Molecular design of a "two-in-one" orthosteric-allosteric chimeric mutant selective EGFR inhibitor

Florian Wittlinger,[a]‡ David E. Heppner,‡[b,g] Ciric To,[c] Marcel Günther,[a] Bo Hee Shin,[c] Jaimin K. Rana,[b] Anna M. Schmoker,[b] Tyler S. Beyett,[b] Pasi A. Jänne,[c,d] Michael J. Eck,[b] and Stefan A. Laufer*[a,e,f]*

[a]Institute for Pharmaceutical Sciences Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 8, 72076 Tübingen
[b]Department of Cancer Biology, Dana-Farber Cancer Institute, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Longwood Center, 360 Longwood Avenue, Boston, MA 02215 (USA)
[c]Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, Department of Medical Oncology, Dana-Farber Cancer Institute, Department of Medicine, Harvard Medical School, Longwood Center, 360 Longwood Avenue, Boston, MA 02215 (USA)
[d]Belfer Center for Applied Cancer Science, Longwood Center, 360 Longwood Avenue, Boston, MA 02215 (USA)
[e]Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", University of Tübingen, 72076 Tübingen, Germany
[f]Tübingen Center for Academic Drug Discovery & Development (TüCAD2), 72076 Tübingen, Germany
[g]Current Address: Department of Chemistry, University at Buffalo, State University of New York, 515 Natural Science Complex, Buffalo, NY 14260-3000

Keywords: EGFR • inhibitor • mutant selective • drug design • antitumor

Abstract

Inhibitors developed to target the epidermal growth factor receptor (EGFR) are an effective therapy for patients with non-small cell lung cancer harbouring drug-sensitive activating mutations in the EGFR kinase domain. Drug resistance due to treatment-acquired mutations within the receptor itself has motivated development of successive generations of inhibitors that bind in the ATP-site, and third-generation agent osimertinib is now a first-line

treatment for this disease. More recently, allosteric inhibitors have been developed to overcome the C797S mutation that confers resistance to osimertinib. In this study, we present the rational structure-guided design and synthesis of a mutant-selective EGFR inhibitor that spans the ATP- and allosteric sites. The lead compound consists of a pyridinyl imidazole scaffold that binds irreversibly in the orthosteric site fused with a benzylisoindolinedione occupying the allosteric site. The compound potently inhibits enzymatic activity in L858R/T790M/C797S mutant EGFR (4.9 nM), with relative sparing of wild-type EGFR (47 nM). Additionally, this compound achieves cetuximab-independent, mutant-selective cellular efficacy on the L858R and L858R/T790M variants.

Introduction

The epidermal growth factor receptor (EGFR) is one of the most investigated receptor tyrosine kinases and its link to non-small cell lung cancer (NSCLC) is well established.[1] However, over 75% of patients succumb to this disease within 5 years after their diagnosis.[2] Tumours driven by activating mutations within the EGFR tyrosine kinase domain, e.g. point-mutation L858R or in-frame exon-19 deletions (ex19del) are initially sensitive to first and second generation EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib, erlotinib, and afatinib,[3,4] but become resistant due to the acquisition of the secondary 'gatekeeper' T790M mutation.[5–9] Third-generation inhibitors overcome T790M-mediated resistance. Osimertinib and other third-generation EGFR TKIs are mutant-selective, and rely on formation of a covalent bond with C797 for their potency.[10,11] Osimertinib was initially approved for treatment of patients whose tumours harboured the T790M resistance mutation, but is now also approved as a front-line therapy in untreated EGFR mutant NSCLC patients.[12] Not surprisingly, patients can

become resistant to osimertinib through the acquisition of the C797S mutation that precludes the ability of the drug to form its essential covalent bond with the receptor.[13]

As an extension of our on-going efforts on inhibitors of p38 MAP kinases, we discovered that trisubstituted imidazole compounds are capable of inhibiting the EGFR kinase.[14–16] Medicinal chemistry optimization aided by molecular docking of these scaffolds allowed for the development of a series of inhibitors that can productively inhibit EGFR and exhibited low nanomolar potency against osimertinib-resistant L858R/T790M/C797S triple-mutant EGFR due to their additional strong noncovalent interactions (e.g. LN2057). Structural characterization of a series of trisubstituted imidazole inhibitors in complex with EGFR indicated that the enhanced reversible binding of these inhibitors is due to intramolecular interactions with the kinase residues K745 and D855 in the αC-helix outward inactive conformation.[17] Although they are potent against L858R/T790M/C797S EGFR, they have limited therapeutic potential because they also potently inhibit wt EGFR. Mutant-selectivity, i.e. relative sparing of wt EGFR, is required for achieving a therapeutic window with EGFR TKIs in NSCLC.[18]

As an alternative to the aforementioned ATP-site inhibitors, highly mutant-selective inhibitors with an allosteric mechanism of action have recently been described.[19,20] These EGFR allosteric inhibitors (EAIs) bind within an allosteric pocket created by the outward displacement of the αC-helix in the inactive conformation of the kinase. Binding of these allosteric agents is antagonized by formation of active EGFR dimers, and the initial allosteric agent EAI045 required co-administration of the dimer-blocking antibody cetuximab for efficacy. A much more potent allosteric inhibitor, JBJ-04-125-02, is effective in vivo without a requirement for cetuximab.[21] Importantly, allosteric inhibitors can bind EGFR simultaneously with certain ATP-site inhibitors,

including osimertinib. As such, JBJ-04-125-02 synergizes in vivo with osimertinib in a potentially cooperative mechanism involving simultaneous binding of both agents.

Considering the adjacency of the orthosteric and allosteric sites (Figure 1) and the potential for development of a more effective therapeutic, we sought to design inhibitors that span both binding pockets. With the goal of developing a compound that was both potent against the T790M and C797S resistance mutations and selective for the mutant over wt EGFR, we designed and synthesized a series of compounds that fused portions of the allosteric inhibitor EAI045 with ATP-site inhibitor LN2057 (Figure 2). Here we describe the structure-guided design, medicinal chemistry optimization, and characterization of these compounds leading to a mutant-selective agent that potently inhibits L858R/T790M/C797S EGFR (4.9 nM) but exhibits relative sparing of the wt kinase (47 nM).

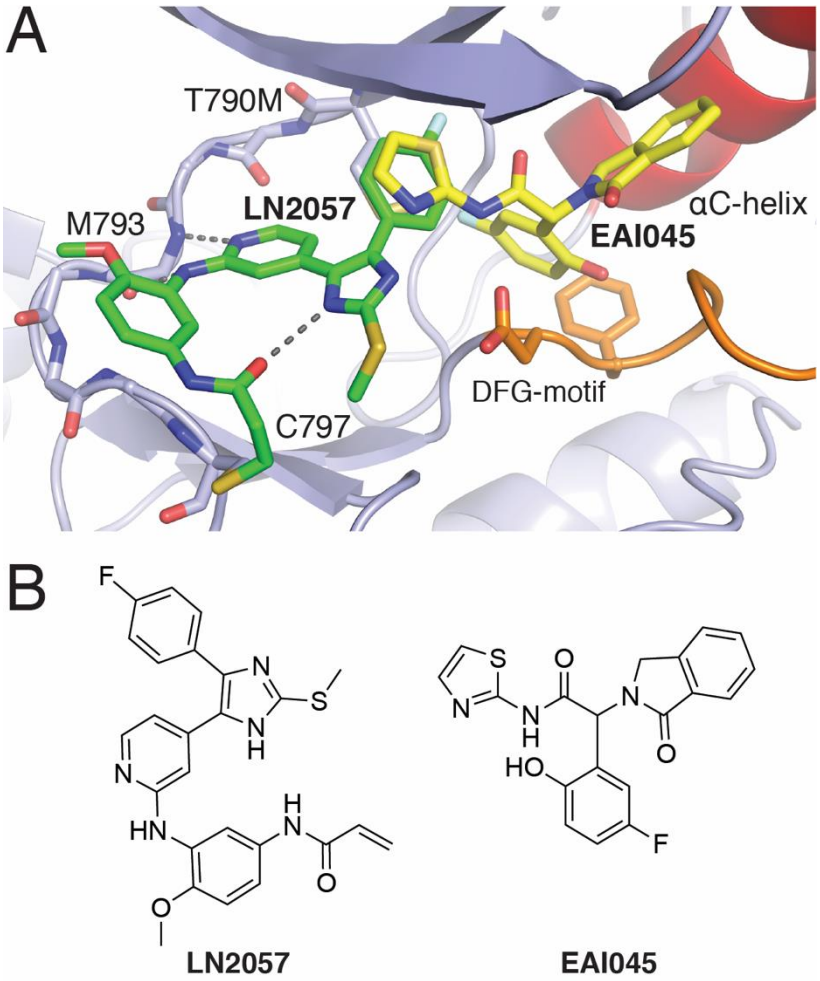

Figure 1: A) Structural superposition of the ATP-site binding LN2057 (PDB code 6V6K) and allosteric inhibitor EAI045 (yellow, PDB code 6P1L) illustrating the overlaying features of the 4-Fluorophenyl of LN2057 with the aminothiazole of EAI045. B) Chemical structures of LN2057 and EAI045

Results

Guided by structural superpositions that showed close correspondence in the binding poses of the 4-fluorphenyl moiety of LN2057 and the thiazole of EAI045 (Figure 1), we prepared a series of compounds fusing successively larger portions of the allosteric inhibitor with the pyridinyl imidazole scaffold (Figure 2). By linking the two scaffolds via the phenyl group of the ATP-site scaffold, rather than the aminothiazole of EAI045, we avoided introducing a chiral center and simplified chemical specifications. Compound **2a** combines the pyridinyl imidazole scaffold with the 2-fluoro-5-hydroxyphenyl moiety of EAI045. In compound **2b**, we installed the

1-oxoisoindolin-2-yl, and in **2c** we introduced instead a 1,3-dioxoisoindolin-2-yl to further probe the structure-activity relationship in the allosteric site. Finally, we incorporated a N-(4-methoxyphenyl) acrylamide to produce compound **1**, a dual-site inhibitor expected to form a covalent bond with C797 (as seen in previous imidazole EGFR inhibitors).[14–16]

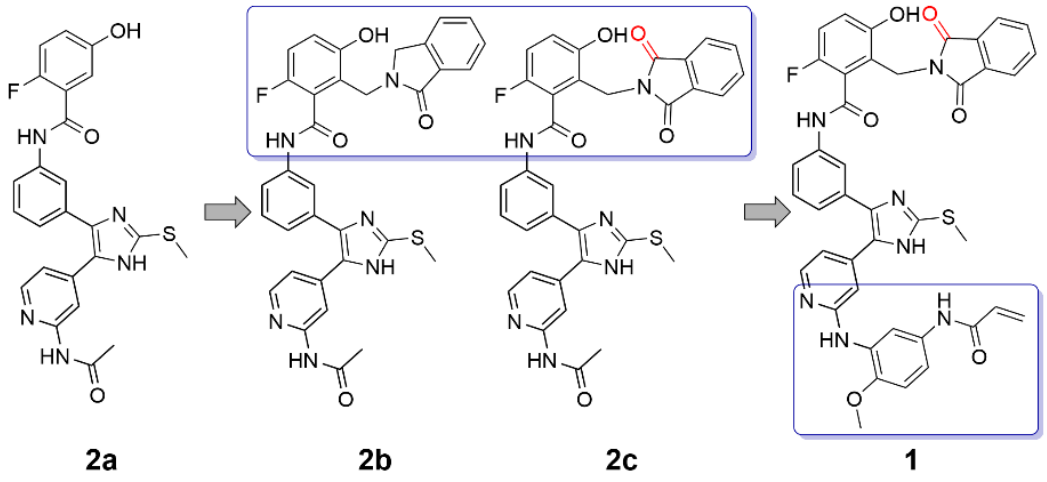

Figure 2: Structural features and the structure-guided design of a novel EGFR inhibitor targeting both ATP and allosteric binding sites.

X-ray crystal structures of **2a**, **24**, and **2c** with EGFR(T790M/V948R) confirmed that these compounds bind as expected (Figure 3, Figure S1). The binding modes of these three compounds are largely comparable, and we focus our discussion on the 1.8 Å resolution structure of **2c** (Figure 3). The pyridinylamide anchors the compound in the ATP site via H-bonds with the kinase hinge (at residue M793). The imidazole is extended and engaged in the expected H-bond with K745 at N3, which is critical for the strong reversible binding of the imidazole scaffold.[17] The phenylamide linkage extending toward the allosteric pocket is positioned directly against the T790M gatekeeper mutation with the amide nitrogen forming hydrogen bonds with the side chains of T854 and D855. The interactions of **2c** in the allosteric pocket are analogous to those of EAI045; the 2-fluoro-5-hydroxyphenyl and 1,3-dioxoisoindolin-2-yl groups closely superimpose with the corresponding regions of EAI045 (Figure 3C).[19,21] To date we have not been able to

obtain co-structures with **1** using an analogous approach, but based on our prior work with trisubstituted imidazole inhibitors [14–16] we do not expect considerable difference in binding modes as compared with **2c**.

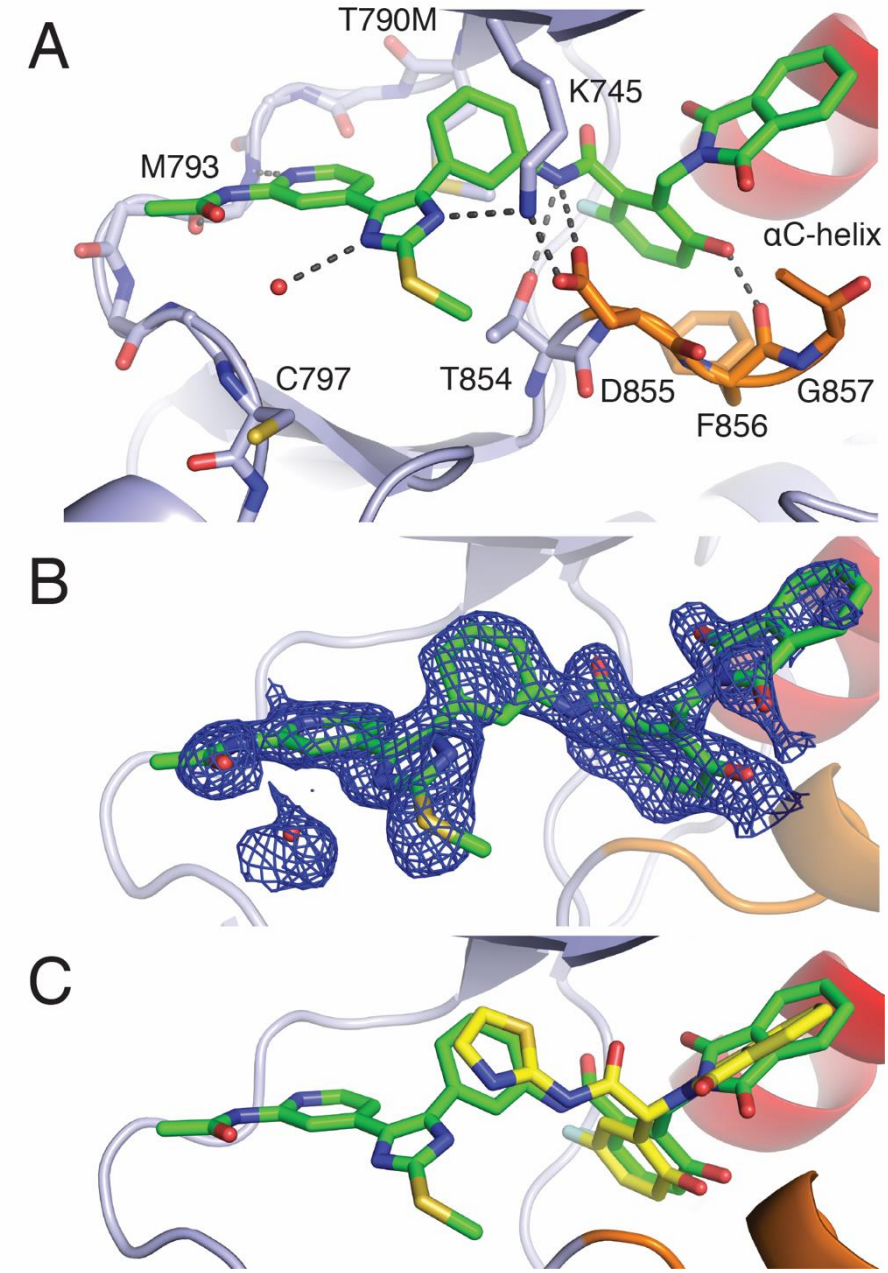

*Figure 3: X-ray crystallographic definition of the binding mode of an ATP-Allosteric chimeric inhibitor. A) Binding mode of **2c** in complex with EGFR(T790M/V948R). The V948R mutation allows for the crystallization of the EGFR kinase domain in the inactive conformation. B) Fo-Fc electron density map generated in PHENIX through molecular replacement from 1.8 Å resolution data collected on EGFR crystals soaked with **2c** and coordinates of an apo EGFR(T790M/V948R) kinase domain. C) Superposition of crystal structures of **2c** (green) with EAI045 (yellow, PDB code 6P1L).*

We assessed inhibitory activity of these compounds against wt EGFR and against the L858R, L858R/T790M and L858R/T790M/C797S mutants using an HTRF-based biochemical assay [Table 1]. **2a** exhibited low nanomolar inhibition across all enzyme variants, indicating that incorporation of the 2-fluoro-5-hydroxyphenyl group alone did not confer mutant-selectivity. The addition of the oxoisoindolin-2-yl moiety in compound **2b** resulted in a decrease in IC_{50}, while exhibiting some potency against the L858R mutant. Introduction of an additional carbonyl in **2c** was found to substantially restored inhibitor potency. Puzzlingly, wt EGFR kinase activity is not fully inhibited by **2c** at concentrations approaching 1000 nM. However, such incomplete inhibition is unique for wt inhibition by **2c** as such effect is not observed in the case of the three mutants (Figure S2) or any other inhibitor featured in Table 1. Furthermore, the inclusion of the acrylamide warhead in **1** resulted in low nanomolar potency for all three EGFR mutants. Though we were not able to obtain a co-crystal structure with **1**, we confirmed formation of the expected covalent bond with C797 using mass spectrometry with the L858R/T790M EGFR (Figure S3). The potency of **1** on wt EGFR (IC_{50} = 47 nM) indicates a moderate degree of mutant-selectivity; it is not clear to us why **1** exhibits mutant-selectivity, while the closely similar reversible compound **2c** does not.

Table 1: Biochemical activities of novel inhibitors on wt and EGFR mutants via FRET-based HTRF assay.

	EGFR IC_{50} [nM][a]			
Compound	wt	LR[d]	LR/TM[d]	LR/TM/CS[d]
1	47 ± 8	2.0 ± 0.5	1.5 ± 0.3	4.9 ± 1.0
2a	6.2 ± 1	5.5 ± 0.3	32 ± 3	8.0 ± 1
2b	> 1000	280 ± 22	> 1000	> 1000
2c	5.8 ± 1.0[c]	1.2 ± 0.4	51 ± 3	32 ± 6
LN2057	6.5 ± 1.0	0.26 ± 0.03	0.27 ± 0.05	130 ± 40
EAI045[b]	> 1000	8.8 ± 0.9	2.0 ± 0.5	13 ± 0.8
AZD9291	17 ± 2	1.5 ± 0.4	0.35 ± 0.04	> 1000

[a] IC_{50} values were measured from a single experiment in triplicate. ATP concentration was 100 μM. Errors are reported as ± standard error. [b] Data from De Clercq and Heppner ACS Med Chem Lett.[20] [c] Value is actually an EC_{50} due to incomplete tyrosine kinase activity inhibition at [**2c**] = 1000 nM. [d] L858R (LR), T790M (TM), C797S (CS).

We next evaluated the anti-proliferative activity of these compounds in Ba/F3 cell lines that are stably infected with wt EGFR, L858R, L858R/T790M and L858R/T790M/C797S EGFR mutants [Table 2]. Our previous studies on allosteric EGFR inhibitors have indicated that Ba/F3, and related cell line models, are potentially resistant to EAIs due to EGFR dimerization, but can be re-sensitized by co-administration of the anti-EGFR antibody, cetuximab (see EAI045 in [Table 2]).[19,21] Therefore, we compared the cellular activity of our inhibitors in the presence and absence of cetuximab. Overall, the reversible inhibitors did not inhibit cell proliferation at concentrations below 10 μM irrespective of cetuximab treatment. However, compound **1** did show anti-proliferative effects in L858R and L858R/T790M mutant cell line with micromolar-level IC_{50} values both with and without cetuximab treatment. The lack of cetuximab sensitivity of **1** likely stems from its irreversible binding mode, which may allow it to overcome formation of the asymmetric dimer interaction of the kinase domain by forcing the outward, inactive conformation of the αC-helix.

Table 2: Inhibitory activities on proliferation of Ba/F3 cell lines of wt EGFR and selected mutants [nM]. Measured without and (with) dimerization inhibiting antibody cetuximab.

	EGFR IC_{50} [nM][a]			
Compound	wt	LR	LR/TM	LR/TM/CS
1	$>1 \times 10^4$ (3700 ± 400)	1200 ± 70 (1100 ± 100)	4400 ± 500 (3600 ± 300)	$>1 \times 10^4$ ($>1 \times 10^4$)
2a	$>1 \times 10^4$ (800 ± 100)	$>1 \times 10^4$ ($>1 \times 10^4$)	$>1 \times 10^4$ ($>1 \times 10^4$)	$>1 \times 10^4$ ($>1 \times 10^4$)
2b	$>1 \times 10^4$ ($>1 \times 10^4$)	$>1 \times 10^4$ ($>1 \times 10^4$)	$>1 \times 10^4$ ($>1 \times 10^4$)	$>1 \times 10^4$ ($>1 \times 10^4$)
2c	$>1 \times 10^4$ ($>1 \times 10^4$)	$>1 \times 10^4$ ($>1 \times 10^4$)	$>1 \times 10^4$ ($>1 \times 10^4$)	$>1 \times 10^4$ ($>1 \times 10^4$)
LN2057	20 ± 6 (2.3 ± 0.6)	< 1[d] (< 1)[d]	22 ± 1 (3 ± 0.5)	1600 ± 200 (780 ± 200)
EAI045[b]*	$>1 \times 10^4$ ($>1 \times 10^4$)	$>1 \times 10^4$ (840 ± 700)	$>1 \times 10^4$ (470 ± 200)	$> 1 \times 10^4$ (250 ± 200)
AZD9291	110 ± 40 (16 ± 4)	3.3 ± 0.6 (3 ± 0)	8 ± 0 (2 ± 0)	1200 ± 130 (800 ± 200)

[a] IC_{50} values are averages of at least three independent experiments with each experiment performed in triplicate. Errors are reported as ± standard deviation. [b] Data from De Clercq and Heppner ACS Med Chem Lett.[20] [c] L858R (LR), T790M (TM), C797S (CS). [d] values below the resolution limit of the assay.

Discussion

Certain first and second-generation EGFR/ErbB2 inhibitors (e.g. lapatinib and neratinib, respectively) also span the orthosteric and allosteric sites, and bind the αC-helix out conformation of the kinase (Figure S4). However, these compounds are not clinically effective against the L858R/T790M or L858R/T790M/C797S variants due to their lack of mutant-selectivity.

Also, most recently growing interest in the development of hybrid compounds for ErbB-family can be observed in literature.[22–24] Examples of the combination of conventional amino quinazoline scaffold plus EAI045-like motifs with reversible binding modus for EGFR showed similar behaviour to our study. While high potency for L858R/T790M/C797S mutation is achieved, they lack efficient selectivity against wt enzyme. Unfortunately, no x-ray crystal of

these structures in EGFR are available.[23,25] A study for targeting a specific ErbB2 mutation with covalent inhibitors fused pyrollopyrimidine-based scaffolds with a diverse set of moieties (including isoindolinone and phthalimide derivatives), which were meant to occupy the back-pocket. Owing to the isostructural position of the cysteine in EGFR and ErbB2 and their high conservation in general, biochemical evaluation of EGFR enzymes was conducted. Yet again, mutant selectivity is not exhibited in these examples. Crystal structures display that these compounds do not reach as far into the back-pocket (Figure S5), what potentially could explain differences to our approach.[24]

While **1** is potent and selective in biochemical assays, this selectivity was achieved at a high cost with respect to molecular weight and number of hydrogen bond donors and acceptors. These medicinal chemistry liabilities limit its cellular potency, and likely explain its lack of cellular efficacy in the context of L858R/T790M/C797S EGFR, with which it cannot bind irreversibly. We note that the increased molecular weight and extensive interactions of our dual-site compounds does not confer higher potency than that achieved with prior ATP-site or allosteric-site inhibitors. The reason(s) for this are unclear, but it could stem from many factors including non-ideal binding geometries of the orthosteric and allosteric portions of our fused inhibitors or constrained access of these large compounds to bind the dual site. Our preparation and characterization of **1** shows that mutant-selectivity can be achieved with an EGFR inhibitor that spans the orthosteric and allosteric sites, but further work will be required to achieve sufficient cellular potency, in particular on the highly resistant L858R/T790M/C797S triple mutant. We are also keenly interested in alternative approaches, including development of customized “pairs” of ATP- and allosteric site inhibitors that bind with high selectivity and cooperativity to mutant EGFR.

Conclusions

In summary, we have rationally designed and synthesized a mutant-selective EGFR inhibitor that simultaneously binds to the EGFR kinase ATP- and allosteric sites as characterized with X-ray crystallography. We reveal that the introduction of structural elements of an ATP-site inhibitor that associate with the allosteric site direct mutant-selectivity of these compounds, and the incorporation of an acrylamide C797-targeting warhead enables anti-proliferative effects in Ba/F3 cell line models. Unlike allosteric site inhibitor EAI045, our lead compound is capable of inducing anti-proliferative activity of the L858R and L858R/T790M mutants in Ba/F3 cells independent of co-administration of cetuximab. Future directions will involve structure-guided medicinal chemistry optimization of this lead compound to improve its cellular activity.

Author Information

Corresponding Authors

Stefan A. Laufer: stefan.laufer@uni-tuebingen.de

Michael J. Eck: eck@crystal.harvard.edu

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

Acknowledgment

This work was supported by the National Institutes of Health grants R01CA201049 and R01CA116020 (to M.J.E), R35CA220497 (to P.A.J.), and postdoctoral fellowship F32CA247198 (to T.S.B). S.L. and iFIT are funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy - EXC 2180 –

390900677. TüCAD2 is funded by the Federal Ministry of Education and Research (BMBF) and the Baden-Württemberg Ministry of Science as part of the Excellence Strategy of the German Federal and State Governments. This work is based upon research conducted at the Northeastern Collaborative Access Team beamlines, which are funded by the National Institute of General Medical Sciences from the National Institutes of Health (P30 GM124165). The Eiger 16M detector on 24-ID-E is funded by a NIH-ORIP HEI grant (S10OD021527). This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357. We thank Kristine Schmidt for the language proof-reading.

References

(1) Russo, A.; Franchina, T.; Ricciardi, G. R. R.; Picone, A.; Ferraro, G.; Zanghì, M.; Toscano, G.; Giordano, A.; Adamo, V. A decade of EGFR inhibition in EGFR-mutated non small cell lung cancer (NSCLC): Old successes and future perspectives. *Oncotarget* **2015**, *6* (29), 26814–26825. DOI: 10.18632/oncotarget.4254.

(2) Howlader N, Noone AM, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds. *SEER Cancer Statistics Review, 1975-2017*. https://seer.cancer.gov/csr/1975_2017/.

(3) Lynch, T. J.; Bell, D. W.; Sordella, R.; Gurubhagavatula, S.; Okimoto, R. A.; Brannigan, B. W.; Harris, P. L.; Haserlat, S. M.; Supko, J. G.; Haluska, F. G.; Louis, D. N.; Christiani, D. C.; Settleman, J.; Haber, D. A. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *The New England journal of medicine* **2004**, *350* (21), 2129–2139. DOI: 10.1056/NEJMoa040938.

(4) Paez, J. G.; Jänne, P. A.; Lee, J. C.; Tracy, S.; Greulich, H.; Gabriel, S.; Herman, P.; Kaye, F. J.; Lindeman, N.; Boggon, T. J.; Naoki, K.; Sasaki, H.; Fujii, Y.; Eck, M. J.; Sellers, W. R.; Johnson, B. E.; Meyerson, M. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science (New York, N.Y.)* **2004**, *304* (5676), 1497–1500. DOI: 10.1126/science.1099314.

(5) Gazdar, A. F. Activating and resistance mutations of EGFR in non-small-cell lung cancer:Role in clinical response to EGFR tyrosine kinase inhibitors. *Oncogene* **2009**, *28 Suppl 1*, S24-31. DOI: 10.1038/onc.2009.198.

(6) Yun, C.-H.; Mengwasser, K. E.; Toms, A. V.; Woo, M. S.; Greulich, H.; Wong, K.-K.; Meyerson, M.; Eck, M. J. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proceedings of the National Academy of Sciences of the United States of America* **2008**, *105* (6), 2070–2075. DOI: 10.1073/pnas.0709662105.

(7) Kobayashi, S.; Boggon, T. J.; Dayaram, T.; Jänne, P. A.; Kocher, O.; Meyerson, M.; Johnson, B. E.; Eck, M. J.; Tenen, D. G.; Halmos, B. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *The New England journal of medicine* **2005**, *352* (8), 786–792. DOI: 10.1056/NEJMoa044238.

(8) Pao, W.; Miller, V. A.; Politi, K. A.; Riely, G. J.; Somwar, R.; Zakowski, M. F.; Kris, M. G.; Varmus, H. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS medicine* **2005**, *2* (3), e73. DOI: 10.1371/journal.pmed.0020073.

(9) Yu, H. A.; Arcila, M. E.; Rekhtman, N.; Sima, C. S.; Zakowski, M. F.; Pao, W.; Kris, M. G.; Miller, V. A.; Ladanyi, M.; Riely, G. J. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2013**, *19* (8), 2240–2247. DOI: 10.1158/1078-0432.CCR-12-2246.

(10) Kwak, E. L.; Sordella, R.; Bell, D. W.; Godin-Heymann, N.; Okimoto, R. A.; Brannigan, B. W.; Harris, P. L.; Driscoll, D. R.; Fidias, P.; Lynch, T. J.; Rabindran, S. K.; McGinnis, J. P.; Wissner, A.; Sharma, S. V.; Isselbacher, K. J.; Settleman, J.; Haber, D. A. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proceedings of the National Academy of Sciences of the United States of America* **2005**, *102* (21), 7665–7670. DOI: 10.1073/pnas.0502860102.

(11) Cross, D. A. E.; Ashton, S. E.; Ghiorghiu, S.; Eberlein, C.; Nebhan, C. A.; Spitzler, P. J.; Orme, J. P.; Finlay, M. R. V.; Ward, R. A.; Mellor, M. J.; Hughes, G.; Rahi, A.; Jacobs, V. N.; Red Brewer, M.; Ichihara, E.; Sun, J.; Jin, H.; Ballard, P.; Al-Kadhimi, K.; Rowlinson, R.; Klinowska, T.; Richmond, G. H. P.; Cantarini, M.; Kim, D.-W.; Ranson, M. R.; Pao, W. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer discovery* **2014**, *4* (9), 1046–1061. DOI: 10.1158/2159-8290.CD-14-0337.

(12) Soria, J.-C.; Ohe, Y.; Vansteenkiste, J.; Reungwetwattana, T.; Chewaskulyong, B.; Lee, K. H.; Dechaphunkul, A.; Imamura, F.; Nogami, N.; Kurata, T.; Okamoto, I.; Zhou, C.; Cho, B. C.; Cheng, Y.; Cho, E. K.; Voon, P. J.; Planchard, D.; Su, W.-C.; Gray, J. E.; Lee, S.-M.; Hodge, R.; Marotti, M.; Rukazenkov, Y.; Ramalingam, S. S. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. *The New England journal of medicine* **2018**, *378* (2), 113–125. DOI: 10.1056/NEJMoa1713137.

(13) Thress, K. S.; Paweletz, C. P.; Felip, E.; Cho, B. C.; Stetson, D.; Dougherty, B.; Lai, Z.; Markovets, A.; Vivancos, A.; Kuang, Y.; Ercan, D.; Matthews, S. E.; Cantarini, M.; Barrett, J. C.; Jänne, P. A.; Oxnard, G. R. Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. *Nature medicine* **2015**, *21* (6), 560–562. DOI: 10.1038/nm.3854.

(14) Günther, M.; Juchum, M.; Kelter, G.; Fiebig, H.; Laufer, S. Lung Cancer: EGFR Inhibitors with Low Nanomolar Activity against a Therapy-Resistant L858R/T790M/C797S Mutant. *Angewandte Chemie (International ed. in English)* **2016**, *55* (36), 10890–10894. DOI: 10.1002/anie.201603736.

(15) Günther, M.; Lategahn, J.; Juchum, M.; Döring, E.; Keul, M.; Engel, J.; Tumbrink, H. L.; Rauh, D.; Laufer, S. Trisubstituted Pyridinylimidazoles as Potent Inhibitors of the Clinically Resistant L858R/T790M/C797S EGFR Mutant: Targeting of Both Hydrophobic Regions and the Phosphate Binding Site. *J. Med. Chem.* **2017**, *60* (13), 5613–5637. DOI: 10.1021/acs.jmedchem.7b00316.

(16) Juchum, M.; Günther, M.; Döring, E.; Sievers-Engler, A.; Lämmerhofer, M.; Laufer, S. Trisubstituted Imidazoles with a Rigidized Hinge Binding Motif Act As Single Digit nM Inhibitors of Clinically Relevant EGFR L858R/T790M and L858R/T790M/C797S Mutants: An Example of Target Hopping. *Journal of medicinal chemistry* **2017**, *60* (11), 4636–4656. DOI: 10.1021/acs.jmedchem.7b00178.

(17) Heppner, D. E.; Günther, M.; Wittlinger, F.; Laufer, S. A.; Eck, M. J. Structural Basis for EGFR Mutant Inhibition by Trisubstituted Imidazole Inhibitors. *J. Med. Chem.* **2020**, *63* (8), 4293–4305. DOI: 10.1021/acs.jmedchem.0c00200.

(18) Chen, L.; Fu, W.; Zheng, L.; Liu, Z.; Liang, G. Recent Progress of Small-Molecule Epidermal Growth Factor Receptor (EGFR) Inhibitors against C797S Resistance in Non-Small-Cell Lung Cancer. *Journal of medicinal chemistry* **2018**, *61* (10), 4290–4300. DOI: 10.1021/acs.jmedchem.7b01310.

(19) Jia, Y.; Yun, C.-H.; Park, E.; Ercan, D.; Manuia, M.; Juarez, J.; Xu, C.; Rhee, K.; Chen, T.; Zhang, H.; Palakurthi, S.; Jang, J.; Lelais, G.; DiDonato, M.; Bursulaya, B.; Michellys, P.-Y.; Epple, R.; Marsilje, T. H.; McNeill, M.; Lu, W.; Harris, J.; Bender, S.; Wong, K.-K.; Jänne, P. A.; Eck, M. J. Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. *Nature* **2016**, *534* (7605), 129–132. DOI: 10.1038/nature17960.

(20) Clercq, D. J. H. de; Heppner, D. E.; To, C.; Jang, J.; Park, E.; Yun, C.-H.; Mushajiang, M.; Shin, B. H.; Gero, T. W.; Scott, D. A.; Jänne, P. A.; Eck, M. J.; Gray, N. S. Discovery and Optimization of Dibenzodiazepinones as Allosteric Mutant-Selective EGFR Inhibitors. *ACS medicinal chemistry letters* **2019**, *10* (11), 1549–1553. DOI: 10.1021/acsmedchemlett.9b00381.

(21) To, C.; Jang, J.; Chen, T.; Park, E.; Mushajiang, M.; De Clercq, Dries J H; Xu, M.; Wang, S.; Cameron, M. D.; Heppner, D. E.; Shin, B. H.; Gero, T. W.; Yang, A.; Dahlberg, S. E.; Wong, K.-K.; Eck, M. J.; Gray, N. S.; Jänne, P. A. Single and Dual Targeting of Mutant EGFR with an Allosteric Inhibitor. *Cancer discovery* **2019**, *9* (7), 926–943. DOI: 10.1158/2159-8290.CD-18-0903.

(22) Niggenaber, J.; Heyden, L.; Grabe, T.; Müller, M. P.; Lategahn, J.; Rauh, D. Complex Crystal Structures of EGFR with Third-Generation Kinase Inhibitors and Simultaneously Bound Allosteric Ligands. *ACS Med. Chem. Lett.* **2020.** DOI: 10.1021/acsmedchemlett.0c00472.

(23) Li, Q.; Zhang, T.; Li, S.; Tong, L.; Li, J.; Su, Z.; Feng, F.; Sun, D.; Tong, Y.; Wang, X.; Zhao, Z.; Zhu, L.; Ding, J.; Li, H.; Xie, H.; Xu, Y. Discovery of Potent and Noncovalent Reversible EGFR Kinase Inhibitors of EGFRL858R/T790M/C797S. *ACS medicinal chemistry letters* **2019**, *10* (6), 869–873. DOI: 10.1021/acsmedchemlett.8b00564.

(24) Lategahn, J.; Hardick, J.; Grabe, T.; Niggenaber, J.; Jeyakumar, K.; Keul, M.; Tumbrink, H. L.; Becker, C.; Hodson, L.; Kirschner, T.; Klövekorn, P.; Ketzer, J.; Baumann, M.; Terheyden, S.; Unger, A.; Weisner, J.; Müller, M. P.; van Otterlo, W. A. L.; Bauer, S.; Rauh, D. Targeting Her2-insYVMA with Covalent Inhibitors-A Focused Compound Screening and Structure-Based Design Approach. *Journal of medicinal chemistry* **2020**, *63* (20), 11725–11755. DOI: 10.1021/acs.jmedchem.0c00870.

(25) Akher, F. B.; Farrokhzadeh, A.; Ravenscroft, N.; Kuttel, M. M. Mechanistic Study of Potent Fluorinated EGFR Kinase Inhibitors with a Quinazoline Scaffold against L858R/T790M/C797S Resistance Mutation: Unveiling the Fluorine Substituent Cooperativity Effect on the Inhibitory Activity. *The journal of physical chemistry. B* **2020**, *124* (28), 5813–5824. DOI: 10.1021/acs.jpcb.0c03440.