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A Review

Design and development of mutant EGFR inhibitors from a structural perspective

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Molecules targeting non-small cell lung cancer driven by activating mutations within the epidermal growth factor receptor (EGFR) are highly effective but acquired drug resistance remains a persistent challenge. Insights from structural pharmacology and medicinal chemistry have aided in detailed understanding of the structural basis for how these inhibitors gain their mutant EGFR selective inhibitory activity and inform state-of-the-art drug design. The novel third-generation EGFR tyrosine kinase inhibitor (TKI) YH25448 (lazertinib) binds to EGFR with T790M-targeting van der Waals interactions and intramolecular hydrogen bonds consistent with improved medicinal chemistry properties compared to AZD9291 (osimertinib). Additionally, fourth-generation TKIs targeting the drug resistant C797S mutation comprise diverse structural features, but all share hydrogen bonding capabilities with the K745 catalytic residue consistent with stronger binding. Finally, inspired by the synergy seen between ATP and allosteric inhibitors, bivalent EGFR inhibitors have emerged showing potential for compounds with structurally diverse binding modes. Insights from these combined structural and functional studies offer key insights into the development of next-generation EGFR TKIs and inspire further exploration of similar binding features more broadly in protein kinases.

Keywords: Cancer biology, Drug design, Kinase inhibitors, Medicinal chemistry, Structural biology

Introduction

Activating mutations within the epidermal growth factor receptor (EGFR) kinase domain, for example L858R and exon19del, occur frequently in non-small cell lung cancer (NSCLC) and are biomarkers for the selection of EGFR tyrosine kinase inhibitors (TKIs) as clinically-effective therapies.[1-3] Prolonged effects of first-generation TKIs (*e.g.*, gefitinib and erlotinib) are eventually made limited due to acquired drug resistance due to an additional T790M "gatekeeper" mutation[4]. Subsequent drug development efforts produced the clinically-approved drug AZD9291 (osimertinib), which is selective for T790M-containing EGFR and made potent by forming an irreversible covalent bond to C797[5-8]. More recently, osimertinib has been shown effective, and clinically-approved as a front-line therapy for untreated patients with EGFR activating mutations[9]. Despite promising indications, drug resistance to osimertinib is caused in part by the acquisition of a third kinase domain mutation (C797S) that thwarts formation of the potency-enabling covalent bond[10,11]. Several next-generation TKIs targeting these triple-mutant kinase domains have been reported with promising pre-clinical results[12,13].

Structural basis of YH25448 (lazertinib) mutant EGFR inhibition

To produce an improved third-generation EGFR TKI, drug development efforts yielded YH25448 (lazertinib), which is structurally related to osimertinib but unique with respect to the substituted pyrazole moiety (Fig. 1A)[14]. Lazertinib is noteworthy in that it shows several improvements when compared to osimertinib such as *in vivo* efficacy, brain penetrance, target specificity, and dose-limiting toxicity[15]. As such, lazertinib has advanced into clinical evaluation in a variety of trials[16,17] such as first-line therapy[18] or in combination with the EGFR antibody amivantamab[19,20], and is currently approved to treat T790M-positive NSCLC in the Republic of Korea[14]. Resistance to treatment by lazertinib isak in to osimertinib involving the acquisition of the additional C797S mutation that prevents covalent bond formation and ablates drug potency[11,21].

To better understand the biochemical and structural aspects of lazertinib that differentiate it from osimertinib, and earlier EGFR third-generation TKIs,

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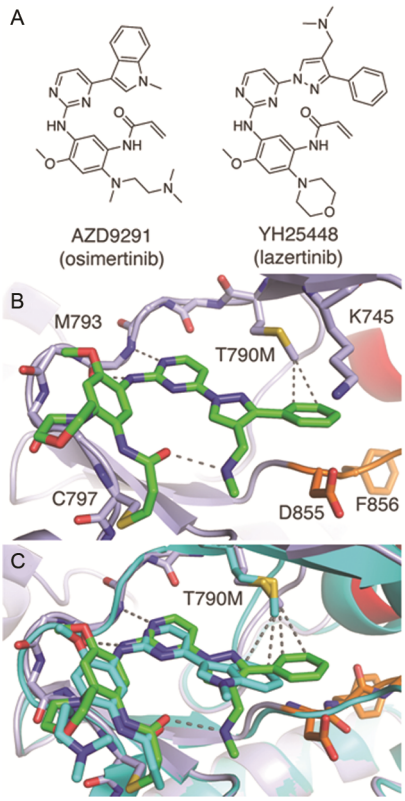

Fig. 1 — Lazertinib in complex with the EGFR (T790M) kinase domain. (A) Chemical structures of AZD9291 (osimertinib) and YH25448 (lazertinib). (B) Binding mode of lazertinib from a cocrystal structure of lazertinib bound to the EGFR(T790M/V948R) kinase domain (PDB ID 7UKW). Overlay of lazertinib (PDB ID 7UKW) and osimertinib (PDB ID 7JX0) cocrystal structures showing similar intermolecular interactions of both compounds with the T790M methionine and distinct intramolecular interaction for lazertinib. Adapted in part with permission from Heppner DE *et al*., Structural Basis for Inhibition of Mutant EGFR with Lazertinib (YH25448). *ACS Med. Chem. Letts.* 2022, *13* (12), 1856-1863. Copyright 2022 *American Chemical Society*

a series of cocrystal structures were determined of lazertinib bound to EGFR[22]. Lazertinib in complex with the T790M-containing kinase domain shows van der Waals intermolecular interactions between the T790M mutant methionine and the pyrazole phenyl and an intramolecular hydrogen bond from the pyrazole amine to the acrylamide carbonyl (Fig. 1B)[22]. The van der Waals interactions are similar to those seen in cocrystal structures of osimertinib whereas the intramolecular hydrogen bond is distinct to lazertinib (Fig. 1C)[23]. Time-dependent inhibition studies of lazertinib against WT and L858R/T790M EGFR kinase domains indicate that lazertinib is selective for the mutant kinase but is superior to osimertinib with respect to overall potency[22]. Structurally, the enhancement in potency is likely due

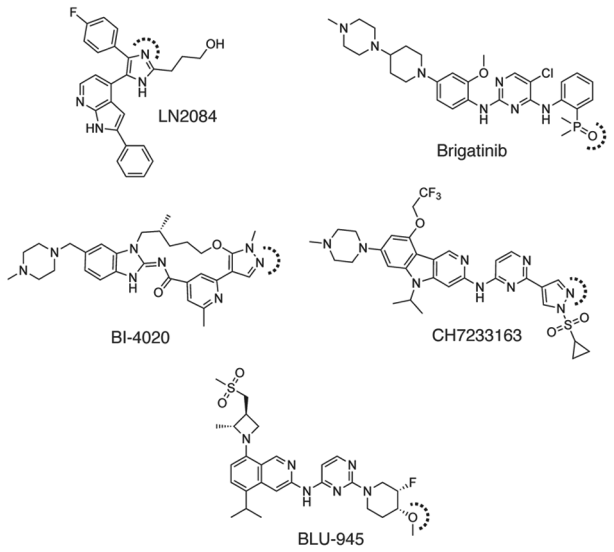

Fig. 2 — Chemical structrures of selected C797S-targeting EGFR TKIs. Dashed arcs indicate atoms that are shown in crystal structures to accept hydrogen bonds from the catalytic lysine (K745)

to the ability for lazertinib to form stable intramolecular interactions consistent with potency-enabling rigidification when it is bound to EGFR, which is not possible in any other third-generation EGFR TKI.

Targeting C797S with ATP-competitive tyrosine kinase inhibitors

The reliance of third-generation inhibitors on covalent bond formation at C797 has motivated the development of novel fourth-generation ATP-competitive inhibitors targeting the mutant drug-resistant C797S kinase domain[10,11,21]. A variety of molecules from academic and pharmaceutical company laboratories have been reported showing promising potency in C797S mutant settings comprising a variety of chemical structures. Initially, explorations of an EGFR off-target effect in trisubstituted imidazole kinase inhibitors led to the serendipitous discovery of inhibitors that are effective against reversible-binding C797S inhibitors[24-26]. Cocrystal structures of a series of imidazole inhibitors (representatively LN2084 in Fig. 2) exhibited H-bond through the imidazole nitrogen to the catalytic K745 residue only in αC-helix outward inactive crystal structures[27]. *N*-methylation of the imidazole nitrogens considerably impaired C797S reversible binding demonstrating that this hydrogen bond is necessary for C797S potency (dashed lines indicate the atom that accepts H-bonds in Fig. 2)[27]. The positioning of the imidazole

heterocycle in these compounds are structurally distinct from third-generation inhibitors showing that designing enhanced reversible binding would benefit new C797S-targeting inhibitors.

Another unexpected EGFR off-target effect is the ALK inhibitor brigatinib that showed C797S potency when combined with the anti-EGFR antibody cetuximab[28,29]. Recent structures of brigatinib and variants bound to EGFR revealed hydrogen bonds from the phosphine oxide to K745[30]. Additionally, Boehringer Ingelheim has recently reported a macrocyclic benzimidazole inhibitor (BI-4020, Fig. 2) [31], related to the third-generation EGF816[32], inhibits C797S-containing EGFR and interacts with K745 through a pyrazole group[31,33]. Similar binding interactions are observed for CH7233163 that, despite the distinct chemical structure compared to BI-4020, is involved in hydrogen bonding with K745 through a pyrazole ring (Fig. 2). More recently, BLU-945 comprises an isoquinoline scaffold and contains a methoxy group within hydrogen bond distance with K745 (Fig. 2)[34]. Since osimertinib is now a front-line therapy, it is expected that patients will likely acquire the C797S mutation in the absence of the T790M gatekeeper[35,36]. Accordingly, drug development efforts are directed toward developing agents with the ability to target diverse C797S-containing kinase domains[37]. While it is difficult to conclude the consequences of any given intermolecular interaction formed between an inhibitor and protein target, it is striking that the majority of ATP-competitive C797S-targeting compounds contain groups that accept hydrogen bonds from the catalytic K745, or other conserved residues such as D855, and showcase a trend in advanced EGFR TKIs across these diverse molecules[38].

Inhibitors of EGFR that simultaneously bind the ATP and allosteric sites

A new class of allosteric inhibitors have recently been described for the EGFR kinase and show promise as T790M and C797S mutant-selective agents[36,39,40]. The results of a targeted high-throughput screening resulted in the discovery of two unique scaffolds: a phenyl glycine "tri-blade" (Fig. 3A)[39] and a dibenzodiazepinone[40]. Since their initial reports, a variety of examples have been reported with promising medicinal chemistry properties[36,41-43]. Structurally, these inhibitors bind to a pocket that is adjacent to the ATP-binding site made accessible by the kinase being stabilized in the αC-helix out inactive conformation. Encouragingly, allosteric inhibitors

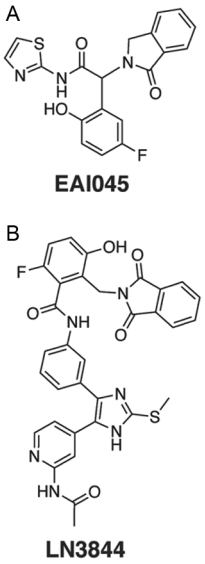

Fig. 3 — Allosteric EGFR and Bivalent ATP-allosteric site inhibitors. Chemical structures of the EGFR allosteric inhibitors (A) EAI045; and (B) the EAI045-inspired bivalent ATP-allosteric inhibitor LN3844

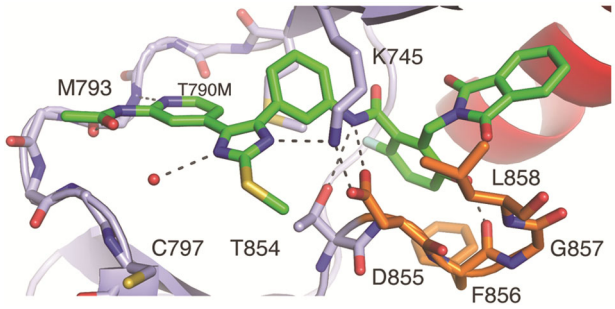

Fig. 4 — Binding modes of imidazole-based ATP-allosteric bivalent inhibitors. A) Cocrystal structure of LN3844 in complex with EGFR(T790M/V948R) (PDB ID 6WXN). Adapted in part with permission from Wittlinger F and Heppner DE *et al*., "Design of a 'Two-in-One' Mutant-Selective Epidermal Growth Factor Receptor Inhibitor That Spans the Orthosteric and Allosteric Sites". *J Med Chem* 2022, 65 (2), 1370-1383. 2022 *American Chemical Society*

have been shown to synergize with ATP-competitive inhibitors *in vivo* and combined within the same kinase domain to elicit structural changes consistent with positive cooperativity[41,44,45].

The structural proximity of these drug binding sites, and the observation of ATP and allosteric inhibitor synergy[41,44], has motivated the exploration of bivalent molecules that simultaneously bind these pockets (Figs 3B & 4)[46-48]. An X-ray cocrystal structure of LN3844, based on the ATP-competitive imidazole inhibitors (*e. g*., LN2084 in Fig. 2) and the tri-blade allosteric inhibitor EAI045 (Fig. 3A), in

complex to EGFR(T790M/V948R) demonstrates the ideal anchoring of functional groups within these pockets including H-bonding interactions with K745 and D855 (Fig. 4). Recently reported examples of dibenzodiazepinone-derived bivalent inhibitors based on DDC4002 show related binding modes with this different allosteric pocket group, but with variable conformations of the ligand depending on the linker structure[40]. An earlier example of a bivalent inhibitor comprising a pyrrolopyrimidine ATP-site scaffolds were designed prior to the discovery of C797S and mutant-selective allosteric inhibitors and anchor a urea-cyclohexane moiety within the EGFR allosteric pocket[49]. Crystal structures show that the bivalent ATP-allosteric binding of these molecule influences the positioning of K745, which could be exploited for unique drug properties[49]. While such bivalent inhibitors are currently underrepresented compared to other EGFR inhibitors, these studies provide motivation for their optimization and targeting in other protein kinases.

Conclusion

Addressing cancers with structural insights that detail how dysregulated targets in growth factor receptor signalling is a major focus in medicinal chemistry with a proven track record in the clinic[50]. The EGFR kinase domain continues to be an important drug target despite the diverse avenues by which NSCLC tumors can acquire drug resistance through additional mutations within the kinase domain protein[12,38,51]. In some respects, the emergence of mutations in response to therapies are opportunities for the development of improved drugs, such as how osimertinib was originally developed as a second-line therapy that was eventually discovered to be effective against untreated NSCLC[9]. The pursuits of novel inhibitors with distinct binding modes and currently unrealized avenues for selectivity may serve purposes beyond their original scope of development. Additionally, future efforts to predict emerging mechanisms of drug resistance coupled to structural and functional insights continue to guide novel developments to address important unmet needs in oncology and other human diseases.

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Conflict of interest

All authors declare no conflict of interest.

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