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Structural elements that enable specificity for mutant EGFR kinase domains with next-generation small-molecule inhibitors

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Abstract

Specificity for a desired enzyme target is an essential property of small-molecule inhibitors. Molecules targeting oncogenic driver mutations in the epidermal growth factor receptor (EGFR) kinase domain have had a considerable clinical impact due to their selective binding to cancer-causing mutants compared to wild type. Despite the availability of clinically approved drugs for cancers driven by EGFR mutants, persistent challenges in drug resistance in the past decades have led to newer generations of drugs with divergent chemical structures. The current clinical challenges are mainly due to acquired resistance to third generation inhibitors, including by the acquisition of the C797S mutation. Several diverse fourth generation candidates and tool compounds that inhibit the C797S mutant have emerged, and their structural characterization has revealed molecular factors that allow for EGFR mutant selective binding. Here, we have reviewed all known structurally-characterized EGFR TKIs targeting clinically-relevant mutations to identify specific features that enable C797S inhibition. Newer generation EGFR inhibitors exhibit consistent and previously underutilized hydrogen bonding interactions with the conserved K745 and D855 residue side chains. We also consider binding modes and hydrogen bonding interactions of inhibitors targeting the classical ATP and the more unique allosteric sites.

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Introduction

Tyrosine kinase inhibitors (TKIs) targeting EGFR represent effective therapies in non-small cell lung cancer (NSCLC) due to their ability to selectively inhibit the cancer-causing mutants.(Lynch et al., 2004; Paez et al., 2004) Selective binding for the disease-causing mutation while simultaneously exhibiting limited inhibition of the wild type EGFR is critical for establishing a therapeutic window that maximizes clinical efficacy and limits toxic side effects.(Eck & Yun, 2010) Various structural and pharmacological studies have established that the prevalent activating EGFR mutations, including in-frame deletions in exon 19 (exon19del), insertions in exon 20, and point mutations such as G719S and L858R, all effectively perturb the structure of the kinase domain to ignore ordinary ligand-dependent activation.(Eck & Yun, 2010) The ATP-competitive first-generation 4-anilinoquinazoline-based drugs gefitinib and erlotinib are approved for the treatment of L858R and exon19 del variants and are selective for these activating mutations due to the fortuitously lower ATP affinity for the mutant kinase domain.(Carey et al., 2006; C.-H. Yun et al., 2007) Second-generation inhibitors have emerged based on the 4-anilinoquinazoline scaffold, such as afatinib, that form a covalent bond to the kinase domain through a Michael acceptor that forms an irreversible covalent bond with C797,(Solca et al., 2012) and inactive conformation binding lapatinib.(Moy, Kirkpatrick, Kar, & Goss, 2007) These compounds lack the needed selectivity for EGFR mutants required for clinical efficacy but are approved for other tumors. The emergence of acquired drug resistance to the first-generation 4-anilinoquinazoline drugs through acquisition of the gatekeeper T790M mutation(Kobayashi et al., 2005; C.-H. Yun et al., 2008) led to the development of third-generation anilinoquinazoline-based TKIs with similar C797-targeting Michael acceptors to second-generation compounds, but with marked mutant selectivity.(Oxnard et al., 2016; Zhou, Ercan, Chen, Yun, Li, Capelletti, Cortot, Chirieac, Iacob, Padera, et al., 2009) Of the many third-generation TKIs, AZD9291 (Osimertinib) has received clinical approval for T790M-positive NSCLC and more recently as a first-line therapy.(Soria et al., 2018) Drug resistance as a result of treatment by AZD9291 occurs in part because of the acquisition of a third point mutation (C797S) preventing inhibitors from establishing their potency-conferring covalent bonds.(Niederst et al., 2015; Thress et al., 2015)

To address drug resistance to AZD9291 where no current therapies are available, several drug discovery programs have generated newer fourth-generation inhibitors that target C797S-containing EGFR and are well-positioned for clinical evaluation.(L. Chen, Fu, Zheng, Liu, & Liang, 2017; H.-Y. Zhao, Xi, Xin, & Zhang, 2022) Expectedly, these new inhibitors are structurally divergent from third-generation inhibitors. Additionally, distinct allosteric site targeting inhibitors offer another avenue to target C797S.(D. J. H. De Clercq et al., 2019; Thomas W. Gero et al., 2022; Jia et al., 2016; To et al., 2019) Here, we review the structures and binding modes of these next-generation EGFR inhibitors and show how their

structures produce previously underutilized intermolecular interactions from the previous third-generation inhibitors.

EGFR kinase architecture in active versus inactive conformations.

Understanding inhibitor binding modes and their role in enabling drug selectivity first requires a short discussion of the structural elements of the EGFR kinase domain. Generally, protein kinases are phosphoryltransferase enzymes targeting alcohol-containing amino acid side chains (Ser, Thr, and Tyr) of protein targets. (Taylor, Knighton, Zheng, Ten Eyck, & Sowadski, 1992) This activity is enabled by hydrolysis of the γ -phosphate of ATP and requires Mg^{2+} ions. Specifically, EGFR is a tyrosine kinase (E.C. 2.7.10.1) often involved in autophosphorylation of the long C-terminal tail of other receptor tyrosine kinases (Figure 1). (Hunter, 2009; Lemmon & Schlessinger, 2010) As common in all kinases, the kinase domain in EGFR consists of a conserved ATP-binding site that is located between the N- and C-lobes of the kinase domain. The short peptide linking the lobes is known as the “hinge” (amino acids 790 to 797) and is where the adenosine ring of ATP makes hydrogen bonds (H-bonds) with Q791 and M793. A Thr residue at position 790, known as the “gatekeeper,” is at the N-terminus of the hinge while Cys at 797, a common target of covalent inhibitors, is at the C-terminus. In the inactive conformation, a conserved α -helix (α C-helix, red Figure 2A) is rotated in the “out” position and held in this position by the folded activation loop (A-loop, orange Figure 2A). Activation of EGFR is regulated by extracellular binding of cognate ligands that induce homo- or heterodimerization of kinase domains that induce structural changes within the kinase domain. (Zhang, Gureasko, Shen, Cole, & Kuriyan, 2006) Kinase dimerization leads to inward rotation of the α C-helix and formation of a H-bond between E762 and K745, known as the K-E salt bridge, that is essential for phosphoryl transferase enzymatic activity (Figure 2B). K745 is conserved in all kinases and often referred to as the “catalytic lysine.” (Carrera, Alexandrov, & Roberts, 1993) Another important conserved residue is D855 of the DFG-motif, which is directly involved in H-bonds with ATP and the protein conformation of this element is important in distinguishing certain types of kinase inhibitors. (Müller, Chaikuad, Gray, & Knapp, 2015) While it seems counterintuitive, several new EGFR inhibitors target these active sites conserved residues with noteworthy selectivity for oncogenic mutants compared to wildtype. (See Table1)

Third Generation Inhibitors (ATP competitive)

To best understand the binding modes of C797S-targeting inhibitors, a brief survey of the binding modes of third-generation inhibitors is necessary. Third-generation EGFR TKIs were developed in response to patents acquiring the gatekeeper T790M resistance mutation as a result of treatment with first-generation inhibitors. (L. Chen et al., 2017; Tan et al., 2018) They are all irreversible inhibitors that form a covalent bond with the thiol of C797, and resistance to these TKIs through the C797S point mutation that renders them essentially inactive. (Niederst et al., 2015; Thress et al., 2015) Generally, their chemical structures contain a hinge binding motif, which is the anilino-pyrimidine, and an acrylamide as originally described in the development of WZ4002 (Scheme 1). (Zhou, Ercan, Chen, Yun, Li, Capelletti, Cortot, Chirieac, Iacob, Padera, et al., 2009)

Due to its clinical success, AZD9291 (osimertinib) has been the subject of intense study with the goal of understanding its selectivity for T790M mutant EGFR. (Yosaatmadja et al., 2015) Recent X-ray crystallography and molecular modeling studies have shown that AZD9291 exhibits T790M-selective binding through a previously underappreciated rotation of the *N*-methylindole group producing favorable van der Waals interactions with the T790M mutant methionine residue (Figure 3A). (Xiao- E. Yan et al., 2020) More recently, a structurally-related inhibitor YH25448 (lazertinib) was shown to possess improved medicinal chemistry properties over AZD9291 due to the presence similar van der Waals interactions with T790M and a unique intramolecular H-bond between amine and amide group in addition to an H-bond with the D855 of the DFG motif (Figure 3B). (D.E. Heppner et al., 2022; J. Yun et al., 2019).

One important point of difference that will be made in the following section is that nearly all the reversible binding fourth-generation inhibitors make H-bonding interactions with K745, which are seen in a minority of third-generation inhibitors (Table 1). One example is the phase 1 metabolite of AZD9291 (AZD5104), which is known to have similar potency against WT EGFR and the demethylation of the indole nitrogen leads to a H-bond with D855 (PDB ID 7JXL). (Tyler S. Beyett, To, et al., 2022) Other inhibitors that show H-bonding interactions with K745 include compound 43 (**1**) (related to EGF816) (Gérald Lelais et al., 2016) through a $-CF_3$ group (Figure 3C). Moreover, CO-1686 (Yan, Zhu, Liang, Zhao, Geun Choi, et al., 2017) shows a dipole-dipole interaction through a $-CF_3$ group with K745. (Table 1) While not seen in this active conformation structure, a potential H-bond is possible via the pyrazole of QL-X138 and could be relevant in the inactive (α C-helix out) conformation where the K-E salt bridge is not formed (Figure 3D).

Fourth Generation Inhibitors (ATP competitive)

The emergence of acquired drug resistance to AZD9291, and other third-generation TKIs, through the C797S mutation presented a variety of opportunities for developing novel inhibitors and the earliest examples of reversible-binding ATP-competitive inhibitors emerged serendipitously. (Niederst et al., 2015; Park et al., 2020; Thress et al., 2015) (Table 2) The first series of ATP-competitive inhibitors that exhibited C797S activity were based on a trisubstituted imidazole core. (Günther, Juchum, Kelter, Fiebig, & Laufer, 2016) Optimization yielded imidazole inhibitors that bound reversibly to C797S-containing EGFR kinases with low nanomolar biochemical potencies. (Günther et al., 2016; Günther et al., 2017; Juchum et al., 2017) A series of reversible and irreversible imidazole inhibitors showed comparable H-bond to the catalytic K745 residue only in α C-helix outward inactive crystal structures (Scheme 2, Figure 4A). (David E. Heppner, Günther, Wittlinger, Laufer, & Eck, 2020) These imidazole-K745 H-bonds are observed in addition to H-bonds between K745 and D855. (David E. Heppner et al., 2020) Selective *N*-methylation of the imidazole nitrogens showed that blocking the K745 H-bond was responsible for the C797S-dependent reversible binding properties. This highlights the functional importance of this H-bonding interaction for enabling stronger reversible binding and implies that additional interactions not ordinarily seen in the majority of third-generation TKIs may be the key to enhancing the potency of small-molecule inhibitors against C797S. However, the imidazole-based

inhibitors are most potent against WT EGFR implying that H-bonds with K745 are necessary for tight reversible binding but not mutant selectivity.

Another compound that unexpectedly showed potency against C797S was brigatinib, which was originally described as an ALK inhibitor (Scheme 2, Figure 4B). This compound as well as chemical derivatives showed off-target inhibition of EGFR(del19/T790M/C797S) that was enhanced if combined with the anti-EGFR antibody cetuximab.(S. Li, Zhang, Zhu, Lei, Lai, Peng, Tong, Pang, Lu, Ding, et al., 2022; Uchibori et al., 2017b) Recent structures of brigatinib and variants bound to EGFR revealed an H-bond from the phosphene oxide to K745, which is analogous to the binding mode as seen to K1150 in ALK (Figure 4B).(Floch, Finlay, Bianco, Bickerton, Colclough, Cross, Cuomo, Guerot, Hargreaves, Martin, et al., 2019) Alternative structures based on optimization of brigatinib have been produced and have been shown to be selective for EGFR C797S.(S. Li, Zhang, Zhu, Lei, Lai, Peng, Tong, Pang, Lu, Ding, et al., 2022)

More recent activity in pharmaceutical companies have generated distinct ATP-competitive reversible inhibitors with activity against C797S. At Boehringer Ingelheim, structure-guided design of a macrocyclic benzimidazole inhibitor (BI-4020), related to the third-generation EGF816, shows tight binding to C797S-containing EGFR and interacts with K745 in both active and inactive conformations in through a novel pyrazole moiety (Figure 4C).(Tyler S. Beyett, Jaimin K. Rana, Ilse K. Schaeffner, David E. Heppner, & Michael J. Eck, 2022; Engelhardt et al., 2019) In another case, an extensive drug development campaign by Blueprint Therapeutics led to the development of Compound 24 (**3**) the analogue of BLU-945 based on an isoquinoline scaffold, which exhibits selectivity for C797S (Figure 4E)(Eno et al., 2022). Like other inhibitors for C797S variants, the crystal structures of analogs of Compound 24 (**3**) show that a critical alcohol is positioned toward the K745 residues in the inactive conformation. Additionally, the Chugai Pharmaceutical Company developed a molecule CH7233163 that overcomes AZD9291 resistance and exhibits H-bonds with K745 through a pyrazole similar to BI-4020 in the active kinase conformation (Figure 4D). These inhibitors, as well as those based on brigatinib, are more selective for the mutant EGFR as compared to WT. Furthermore, molecules published prior to the emergence of C797S that have structural resemblance to BLU-945 and analogues, show binding modes that also show H-bonds to K745 but to our knowledge have yet to be assayed for C797S activity (Scheme 2 compounds 41a-b).(Heald, Bowman, Bryan, Burdick, Chan, Chan, Chen, Clausen, Dominguez-Fernandez, Eigenbrot, et al., 2015) Since the imidazole-based molecules are not selective while these scaffolds are, it is difficult to generalize that H-bonds to K745 are needed for mutant selectivity but appear to drive stronger reversible binding. A distinguishing feature of the imidazoles (Figure 4A) versus the other mutant-selective scaffolds (Figure 4B–F) could be variations in interactions near the gatekeeper T790 residue, but further studies are needed to confirm such a structure-activity relationship. Overall, reversible-binding ATP-competitive inhibitors with promise against C797S-containing EGFR are distinct from earlier third-generation TKIs and suggest new mutant-selective molecules can gain needed potency through binding conserved residues.

Fourth-Generation Allosteric Inhibitors (Type 3)

An alternative approach to target C797S and T790M EGFR mutations is to bind a drug molecule within a pocket distinct and distant from the ATP binding site. Such allosteric inhibitors have recently been discovered through a targeted high-throughput screen that selected for molecules with selectivity for the L858R/T790M mutant.(D. J. H. De Clercq et al., 2019; Jia et al., 2016) Two scaffolds identified, a phenyl glycine (triblade)(Jia et al., 2016; To, Beyett, Jang, Feng, Bahcall, Haikala, Shin, Heppner, Rana, Leeper, et al., 2022; To et al., 2019) and dibenzodiazepinone,(D. J. H. De Clercq et al., 2019) have led to drug development efforts including quinazolinones (Thomas W. Gero et al., 2022) and related molecules (Table 3).(Obst-Sander, Ricci, Kuhn, Friess, Koldewey, Kuglstatter, Hewings, Goergler, Steiner, Rueher, et al., 2022) Generally from a variety of X-ray cocrystal structures (Scheme 3, Figure 5A–B), these inhibitors are ATP non-competitive and bind to a site that is made accessible due to the outward α C-helix conformation that is adjacent to the ATP site.(Jia et al., 2016) Inhibitors that bind this pocket are intrinsically mutant selective as the L858R point mutation allows for access to this site via permanent unfolding of the A-loop.(Tyler S. Beyett, To, et al., 2022; Jia et al., 2016) Cocrystal structures containing the L858R mutation, which are underrepresented due to being relatively difficult to crystallize, show H-bonding interactions between the mutant Arg and the 2-isoindolinone of JBJ-09-063 potentially also contributing to L858R selectivity (Figure 5C).(Tyler S. Beyett, To, et al., 2022) Additionally, selectivity for T790M is enabled by thioether-aromatic ring interactions and otherwise insensitive to the C797S mutation due to the relatively long distance from the allosteric pocket to this amino acid side chain.

With respect to interactions with conserved residue side chains, the aminothiazole-containing variants (e.g., EAI001 and related derivatives) all form H-bonds with D855 that bridges the allosteric pocket and H-bonds with ATP (Figure 5A,C). Such an H-bond is not possible with the benzodiazepinone inhibitors (Figure 5B).(D. J. H. De Clercq et al., 2019) Notably, the catalytic K745 does not interact with the allosteric inhibitors but is involved in H-bonds with ATP (AMP-PNP in the crystal structures, Figure 5A–B) in all scaffolds crystallized as well as interacting with L858R that include this mutation (Figure 5C).(To, Beyett, Jang, Feng, Bahcall, Haikala, Shin, Heppner, Rana, Leeper, et al., 2022) Recent cocrystal structures of allosteric inhibitors simultaneously bound with ATP-competitive third generation inhibitors, such as AZD9291 (Figure 5D), show a “swing” of K745 from the ATP side and over to the allosteric inhibitor now H-bonding with the 2-isoindolinone ketone (Figure 5D–E).(Tyler S. Beyett, To, et al., 2022; Niggenaber et al., 2020) Additionally, structural rearrangement of the P-loop F723 into a pi-stack with certain phenyl-containing allosteric inhibitors (e.g., JBJ-04-125-02, JBJ-09-063) are the source of positive cooperativity of ATP-competitive and allosteric inhibitors,(Tyler S. Beyett, To, et al., 2022) which is an important insight given that these inhibitors synergize with TKIs *in vivo*.(To, Beyett, Jang, Feng, Bahcall, Haikala, Shin, Heppner, Rana, Leeper, et al., 2022; To et al., 2019) Both the structural rearrangements of F723 and K745 toward the allosteric pocket indicate that displacement of ATP can lead to binding differences of amino acid side chains toward the allosteric site, which likely impact pharmacological efficacy of these next-generation inhibitors.

Bivalent ATP-allosteric Inhibitors

The proximity of the ATP and allosteric sites has motivated the design of spanning molecules that bind extensively within these sites (Table 3). (Q. Li et al., 2019; Wittlinger et al., 2022) Such spanning inhibitors based on 4-anilinoquinazoline and trisubstituted imidazole ATP-site scaffolds hybridized with groups resembling EAI045 have offered proof-of-concept examples of such molecules, however structurally and functionally much remains unexplored. X-ray cocrystal structures of the imidazole bivalent molecules indicate that these molecules bind to both allosteric and ATP sites with H-bonding interactions with K745 and D855 mostly resembling the parent ATP-site imidazole inhibitor (Figure 6A, cf. Figure 4A). Interestingly, inhibitors based on pyrrolopyrimidine ATP-site scaffolds were designed prior to the discovery of C797S and anchor a urea-cyclohexane moiety within the EGFR allosteric pocket. (Sogabe et al., 2013) Crystal structures show that urea group in the allosteric pocket directs the K745 “swing” toward the allosteric pocket since the ATP site part of this molecule cannot accept H-bonds from K745 (Figure 6B). While few examples have been studied, spanning ATP-allosteric inhibitors do not appear to guarantee mutant-selective. Distinctly the 4-anilinoquinazolines are similarly potent between WT and mutants (Q. Li et al., 2019) and the trisubstituted imidazoles exhibit modest selectivity. (Wittlinger et al., 2022)

Concluding Thoughts

Over the past decades, designing drugs with sufficient specificity against activating mutations in EGFR has been a persistently “moving target”. Throughout, structural biology has remained a critical asset in directing new inhibitor mechanisms (covalent versus reversible binding) as well as the exploitation of unique intermolecular interactions. As the newer generation of ATP-competitive C797S-targeting EGFR inhibitors have been reported, distinct H-bonding interactions with conserved catalytic residues K745 and D855 drive needed potency, although not guaranteeing mutant selectivity. Allosteric inhibitors, by virtue of their distinct binding pocket, are robustly mutant selective and make H-bonding interactions with K745 in circumstances where ATP is replaced with an ATP site TKI. Moreover, simultaneous binding of both allosteric inhibitors and ATP-competitive inhibitors have shown synergize effect against mutant EGFR. Lessons from these examples are valuable given the need for C797S-selective therapies as well as the recent emergence of interest in targeting Lys residues with covalent warheads. (Arafet et al., 2023; P. Chen et al., 2022; Liu, Yue, Tsai, & Shen, 2019; Pettinger, Jones, & Cheeseman, 2017; Reja, Wang, Lyu, Haeffner, & Gao, 2022) Future studies and drug design of spanning ATP-allosteric inhibitors as well as Lys-targeting (covalent and non-covalent) in kinases may offer valuable insights to how producing strong interactions to conserved residues may aid in producing inhibitors with selective binding to activating mutations.

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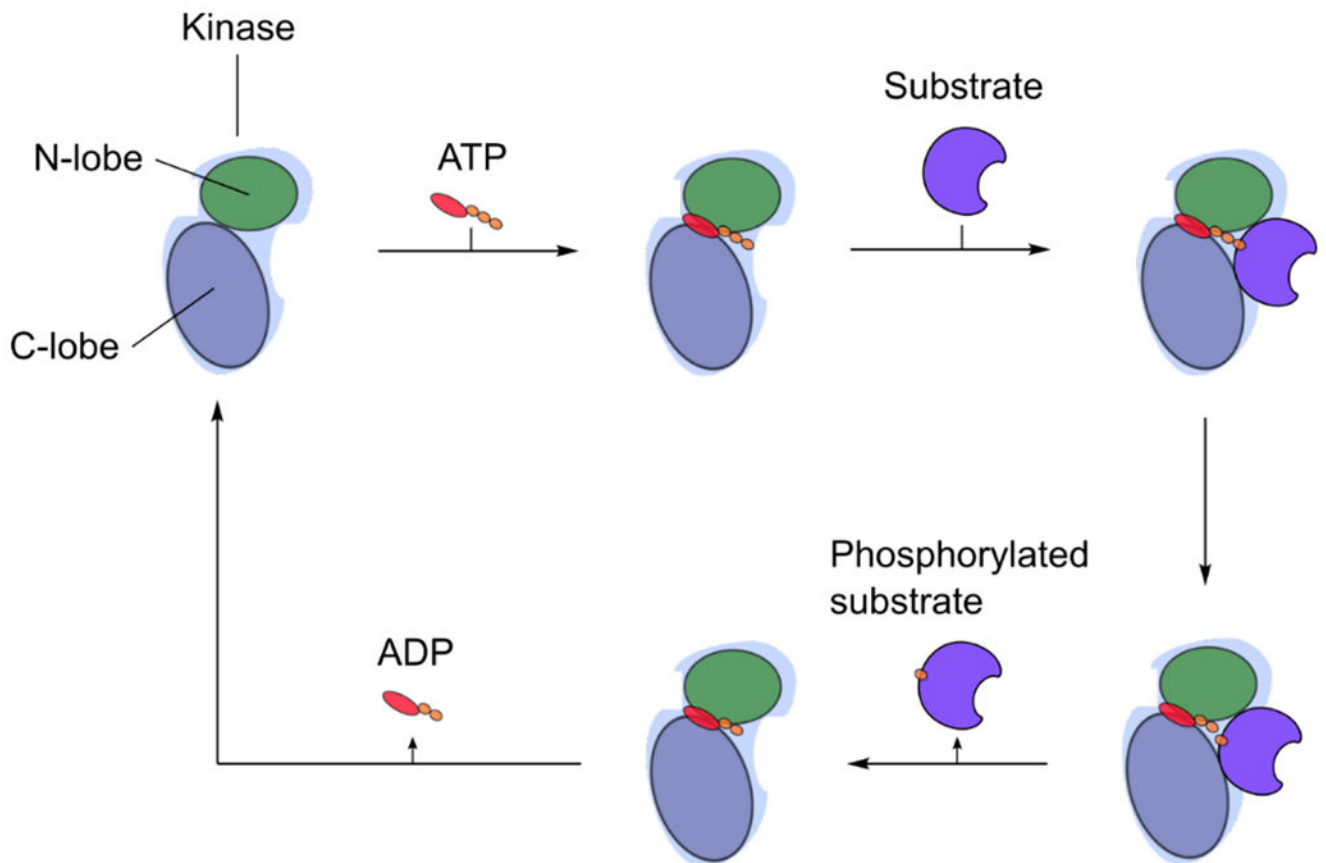


Figure 1. The catalytic cycle of a protein kinase.

ATP binds to the active site of the kinase, which consists of an N-terminal and C-terminal lobe. The γ -phosphate of ATP is hydrolyzed and transferred to the hydroxyl oxygen of a protein target residue side chain (Ser, Thr or Tyr) and concomitant production of ADP.

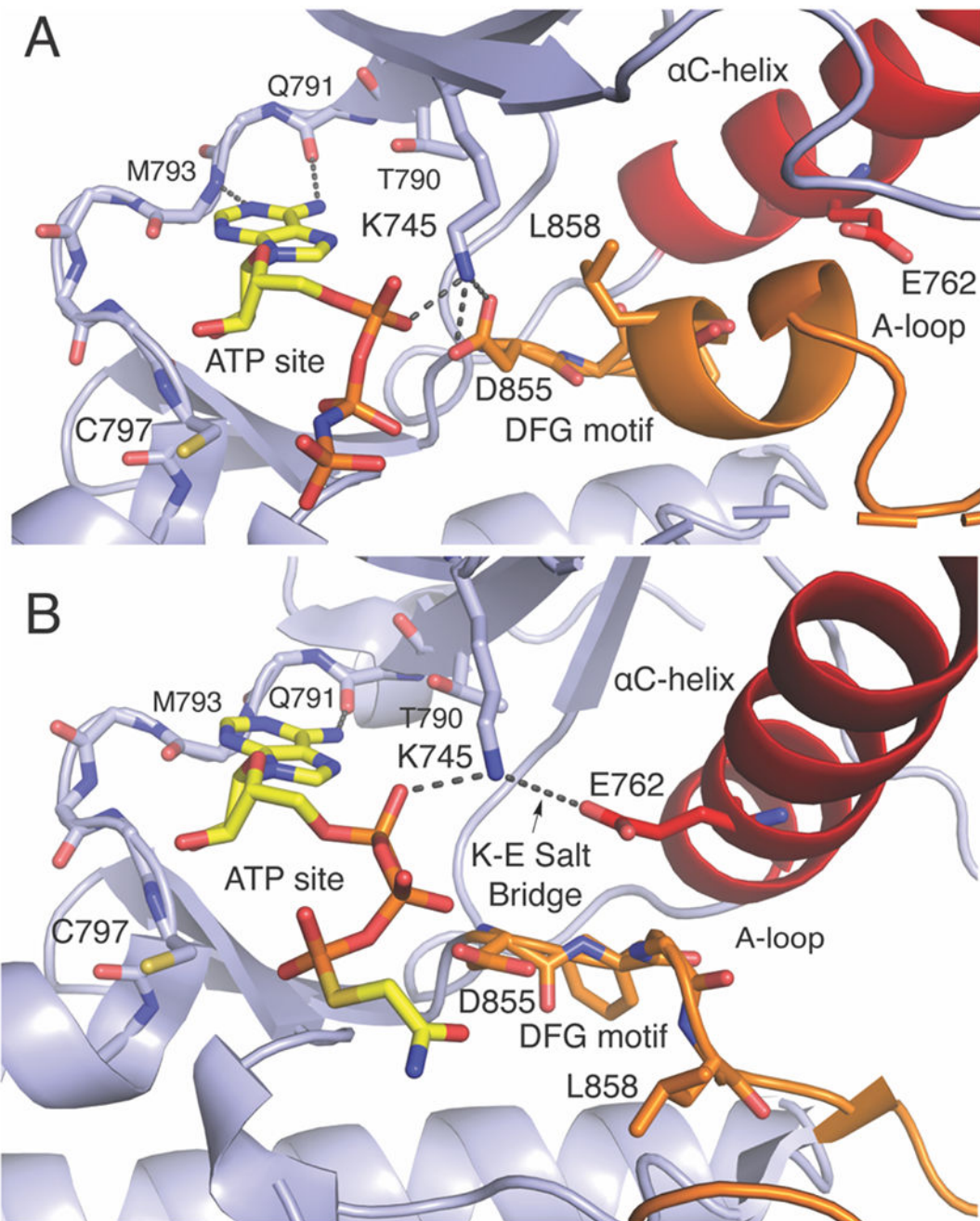


Figure 2. The interplay of residues and binding pockets in the inactive and active EGFR kinase domain.

The EGFR kinase domain in the A) inactive (PDB ID 2GS7, ATP site defined by AMP-PNP is colored yellow) and B) active conformations (PDB ID 2GS6, ATP site defined by an ATP analog-peptide conjugate). Activation loop (A-loop) is colored orange and α C-helix colored red. Some hinge region protein side chains are omitted for clarity

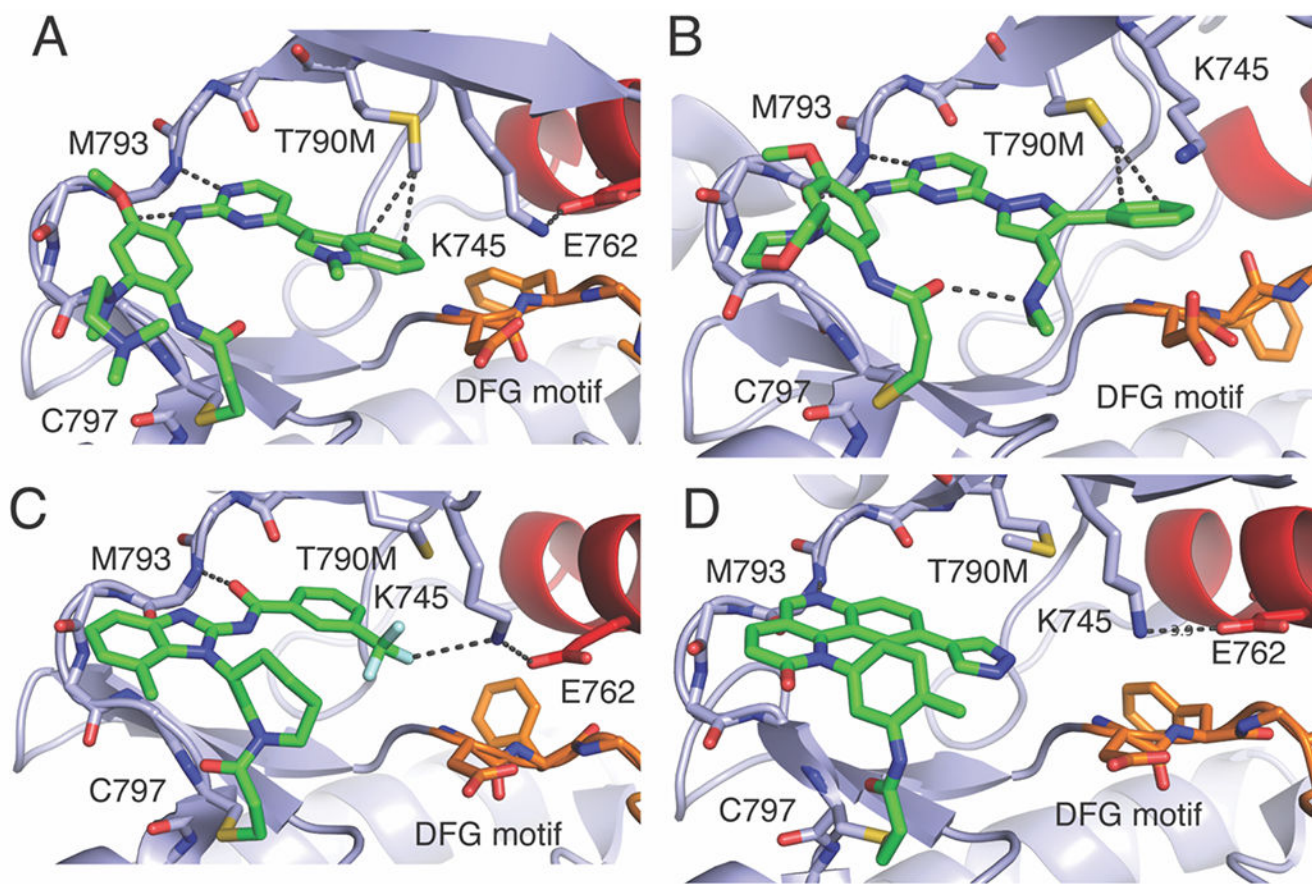


Figure 3. Structures and binding modes of selected third-generation EGFR TKIs.

A) AZD9291 in complex with EGFR(T790M) in the active conformation PDB 6JX0. **B)**

YH25448 in complex with T790M/V948R in the inactive conformation PDB ID 7UKW.

C) Compound 43 (**1**) (Gérald Lelais et al., 2016)(related to EGF816) in complex with EGFR(T790M) active conformation with $-CF_3$ in a dipole-dipole interaction with K745 H-bond PDB ID 5FEE. **D)** QL-X138 in complex with EGFR(T790M) in active conformation with pyrazole directed toward K745 PDB ID 7A6I.

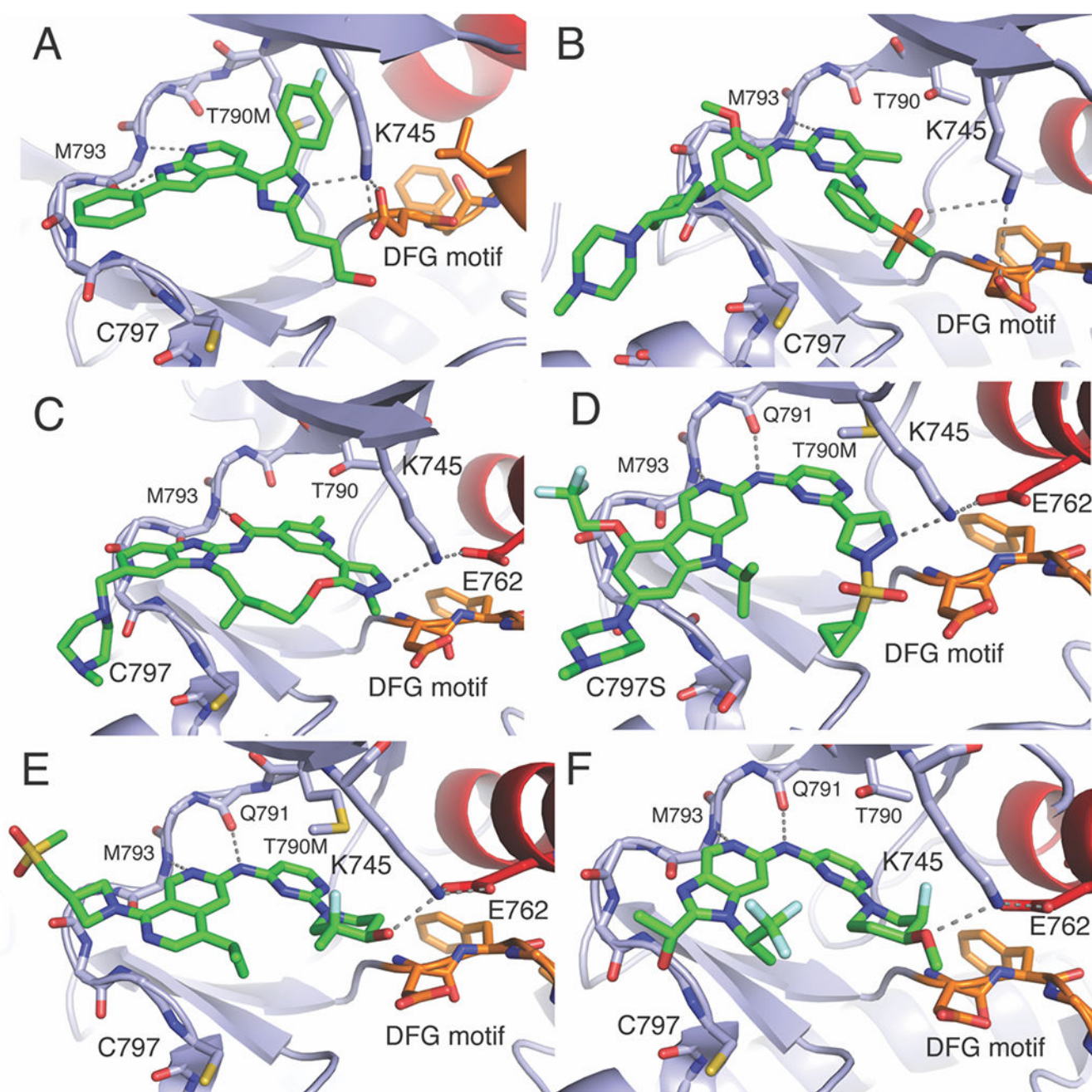


Figure 4. Structures and binding modes of reversible-binding fourth generation EGFR TKIs that inhibit C797S.

A) The reversible-binding trisubstituted imidazole LN2084 in complex with EGFR(T790M/V948R) PDB ID 6V5N **B)** Brigatinib bound to WT EGFR 7AEM. **C)** BI-4020 in complex with WT EGFR PDB ID 7KXZ. **D)** CH7233163 in complex with T790M/C797S PDB ID 6LUB. **E)** Compound 24 (**3**) in complex with T790M EGFR (Note that this analogue contains an alcohol where this group is methylated in BLU-945) PDB ID 8D76). **F)** Compound 41a (**13**) in complex with WT EGFR PDB ID 5CAV.

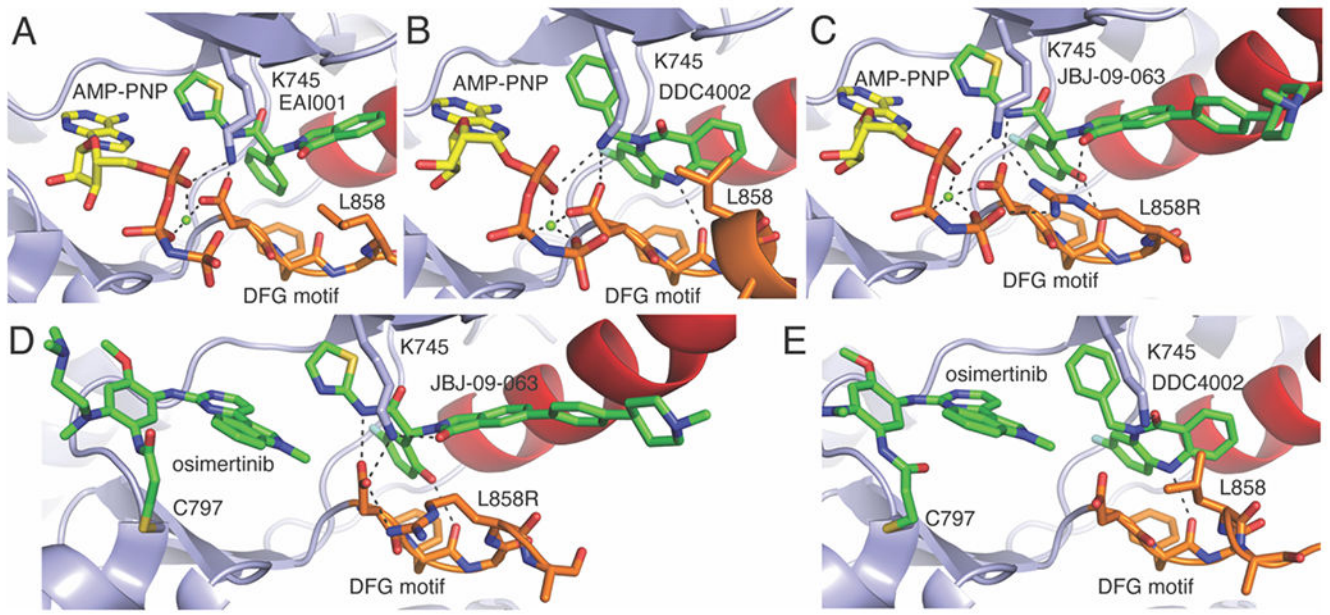


Figure 5. Binding modes of mutant-selective allosteric inhibitors.

A) EAI001 bound in the allosteric pocket adjacent to the ATP site (AMP-PNP is colored yellow) PDB ID 5D41. **B)** DDC4002 bound in the allosteric pocket adjacent to ATP (AMP-PNP) PDB ID 6P1D. **C)** JBJ-09-063 bound in the allosteric pocket showing L858R H-bonding interactions with allosteric inhibitor PDB ID 7K1I. **D)** Cocrystal structure of JBJ-09-063 bound simultaneously with AZD9291 PDB ID 7JXW. **E)** Cocrystal structure of DDC4002 bound simultaneously with AZD9291 PDB ID 6XL4.

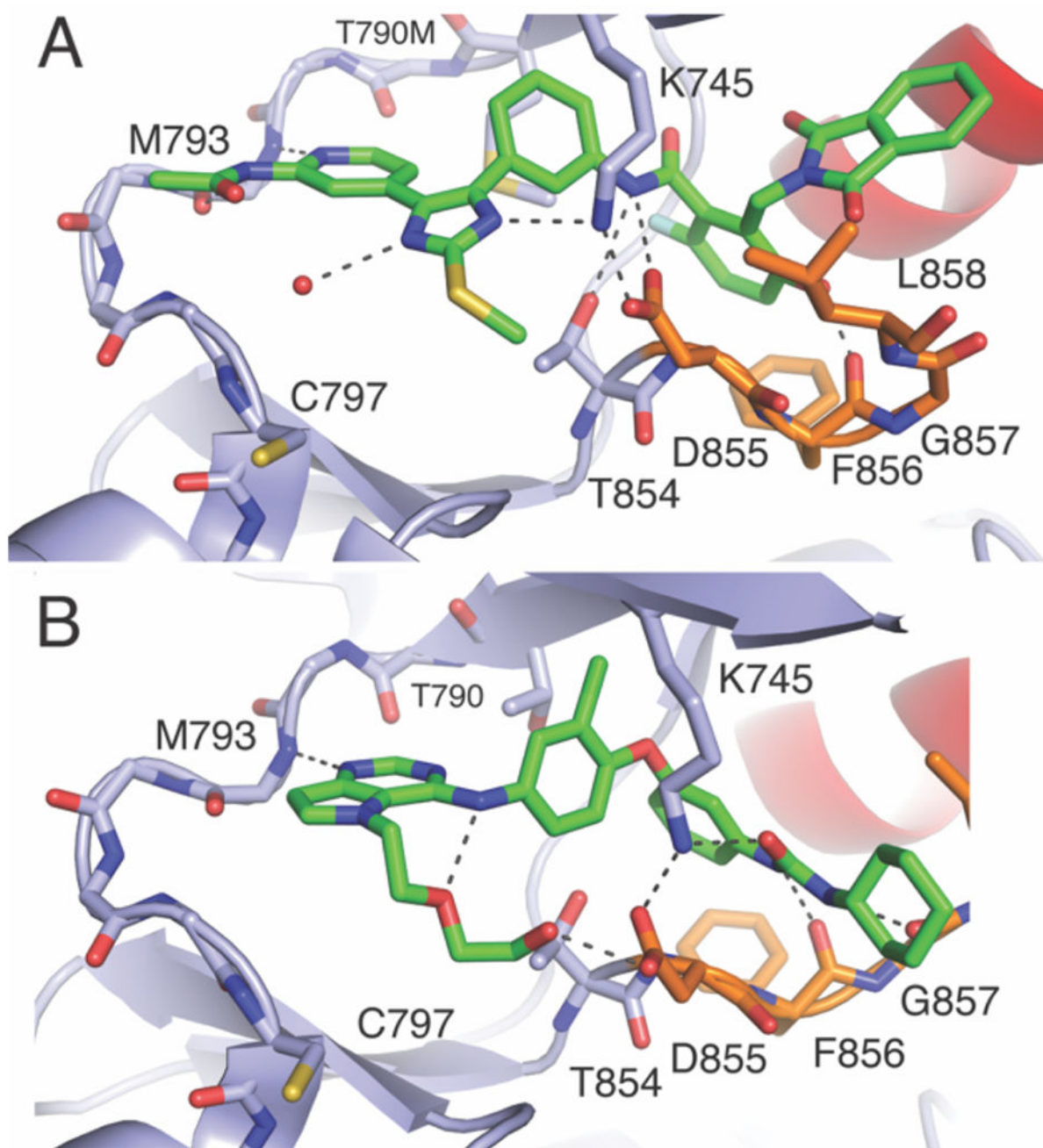
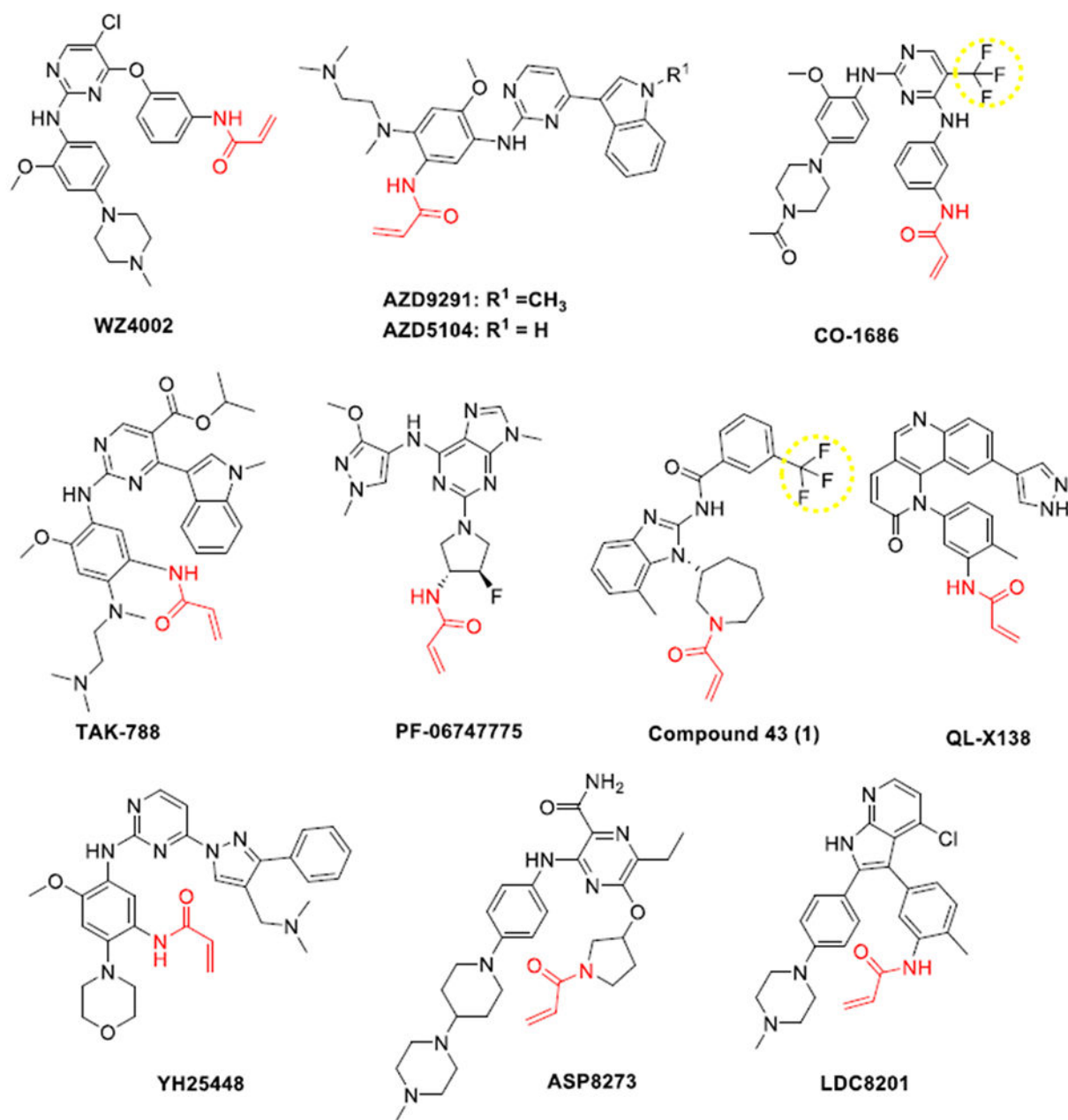
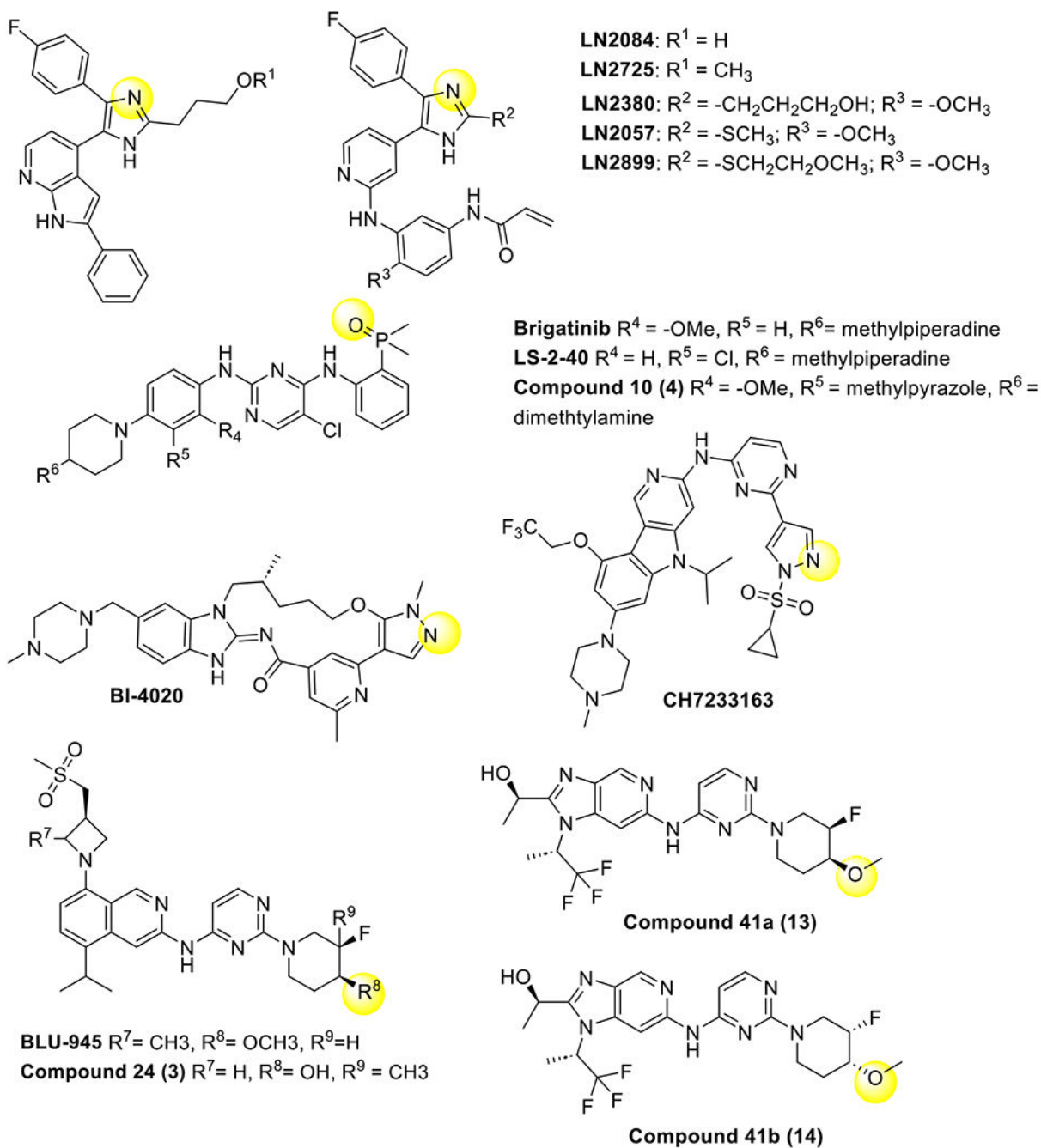


Figure 6. Structurally-characterized spanning inhibitors covalently linking the ATP and allosteric pockets.

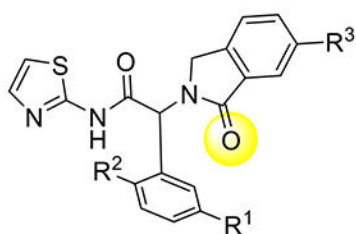
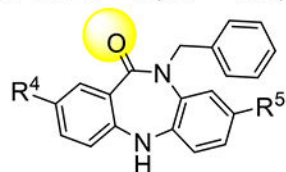
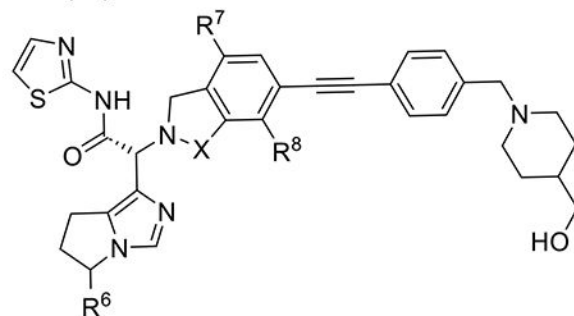
A) Trisubstituted imidazole (PDB ID 6WXN) and **B)** pyrrolopyrimidine (PDB ID 3W2S) ATP-site inhibitors.

**Scheme 1.**

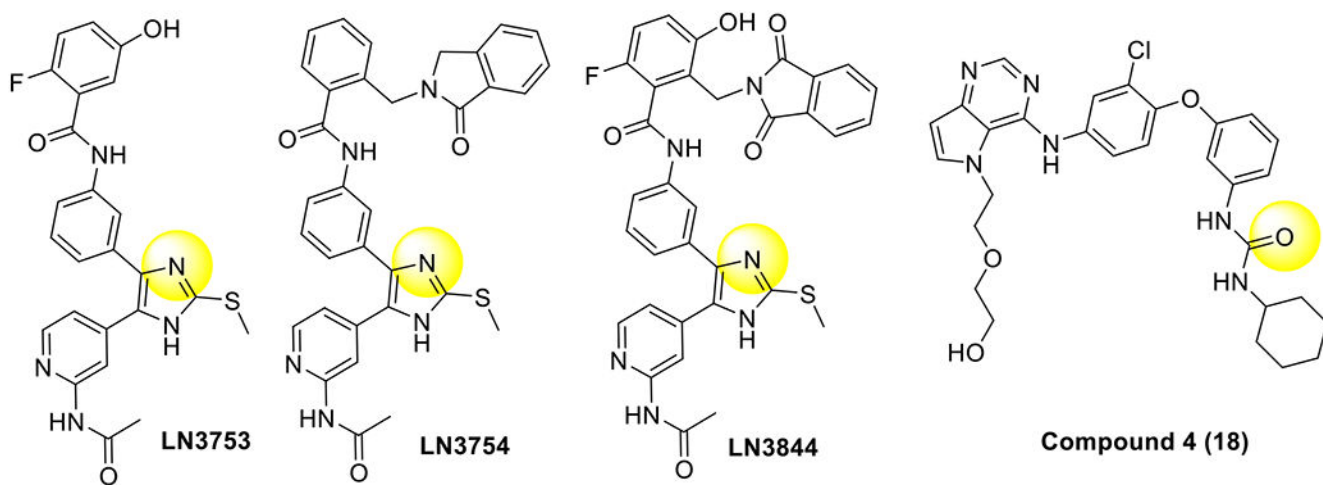
Structurally characterized third-generation EGFR TKIs. Groups that are observed in dipole-dipole interactions with K745 are highlighted with dash yellow circles and the C797-targeting acrylamide warheads are colored red.

**Scheme 2.**

Structurally characterized ATP-competitive fourth-generation EGFR TKIs and related analogues (Table 2). Groups that are engaged in H-bonds with K745 are highlighted with yellow circles.

**Compound 34 (15)****EAI001** $R^1 = H, R^2 = H, R^3 = H$ **EAI045** $R^1 = F, R^2 = OH, R^3 = H$ **JBj-04-125-02** $R^1 = F, R^2 = OH, R^3 =$ **JBj-09-063** $R^1 = F, R^2 = OH, R^3 =$ **EAI002** $R^4 = F, R^5 = H$ **DDC4002** $R^4 = H, R^5 = F$ **Compound 38 (16)** $R^6 = -H, R^7 = -CF_3, R^8 = -H, X = CO$ **Compound 57 (17)** $R^6 = -CH_2CH_2, R^7 = -CHF_2,$
 $R^8 = -CH_3, X = N$ **Scheme 3.**

All structurally characterized allosteric EGFR TKIs and related analogues (Table 3). Groups that form H-bonds with K745 are highlighted with yellow circles.



Scheme 4.
All structurally characterized bivalent EGFR TKIs.

Table 1.

Structurally characterized third-generation EGFR inhibitors targeting Ex19del/L858R/T790M mutations.

	Compound Name ^a	PDB ID	EGFR Kinase Domain α C-helix	Interactions with K745 and D855	Ref
1	AZD9291 (Osimertinib)	6JX4	in	None	(Xiao-E Yan et al., 2020)
2		6JX0	out	None	(Xiao-E Yan et al., 2020)
3		6JWL	in	None	(Xiao-E Yan et al., 2020)
4		6JXT	out	None	(Xiao-E Yan et al., 2020)
5		4ZAU	in	None	(Yosaatmadja et al., 2015)
6	AZD5104 (AZD9291 metabolite)	7JXL	out	Indole H-bond between D855	(Tyler S Beyett, To, et al., 2022)
7	CO-1686 (Rociletinib)	5XDK	in	None	(Yan, Zhu, Liang, Zhao, Choi, et al., 2017)
8		5XDL	in	None	(Yan, Zhu, Liang, Zhao, Choi, et al., 2017)
9		5UWD	out	-CF ₃ dipole-dipole between K745	(Niessen et al., 2017)
10	WZ4002	3IKA	in	None	(Zhou, Ercan, Chen, Yun, Li, Capelletti, Cortot, Chirieac, Iacob, & Padera, 2009)
11	WZ4003	5X2K	in	None	(Zhu et al., 2018)
12	ASP8273 (Naquotinib)	5Y9T	in	None	(Hirano et al., 2018)
13	EGF816 (Nazartinib)	5FEQ	in	None	(Gerald Lelais et al., 2016)
14	Compound 43 (Related to EGF816) (1)	5FEE	in	-CF ₃ dipole-dipole between K745	(Gerald Lelais et al., 2016)
15		5FED	in	None	(Gerald Lelais et al., 2016)
16	PF-06747775 (Mavelertinib)	7JXI	out	None	(Tyler S. Beyett, To, et al., 2022)
17	YH25448 (Lazertinib)	7UKW	out	H-bond between D855	(D. Heppner et al., 2022)
18		7UKV	in	H-bond between D855	(D. Heppner et al., 2022)
19	LDC8201	7A6I	in	None	(Lategahn et al., 2022)
20	TAK-788	7B85	in	None	(Lategahn et al., 2022)
21	QL-X138	4WD5	in	None	<i>To be published.</i>

^aNote that we have listed all compounds according to their generic name or compound code included in the original reference. We have elected to enumerate all of the compounds here that are reported by numeral in the original reference for clarity.

Table 2.

Structurally characterized fourth-generation EGFR inhibitors targeting C797S mutants.

	Compound Name ^a	PDB ID	EGFR Kinase Domain α C-helix	Interactions with K745 and D855	Ref
1	LN2057	6V6K	out	H-bond between imidazole and K745	(David E Heppner, Günther, Wittlinger, Laufer, & Eck, 2020)
2		6VHN	in	none	(David E Heppner et al., 2020)
3	LN2899	6V66	out	H-bond between imidazole and K745	(David E Heppner et al., 2020)
4		6VHP	in	none	(David E Heppner et al., 2020)
5	LN2380	6V6O	out	H-bond between imidazole and K745	(David E Heppner et al., 2020)
6		6VH4	in	none	(David E Heppner et al., 2020)
7	LN2084	6V5N	out	H-bond between imidazole and K745	(David E Heppner et al., 2020)
8	LN2725	6V5P	out	H-bond between imidazole and K745	(David E Heppner et al., 2020)
9	AP-26113 (Brigatinib)	7AEM	in	H-bond between phosphine oxide and K745	(Floch, Finlay, Bianco, Bickerton, Colclough, Cross, Cuomo, Guerot, Hargreaves, & Martin, 2019; Uchibori et al., 2017a)
10	BI-4020	7KY0	out	H-bond between pyrazole oxide and K745	(Tyler S. Beyett, Rana, et al., 2022)
11		7KXZ	in	H-bond between pyrazole oxide and K745	(Tyler S Beyett, Jaimin K Rana, Ilse K Schaeffner, David E Heppner, & Michael J Eck, 2022)
12	Compound 7 (Related to BLU-945) (2)	8D73	In	H-bond between alcohol and K745	(Eno et al., 2022)
13	Compound 24 (Related to BLU-945) (3)	8D76	in	H-bond between alcohol and K745	(Eno et al., 2022)
14	LS_2_40	7ER2	in	H-bond between phosphine oxide and K745	(S. Li, Zhang, Zhu, Lei, Lai, Peng, Tong, Pang, Lu, & Ding, 2022)
15	Compound 10 (4)	7AEI	in	H-bond between phosphine oxide and K745	(Finlay et al., 2021; Floch, Finlay, Bianco, Bickerton, Colclough, Cross, Cuomo, Guerot, Hargreaves, & Martin, 2019)
16	CH7233163	6LUB	in	H-bond between pyrazole and K745	(Kashima et al., 2020)
17	Compound 1 (5)	5C8K	in	H-bond between methoxy and K745	(Heald, Bowman, Bryan, Burdick, Chan, Chan, Chen, Clausen, Dominguez-Fernandez, & Eigenbrot, 2015)
18	Compound 17 (6)	5C8M	in	H-bond between sulfone oxygen and K745	(Heald, Bowman, Bryan, Burdick, Chan, Chan, Chen, Clausen, Dominguez-Fernandez, & Eigenbrot, 2015)
19	Compound 23 (7)	5C8N	in	H-bond between amin and D855, H-bond between methoxy and K745	(Heald, Bowman, Bryan, Burdick, Chan, Chan, Chen, Clausen, Dominguez-Fernandez, & Eigenbrot, 2015)
20	Compound 24 (8)	5CAL	in	H-bond between K745 and E762 in α C-helix	(Heald, Bowman, Bryan, Burdick, Chan, Chan, Chen, Clausen, Dominguez-Fernandez, & Eigenbrot, 2015)

	Compound Name ^a	PDB ID	EGFR Kinase Domain α C-helix	Interactions with K745 and D855	Ref
21	Compound 27 (9)	5CAN	in	H-bond between K745 and E762 in α C-helix in and interact with D855	(Heald, Bowman, Bryan, Burdick, Chan, Chan, Chen, Clausen, Dominguez-Fernandez, & Eigenbrot, 2015)
22	Compound 29 (10)	5CAO	in	H-bond between sulfone oxygen and K745	(Heald, Bowman, Bryan, Burdick, Chan, Chan, Chen, Clausen, Dominguez-Fernandez, & Eigenbrot, 2015)
23	Compound 30 (11)	5CAP	in	H-bond between sulfone oxygen and K745	(Heald, Bowman, Bryan, Burdick, Chan, Chan, Chen, Clausen, Dominguez-Fernandez, & Eigenbrot, 2015)
24	Compound 33 (12)	5CAQ	in	H-bond between methoxy and K745	(Heald, Bowman, Bryan, Burdick, Chan, Chan, Chen, Clausen, Dominguez-Fernandez, & Eigenbrot, 2015)
25	Compound 41a (13)	5CAV	in	H-bond between methoxy and K745	(Heald, Bowman, Bryan, Burdick, Chan, Chan, Chen, Clausen, Dominguez-Fernandez, & Eigenbrot, 2015)
26		5CAS	in	H-bond between methoxy and K745	(Heald, Bowman, Bryan, Burdick, Chan, Chan, Chen, Clausen, Dominguez-Fernandez, & Eigenbrot, 2015)
27	Compound 41b (14)	5CAU	in	H-bond between methoxy and K745	(Heald, Bowman, Bryan, Burdick, Chan, Chan, Chen, Clausen, Dominguez-Fernandez, & Eigenbrot, 2015)

^aNote that we have listed all compounds according to their generic name or compound code included in the original reference. We have elected to enumerate all compounds here that are reported by numeral in the original reference for clarity.

Table 3.

Structurally characterized allosteric and spanning ATP-allosteric hybrid inhibitors effective against L858R, T790M, and C797S mutants

	Compound Name ^a	PDB ID	EGFR Kinase Domain α C-helix	Interactions with K745 and D855	Ref
1	EAI001	5D41	out	H-bond between aminothiazole and D855, H-bond between AMP-PNP and K745	(Jia et al., 2016)
2	EAI002	6P8Q	out	H-bond between carbonyl and K745.	(D. J. H. De Clercq et al., 2019)
3	EAI045	5ZWJ	out	H-bond between aminothiazole and D855, H-bond between isoindolinone and K745	(P. Zhao, Yao, Zhu, Chen, & Yun, 2018)
4		6P1L	out	H-bond between aminothiazole and D855, H-bond between AMP-PNP and K745	(D. J. De Clercq et al., 2019)
5	JBj-09-063	7JXQ	out	H-bond between aminothiazole and D855, H-bond between AMP-PNP and K745	(To, Beyett, Jang, Feng, Bahcall, Haikala, Shin, Heppner, Rana, Leeper, et al., 2022)
6	JBj-09-063	7K1I	out	H-bond between aminothiazole and D855, H-bond between AMP-PNP and K745. H-bond between L858R and K745	(Tyler S Beyett, To, et al., 2022; To, Beyett, Jang, Feng, Bahcall, Haikala, Shin, Heppner, Rana, & Leeper, 2022)
7	JBj-04-125-02	6DUK	out	H-bond between aminothiazole and D855, H-bond between AMP-PNP and K745	(To et al., 2019)
8	DDC4002	6PID	out	H-bond between aminothiazole and D855, H-bond between AMP-PNP and K745	(D. J. H. De Clercq et al., 2019)
9	Compound 34 (15)	7LTX	out	H-bond between NH of amide and D855.	(Thomas W Gero et al., 2022)
10	Compound 38 (16)	8A2B	out	H-bond between aminothiazole and D855. H-bond between inhibitor ketone and K745	(Obst-Sander, Ricci, Kuhn, Friess, Koldewey, Kuglstatler, Hewings, Goergler, Steiner, & Rueher, 2022)
11	Compound 57 (17)	8A2D	out	H-bond between aminothiazole and D855	(Obst-Sander, Ricci, Kuhn, Friess, Koldewey, Kuglstatler, Hewings, Goergler, Steiner, & Rueher, 2022)
Simultaneously Bound ATP Site TKIs and allosteric inhibitors					
12	AZD9291 and EAI045	7JXM	out	H-bond with aminothiazole and D855. H-bond between isoindolinone ketone and K745.	(Tyler S Beyett, To, et al., 2022)
13	AZD9291 and DDC4002	6XL4	out	H-bond between benzodiazepinone ketone and K745.	(Tyler S Beyett, To, et al., 2022)
14	AZD9291 and JBj-04-125-02	7JXP	out	H-bond with aminothiazole and D855. H-bond between isoindolinone ketone and K745.	(Tyler S Beyett, To, et al., 2022)
15	PF-06747775 and JBj-04-125-02	7JXK	out	H-bond with aminothiazole and D855. H-bond between isoindolinone ketone and K745.	(Tyler S Beyett, To, et al., 2022)
16	AZD9291 and JBj-09-063	7JXW	out	H-bond with aminothiazole and D855. H-bond between isoindolinone ketone and K745.	(Tyler S Beyett, To, et al., 2022)
17	ASP8273 and JBj-09-063	7LG8	out	H-bond with aminothiazole and D855. H-bond between isoindolinone ketone and K745.	(Tyler S. Beyett, To, et al., 2022)
18	AZD9291 and JBj-09-063	7K1H	out	H-bond between aminothiazole and D855. H-bond between isoindolinone ketone and K745. H-bond between L858R and K745	(Tyler S Beyett, To, et al., 2022)
19	PF-06747775 and JBj-04-125-02	7JXK	out	H-bond with aminothiazole and D855. H-bond between isoindolinone ketone and K745.	(Tyler S Beyett, To, et al., 2022)

	Compound Name ^a	PDB ID	EGFR Kinase Domain α C-helix	Interactions with K745 and D855	Ref
20	PF-06747775 and EAI001	6Z4D	out	H-bond with aminothiazole and D855. H-bond between isoindolinone ketone and K745.	(Niggenaber et al., 2020)
21	AZD9291 and EAI045	6Z4B	out	H-bond with aminothiazole and D855. H-bond between isoindolinone ketone and K745.	(Niggenaber et al., 2020)
22	Spebrutinib and EAI001	7A2A	out	H-bond with aminothiazole and D855. H-bond between isoindolinone ketone and K745.	(Niggenaber et al., 2020)

^aNote that we have listed all compounds according to their generic name or compound code included in the original reference. We have elected to enumerate all of the compounds here that are reported by numeral in the original reference for clarity.

Table 4.

Structurally characterized of bivalent ATP-allosteric EGFR inhibitors

	Name ^a	PDB ID	EGFR Kinase Domain α C-helix	Interactions with K745 and D855	Ref
1	LN3753 (2a)	6WA2	out	H-bond between imidazole and K745. H-bond between D855 and amide and K745.	(Wittlinger et al., 2022)
2	LN3754	6WAK	out	H-bond between imidazole and K745. H-bond between D855 and amide and K745.	(Wittlinger et al., 2022)
3	LN3844 (2c)	6WXN	out	H-bond between imidazole and K745. H-bond between D855 and amide and K745.	(Wittlinger et al., 2022)
4	Compound 4 (18)	3W2S	out	H-bond between urea and K745.	(Sogabe et al., 2013)

^aNote that we have listed all compounds according to their generic name or compound code included in the original reference. We have elected to enumerate all of the compounds here that are reported by numeral in the original reference for clarity.