In Silico Identification of Novel Erlotinib Analogues Against Epidermal Growth Factor Receptor

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Abstract. Cancer is one of the major health challenges in modern times. Considering its high mortality rate, many proteins that are linked to cancer have been targeted for therapy, with one of them being the epidermal growth factor receptor (EGFR). A drug that is currently in the market for the treatment of non-small cell lung cancer and targets EGFR is erlotinib. In a quest for improved efficacy of erlotinib, herein we report molecular docking studies of thirteen erlotinib analogues by modification of the alkyne and anilino groups, all of which displayed better binding affinity than erlotinib. We identified aziridinyl analogue (S)-**13b** with the best binding energy of all the analogues studied.

Cancer remains one of the major health problems that affects millions of lives yearly. Over the past decade, cancer has become the second disease that leads to death with an increasing mortality rate on a global scale (1). There are many proteins associated with cancer development and survival that are currently targeted for therapeutic intervention. One of the therapeutic targets for cancer therapy is the epidermal growth factor receptor (EGFR) that has received considerable attention from the scientific community. EGFR belongs to the ErbB family of receptor tyrosine kinases (2). EGFR is one of the most frequently studied signaling networks as it is involved in various vital cellular events such as proliferation, migration and apoptosis (3). One of the success stories in small-molecule anticancer drug discovery is the discovery of the “tinib” drugs such as those that are currently in the market for the treatment of various cancers (**1-3**; in Figure 1). These drugs act as EGFR inhibitors by blocking signalling pathways. Among them is erlotinib, a reversible tyrosine kinase inhibitor that is used to treat non-small cell lung cancer (4). Since current drugs lack

selectivity and efficacy, the search for anticancer agents with enhanced selectivity and potency through better binding to the target protein remains actively pursued for the continued fight against cancer. Towards this end, we were interested in using computational chemistry by conducting *in silico* molecular docking studies to discover erlotinib analogues that have better binding to EGFR than erlotinib itself. In this work, we designed various analogues of erlotinib by bringing modifications in the alkyne and anilino groups (Figure 2). We subjected these analogues to induced fit docking against EGFR and calculated their binding affinity values.

Materials and Methods

The *in silico* docking studies of EGFR with erlotinib and its analogues was carried out using the Schrodinger 2015 suite (Schrodinger, LLC, New York, NY, USA), according to Sheikh (5).

Protein selection and preparation. The three dimensional structure of EGFR complex with erlotinib was retrieved from the Protein Data Bank (PDB) (<http://www.rcsb.org>) and has a resolution of 2.60 Å (PDB code: 1M17) (6). This PDB complex structure was developed using preparation wizard workflow of Schrodinger.

Ligand preparation. Maestro 10.3 was used for drawing ligand structures (Maestro, version 10.3; Schrodinger). For further analysis, ligands were prepared using LigPrep module (LigPrep, version 3.1; Schrodinger).

Induced fit docking. Prime module of Schrodinger 2015 suite was used for the execution of induced fit docking.

Binding energy calculations. Molecular mechanics with generalised Born and surface area solvation (MM-GBSA) continuum solvent model present in Prime module of Schrodinger 2015-3 was utilized for calculating the binding affinity of erlotinib analogues against EGFR.

Results

Molecular docking. The interacting residues of EGFR with **1** (erlotinib) and all of its analogues **4-13** are shown in Figures 3-7. In the EGFR-analogue complexes, 19-26 residues are engaged in interactions with their respective analogue ligands.

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Figure 1. Structure of some of the marketed “tinib” drugs. Erlotinib is used in this work.

The parent ligand **1** displayed interactions with 22 amino acid residues of EGFR and there are 12 common interactions between **1** and analogues **4-13**. The common 12 interacting residues are Leu-694, Val-702, Ala-719, Lys-721, Met-742, Thr-766, Gln-767, Leu-768, Met-796, Gly-772, Thr-830 and Asp-831. In addition, there are some interactions observed in **1** and shared between all the analogues but are not present in analogues **5**, **7b**, **11**, **12**, **13a** and **13b**. These interacting residues include Glu-738, Leu-753, Leu-764, Pro-770, Cys-773 and Leu-820. Moreover, some of the docked complexes of EGFR with analogues **4**, **5**, **7a**, **7b**, **8a**, **8b**, **11**, **12**, **13a** and **13b** displayed specific interactions that are not observed in **1** (Gly-695, Ser-696, Gly-697, Ala-698, Phe-699, Thr-701, Cys-751, Arg-752, Asp-776 and Lys-828). Binding affinity values, glide score and dock score for **1** and all of its analogues are shown in Table I.

Discussion

A number of quinazoline-based inhibitors have been synthesized that target EGFR and molecular docking studies have been carried out. Nakamura and co-workers reported a novel boron-conjugated 4-anilinoquinazoline in complex with EGFR (7). Zhu and co-workers reported the docking of several 4-anilinoquinazoline derivatives, bearing dioxxygenated ring systems, into EGFR (8). Zhang and co-workers have

Figure 2. Structure of erlotinib and illustration of the modification parts of the molecule for molecular docking studies. (a) modification of alkyne group and (b) modification of anilino group.

identified and docked a novel oxazolo[4,5-g]quinazolin-2(1H)-one against EGFR (9). Wang *et al.* disclosed a number of 4-anilinoquinazoline derivatives docked into EGFR with modifications on the aniline ring or at C-6 position of the quinazoline scaffold (10). Depreux and co-workers demonstrated an anilinoquinazoline derivative substituted by

Figure 3. Erlotinib **1**, cyclopropyl analogue **4**, nitrile analogue **5**, alkenyl analogue **6** and their residue interactions with EGFR.

carbamic acid ester in complex with EGFR (11). Kitano *et al.* reported docking of a 4-alkynylquinazoline derivative into EGFR (12). We embarked in using molecular docking studies on **1** (erlotinib) and some of its closely related designed analogues against EGFR. We chose **1** for our endeavours as it has a highly interesting terminal alkyne handle, which could easily be elaborated into a number of useful structural analogues for carrying out molecular docking studies. We were also interested in modifying the aromatic ring of the anilino group connecting the alkyne group and the quinazoline part of **1** (Figure 2). We intended to design erlotinib analogues that could potentially have better binding to the target EGFR protein than **1**, thus providing better understanding on its structure-binding activity.

Firstly, we calculated the binding energy value of **1** against EGFR protein and analysed its binding pattern.

Docking of **1** with EGFR gave a binding energy of -97.07 kcal/mol. Replacement of the alkyne group with cyclopropyl, nitrile and alkenyl groups was then performed. The resulting three erlotinib analogues **4**, **5**, and **6** were docked against EGFR and binding energies of -111.09, -99.76, -98.97 kcal/mol generalised Born and surface area solvation were achieved, respectively. Cyclopropyl analogue **4** gave the best binding energy from these three analogues (Figure 3). One of the reasons for the improved binding efficiency when the alkyne group was replaced with a cyclopropyl group could be due to an increased hydrophobic interaction between the hydrophobic cyclopropane ring and the surrounding hydrophobic residues present in the pocket.

We carried out a further modification of the alkyne group of **1** to an aziridinyl group. We initially docked one of the possible enantiomers (aziridinyl analogue, (*R*)-**7a**) and obtained a

Figure 4. Residue interactions of erlotinib (*R*)-aziridinyl analogue **7a** (left) and (*S*)-aziridinyl analogue **7b** (right) with EGFR.

Figure 5. Erlotinib analogues (*R*)-**8a** and (*S*)-**8b** and their interactions with EGFR's amino acid residues.

further improved binding energy (-119.49 kcal/mol). We then performed docking of the other possible enantiomer (aziridinyl analogue, (*S*)-**7b**) and obtained a similar binding energy of -118.28 kcal/mol (Figure 4).

The four erlotinib analogues **4**, **5**, **6**, **7a** and **7b** displayed more efficient binding than **1**. In particular, the binding efficiency was improved upon replacement of the cyclopropyl group (analogue **4**) to an aziridinyl group (analogues **7a** and **7b**). We then attempted the introduction of a methyl group to the nitrogen atom of the aziridine ring

of (*R*)-**7a** to give the *N*-methylated analogue (*R*)-**8a** to assess if such analogue will provide better binding. However, the binding energy of analogue (*R*)-**8a** was reduced to -106.99 kcal/mol (from 119.49 kcal/mol), thus indicating that the free nitrogen atom of (*R*)-**7a** is possibly engaged in H-bonding and, therefore, improving binding to EGFR (Figure 5). We then prepared and docked the other enantiomer (*S*)-**8b** to evaluate if a change in binding energy could possibly happen due to change of stereochemistry of the molecule; however, a similar binding energy was obtained (-106.54 kcal/mol).

Table I. Structure of erlotinib [1] and its analogues [4-13] with their binding energy, glide score and dock score against EGFR.

Ligand	Structure	Binding energy (kcal/mol)	Glide score (kcal/mol)	Dock score (kcal/mol)
1		-97.07	-7.85	-7.85
4		111.09	-9.47	-9.47
5		-99.76	-8.9	-8.9
6		-98.97	-6.2	-6.2
7a		-119.49	-9.14	-9.14
7b		-118.28	-8.67	-8.67
8a		-106.99	-8.25	-8.25

Table I. Continued

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Ligand	Structure	Binding energy (kcal/mol)	Glide score (kcal/mol)	Dock score (kcal/mol)
8b		-106.54	-8.56	-8.56
9		-106.83	-10.33	-10.33
10		-105.07	-8.89	-8.89
11		-104.84	-8.14	-8.14
12		-101.93	-7.18	-7.18
13a		-119.74	-10.97	-10.97
13b		-124.54	-8.89	-8.89

We then shifted our attention to the aromatic ring of the anilino group that connects the alkyne group with the quinazoline part of **1**. Here, we decided to keep the alkyne group of **1** and introduced halides, hydroxy and methoxy substituents at C-4 and C-6 of the anilino group to assess if such modification would improve the binding of the resulting analogues **9**, **10**, **11** and **12**. When we docked analogues **9**, **10**, **11** and **12**, binding energies of -106.83, -105.07 -104.84 and -101.93 kcal/mol were obtained indicating that such modifications did improve binding in comparison to **1** but were somewhat lower than the binding energy found for the aziridinyl analogue (*R*)-**7a** (-119.49 kcal/mol) (Figure 6).

Finally, we decided to apply a modification of analogue (*R*)-**7a** by introducing hydroxyl groups at C-4 and C-6 of the anilino group for obtaining analogue (*R*)-**13a** and examine if such alteration would provide better binding. We have been motivated to select such modification over the other examined alterations on the aromatic ring because we have observed the best improvement in binding energy in analogue **9** compared with analogues **10**, **11** and **12**. In the case of analogue (*R*)-**13a**, a binding energy of -119.74 kcal/mol was obtained, which is similar to analogue (*R*)-**7a**. Interestingly, docking the other enantiomer (*S*)-**13b** into EGFR gave the best binding of all the analogues studied with a binding energy of -124.54 kcal/mol (Figure 7). Thus, analogue (*S*)-**13b** could be regarded as a more potent compound than **1** according to our *in silico* studies.

Conclusion

In this work, we reported the molecular docking of **1** (erlotinib) and thirteen analogues against EGFR. All the designed analogues showed similar or better binding than **1** (-97.07 kcal/mol), with the aziridinyl analogue (*S*)-**13b** displaying the best binding to EGFR (-124.54 kcal/mol). This suggests that (*S*)-**13b** could be a more potent compound than **1**, exhibiting a better fit into EGFR's active site.

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