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Free energy perturbation guided Synthesis with Biological Evaluation of Substituted Quinoline derivatives as small molecule L858R/T790M/C797S mutant EGFR inhibitors targeting resistance in Non-Small Cell Lung Cancer (NSCLC)

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Highlights

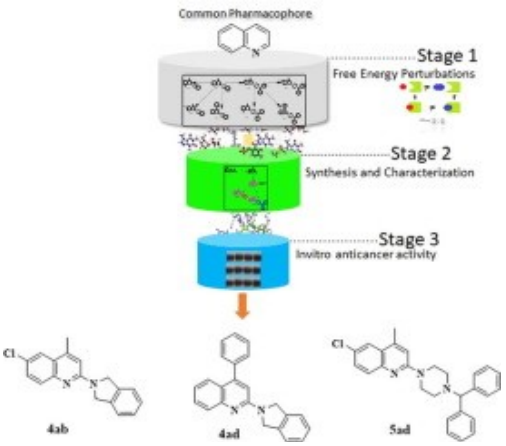
- Novel substituted quinoline derivatives were designed and synthesized which can serve as mutant L858R/T790M/C797S EGFR-TKIs.
- Free energy perturbations were carried out to determine the absolute binding free energy of a protein–ligand complex in the form of $\Delta G_{binding}$.
- The compounds **4ab, 4ad** and **5ad** showed significantly potent anti-proliferation and EGFR L858R/T790M/C797S enzyme-based inhibition.
- The most potent compound **4ad**, inhibited the EGFRL858R/T790M/C797S with an IC_{50} value of 124 nM. **4ad** inhibited HCC827 and H1975 cells *in vitro* with IC_{50} values of 0.0082 µM and 0.91 µM respectively.
- It was clear that compound **4ad** induced early apoptosis (23.7%) and late apoptosis (3.1%) in comparison with control (early apoptosis 1.1%, late apoptosis

1.0%). It has also shown cell cycle arrest at G0/G1 phase.

Abstract

Two different schemes of novel substituted quinoline derivatives were designed and synthesized via simple reaction steps and conditions. A comparative molecular docking study was carried out on two different types of EGFR enzymes which include wild-type (PDB: 4I23) and T790M mutated (PDB: 2JIV) respectively. Compounds were also validated upon T790M/C797S mutated (PDB ID: 5D41) EGFR enzyme at the allosteric binding site. Free energy perturbations were carried out to determine the absolute binding free energy of a protein–ligand complex in the form of $\Delta G_{binding}$, which in turn provided **4ab** and **5ad** as the most potential contenders through the structural enhancement in the determined initial scaffolds. Anticancer activity of the synthesized derivatives was examined against HCC827, H1975 (L858R/T790M), A549, and HT-29 cell lines by standard MTT assay. Compound **4ad** (6-chloro-2-(isoindolin-2-yl)-4-methylquinoline) has shown excellent inhibitory activities against mutant EGFR kinase with IC_{50} value 0.91 μM. The potency of compounds 4ab, 4ad and 5adwas compared throughan insilicoADMET study.

Graphical abstract



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Introduction

Epidermal Growth Factor Receptor (EGFR) is one of the most enzyme belonging to the ErbB family of receptor tyrosine kinase which also includes ErbB2 (HER-2 or Neu), ErbB3 (HER-3), and ErbB4

(HER-4) [1], [2]. Overexpression of EGFR may lead to adverse conditions, mostly in lung cancer [3]. The activation of epidermal growth factor pathways results in the initiation of cancer proliferation, increased metastasis potential, and neoangiogenesis [4]. Overexpression of EGFR triggers many downstream signalling pathways which cause more aggressive growth and invasiveness characteristics [5]. Activating mutations in the EGFR gene left a tremendous impact on treatment procedures in Non-Small Cell Lung Cancer (NSCLC). To overcome such mutations, the recent discovery of new EGFR inhibitors played an important role [6].

EGFR enzymes have certainly been undergoing missense mutations. Such mutations leave a tremendous impact on the drugs by showing resistance [7]. Changes in functions of the protein have forced the researchers to discover new drugs to avoid as well as inhibit the resistance. EGFR mutations are categorized according to their nucleotide changes. The initial mutation was found in the form of exon19 deletion [8]. Drugs like erlotinib and gefitinib which were extensively used for the treatment of lung cancer, initiated such resistance [9].

The emergence of mutation provided new targets and compounds named in the form of generations. Such drugs inhibiting the target enzyme were considered as 1st generations. Continuing a similar quinazoline scaffold, 2nd generation drugs were designed by changing the residing R-groups. Drugs like afatinib and neratinib showed potential inhibition towards the ongoing mutations [10]. It was all good until the wild-type EGFR enzyme exhibited a new missense mutation in the form of replacement of specific AUG gene to UAU gene [11]. This resulted from a change in amino acid of threonine to methionine at position 790. This new T790M mutation also changed the binding property functions which were observed in the form of toxicity [12].

To overcome mutations and observed toxicity, a new set of drugs came into emergence with a change in scaffold from quinazoline to pyrimidine ring [13]. Most recent development in the form of third-generation molecules were rociletinib and osimertinib which showed improved action against mutations such as exon 19 deletion and T790M mutation [14]. Though effective, the molecules had several drawbacks with the development of new resistance in the form of change in genetic code resulting in C797S mutation [15]. Due to the emergence of the C797S mutation, protein exhibited functional and conformational changes. These changes shifted the attention of many chemists towards already existing allosteric binding site of the enzyme [16]. The study of allosteric binding pocket was found to be helpful enough to overcome resistance as well as inhibit the protein. Compounds like EAI001 and EAI045 had shown their potential towards inhibition of the EGFR enzyme by binding to the allosteric site [17]. Fig. 1 shows the evolution of 4th generation allosteric binding EGFR inhibitors due to new mutations.

Quinoline exhibit a wide range of pharmacologic properties such as antibacterial [18], anti-inflammatory [19], antifungal [20], [21], anti-trypanosomal [22], anti-bacterial [23], antimalarial [24], anticonvulsant [25], antihypertensive [26], anti-HIV [27], and anticancer [28], [29] activities which is a frequently encountered heterocycle in medicinal chemistry literature [30]. Different biological activities have been shown by medicinal chemists through installing various active

groups to quinoline moiety via different synthetic protocols [31].

The emergence of the new era of computational drug designing has become an easier pathway to produce high-potency drugs within less period. To improve and understand newer techniques of drug designing, we have come up with a set of highly potent quinoline scaffold molecules. Extending our previous work further in terms of application of newer molecular modelling techniques [32], [33], the designed quinoline scaffold compounds were subjected to Free Energy Perturbations to construct, refine and generate a new potent set of molecules by analyzing the substituent transformations. Further, the generated novel molecules were subjected to molecular docking and ADMET studies to understand binding pockets and predict their potency. Finally, the designed compounds were synthesized and subjected for biological evaluation.

Section snippets

Chemistry

Overall synthetic route of compounds **4(aa-ad)** and compounds **5(aa-ad)** is outlined in Scheme 1. The complete multi-step reaction resulted high yields in each step. Synthesized compounds with their yields are shown in Table 1.

As depicted in scheme 1, initially substituted acetoacetanilide (**1**) was cyclised using polyphosphoric acid to form hydroxy compound (**2**). The substituted compound (**2**) was chlorinated using $POCl_3$ to form substituted chloride compound (**3**). Substituted chloride compound (**3**) was…

Conclusion

In this work, the novel substituted quinoline derivatives were designed and synthesized which can serve mutant L858R/T790M/C797S allosteric EGFR-TKIs. The computational studies of the compounds gave a preliminary idea of the potency which also proved to be positive during the biological activity. Free energy perturbations played an important role in characterizing the right substituent transformation. Among the synthesized compounds, **4ab, 4ad** and **5ad** showed noteworthy potent anti-proliferation…

General procedures

Chemicals were purchased from commercial sources, viz. TCI and Avra Labs, and used without further purification. Progress of the reaction was monitored by thin-layer chromatography (TLC) on pre-coated silica gel F254 aluminium sheets (Merck), and visualization was done by UV light. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on Bruker AVANCE II NMR

spectrometer in Dimethylsulfoxide-d_6 solution. Tetramethyl silane was used as an internal standard. Chemical shift values are given …

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.…

Acknowledgement

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PDB codes

PDB: 4I23, PDB: 2JIV, PDB ID: 5D41.…

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> ...The key interacting residues GLU762, PHE856 came into highlight at the allosteric binding site [52]. Karnik et al. [53] designed compounds show a positive conformational fit with the allosteric bag. In addition, in the enzyme activity test, most potent compounds 43, 44 and 45 inhibited the EGFRL858R/T790M/C797S with an IC50 values of 130 nM, 124 nM and 136 nM respectively (Fig. 19)....

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Design, synthesis, and biological evaluation of novel quinoline derivatives as small molecule mutant EGFR inhibitors targeting resistance in NSCLC: In vitro screening and ADME predictions

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> ...Sometimes, mutations (changes) in the EGFR gene cause EGFR proteins to be made in higher-than-normal amounts on some types of cancer cells. This causes cancer cells to divide more rapidly [65]. Therefore, it's very

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> ...As the fourth-generation EGFR-TKI development is still in its infancy, other drug discovery scientists also reported their developments at the pre-clinical stage. For example, Karnik et al. used computer-aided drug design of quinoline derivatives to target triple mutant EGFR (L858R/T790M/C797S) and discovered a quinoline derivative having inhibitory activity at nanomolar levels using enzymatic assays [135]. They further refined the derivative and tested it on NSCLC cells with the triple mutation, which yielded a half-maximal inhibitory concentration (IC50) of 1.9 μM....

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