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Research Article

Designing targeted drugs against EGFR protein in the treatment of lung cancer

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A B S T R A C T

Third-generation covalent EGFR inhibitors, including WZ4002, Osimertinib (AZD9291), and PF-06459988, have been developed to overcome drug resistance by irreversibly binding to specific residues within the EGFR active site, particularly Cys797 and Met790. To investigate these interactions and resistance mechanisms, we performed molecular docking analyses to model and evaluate the binding behavior of these inhibitors with EGFR. Structural results demonstrated that these compounds form highly specific covalent interactions that block EGFR activation and reduce cancer cell signaling. Additionally, second-generation inhibitors such as Afatinib and PD168393 were also shown to covalently bind to Cys797, with 3D structural models highlighting the stable interactions at this critical residue. Comparative docking-based analysis of first-, second-, and third-generation EGFR inhibitors revealed that while early-generation agents like Gefitinib and Afatinib rely on reversible or irreversible binding, newer inhibitors such as WZ4002 and Osimertinib are structurally optimized for enhanced activity against resistant EGFR mutations. The clear distinction between covalent irreversible and covalent-reversible inhibitors further illustrates differences in therapeutic potential. These docking-based findings emphasize the importance of precise molecular design in developing effective treatments against EGFR-driven drug-resistant cancers.

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1. Introduction

In today's societies, non-communicable diseases (NCDs) and cancer are recognized as the most challenging issues globally [1]. Among them, lung cancer has been identified as the leading cause of cancer and mortality, which unfortunately has been the most common type of cancer in the past few decades [2]. Factors such as smoking, obesity, diet, genetics, and environmental factors such as air pollution play a major role in the development of lung cancer. [3]. Lung cancer includes two main subtypes, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), which respond differently to treatments such as radiation therapy, chemotherapy, and complementary methods [4]. One of the most effective treatment options is surgical removal of the tumor, which unfortunately comes with severe side effects. This underscores the urgent need for new and targeted drugs [5]. For NSCLC, a promising therapeutic approach has been found that focuses on inhibiting specific tyrosine kinase receptors that contribute significantly to cell growth and survival, including hepatocyte growth factor receptor (c-Met), epidermal growth factor receptor (EGFR), and anaplastic lymphoma kinase (ALK) [6].

For patients who have shown resistance to previous therapies, covalent inhibitors that selectively target EGFR have become of great importance [7]. First-generation EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, are reversible, while second-generation inhibitors, such as afatinib and dacomitinib, are designed to be irreversible and overcome resistance mechanisms, particularly T790M, a common mutation in more than half of resistance cases [8]. Poziotinib, Dacomitinib, Allitinib, and Neratinib, second-generation EGFR inhibitors, use an electrophilic group and a specific cysteine residue (Cys797) around the ATP-binding site of EGFR to form a covalent bond. This covalent interaction increases target occupancy and improves selectivity over reversible inhibitors [9]. Engel et al. demonstrated a useful formulation of irreversible EGFR inhibitors through structural and biochemical analyses [10]. In another study, Schwart et al. proposed a kinetic framework for covalent EGFR inhibitors, pointing out the roles of reversible binding, chemical modification of cysteine, and resistance mechanisms in

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modulating potency [11]. Early clinical trials by Jiang et al. revealed that third-generation EGFR-TKIs were effective in patients with double-mutated tumors (EGFR L858R / T790M or ex19del / T790M) and also showed high selectivity for mutant EGFR, thus reducing side effects in the skin and gastrointestinal system by non-selective inhibition of WT-EGFR [12]. Research by Fry et al. has shown that specific inhibitors irreversibly inactivate the receptor tyrosine kinases EGFR and erbB2 and have potent antitumor activity without toxicity, indicating that these compounds represent a promising new generation of drugs for proliferative diseases [13]. Moreover, Tan et al. designed next-generation covalent inhibitors of FIIN-2 and FIIN-3 based on a structure that targets resistant FGFR1 / 2 mutations. although resistant mutations limit the efficacy of treatments, FIIN-3 simultaneously inhibits EGFR by binding to different cysteines [14].

Recently, the development of third-generation inhibitors, such as osimertinib (AZD9291), rociltinib, olmotinib, and other similar drugs, has focused specifically on targeting tumors harboring the T790M mutation [9]. Clinical trials have demonstrated that patients with EGFR mutations exhibit high response rates to treatment. In particular, first- and second-generation TKIs have achieved response rates exceeding 70%, along with marked improvements in progression-free survival [15]. Mutations in the EGFR kinase domain, such as L858R and exon 19 deletions, increase cancer activity and growth and have therefore become important therapeutic targets. Patients with these mutations usually show a positive clinical response, with response rates between 50% and 80% to first-generation inhibitors, such as gefitinib and erlotinib [7]. The main goal of this study was to design and evaluate targeted covalent inhibitors to overcome mutated EGFR proteins, which are connected with resistance to lung cancer therapy. By using advanced molecular modeling and working with high-quality, accurate structural data from the PubChem database, detailed simulations were performed to investigate the attachment behavior and covalent binding mechanisms of the novel inhibitors to EGFR. This process allowed the identification of key molecular features that could improve the selectivity and effectiveness of the inhibitors. The study also provided innovative insights into approaches to drug creation aimed at improving therapeutic efficacy and reducing off-target side effects.

2. Computational Methods

In this study, all simulations and molecular modeling were performed using data and resources obtained from the PubChem database. The chemical structures of EGFR and its inhibitors were retrieved from PubChem, which provided detailed information on molecular properties, bioactivities, and relevant literature. Molecular docking and structural analysis were conducted to evaluate the binding interactions between EGFR and selected inhibitors. All computational procedures, including ligand preparation and target protein modeling, were based on the chemical and structural data available through PubChem, ensuring the accuracy and reliability of the simulation results.

3. Results and discussions

Fig. 1. illustrates the chemical structures and binding interactions of several covalent inhibitors WZ4002, osimertinib, and PF-06459988 with the EGFR protein. These inhibitors specifically target two critical amino acid residues, Cys797 and Met790, within the EGFR kinase domain. WZ4002 and osimertinib are third-generation inhibitors developed to overcome resistance mutations, particularly the T790M mutation, which commonly reduces the effectiveness of earlier therapies. PF-06459988 shares similar covalent binding characteristics, reinforcing its role as a potent EGFR inhibitor.

At the molecular level, Cys797 and Met790 are essential for EGFR activation and function. The covalent bond formed between these inhibitors and Cys797 effectively locks the receptor in an inactive state, thereby blocking downstream signaling pathways that promote cancer cell proliferation. The image highlights the precise distances and orientations of these covalent bonds, underscoring the meticulous design that ensures high specificity and strong binding affinity to the active site of EGFR. Compared to first and second-generation reversible EGFR inhibitors such as gefitinib and erlotinib, which often fail against T790M-mediated resistance, these third-generation covalent inhibitors demonstrate significantly improved efficacy and selectivity [16, 17]. For example, osimertinib exhibits over 200-fold greater potency against T790M mutants relative to wild-type EGFR, which translates into enhanced clinical outcomes and reduced side effects [18]. WZ4002 was among the pioneering compounds to show selective covalent binding to mutant EGFR, providing a structural basis for overcoming resistance [19]. PF-06459988 further advances this approach by optimizing binding affinity and minimizing off-target proteome reactivity [20].

Fig. 2A shows the 3D structure of the EGFR kinase domain, emphasizing key regions. The catalytic loop and cysteine residues (C797, C775, C818, C939, C950) are labeled, indicating the active site and potential covalent modification sites.

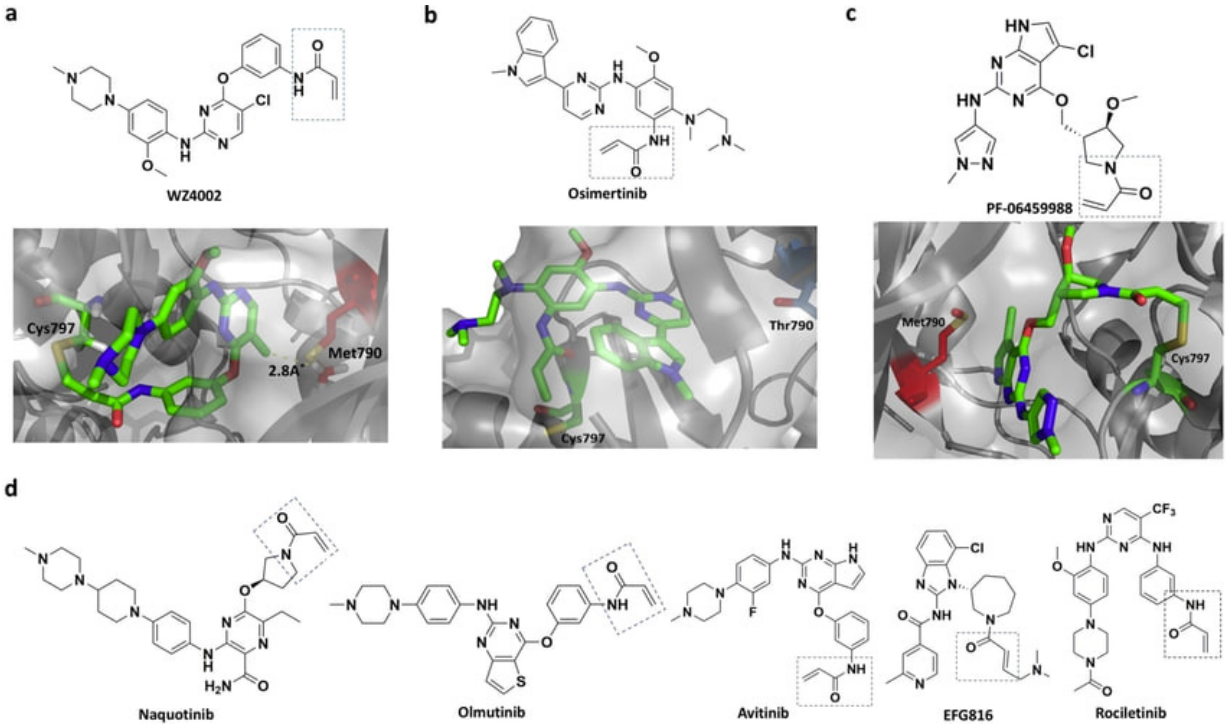

Fig. 1. Covalent Inhibitors and Their Interactions with EGFR.

Based on the figure, AMNP (which appears to be a non-covalent inhibitor or ligand) does not seem to form a covalent bond with Cys797. Instead, it likely interacts through non-covalent interactions such as hydrogen bonds, van der Waals forces, or electrostatic interactions within the binding pocket. The figure shows adenylylimidodiphophate (AMPPNP) positioned near Cys797, but there is no indication of a covalent linkage, which is consistent with the typical behavior of non-covalent inhibitors. In contrast, Afatinib's structure in Fig. 2B clearly bonds covalently with Cys797, demonstrating a different binding mode used by covalent inhibitors. The C775 and L858 are near the active site, indicating their roles in inhibitor interactions. It was reported that AMPPNP is a form of ATP that cannot be hydrolyzed and linked to the wild-type EGFR due to the later crystal structure; thus, AMPPNP has to form conventional hydrogen bonds with Met793 and Gln791 [21]. This triggers the downstream signaling and cascade of process in the cytoplasm contain cell growth, and apoptosis inhibition [22]. The structure of Afatinib and PD168393, which covalently bind to Cys797 in EGFR, can be seen in Fig. 2B. Afatinib is a second-generation inhibitor that binds irreversibly to EGFR, preventing its activation. PD168393 also binds to the same region of EGFR. As can be seen, Cys797 is highlighted in blue in Fig. 2B, representing the location of the covalent bond between the inhibitors and EGFR. These figures show the direct and stable interaction between the inhibitors and EGFR, which is crucial for treating resistant cancers. The purpose of designing these inhibitors is to block EGFR activation and prevent cancer cell proliferation. Afatinib and PD168393 are especially useful for treating cancers resistant to previous treatments.

Fig. 3. compares different types of EGFR inhibitors according to their generation. Gefitinib and Afatinib are first and second-generation inhibitors that bind reversibly and irreversibly to EGFR, respectively. Second-generation inhibitors feature electrophilic Michael-acceptor groups designed to target a rare cysteine (Cys797) located at the edge of the ATP-binding cleft of EGFR. These new inhibitors are believed to overcome T790M-mediated drug resistance through covalent binding to Cys797, which enhances target residence time and drug efficacy [10].

WZ4002 and Osimertinib are third-generation inhibitors, designed specifically to target specific mutations in EGFR. WZ4002 and Osimertinib, due to their molecular design, are much more effective at binding to Cys797 and blocking EGFR activation. Covalent Inhibitor (COV) and Covalent-Reversible Inhibitors (CRI) are clearly distinguished in Fig. 3. to highlight the fundamental differences between these two types of inhibitors. From Clinical Differences view, COV inhibitors bind permanently to EGFR, blocking its function, while CRI inhibitors can be reversible, which can have specific advantages in treating diseases. These differences may influence treatment choices for patients with cancers that have become resistant to earlier therapies.

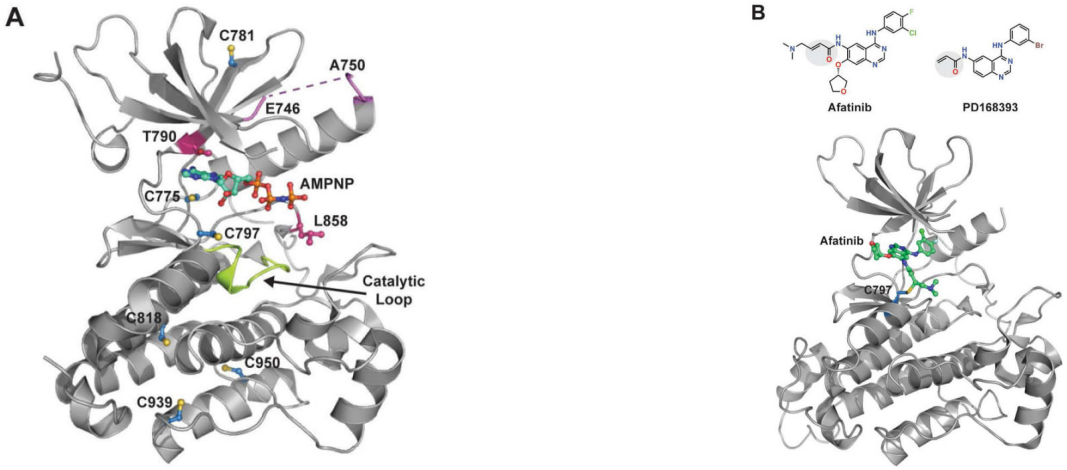

Fig. 2. EGFR structure and interaction with Afatinib and PD168393.

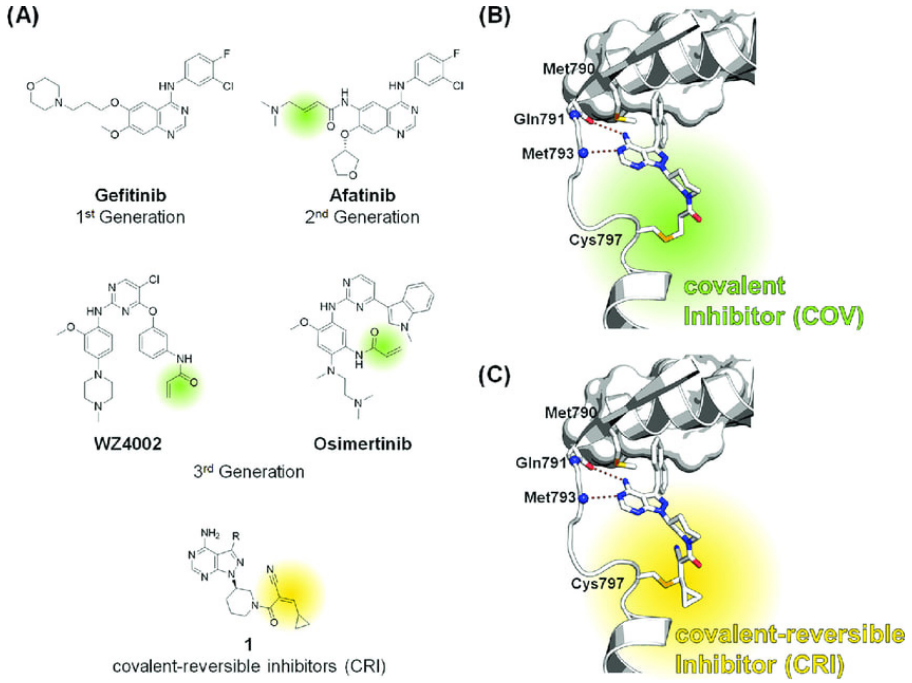

Fig. 3. Comparison of first, second, and third Generation EGFR Inhibitors.

4. Conclusions

The development and structural analysis of third-generation covalent EGFR inhibitors, such as WZ4002 and Osimertinib, have significantly advanced targeted cancer therapy by overcoming resistance mechanisms associated with earlier treatments. These inhibitors are precisely designed to form covalent bonds with critical residues like Cys797 within the EGFR kinase domain, effectively locking the receptor in an inactive state and disrupting downstream proliferative signaling pathways. Molecular docking studies highlight their high specificity and strong binding affinity, especially against resistant mutations such as T790M, thus exhibiting superior efficacy compared to first- and second-generation agents. The distinction between covalent irreversible and covalent-reversible inhibitors further emphasizes the nuanced approach required for optimizing therapeutic outcomes. Overall, these structural insights underscore the importance of meticulous molecular design in developing next-generation EGFR inhibitors capable of addressing drug resistance and improving clinical responses in EGFR-driven cancers.

Author Contributions

Michael Askari: Conceptualization, Writing – original draft, Writing – review & editing. The author read and approved the final version of manuscript.

Declaration of competing interest

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

Data Availability

No data is available.

Ethical issues

The author confirms full adherence to all ethical guidelines, including the prevention of plagiarism, data fabrication, and double publication.

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