibly to the ATP-binding pocket of the EGFR tyrosine kinase domain, gefitinib can block phosphorylation and downstream signal pathway.

Due to its high membrane permeability but poor aqueous solubility, gefitinib is administered orally only. However, oral delivery requires the drug to go through the first-pass metabolism, which can form toxic reactive intermediates that contribute to hepatocellular injury by the oxidation process performed by liver enzymes like CYP2D6 and CYP3A4.

Given these concerns, this study aimed to reduce hepatotoxicity, improve solubility, and explore the potential for dual-route administration (oral and intravenous) by designing five derivatives of gefitinib.

Our findings showed that all derivatives successfully reduced hepatotoxicity from active to inactive status and had significant reductions in LogP values. We propose that derivative 2 is the best candidate in these five derivatives. It demonstrates a favorable LogP (2.91), strong EGFR binding affinity (-8.76 kcal/mol), more polar interactions, and reduced toxicities. However, derivative 3 achieves significant reductions in hepatotoxicity, specifically, and has the least number of active toxicity endpoints. It can only be considered as a capable future research target as it exhibits lower LD_{50} value, indicating higher acute toxicity, and has a LogP value of 1.08, which may be too soluble for oral drugs.

These findings provide a computational basis for the development of gefitinib with improved pharmacological profiles. Still, we strongly recommend further experiments and clinical trials to validate the efficacy and safety of these five novel derivatives.

2. Introduction

Lung cancer, which includes small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), remains the leading cause of cancer-related mortality worldwide. The causes vary, including family history, chemical exposure, and alcohol use, but the most well-known cause is tobacco use (1). NSCLC accounts for approximately 85% of lung cancer cases, consisting of adenocarcinoma (ADC), large cell carcinoma (LCC), and squamous cell lung carcinoma (SCC), three subtypes (2).

A key biomarker in ADC is the epidermal growth factor receptor (EGFR), a transmembrane tyrosine kinase that, when mutated or overexpressed, promotes uncontrolled cell proliferation, survival, and metastasis (3). In the normal state, a ligand such as epidermal growth factor (EGF) or transforming growth factor-alpha (TGF-α) binds to the EGFR, causing dimerization. Then, the Asp-Phe-Gly (DFG) loop and C-helix move inward, the activation loop moves aside, so ATP can bind to phosphorylate the next enzyme along the pathway.

Mutations in the EGFR gene, on the other hand, such as the most common exon 19 deletions and exon 21 missense mutations near the binding site of the adenosine triphosphate (ATP), lead to the activation of receptors regardless of ligand binding (4). The activation results in downstream signaling through pathways such as PI3K/AKT and RAS/RAF/MEK/ERK, promoting tumor survival (5).

Small-molecule tyrosine kinase inhibitors (TKIs), such as 4-Anilinoquinazoline, have been developed to selectively block EGFR activity by binding to its ATP binding site, thereby inhibiting downstream signaling pathways. Gefitinib (Iressa) is a first-generation TKI, approved by the US Food and Drug Administration (FDA) in 2003 for the first time, that competitively binds to the TK domain of EGFR in a reversible manner (6). It was withdrawn from the US market by the manufacturer voluntarily in 2011 due to poor post marketing studies. However, it was approved by the FDA again in 2015, but only for the initial treatment of patients who have metastatic EGFR-positive NSCLC (7). It is still available in many European countries and some Asian countries.

Compared to another commonly used first-generation TKI, erlotinib, gefitinib shows a better safety level and is more cost-effective (8). The adverse events associated with gefitinib treatment may vary, including rash, diarrhea, nausea, vomiting, and decreased appetite. One main cause of these symptoms is gefitinib's high hepatotoxicity, which sometimes can induce severe liver damage, further requiring dose reduction or discontinuation. Gefitinib has been reported to have a higher frequency of causing liver-related adverse events compared to other TKIs, like erlotinib and afatinib (9). Some studies have been conducted to evaluate the efficacy of hepatoprotective drugs in alleviating liver damage caused by gefitinib, and their results show either a restoration of liver function or a successful decrease in the level of injury in patients with mild hepatotoxicity (grades II, III) (10,11). However, these drugs may also cause AEs if used improperly, which means that if we can modify the gefitinib structure to decrease hepatotoxicity, there will be less chance for other drugs to add to the side effects.

Since gefitinib is a Biopharmaceutical Classification Class II drug, meaning that it has high permeability and low solubility, it is generally administered orally. However, even though oral drug delivery is usually preferred, there are problems with drug absorption depending on the drug itself and patients' different gastrointestinal conditions. Therefore, we also aim to investigate the effect of increasing solubility while maintaining permeability on gefitinib and its administration route.

3. Objective

The primary objective of this investigation was to reduce the hepatotoxicity of gefitinib, along with other toxicities such as respiratory toxicity and neurotoxicity. In addition, we also aimed to decrease the solubility while maintaining the good oral bioavailability and stability in the GI system to achieve oral/IV dual route administration. Therefore, we modified the structure of gefitinib to create five novel derivatives and performed docking to evaluate relative data. The 2D structures of gefitinib and its derivatives which we created are shown in Figures 1 and 2.

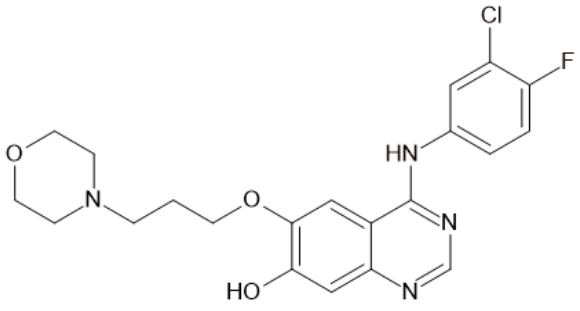
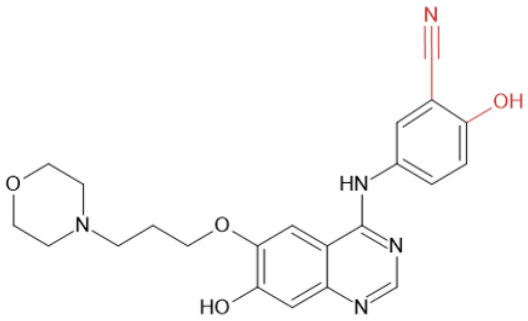
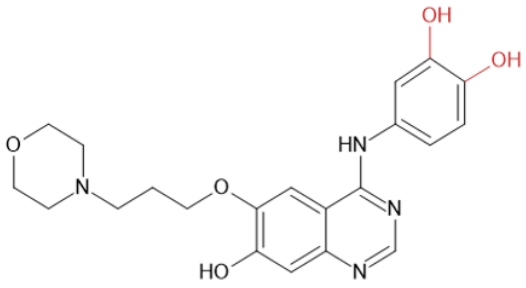


Figure 1. The 2D structure of gefitinib



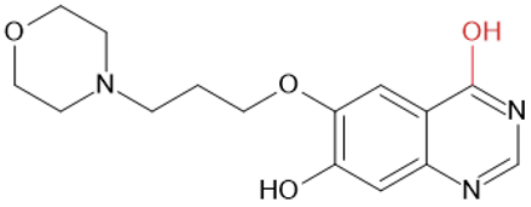
(A) Derivative 1

Changing the chlorin to -CN and changing fluorine to -OH.



(B) Derivative

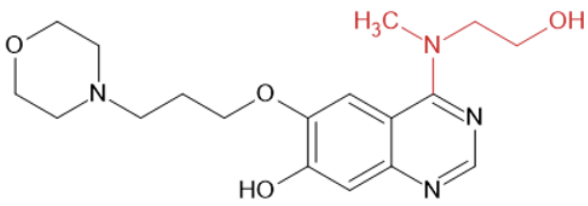
Changing both the chlorine and fluorine to -OH.



(C) Derivative 3

Changing the 3-Chloro-4-fluoroaniline to

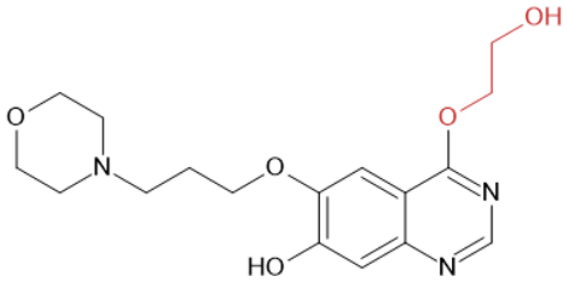
-OH, and deleting -NH



(D) Derivative 4

Changing the 3-Chloro-4-fluoroaniline to a

N-methyl ethanolamine, and deleting -NH



(E) Derivative 5

Changing the 3-Chloro-4-fluoroaniline to a diol chain, and deleting -NH

Figure 2. The five derivatives of gefitinib. (A) Derivative 1, (B) Derivative 2, (C) Derivative 3, (D) Derivative 4, and (E) Derivative 5.

4. Methodology

Computational-based data analysis is the best way to evaluate the efficacy of gefitinib and its derivatives. We obtained gefitinib's structure in SDF file format from PubChem, an open-source chemistry database developed by the National Institutes of Health (NIH). By putting the gefitinib into Pro Tox 3.0, an online toxicity predictor developed by the Charité University of Medicine, we were able to get its data on toxicities and the degree of compliance with Lipinski's four requirements, especially on the LogP values and the number of hydrogen donors/acceptors (12). To decrease toxicity and increase solubility, we computationally modeled five derivatives of this drug using PubChem Sketcher V2.4 and ChemSketch (13).

We first input the SMILES of gefitinib into PubChem Sketcher and modified part of the structure. Then, copied the new SMILES into the PubChem database to ensure that no one had already done this derivative. Putting the derivatives into Pro Tox to look for their toxicity information and comparing them with the information we got from the original drug helped us determine the research value of continuing to investigate this derivative. Chemsketch, managed by ACD/Labs (Advanced Chemistry Development, Inc.), was used to draw the 2D structure of the new chemicals and to easily save the SDF files. We used Pymol, an open-source molecular visualization system, to convert the SDF files to PDB files (14).

After determining the ligands, we took the structure of their target protein, EGFR (2ITY), from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) in PDB file format (15). Swiss-Pdb Viewer 4.1.0 was used to optimize structures, including ligand energy minimization and protein completeness checking (16).

The newly saved EGFR file was then imported into AutoDock Tools 4.0, an open-source automated docking tool developed by the Scripps Research Institute, to perform the necessary preparations before docking (17). Next, we added gefitinib as the ligand and saved the resulting file as a PDBQT file. Reimporting EGFR, we then launched AutoDock Vina 1.2.7 to perform molecular docking and calculate the binding affinity (18). Pymol was used again to check any polar interactions between gefitinib and the EGFR active site, and employed the same procedures to test our derivatives.

By holistically analyzing binding affinity, hydrogen bonds, toxicity, and LogP value, we concluded on which derivative of gefitinib would be the best at improving the efficacy in treating EGFR-mutated NSCLC patients.

5. Results and Discussion

5.1 Toxicities

We first analyzed the toxicity of gefitinib and each derivative by looking at toxicity endpoints since reducing the toxicity is the primary aim. Comparing the endpoints allow us to eliminate derivatives that didn't show significant improvement, prioritizing safe structures for further analysis.

Gefitinib Derivative 1

Target	Prediction	Probability	Prediction	Probability
Hepatotoxicity	Active	0.73	Inactive	0.63
Neurotoxicity	Active	0.83	Active	0.69
Nephrotoxicity	Inactive	0.51	Active	0.55
Respiratory toxicity	Active	0.98	Active	0.94
Cardiotoxicity	Inactive	0.81	Inactive	0.79
Carcinogenicity	Inactive	0.52	Inactive	0.54
Immunotoxicity	Active	0.99	Active	0.99
Mutagenicity	Inactive	0.50	Inactive	0.55
Cytotoxicity	Inactive	0.83	Inactive	0.56
BBB-barrier	Active	0.78	Active	0.60
Ecotoxicity	Active	0.63	Inactive	0.60
Clinical toxicity	Active	0.87	Active	0.71
Nutritional toxicity	Inactive	0.68	Inactive	0.57
Aryl hydrocarbon Receptor (AhR)	Active	1.0	Active	0.60
Cytochrome CYP2C9	Inactive	0.58	Inactive	0.74
Cytochrome CYP2D6	Active	0.78	Active	0.67
Cytochrome CYP3A4	Active	0.62	Inactive	0.56

Derivative 2 Derivative 3

Target	Prediction	Probability	Prediction	Probability
Hepatotoxicity	Inactive	0.60	Inactive	0.78
Neurotoxicity	Active	0.65	Active	0.73
Nephrotoxicity	Active	0.53	Active	0.50
Respiratory toxicity	Active	0.95	Active	0.85
Cardiotoxicity	Inactive	0.73	Inactive	0.76
Carcinogenicity	Inactive	0.55	Inactive	0.60
Immunotoxicity	Active	0.99	Active	0.89
Mutagenicity	Inactive	0.56	Inactive	0.64
Cytotoxicity	Inactive	0.57	Inactive	0.65
BBB-barrier	Active	0.52	Active	0.53
Ecotoxicity	Inactive	0.62	Inactive	0.58
Clinical toxicity	Active	0.72	Active	0.71
Nutritional toxicity	Inactive	0.58	Inactive	0.54
Aryl hydrocarbon Receptor (AhR)	Active	0.56	Inactive	0.66
Cytochrome CYP2C9	Inactive	0.73	Inactive	0.51
Cytochrome CYP2D6	Active	0.59	Inactive	0.56
Cytochrome CYP3A4	Inactive	0.63		

Derivative 4 Derivative 5

Target	Prediction	Probability	Prediction	Probability
Hepatotoxicity	Inactive	0.67	Inactive	0.82
Neurotoxicity	Active	0.69	Active	0.79
Nephrotoxicity	Inactive	0.51	Inactive	0.55
Respiratory toxicity	Active	0.96	Active	0.85
Cardiotoxicity	Inactive	0.85	Inactive	0.79
Carcinogenicity	Inactive	0.51	Inactive	0.63
Immunotoxicity	Active	0.99	Active	0.99
Mutagenicity	Active	0.51	Inactive	0.58
Cytotoxicity	Inactive	0.66	Inactive	0.57
BBB-barrier	Active	0.78	Active	0.66
Ecotoxicity	Active	0.55	Inactive	0.57
Clinical toxicity	Active	0.54	Active	0.64
Nutritional toxicity	Inactive	0.62	Inactive	0.61
Aryl hydrocarbon Receptor (AhR)	Inactive	0.55	Inactive	0.75
Cytochrome CYP2C9	Inactive	0.68	Inactive	0.58
Cytochrome CYP2D6	Active	0.74	Active	0.50
Cytochrome CYP3A4	Active	0.65	Active	0.55

Figure 3. Toxicity predictions of gefitinib and its derivatives. The red indicates active toxicity, and green indicates inactive toxicity. The darker the color of each bar, the more confidence in the prediction.

Based on Figure 3, which is an incomplete list of toxicity target predictions, the number of active toxicity targets for gefitinib is 9, with 8 in dark red and 1 in light red. These are major toxicity points related to gefitinib and patient safety since the listed points fall under organic toxicity, toxicity endpoints, and metabolism categories. The dark red bars include hepatotoxicity, neurotoxicity, respiratory toxicity, immunotoxicity, BBB-barrier, clinical toxicity, Aryl hydrocarbon receptor (AhR), and cytochrome CYP2D6.

In this study, one of our primary focuses was to decrease hepatotoxicity and reduce drug-induced liver injury (DILI). The hepatotoxicity of gefitinib was active with a probability of 0.73, indicating a high chance of liver damage. So we modified the structures to make them inactive.

Although only derivatives 3 and 5 showed a dark green color, indicating a higher probability of inactivity in hepatotoxicity, all derivatives changed the highly active status to an inactive status. Looking at the hepatotoxicity among these two derivatives, derivative 5 had a probability of 0.82, while derivative 3 had only 0.78. Therefore, derivative 5 showed a better efficacy in reducing DILI from only the perspective of hepatotoxicity. However, there are other factors we should also look at. Cytochrome P450 (CYP) enzymes located in the liver, specifically CYP3A4 and CYP2D6, are crucial in metabolizing drugs like gefitinib (19). As a substrate of the most abundant human hepatic CYP, CYP3A4, gefitinib can be oxidized by this enzyme and form reactive intermediates that may be toxic to liver cells (20). That to be said, an inactive CYP3A4 and CYP2D6 is important in deciding the overall reduction effect of a new derivative. Derivative 3 was the only one with inactive status in both enzyme toxicity predictions, outweighing derivative 5.

Another thing we noticed was that the respiratory toxicity and immunotoxicity in all ligands, including gefitinib, were confidently activated. Respiratory toxicity decreased slightly in derivatives, particularly in derivatives 3 and 5 (0.85 active for both derivatives). However, the overall effect on patients is unlikely to change since it remained in the dark red range. On the other hand, immunotoxicity maintained active with a high probability of 0.99, evidencing why skin rash is the most common side effect of gefitinib treatment (21). Derivative 3 lowered the immunotoxicity to the lowest probability among the five derivatives, which was 0.85. This big reduction is potentially because we simplified the gefitinib structure by replacing the whole 3-Chloro-4-fluoroaniline with a hydroxyl group and deleting the amino group. The 3-Chloro-4-fluoroaniline originally connects to the quinazoline core via this -NH-, forming a well-constructed site for CYP oxidation, which induces immune responses. A reasonable explanation is that the hydroxyl group interrupts the aromatic conjugation, so the CYP metabolic pathway is therefore theoretically being blocked or hindered (22). This blockage means a key source for immunotoxicity has been reduced, though not completely removed. However, this hypothesis requires a substantial number of studies to verify and adjust.

Derivatives 4 offered limited improvements and remained 9 active toxicity endpoints, which was the most number of endpoints among five derivatives, so this paper would not focus on this derivative.

To ensure the clinical safety, we continued to examine the LD_{50} values to quantify acute toxicity risk as presented in Table 1.

Table 1. LD_{50} values for gefitinib and its derivatives

	LD_{50} (mg/kg)
Gefitinib	2935
Derivative 1	3550
Derivative 2	3550
Derivative 3	400
Derivative 4	2935
Derivative 5	400

Median lethal dose (LD_{50}) is the measurement of the estimated amount of a substance that, when administered in a single dose, is expected to cause death in 50% of a test population, meaning that the toxicity level will increase as LD_{50} decreases (23). Derivatives 1 and 2 both showed an increase in the LD_{50} value (both are 3550 mg/kg), meaning that they are

less toxic than the original drug. Whereas the LD_{50} for derivatives 3 and 5 drops in a great amount, both from 2925 mg/kg to 400 mg/kg, indicating an increase in the acute toxicity. These two derivatives with favorable toxicity predictions but low LD_{50} values were flagged for interpretation.

Since LD_{50} may vary based on administration route, and the solubility for derivatives 3 and 5 became very low, which we will discuss later in the paper, the decrease in LD_{50} does not necessarily mean they are disqualified from viable candidates for future investigations (24). Also, since derivatives 3 and 5 have very low LD_{50}, doctors can only prescribe them to the patients in limited doses if further studies apply them to clinical use. This property matches IV drug requirements of administering in a smaller dose than the oral dose, as a result of reduced drug circulation in our body (25).

Overall, according to the predicted toxicity reports, all derivatives we created showed an improvement over gefitinib, with derivative 2 being the most stable and safe option, and derivative 3 showing potential in most of the areas we particularly look at. Nevertheless, further investigations are needed to explore more modifications that can significantly decrease multiple toxicities at the same time while maintaining a moderate toxicity class.

5.2 Binding Affinities

After analyzing the toxicities of the five derivatives, especially the ones we evaluated to have better safety profiles, the next priority was to confirm the derivatives still bind with EGFR properly. Thus, we took the binding affinities and compared them with the binding affinity of gefitinib, to see if these derivatives can maintain drug potency. The findings are presented in Table 2 below.

Table 2. The binding affinity of each ligand when binding to EGFR.

	Binding affinity (kcal/mol)
Gefitinib	-7.608
Derivative 1	-9.222
Derivative 2	-8.758
Derivative 3	-7.518
Derivative 4	-7.720
Derivative 5	-7.522

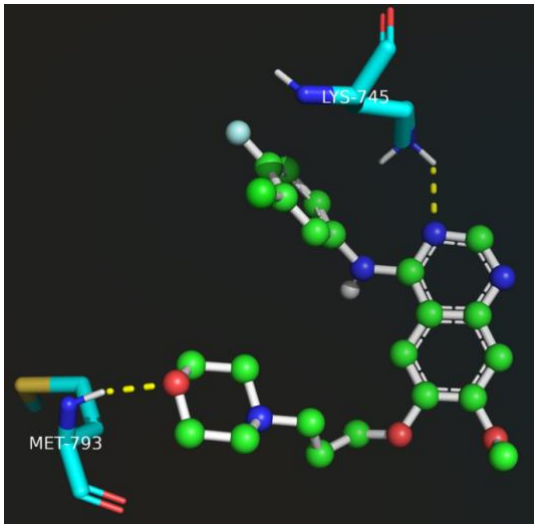
Although there is no particular value for the optimal binding affinity, a more negative number is generally considered better for bond strength (26). Most of our derivatives achieved our goal of enhancing binding affinity. Derivatives 1 demonstrated the greatest improvements in lowering the binding affinity, with a binding score of -9.222 kcal/mol (approximately 22% reduction). It was followed by derivative 2, which had a binding score of -8.758 kcal/mol (approximately 15% reduction).

Derivative 4 improved by only 1.4% (binding score -7.720 kcal/mol), while the binding affinities of derivatives 3 and 5 were around the same as or even slightly higher than the original drug (-7.518 kcal/mol for derivative 3 and -7.522 kcal/mol for derivative 5). The changes were negligible, and a safer drug with slightly lower binding affinity is better than a toxic drug with high binding affinity.

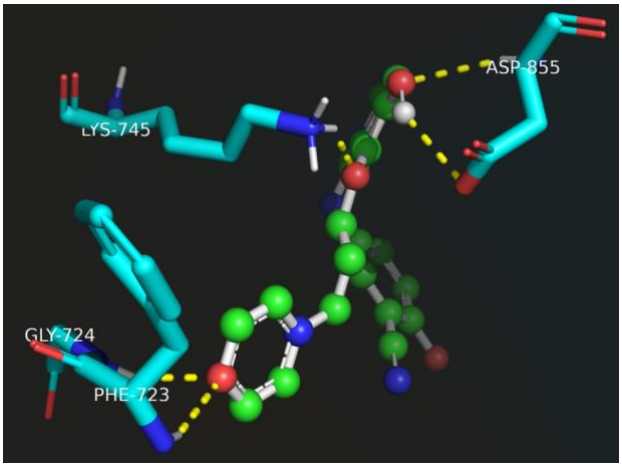
From this analysis, we cannot tell which derivative performs significantly better than the others, but the binding affinity indeed provides insight into the modification for our consideration.

5.3 Polar Interactions and Solubility

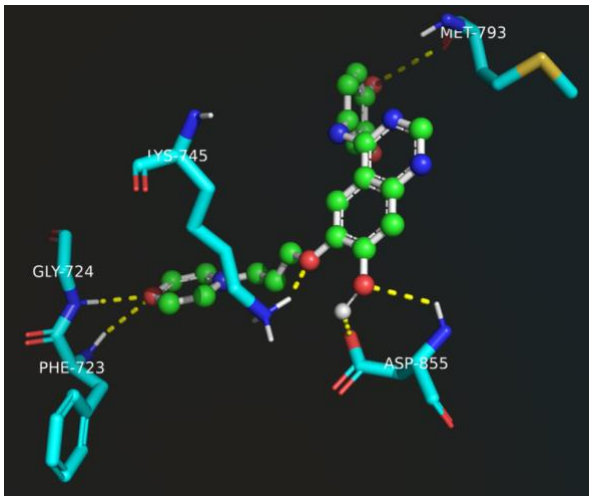
Polar interaction occurs between the polar functional group on the ligand and the polar amino acids on the protein. The amino acids directly participating in the interactions are called active residues. By identifying the name and number of these residues, we can better explain why certain derivatives maintained or enhanced the binding affinity and the change in LogP values.



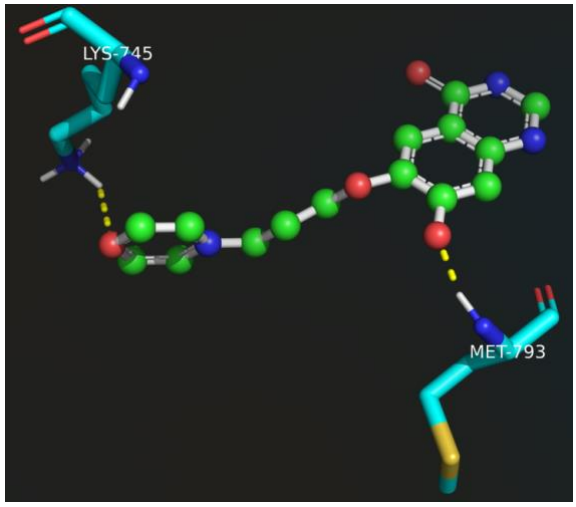
(A). Gefitinib bound with EGFR



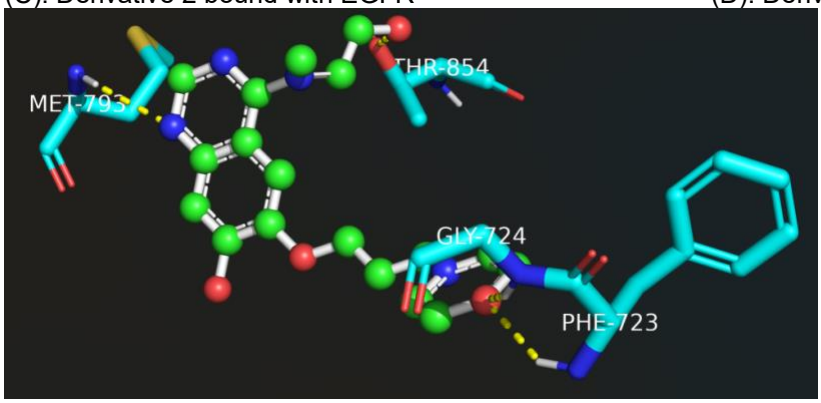
(B). Derivative 1 bound with EGFR



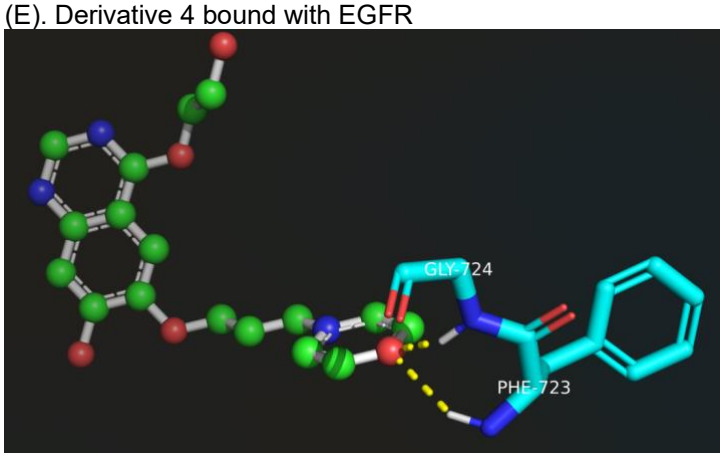
(C). Derivative 2 bound with EGFR



(D). Derivative 3 bound with EGFR



(E). Derivative 4 bound with EGFR



(F). Derivative 5 bound with EGFR

Figure 4. The binding between gefitinib/its derivatives and the ATP binding site of the EGFR protein.

Among all six ligands, four of them included polar interactions with either Met-793, Gly-724, Lys-745, or Phe-723. Derivative 2 could bind with all four of these residues. Derivatives 1 and 2 had an additional Asp-855, and derivative 4 had Thr-854. Due to these changes, the functions of the drug may slightly vary. Derivatives 2 and 3 maintained interactions with the residues that bind with gefitinib, showing their similar mechanism to that of gefitinib when binding with EGFR, which in turn demonstrating their stability to function as a drug.

Notably, we observed that Met-793 bound to different elements and positions in different EGFR-derivative combinations. It lies on the hinge region of EGFR and was reported to form a hydrogen bond with the 1-N on the quinazoline ring of gefitinib previously (27). However, in our investigation, it binds to the oxygen on the propyl-morpholino in gefitinib, the oxygen on the added hydroxyl group in derivative 2, and the 3-N on the quinazoline ring in derivative 4. A similar situation happened to other residues that can form polar interactions with multiple ligands. This situation happened because ligands can alter the binding mode or preferred orientation within the same binding site as the original drug to find a better fit in the binding site due to the modifications, leading to different binding shapes (28).

Table 3. The LogP values of gefitinib and its derivatives, calculated by Protox 3.0.

	LogP
Gefitinib	4.29
Derivative 1	3.07
Derivative 2	2.91
Derivative 3	1.08
Derivative 4	1.76
Derivative 5	1.05

The octanol-water partition coefficient (LogP) measures the hydrophilicity or hydrophobicity of a molecule and can determine the solubility of a drug. A lower value indicates greater solubility, meaning the drug can dissolve more easily in the body's aqueous environment. One previous study said that an optimal LogP value for oral absorption is between 1.35 and 1.8, but most of the time, there is no strict line dividing good and bad values (29).

Gefitinib, as an orally administered only drug, has a LogP value of 4.29, which follows Lipinski's five rules but still approaches the maximum value. This high LogP value causes gefitinib to exhibit high lipophilicity, allowing it to transfer through the membrane more easily, but it hardly dissolves in our bloodstream. Intravenous injection makes the delivery more precise to the target area and is faster than PO administration, so it can serve as a "plan B" for patients who have severe nausea or vomiting or are unable to tolerate oral medications (30).

If intravenous (IV) injection becomes an alternative route for gefitinib administration, the drug can bypass the first-pass effect that happens in the liver. The drugs given intravenously would not metabolized by liver enzymes, causing much less oxidative stress for the liver before

entering the gastrointestinal tract (31). Based solely on the LogP values, derivatives 3 and 5 are the most suitable candidates for IV, since derivative 3 has a LogP of 1.08, and derivative 5 has a LogP of 1.05, respectively. Their pronounced increase in aqueous solubility is needed for IV drugs to function.

Nevertheless, making the drug available in both routes is a more comprehensive approach, as it takes into account more patients with special conditions. To make it a dual-route administered drug, decreasing the LogP to around 1-3 is essential. Referring back to Table 3, all derivatives fall between this range, but most of them go into extremes: derivatives 1 has a LogP of 3.07, which is on the borderline of the recommended range, and the values for derivatives 3 and 5 reach the lower end of the range, making them too soluble to be a good oral drug. Also, derivatives 1, 2, and 4 had more polar interactions with EGFR, but the one with the lowest LogP was derivative 5, suggesting no direct correlation between the number of polar residues the ligand bind to and the solubility.

Therefore, derivative 2, with a LogP of 2.91, is considered to be the best candidate here, because it maintains a neat balance between lipophilicity and aqueous solubility. Derivative 1 can be the second choice

6. Future Development and Limitations

Though the hepatotoxicity decreases in each derivative compared to the original drug, there are many other toxicities still in the active state, especially the respiratory toxicity and immunotoxicity, which none of the derivatives significantly improved. The acute toxicity of derivatives 3 and 5, interpreted from the LD50 value, is a concerning issue in drug development. The toxicity level ranges between 50 mg/kg and 500 mg/kg are considered mild. So, 400 mg/kg is an acceptable value for drugs, but more careful calculations on how much patients can handle with minimized side effects should be performed.

In addition, therapeutic IV delivery of gefitinib has not been pursued clinically for human treatment before, meaning that the study on the IV route was limited to some animal studies and experimental pharmacokinetics analysis. Some recent studies indeed tested IV administration or compared PO with IV, but their results only provide insight into the metabolic and pharmacolipidodynamic effects instead of pushing it toward clinical implications (32,33).

We did not make changes to the propyl morpholino group because of three reasons. First, many studies have been conducted on this substructure compared to the 3-Chloro-4-fluoroaniline or the quinazoline backbone. Secondly, the toxicities increased or remained relatively the same after changing this structure. Thirdly, gefitinib and all five derivatives in this study had a polar interaction with the oxygen atom on the morpholine ring, making it a crucial structure for both binding affinity and solubility. As a result, more studies should be conducted on the morpholine ring to evaluate its function on drug efficacy.

7. Conclusion

The primary objective of this study was to mitigate the hepatotoxicity of gefitinib, a lung cancer drug that targets the mutated EGFR protein, thereby reducing the incidence of DILI. The secondary objective was to investigate the chance of dual-route administration of this drug. We worked toward these goals by creating five derivatives from gefitinib.

From the results obtained from the derivatives, we saw an overall decrease in hepatotoxicity, significant decrease in LogP, some increase in the number of polar interactions, and some increase in LD_{50}, marking the progress we made toward the goal of this study.

Among the derivatives in this study, derivative 2 demonstrated an optimal balance: exhibiting moderate LogP value (2.91), which support good membrane permeability for oral absorption while remaining within the acceptable range for IV delivery; strengthening the binding affinity with EGFR; maintaining the same residue binding sites as the original drug, improving safety profile by increasing LD_{50} by around 21%; inactivating the hepatotoxicity; and decreasing the confidence level of clinical toxicity by 17%.

Meanwhile, derivative 3 provided a better future research value. Despite its higher acute toxicity and a slightly excessive increase in solubility that are currently limiting its direct therapeutic application, it demonstrated notable reduction in several toxicity endpoints, especially a highly confident hepatotoxicity inactivation. The residues derivative 3 bound to are the same as gefitinib, indicating that their properties as a drug would be more similar than other derivatives. As such, if future studies can increase the LogP to a small extent and introduce substructures to mitigate the LD_{50}, this compound could potentially perform better that what we got.

Taken together, it is recommended to pursue subsequent laboratory synthesis of these derivatives, with evaluation through appropriate pharmacological and clinical studies.

8. Acknowledgment

I would like to thank Prof. Thomas Bakupog for his invaluable guidance and constructive feedback that greatly contributed to the completion of this paper. I would also like to thank the Pioneer Research Program for providing the opportunity and resources that made this research possible.

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