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In-silico Interaction Studies of Quinazoline Derivatives for their Inhibitory Action on Both Wild and Mutant EGFRs

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Abstract

Epidermal growth factor receptor (EGFR) family has gained importance as a target for cancer therapy. However, somatic mutations in the tyrosine kinase domain of EGFR alter its sensitivity to anti-EGFR tyrosine kinase (TK) drugs like gefitinib (Iressa™). Docking studies of a few newly synthesized 6, 7-dialkoxy-4-anilinoquinazoline derivatives which showed EGFR-TK inhibitory activity were conducted. It has been found that the docking energies of these novel quniazolines are comparable with the IC50 values against A431 and MCF-7 tumor cell lines. Though the compounds with benzoxazole (1 & 2) and imidazole side chain (4) exhibited low binding energy to wild-type, but compound 3 had the lowest binding energy to its mutants as well (T790M, L858R and double-mutant). Compounds 1, 2, 4 and gefitinib showed affinity only for selective EGFR variants.

Introduction

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein comprising an extracellular ligand-binding domain and an intracellular signal-transducing domain with tyrosine kinase (TK) activity. EGFR is over expressed in a large number of tumors (Kim et al., 2001) and hence been studied as a key target for cancer therapy (Bendelsohn et al., 2000]. Of many drugs targeted at EGFR-TK domain, gefitinib (Iressa, ZD1839) is the first EGFR-TK targeted inhibitor that received approval for the treatment of non-small cell lung cancer (NSCLC) (Cohen et al., 2004)

Recently it has been observed that, a sub group of gefitinib treated patients with NSCLC were identified to have somatic mutations in TK domain of EGFR (Thomas et al., 2004; Guillermo et al., 2004). Mutations have often led to resistance against drugs. Mutations Mutations might selectively resist the binding of inhibitors or might favor better binding of natural ligands giving it the competitive edge over inhibitors or even both. The L858R mutation in the TK domain of EGFR (EGFR-L858R) confers enhanced activity and sensitivity to gefitinib (Thomas et al., 2004; William et al., 2004) than observed in wild-type EGFR (EGFR-WT) (Thomas et al., 2004). Where as, EGFR T790M mutation (EGFR-T790M) was shown to desensitize the EGFR to gefi -tinib (Blencke et al., 2004) reducing the efficacy of these TK inhibitors for a limited treatment period. The T790M mutation is an important step in gaining resistance to TKIs as it accounts for about half of all resistance to gefitinib (Takayuki et al., 2006; Marissa et al., 2006).

Of several 6, 7-dialkoxy-4-anilinoquinazoline derivatives synthesized in our group (Venkateshappa et al., 2008) we have taken benzoxazole (compound1, 2) and imidazole (compound 3, 4) side chain derivatives (Figure 1) based on their inhibitory activity against A431 and MCF-7 tumor cell line and studied their binding affinity to EGFR-TK using in-silico approach. The compounds were docked using AutoDock 4.0 (Marissa et al.,

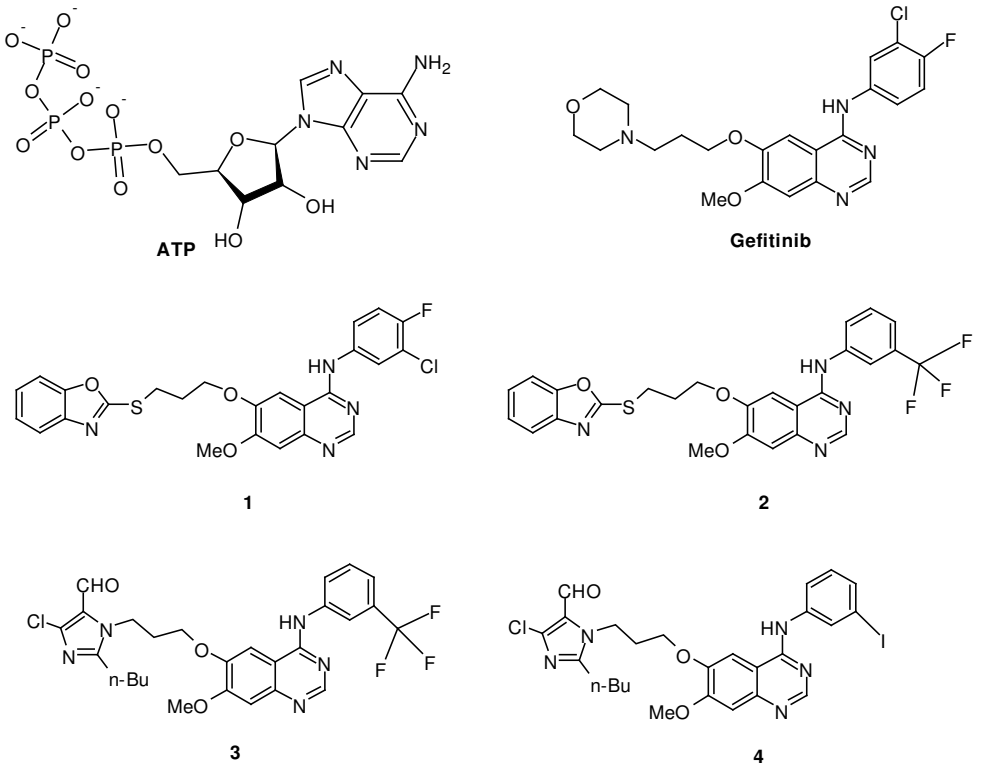

Figure1: Structure of ATP, gefitinib and novel qunizolines **1, 2, 3** and **4**.

1998) to EGFR-WT (PDB code: 1m17), EGFR-T790M mutant (PDB code:2jit), EGFR-L858R mutant (PDB code: 2itu) and modeled double mutant EGFR-DM with both T790M and L858R mutations. Molecular docking is an important concept which reveals the most populated alternatives from an ensemble of solutions comprising several different binding conformations for a given ligand and receptor model. AutoDock 4.0 uses a Lamarckian genetic algorithm (LGA), but encompasses also a Monte Carlo simulated annealing and a traditional genetic algorithm.The free academic licence of AutoDock and the good accuracy and high versatility shown by the program have promoted the widespread use of AutoDock. (Sousa et al., 2006). De Graaf et al. (2005) have used AutoDock to predict binding modes of ligands in 19 Cytochrome P450 and 19 thymidine kinase protein–ligand crystallographic structures which led to better results in terms of root-mean-square deviation (RMSD).

Methods

Modeling of double mutant

The pdb crystal structure 2itu with L858R mutation was taken for creating double mutant. All the hetero atoms and ligands were removed, the structure was cleaned and Tyrosine at 790th position was mutated and replaced with methionine using SPDBV 4.0 (Guex et al., 1997). The M790 side-chain having lowest energy and torsion similar to its posi -tion in T790M mutant crystal structure (PDB code: 2jit) was manually selected. This structure was further used in all the double mutant receptor studies.

Preparation of docking structures

All the ligands were drawn and geometrically optimized using ACD/ChemSketch 10.0. MGLTools 1.5.1 was used to prepare both ligands and receptors for the docking. All the allowed torsions in the ligands were set as flexible. Gefitinib bound wild-type crystal structure (PDB code: 2ity) was used as EGFR-WT, A-chain in PDB structure of T790M (2jit) was used as EGFR-T790M mutant, L858R PDB structure (2itu) was used as EGFR-L858R mutant and the modeled double mutant with both T790M and L858R mutations was used as EGFR-DM. All the hetero atoms including water molecules and bound ligands in PDB crystal structures were removed from the receptors. After adding polar hydrogen and charges, the receptor was set as rigid with no flexible bonds.

Docking studies using autodock 4.0

Using MGLTools 1.5.1, a grid spacing of 0.374 Å with

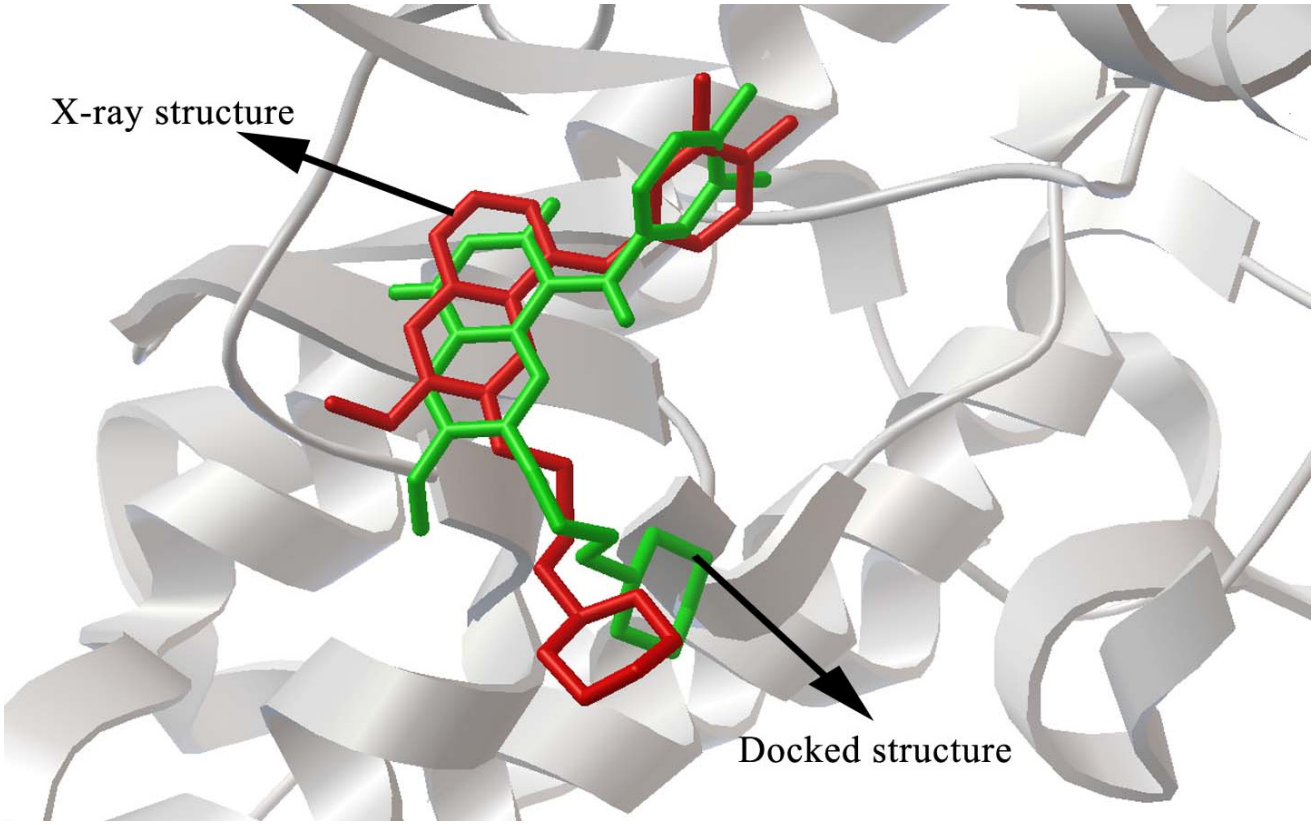

Figure 2: Orientation of gefitinib in X-ray structure (red) and docked structure (green) to EGFR.

Compound	IC50*		Binding energy (kcal/mol)			
	A431	MCF7	EGFR-WT	EGFR-T790M	EGFR-L858R	EGFR-DM
1	4.05	36.95	-8.37	-5.61	-6.92	-5.96
2	4.03	37.26	-6.55	-7.05	-6.75	-5.7
3	3.51	38.83	-6.14	-6.44	-5.75	-6.05
4	3.0	32.65	-6.03	-5.76	-4.87	-6.4
Gefitinib	1.0	12.05	-5.33	-6.39	-4.73	-5.18
ATP	NA	NA	-3.39	-4.89	-5.58	-5.98

*The IC50 values (μM) of these compounds are taken from Chandregowda V., et al., [10]. NA- not available.

Table 1: Binding energies of compounds**1, 2, 3, 4**, gefitinib and ATP with wild-type and mutated EGFRs.

60x60x60 points for EGFR-WT and 80x80x80 points for all other mutant receptors was prepared. The grid was centered around the catalytic clef of the enzyme for docking. Docking for 100 number of GA run was carried out using Lamarckian Genetic Algorithm (LGA) and all other parameters set to default. The top ranked model in the lowest energy cluster with maximum cluster size was considered for all further interaction studies. Test docking runs with geftinib using EGFR-WT with above mentioned parameters yielded a model for ligand binding highly similar to that seen in its crystal structure PDB code: 2ity (Figure 2). Hence, the same protocol was used for all further docking studies.

Results

Docking studies

All 6, 7-dialkoxy-4-anilinoquinazoline derivatives synthesized (Venkateshappa et al., 2008) were docked to EGFR-WT, of which compounds 1, 2, 3 and 4 which not only had low IC50 values but also showed better binding affinity with EGFR-WT were considered for further studies. Compounds 1, 2, 3, 4 along with Gefitinib and natural ligand Adenosine Tri-phosphate (ATP) were docked to EGFR-WT, single mutants EGFR-T790M, EGFR-L858R and modeled double-mutant EGFR-DM (Table 1).

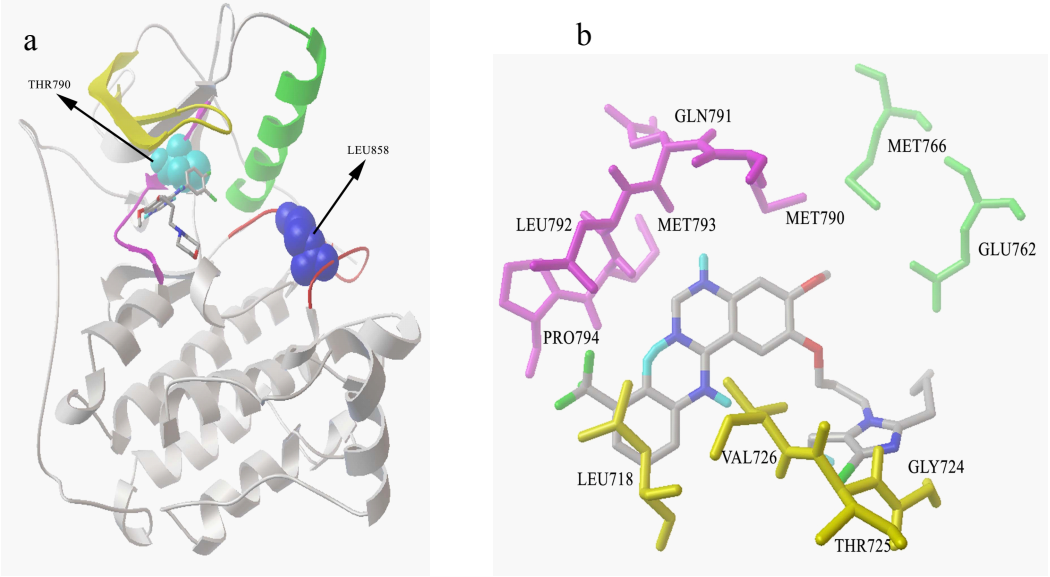

Figure 3: Active site represented as explained by Liu B et al., (2006). Hinge region (L788-C797: pink), P loop (F712-W731: yellow), C helix (S752-A76: Green) Activation loop (D855-p877: Red).
(**a**) Binding mode of gefitinib (shown in CPK-stick model) to EGFR-WT. Two important amino acids T790 (shown in cyan) and L858 (shown in blue) whose mutation plays crucial role in TK inhibitors' affinity are represented.
(**b**) Binding mode of compound 3 in the active site (top view) of EGFR-DM along with interacting amino acids from their respective regions of active site.

Interactions with active site amino acids were studied based on four important regions of the TK domain as explained by Liu B et al., (2006) which are, Hinge region (L788-C797), P loop (F712-W731), C helix (S752-A76) Activation loop (D855-p877) (Fig: 3a).

It was observed that the ATP's binding energy decreased from wild-type to single mutants and was lowest in the double mutant showing its greatest affinity to double mutant among other EGFR variants. Hydrogen bond was formed with arginine at 858th position in both EGFR-L858R and EGFR-DM but not with Leucine at the same position in EGFR-WT and EGFR-T790M showing that the point mutation at 858th position from Leucine to Arginine is favoring ATP binding over other TK inhibitors (see supplementary material). Though gefitinib's binding energy was much lower than ATP in wild-type, its energy did not increase greatly in EGFR-DM showing that the resistance caused by these mutations might be because of competitive affinity of ATP over Gefitinib.

Compound 1

Compound 1 had least energy to EGFR-WT but, it was not effective against EGFR-T790M, though its affinity to EGFR-DM was almost same as ATP.

Compound 2

Compound 2 showed lower binding energies in wild type and single mutants. Though it had very high affinity to EGFR-T790M, its affinity to EGFR-DM was lesser in comparison with ATP.

Compound 3 (Fig: 3b)

Compound 3 was the only compound among various quinazoline derivatives studied, to have lower binding energy than Gefitinib in all the EGFR variants. Though the lowest binding energy was not observed in every case there was consistency to EGFR-TK in both wild-type and its mutants.

Compound 4

Though compound 4 showed least energy in EGFR-DM but it had higher binding energies than either Gefinitib or ATP in other single-mutants. However it showed better affinity in wild-type.

Conclusion

Our analysis with EGFR-TK domain shows that the binding energy of ATP drastically decreased from wild-type to

single mutants and had least energy in double-mutant which was almost half as that of wild-type. Thus binding of ATP is more favorable over TK inhibitors like gefitinib, thereby leading to resistance in double mutant types.

Compounds 1, 2, 3 and 4 exhibited lesser binding energies than gefitinib against EGFR-WT. Compound 1 had best affinity with least binding energy to EGFR-WT and EGFR-L858R. Compound 2 being next best against EGFR-WT had least binding energy to EGFR-T790M. Though, compound 3 did not show the lowest energy in any docking studies, it was consistently showing better affinity to all the EGFR variants over gefitinib. Compound 4 had comparable energies to gefitinib in EGFR-WT and EGFR-T790M, and had the least energy in EGFR-DM.

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