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4-Aminoquinazoline-6, 7-diol Derivatives for Enhanced EGFR Binding (as Inhibitor) Against Lung Cancer

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Abstract: Epidermal growth factor receptor (EGFR) is one of the potential targets for cancer therapy because it regulates the proliferation and survival of cells. In the present study, four '4-aminoquinazoline-6, 7-diol' derivative molecules were computationally investigated for their inhibitory activity against EGFR protein. Methods: Molecules were evaluated based on (i) in-silico cytotoxic assay defined for human cancer cell lines expressing EGFR (A549 and A431), (ii) in-silico kinase assay, (iii) impact of molecular interaction on EGFR, (iv) molecular interaction stability, (v) inhibitory impact of molecules on the combined expression of EGF-EGFR and (v) ADME observations. Studies were observed on the scale of two known EGFR inhibitors. In-silico cytotoxicity screening results demonstrated that the A431 lung cancer cell line was affected by compound Cmp1-4. However, A549 cells were less sensitive to Cmp1-4. Kinase inhibition assay resulted in compounds Cmp1 and 2 inhibiting EGFR. According to the molecular interaction analysis, compound Cmp1-4 was performed similarly to positive control, acting at the catalytic site of EGFR. Altogether, with compromised bioavailability, these potent compounds could likely be developed as promising EGFR-targeted drug(s) for cancer therapy.

Keywords: cancer; EGFR; inhibitor; lung; quinazoline.

1. Introduction

Cancer disease is known for features including uncontrolled cell division and malignant behavior. It is the second leading cause of mortality worldwide. Among the known cancer types, lung cancer is a leading cause of death globally. Epidermal growth factor receptor (EGFR) is a well-known drug target for treating lung cancer because it regulates the proliferation and survival of cells.

EGFR is widely overexpressed in multiple cancers. Various cell lines are used for the study of EGFR-expressed cancers. A549 and A431 are well-known cell lines for studying lung cancer [1-10]. EGFR belongs to the ErbB family of receptor tyrosine kinases. It is composed of four sub-sections: (i) extracellular domain, (ii) transmembrane region, (iii) intracellular tyrosine kinase domain, and (iv) tyrosine-rich C-terminal [11]. Growth factors (such as EGF and TGFα) trigger the extracellular domain to induce EGFR signaling pathways, which activate autophosphorylation and further downstream signaling. Since most non-small lung cancer over-expresses EGFR, it becomes an important strategy for drug targets. Several EGFR inhibitors are known, e.g., Erlotinib and Gefitinib [12]. Erlotinib is the first-line drug for lung cancer (IC_{50} against EGFR is 2.6 nM) with many side effects, such as anemia and dizziness.

Erlotinib also acquires drug resistance within 1.5 years. These points raise the requirement for novel/improved EGFR inhibitors. Common mutations (at extracellular and kinase domains) and truncations (at exon 19) contribute to increased EGFR activity. RAS-RAF-MEK-ERK, MAPK, and AKT-PI3K-mTOR are two downstream pro-oncogenic signaling pathways activated by these EGFR mutations, which results in activation of persistent initiation and proliferation regulating G1 cell cycle advancement. These become the basis for studies of EGFR signaling pathways through stimulation of CYCLIN D, CDK4/6, and inhibition of cyclin-dependent kinase [13]. 4-anilinoquinazoline is a versatile template for designing inhibitors against tyrosine kinases. Gefitinib is the first derived successful EGFR inhibitor through this template. Further research is continued to explore Structure-Activity-Relationship (SAR) for discovering EGFR inhibitors [14-20]. EGFR inhibitors compete with ATP to reach the catalytic domain of tyrosine kinases. Structurally diverse classes of highly potent and selective ATP-competitive inhibitors are known. Among them, 4-anilinoquinoline-3-carbonitriles and others mimic the adenine portion of ATP [21].

The present study designs the possible EGFR inhibitors (Cmp1-4) and aligns sequential computational studies to evaluate Cmp1-4. Evaluation studies involve lung cancer cell-line-based assays and kinase assays, exploration of the molecular interaction mechanism through analysis of binding mode and affinity through docking studies, and impact on systems models and ADME analysis.

2. Materials and Methods

The present study is intended for the computationally designing of molecules as well as their evaluation for EGFR inhibition.

2.1. Designing of structure.

Molecules were designed based on the evolutionary repositioning of fragments from known EGFR binders. The aim was to redefine the molecules with more molecular interactions.

2.2. In-silico cell line assay.

At the first step, cytotoxic activity was evaluated through two lung cancer cell line models for A549 & A431 [1]. Two known EGFR binders (Erlotinib & PDB 4i23 Co-crystallized ligand) were considered positive controls.

2.3. In silico kinase assay.

The consistency of molecular interaction of designed molecules and known EGFR binders was evaluated with the *in-silico* kinase assay model [22].

2.4. Molecular interaction.

Molecular interaction at the atomic level was observed through molecular docking studies with PDB 4i23 [23] and AutoDock Vina [24].

2.5. System model.

The impact of molecules was observed in extrapolation through the existing system models for normal EGFR. It was used to evaluate the inhibitory impact of the molecule on the interaction of EGF-EGFR under the influence of other system components [25].

2.6. ADME evaluation.

Designed molecules were finally processed for the evaluation of bioavailability through the SwissADME [26] online server.

3. Results and Discussion

3.1. Design of molecules.

Compound '4-aminoquinazoline-6, 7-diol' derivatives (Cmp1-4) were designed on the basis of repositioning fragments from existing potential molecules (Figures 1 and 2). Four molecules showed the possible enhanced potency over the existing ones. Here, the designed molecules were evaluated for their molecular performance. Two positive controls, i.e., known EGFR binders, were used: the first was Erlotinib, and the second was a co-crystallized PDB ligand: 4i23. Molecules were evaluated based on (i) *in-silico* cytotoxic assay, (ii) *in-silico* kinase assay, (iii) impact of the molecule on EGFR, (iv) molecular interaction stability, (v) inhibitory impact of the molecule on the interaction of EGF-EGFR and (v) ADME observations.

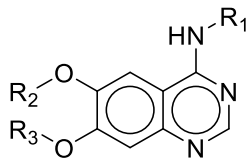
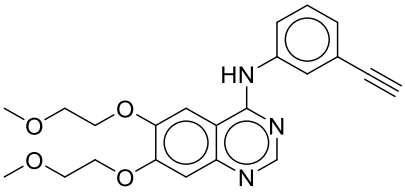
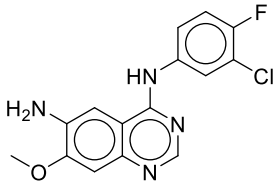


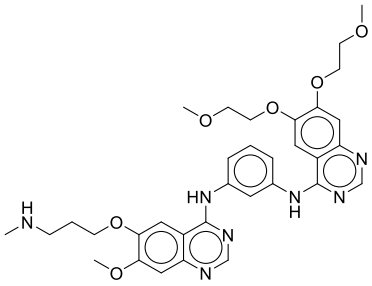
Figure 1. Basic scaffold (4-aminoquinazoline-6,7-diol) for EGFR binding.



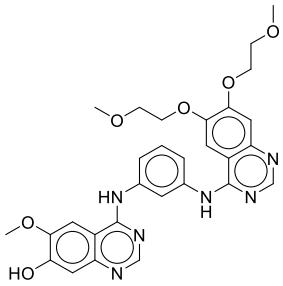
Erlotinib



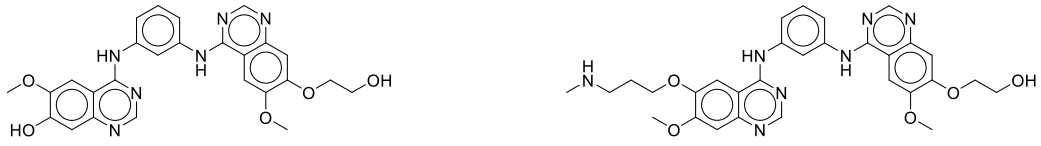
CoLig



Cmp1



Cmp2



Cmp3 **Cmp4**

Figure 2. Known EGFR inhibitors and designed molecules.

3.2. Cytotoxicity.

Initially, Cmp1-4 and known EGFR binders (Erlotinib and CoLig) were subjected to *in silico* cytotoxicity screening against A549 and A431 cell lines. A431 was found to be vulnerable to compound Cmp1-4. However, the A549 cell was less sensitive to the derivatives. Cmp1-4 was evaluated through their half-maximal inhibitory concentration (IC_{50}) values. The IC_{50} values of Cmp1-4 and the known drugs towards the two cancer cell lines are summarized in Table 1. The results revealed that in the case of EGFR-expressing cells, the anti-lung cancer potential of the derivatives ranges between IC_{50} of 7.1 µM to 8.0 µM for A549 and between 2.2 µM to 3.4 µM for A431. In addition, it was found that the IC_{50} value of the A431 cell line treated with compounds Cmp1-4 (IC_{50} of approximately 2.5 µM) was similar to CoLig (IC_{50} of approximately 2.2 µM), while for compounds Cmp2-3 (IC_{50} of approximately 3.3 µM) was similar to Erlotinib (IC_{50} of approximately 3.0 µM). Similarly, in the case of the A549 cell line, it was found that the performance of compound Cmp1-4 (IC_{50} of approximately 7.0 µM) was similar to Erlotininb (IC_{50} of approximately 7.9 µM). Furthermore, these compounds were processed in silico kinase inhibitory activity assay against the EGFR Kinase.

Table 1. Cytotoxic performance of molecules.

Molecules	IC_{50} nM against A549 (Active-Inactive class values ranging between 1-0)	IC_{50} nM against A431 (Active-Inactive class values ranging between 1-0)
Erlotinib	7926.6 (0.677)	3007.65 (1.099)
CoLig	9742.4 (0.275617)	2244.2 (0.5426)
Cmp1	7178.0 (0.4955)	2516.88 (0.711826)
Cmp2	7184.95 (0.501696)	3304.99 (1.084)
Cmp3	7160.75 (0.558)	3359.76 (1.01457)
Cmp4	7160.4 (0.5250)	2553.72 (0.681155)

3.3. Kinase inhibition.

To further evaluate that compound Cmp1-4 can inhibit the EGFR-TK proteins, kinase inhibitory activity assays were conducted compared with the known inhibitors (Erlotinib). As shown in Table 2, the kinase inhibitory scores against A431 of the compounds were: Erlotinib (1.5006), CoLig (1.441882), Cmp1 (1.700237), Cmp2 (2.310034), Cmp3 (0.078865), Cmp4 (-0.24669). Results suggested the potency of Cmp1 &Cmp2 against EGFR. Here, kinase inhibitory activity in cell line A431 was represented by 'C0_K1/C1_K0' with higher values as positive control.

Table 2. Kinase assay output for tested compounds.

Molecules	A431 (C0_K1)	A431 (C1_K0)	Kinase assay observation as C0_K1/C1_K0
Erlotinib	0.89973	0.59958	1.5006
CoLig	0.716105	0.496646	1.441882
Cmp1	0.951439	0.559592	1.700237
Cmp2	0.962522	0.41667	2.310034
Cmp3	0.0371982	0.471667	0.078865
Cmp4	-0.134229	0.544112	-0.24669

3.4. Impact of molecules on EGFR using molecular docking.

Molecular docking was conducted to investigate the binding mechanism of Cmp1-4 towards EGFR-TK in reference to known EGFR binders Erlotinib and CoLig. It was found that the Cmp-EGFR-TK complexes gave a binding affinity approximately similar to or higher than the Erlotinib and CoLig-EGFR complexes. The binding affinity and residual patterns of the underlying interactions of all focused ligands are shown in Table 3 and Figure 3. In the case of Erlotinib complexed with EGFR-TK, its residues 718 and 793 were hydrogen-bound. Meanwhile, the main H-bond interaction was found with residue-745. All derivatives consistently bore H-bonds with residue-745. 'Cmp2' and 'Cmp3' showed additional interaction with residue-793 as Erlotinib. Besides these, residues 720 and 724 were also found to be involved in the interaction with ligands.

Table 3. The binding affinity of the ligand.

Molecules	Binding affinity (kcal/mol)
Erlotinib	-6.9
CoLig	-8.0
Cmp1	-6.6
Cmp2	-7.7
Cmp3	-8.3
Cmp4	-7.5

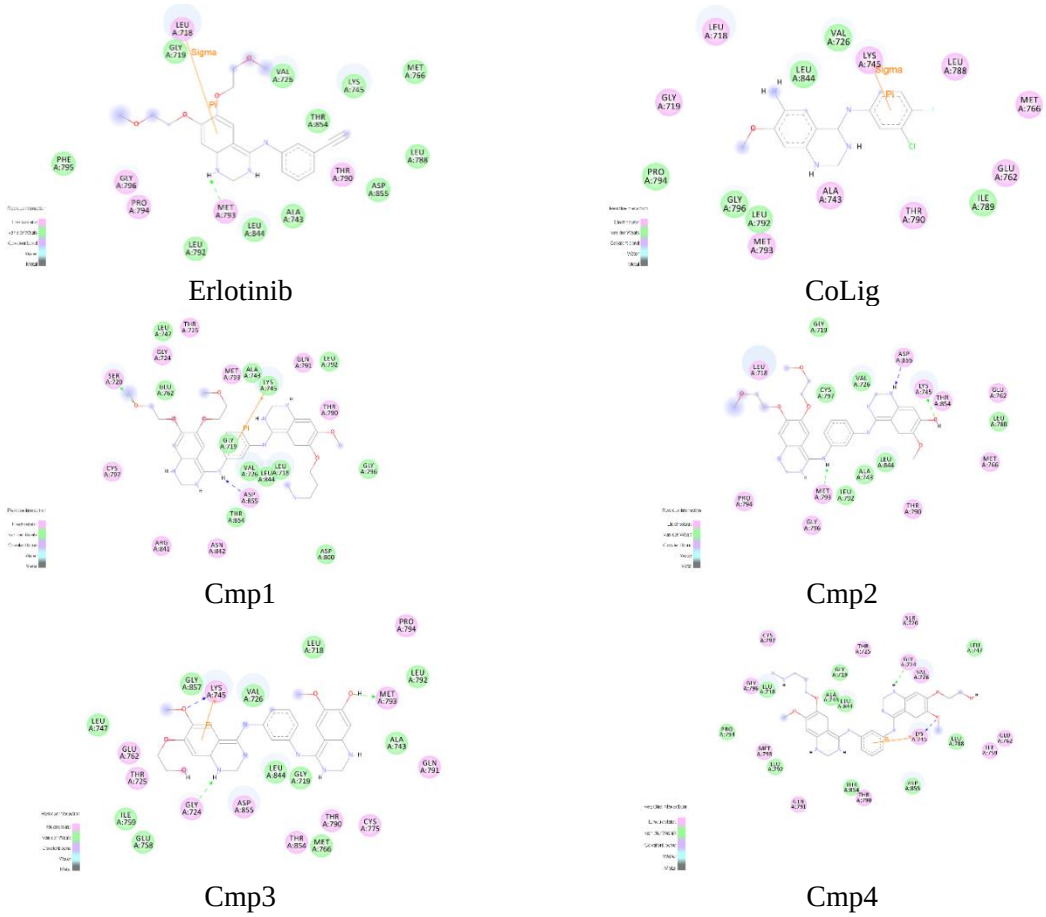


Figure 3. Molecular interactions between ligands & PDB 4i23.

These results were further used to investigate ligands' impact pattern on EGFR. The docking force of the protein by ligand due to protein-ligand interaction was observed as the positive control compounds defined the lower limit as 0.2436. Compounds with values greater than 0.2436 were considered more significant, which showed the required impact on the EGFR

for inhibition. About Erlotininb, it was found that all four Cmp1-4 showed the required force on EGFR for inhibition. While in reference to CoLig, Cmp2 and 4 showed potential impact (Table 4, Figure 4).

Table 4. Impact of ligands on molecular interaction with EGFR.

	Score for impact of ligand on molecular interaction with EGFR					
Molecules	Erlotinib	CoLig	Cmp1	Cmp2	Cmp3	Cmp4
Erlotinib	-	0.2436	0.3502	1	1	0.6493
CoLig	0.2436	-	0.1653	0.2436	0.3683	0.2724

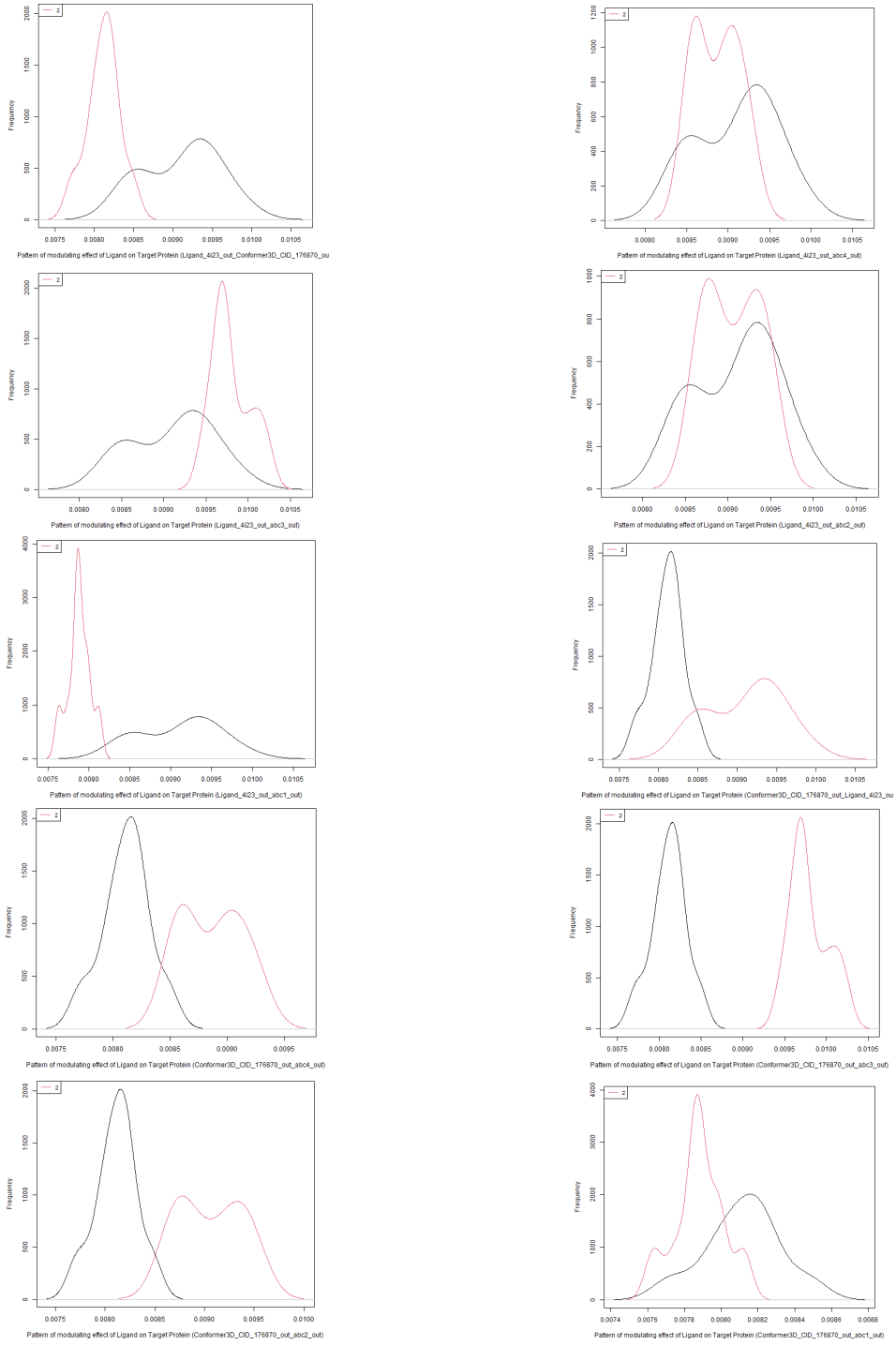


Figure 4. Impact of ligands on EGFR.

3.5. Inhibitory impact of the molecule on the interaction of EGF-EGFR under the influence of other system components.

This was performed through the system model. The normal EGFR-system model was borrowed, and the cancer EGFR-system model was developed. Treatment of compounds Cmp1-4 and controls were observed with a concentration of 1-10 µM. The default k3 value of the normal EGFR system was 2.014. It was decreased sequentially to increase the expression of EGF-EGFR. At the point of k3, the value with the highest EGF-EGFR expression was considered the EGFR cancer system model. Firstly, a ligand concentration of 10 µM was used to evaluate treatment's impact. Derivatives Cmp1 and Cmp4 were found to potentially inhibit EGF-EGFR expression. Furthermore, the ligand concentration gradient of 1 to 10 µM was applied to normal as well as cancer EGFR models. Gradient treatment also showed the behavior of derived Cmp1 and Cmp4 (Table 5, Figures 6 and 7).

Table 5. Treatment of 10 µM ligands in the system model.

Molecules	Binding affinity (kcal/mol)	ki (nM)	Model Cell line without treatment	Cell line model coefficient (Normal)	Cell line model coefficient (cancer)	Model Cell line treated with molecules at 10 µM
Erlotinib	6.9	114227.7	1.087544449	2.014	0.5	0.543772224
CoLig	8	731278.4	1.013674682	2.014	0.5	0.506837341
Cmp1	6.6	68844.35	1.145255209	2.014	0.5	0.572627604
Cmp2	7.7	440737.2	1.02268926	2.014	0.5	0.51134463
Cmp3	8.3	1213349	1.008241649	2.014	0.5	0.504120825
Cmp4	7.5	314468.8	1.031799655	2.014	0.5	0.515899827

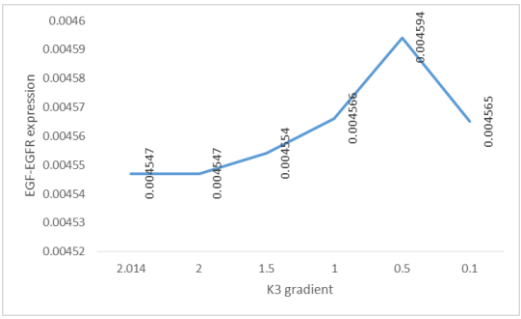


Figure 5. Preparation of cancer EGFR-system model.

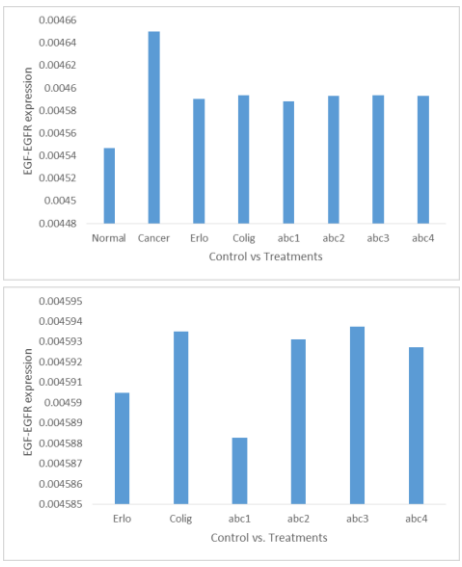


Figure 6. EGF-EGFR expression in normal-cancer model with control and treatments.(here Cmp1-4 have been renamed as abc1-4).

Performance of the ligand was observed with a concentration gradient of 01-10 micromolar. Molecule 'Cmp1' performed better than Erlotinib. Molecules Cmp2 and 4 performed in an approximately similar fashion but not better than Erlotinib (Table 6, Figure 7).

Table 6. Treatment with a concentration gradient of 1-10 µM.

	Model Cell line treated with molecules of concentration of					
Molecules	**1 µM**	**2 µM**	**4 µM**	**6 µM**	**8 µM**	**10 µM**
Erlo	0.004594	0.004593	0.004593	0.004592	0.004591	0.004591
CoLig	0.004594	0.004594	0.004594	0.004594	0.004594	0.004594
Cmp1	0.004593	0.004593	0.004592	0.004592	0.004589	0.004588
Cmp2	0.004594	0.004594	0.004594	0.004594	0.004593	0.004593
Cmp3	0.004594	0.004594	0.004594	0.004594	0.004594	0.004594
Cmp4	0.004594	0.004594	0.004594	0.004593	0.004593	0.004593

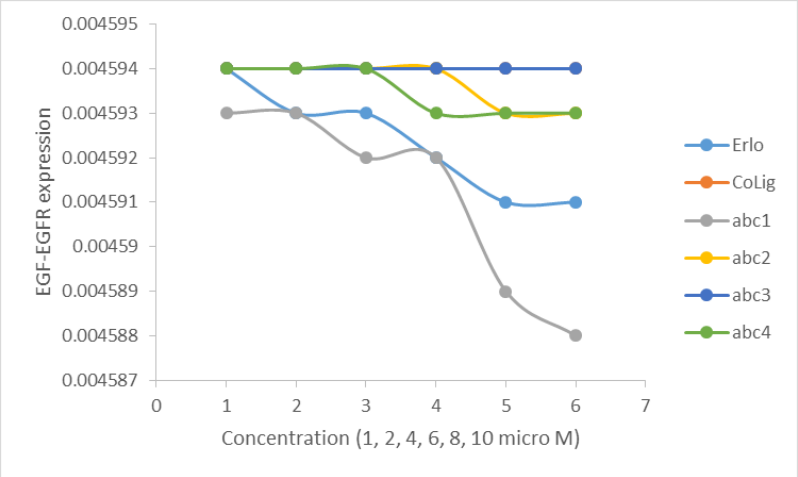


Figure 7. Performance of Ligands. (here Cmp1-4 have been renamed as abc1-4)

3.6. ADME observation.

Overall, aromatic heavy atoms, rotatable bonds, and hydrogen bond interactions were increased in the new compounds. However, due to the increase in TPSA, new compounds are not accessible to BBB. Solubility also decreased in comparison to reference compounds. Synthetic accessibility increased. In this way, the two newly designed molecules showed enhanced target activity but compromised bioavailability compared to the reference control compounds.

3.7. Discussion.

4-aminoquinazoline was previously known for its antitumor activity through binding EGFR against several types of cancer. Therefore, we expected the newly designed 4-aminoquinazoline derivative to inhibit EGFR-TK activity. 4-aminoquinazoline and quinoline scaffold-based derivatives were defined as enhanced selectivity towards EGFR. Various ligands were designed by adding alternative binding groups, such as sulphonamide and carboxylic groups in the aniline moiety. These attempts provided patterns to derivatives for targeting different regions of the ATP-binding site at the protein kinase domain. These patterns were used based on SAR information from previous existing quinazoline-based inhibitors of EGFR, which had shown the pivotal interactions between the receptor and the inhibitors [14]. The discovery of lapatinib revealed that 4 positions of aniline have a lot of capacity to include fundamental changes in pharmacophore to target ErbB-2 but not EGFR. These positions also have the capacity to contain bulky replacements. The designed molecules also showed the

capacity of dual inhibition. As an approach for enhancing the selective targeting towards Erb2, we introduced larger moieties at 4 positions of the aniline, such as heterocyclyl sulfonamide moiety, as done in lapatinib, which binds in the ATP-binding cleft. It enhanced the hydrophobic interactions with the protein by mimicking the aniline derivative group of lapatinib [21]. Moreover, in two compounds, aniline moiety was substituted with piperazine fragments, which made dramatic changes in the pharmacophoric model of anilinoquinolines or quinazoline due to their appreciable effect on the activity.

In silico cytotoxicity evaluation of 4-aminoquinazoline derivatives against lung cancer cell lines showed that Cmp1-4 was more susceptible to EGFR-expressing cell lines A431 than A549 cells. This difference was found because (i) the EGFR expression level found in A431 cells is dramatically higher than that in A549, and (ii) A549 cells exhibit KRAS mutation, which constitutively activates downstream MAPK signaling pathways. The cytotoxic effect of Cmp1-4 on cancer cell lines was similar to that of known inhibitors. In addition, these derivatives showed approximately similar IC_{50} to the known drugs, suggesting that these 4-aminoquinazoline derivatives could be safe and similar to the known drugs. However, the results from the cell-based assay were inconsistent with kinase inhibition in which compounds Cmp1 and Cmp2 were potent against kinases, whereas compounds Cmp3 &Cmp4 showed less inhibitory activity towards kinases. This is because cells may have several factors involved in cell growth inhibition, such as cell permeability and compound degradation within the cell. Therefore, compounds with good inhibitory activity on both kinase inhibition and cell-based inhibition were selected and marked for key attention in studying the binding patterns at the atomic level.

Although the docking binding affinity of compounds Cmp2 and Cmp4, in complex with EGFR, was found to be approximately the same as that of the known inhibitors, their ligand–protein interactions were also similar. It was found that all ligands, including controls, showed consistency in interaction with two residues, LYS745 and MET793. Besides, the derived showed more interaction with other residues. Overall, derivates Cmp1 and Cmp4 were observed to be more consistent with known EGFR inhibitors. Molecular interaction stability analysis showed that all derivatives are less stable than known drugs at the molecular interaction level. Among the derivatives, Cmp3 is the most stable, Cmp4 is the least stable, while Cmp1 and Cmp2 were found to be approximately similar instability.

The inhibitory impact of the molecule on the interaction of EGF-EGFR, under the influence of other system components, was accomplished using the system model. The normal EGFR-system model was adapted, and a cancer EGFR-system model was created. Compounds Cmp1-4 and controls were treated with concentrations ranging from 1 to 10 µM. The normal EGFR system's default k3 value was 2.014. It was gradually reduced to increase the expression of EGF-EGFR. The EGFR cancer system model was chosen at the k3 value with the highest expression of EGF-EGFR. To begin, a ligand concentration of 10 µM was used to assess the impact of treatment. The derivatives Cmp1 and Cmp4 were discovered to have the greatest potential for inhibiting EGF-EGFR expression. Furthermore, a ligand concentration gradient of 1 to 10 µM was used in both the normal and cancer EGFR models. Gradient treatment also demonstrated the same behavior of derived Cmp1 and Cmp4.

Finally, the ADME study showed that the number of aromatic heavy atoms, rotatable bonds, and hydrogen bond interactions in novel compounds increased. However, novel compounds are not accessible to BBB because of increased TPSA. In comparison to the reference compounds, the solubility was also reduced. The availability of synthetics has

improved. In this fashion, the two newly developed molecules outperformed the reference control compounds in terms of target activity but at the expense of bioavailability.

4. Conclusions

Computational methods were utilized to discover new EGFR inhibitors in this study. The A431 lung cancer cell line was sensitive to compound Cmp1-4, according to in-silico cytotoxicity screening data. These compounds' lethal effect on cancer cell types was owing to their EGFR-inhibiting action. The binding interaction analysis revealed that compounds Cmp1-4 hit protein EGFR with adequate force. It was identified that the advanced EGFR inhibitors Cmp1 and Cmp4 can be used as promising starting points for the future development of lung cancer therapeutics.

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Conflicts of Interest

The authors declare no conflict of interest.

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