ACS Medicinal Chemistry Letters

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Letter

Optimization of Brigatinib as New Wild-Type Sparing Inhibitors of EGFR$^{T790M/C797S}$ Mutants

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Cite This: *ACS Med. Chem. Lett.* 2022, 13, 196−202

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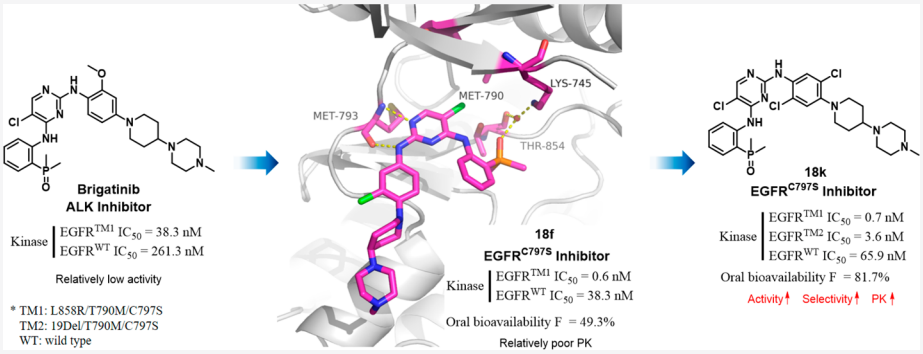


ABSTRACT: A series of brigatinib derivatives were designed and synthesized as new potent and selective EGFR$^{T790M/C797S}$ inhibitors. One of the most potent and selective compounds **18k** strongly suppressed the EGFR$^{L858R/T790M/C797S}$ and EGFR$^{19Del/T790M/C797S}$ kinases with IC$_{50}$ values of 0.7 and 3.6 nM, respectively, which were over 54-fold more potent than the lead compound. **18k** also demonstrated promising EGFR$^{T790M/C797S}$ mutant selectivity, and was 94-fold less potent against the wild type EGFR. A cocrystal structure of EGFR$^{T790M/C797S}$ with a close derivative **18f** was solved to provide insight on the inhibitor's binding mode. Moreover, compound **18k** was orally bioavailable and demonstrated highly desirable PK properties, making it a promising lead compound for further structural optimization.

KEYWORDS: *Epidermal Growth Factor Receptor (EGFR), Resistant Mutation, C797S, Antitumor*

Epidermal growth factor receptor (EGFR) is a well validated target for non-small cell lung cancer (NSCLC) drug discovery.[1] Five small molecular EGFR inhibitors (i.e., gefitinib (**1**),[2] erlotinib (**2**),[3] afatinib (**3**),[4] dacomitinib (**4**),[5] and osimertinib (AZD9291, **5**),[6,7] Figure 1) have been approved by the U.S. Food and Drug Administration (FDA). The first generation drugs **1** and **2** clinically apply to the treatment of NSCLC patients with EGFR activating mutations (e.g., exon 19 deletion (19 Del) and a Leu^{858} → Arg^{858} point mutation (L858R)). Irreversible compounds **3** and **4** belong to the second generation EGFR inhibitor drugs which achieve significant clinical benefit for metastatic and advanced NSCLC patients harboring "gain of function" mutated EGFRs. Although drugs **3** and **4** exhibit strong suppression on the resistant Thr^{790} → Met^{790} (T790M) mutants in preclinical models, the low clinical maximal tolerated dose (MTD) attributing to the strong wild-type EGFR (EGFR^{WT}) inhibition limited their application in resistant patients with the EGFR^{T790M} mutant. Wild-type sparing EGFR^{T790M} inhibitor **5** is the first FDA approved third generation drug for resistant NSCLC patients with EGFR^{T790M} mutations, and it was later approved as a first-line treatment for metastatic NSCLC patients with positive EGFR mutations. Following the success of **5**, two structurally close derivatives, i.e., almonertinib (**6**)[8] and furmonertinib (**7**),[9] were approved by National Medical Products Administration of China (NMPA) in 2020 and 2021, respectively. Several other third generation EGFR inhibitors, e.g., lazertinib, rezivertinib, and abivertinib, are in late stages of clinical investigation.[10] We have also developed ASK120067 (**8**) as a highly potent and selective wild-type sparing EGFR^{T790M} inhibitor. A New Drug Application (NDA) was filed recently in China based on its highly promising efficacy in advanced and multiple brain metastatic NSCLC patients harboring EGFR^{T790M} mutations.[11]

Structurally, all of the third generation EGFR inhibitors possess an acrylamide warhead to covalently react with the

Received: October 10, 2021
Accepted: January 5, 2022
Published: January 7, 2022

https://doi.org/10.1021/acsmedchemlett.1c00555
ACS Med. Chem. Lett. 2022, 13, 196−202

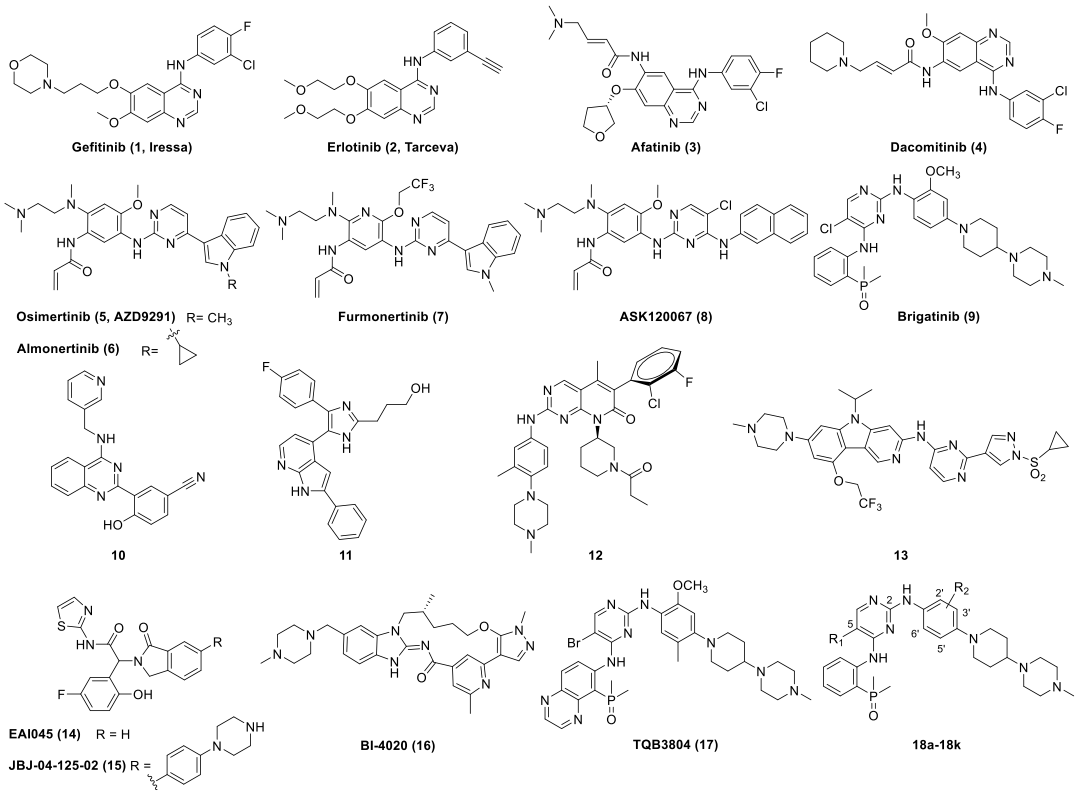


Figure 1. Chemical structures of approved or literature reported EGFR inhibitors.

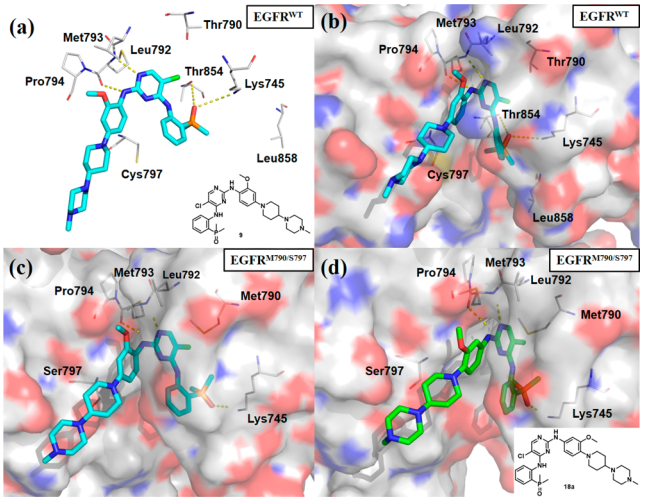
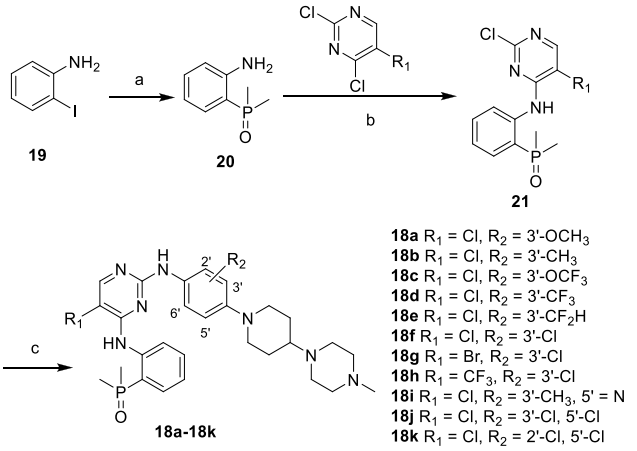


Figure 2. X-ray crystal structure of brigatinib (**9**) with $EGFR^{Thr790/Cys797}$($EGFR^{WT}$) kinase domain (PDB 7AEM) shown in cyan and gray in stick and line representation (a) or in surface representation (b). Preliminary computational docking complex of **9** (c) and **18a** (d) with $EGFR^{Met790/Ser797}$ (PDB 5ZTO). Hydrogen bonds are indicated by yellow hatched lines to key amino acids.

Scheme 1. Synthesis of Compounds 18a−18k[a]



[a]Reagents and conditions: (a) dimethylphosphine oxide, $Pd(OAc)_2$, xantphos, K_3PO_4, DMF, 120 °C, overnight, 82%; (b) R_1 substituted pyrimidine, K_2CO_3, *n*-Bu_4NHSO_4, DMF, 65 °C, overnight, 63−84%; (c) R_2 substituted aniline, 2.5 M HCl in EtOH, 2-methoxyethanol, 120 °C, overnight, 37−75%.

Cys^{797} residue to achieve improved competitiveness with the high concentration ATP (mM level) in cells. A tertiary C797S (Cys^{797} → Ser^{797}) mutation would impair the covalent formation between Cys^{797} and the acrylamide warhead and induce the resistance against the third generation drugs in approximately 20% of the pretreated NSCLC patients.[12] Although the patients with *trans* $EGFR^{T790M/C797S}$ mutations in which the T790M and C797S mutations are located on different alleles could be efficiently treated by combinational therapies of a first generation drug and **5**, the *cis*-$EGFR^{T790M/C797S}$ mutation (mutations are on a same allele) mediated resistance remains an unmet clinical need.[13,14] Therefore, development of the fourth generation $EGFR^{T790M/C797S}$ inhibitors is highly desirable for overcoming the new mutation mediated resistance.[15]

Several classes of $EGFR^{T790M/C797S}$ inhibitors have been reported. Examples include the traditional ATP competitive inhibitor brigatinib (**9**) and derivatives,[16] 4-hydroxy-3-(4-aminoquinazolin-2-yl)benzonitrile derivative **10**,[17] trisubsti-

https://doi.org/10.1021/acsmedchemlett.1c00555
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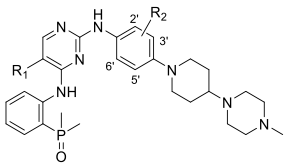
Table 1. In Vitro EGFR Inhibition and Antiproliferation Data of Compounds 18a−18k[a]



compd	substituents		kinase activity IC_{50} (nM)[b]			
	R_1	R_2	$EGFR^{WT}$	$EGFR^{DM}$	$EGFR^{TM1}$	WT/TM1 ratio[c]
18a	Cl	2′-H, 3′-OCH_3	51.2 ± 21.2	2.4 ± 0.4	2.4 ± 1.0	21.3
18b	Cl	2′-H, 3′-CH_3	62.3 ± 11.5	2.7 ± 0.7	0.7 ± 0.3	89.0
18c	Cl	2′-H, 3′-OCF_3	157.0 ± 26.4	9.4 ± 3.1	12.9 ± 3.2	12.2
18d	Cl	2′-H, 3′-CF_3	538.1 ± 62.7	32.8 ± 3.6	3.5 ± 0.9	153.7
18e	Cl	2′-H, 3′-CF_2H	198.7 ± 41.2	13.7 ± 3.3	23.1 ± 1.6	8.6
18f	Cl	2′-H, 3′-Cl	38.3 ± 5.6	1.8 ± 0.4	0.6 ± 0.3	63.8
18g	Br	2′-H, 3′-Cl	113.1 ± 24.2	1.8 ± 0.5	3.3 ± 0.4	34.3
18h	CF_3	2′-H, 3′-Cl	>1000	>1000	>1000	
18i	Cl	2′-H, 3′-CH_3, 5′ = N	337.6 ± 141.6	25.3 ± 6.1	2.1 ± 0.7	160.8
18j	Cl	3′-Cl, 5′-Cl	299.8 ± 169.7	29.4 ± 8.0	33.5 ± 3.5	8.9
18k	Cl	2′-Cl, 5′-Cl	65.9 ± 10.7	0.7 ± 0.1	0.7 ± 0.3	94.1
9	Cl	2′-OCH_3, 3′-H	261.3 ± 78.7	50.9 ± 12.8	38.3 ± 7.0	6.8
5			207.5 ± 93.6	2.0 ± 0.1	236.6 ± 96.0	0.88

[a]$EGFR^{WT}$, $EGFR^{DM}$ and $EGFR^{TM1}$ kinase inhibition was tested by ELISA assay. The data are mean values ± SD from at least three independent experiments. [b]TM1, L858R/T790M/C797S; DM, L858R/T790M. [c]WT/TM1 ratio was calculated by $IC_{50}(EGFR^{WT})/IC_{50}(EGFR^{TM1})$, which reflected the selectivity of the compounds to $EGFR^{WT}$ and $EGFR^{TM1}$.

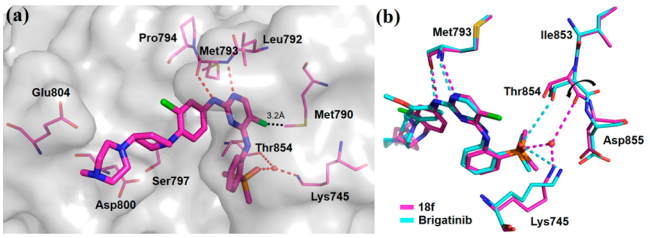


Figure 3. (a) X-ray crystal structure of $EGFR^{T790M/C797S}$ in complex with **18f** (PDB 7ER2). Key residues and **18f** are shown in magenta by the element in lines and in sticks, respectively. Hydrogen bond interactions are showed as red dotted lines, and the water molecule is indicated by a red dot. (b) Superposition of **18f** (PDB 7ER2; compound and protein shown as magenta sticks and lines, respectively) and brigatinib complex (PDB 7AEM; compound and protein shown as cyan sticks and lines, respectively).

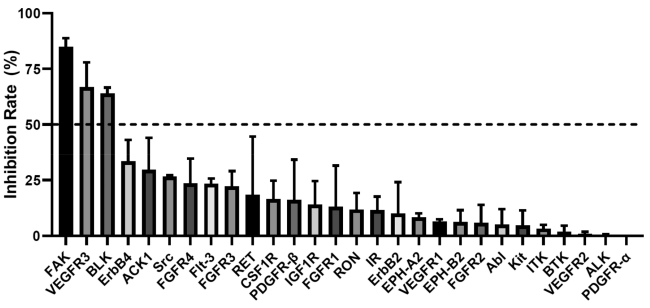


Figure 4. Kinase inhibitory activity of **18k** against 28 different kinases at a concentration of 100 nM. Kinase inhibition was tested by ELISA assay. Inhibition rate are shown as mean ± SD.

tuted imidazole derivative **11**,[18] 5-methylpyrimidopyridone derivative **12**,[19] *N*-(pyrimidin-4-yl)-5*H*-pyrido[4,3-*b*]indol-3-amine analogue **13**,[20] allosteric inhibitors EAI045 (**14**)[21] and JBJ-04-125-02 (**15**),[22] and macrocyclic molecules BI4020 (**16**).[23] To the best of our knowledge, only the brigatinib derivative TQB3804 (**17**) was advanced to clinical investigation in resistant NSCLC patients with $EGFR^{T790M/C797S}$ mutations (NCT04128085).[24,25] Herein, we would like to report a straightforward structural optimization of compound **9**. The effort eventually yielded the discovery of a new highly potent and selective $EGFR^{T790M/C797S}$ inhibitor **18k** with approximatly 54-fold improved kinase inhibitory potency.

A cocrystal structure of $EGFR^{WT}$ with inhibitor **9** was recently reported (PDB 7AEM, Figure 2a in stick and line representation and Figure 2b in surface representation).[26] The results showed that NH group of the aniline in **9** made a donor interaction with the backbone carbonyl of Met^{793}, while the pyrimidine N accepted a hydrogen bond from the backbone NH of Leu^{792}, in the hinge region. An oxygen atom of the dimethylphosphine oxide (DMPO) group formed acceptor interactions with the side chain NH_3 of the conserved catalytic Lys^{745} and the side chain OH of Thr^{854}, respectively, whereas the hydrophilic piperazinylpiperdine moiety was oriented to the solvent accessible surface, indicating it might not contribute much to the protein binding. A preliminary computational study suggested it might bind with $EGFR^{T790M/C797S}$ in a similar mode (Figure 2c). The structural information also suggested that the 2′-methoxyl group might cause potential steric clash with Pro^{794} although it could occupy a small hydrophobic cavity in EGFR to improve the target selectivity. This was consistent with the previous observation that removal of the corresponding methoxyl group resulted in improved EGFR kinase inhibitory potency for the third generation inhibitors.[27,28]

A preliminary docking study also suggested that a change of the 2′-methoxyl group to 3′- position would increase the EGFR inhibitory activity by avoiding the potential steric clash (Figure 2d). Therefore, a series of brigatinib derivatives with substituents at the 3′- position of 2-aniline group were synthesized by following the reported procedures (Scheme

https://doi.org/10.1021/acsmedchemlett.1c00555
ACS Med. Chem. Lett. 2022, 13, 196−202

Table 2. Antiproliferative Activity of 18k in BaF3, PC9, PC9-EGFR^{TM2}, and H1299 Cell Lines[a]

	BaF3					
IC_{50} (μM)	EGFR^{TM1}	EGFR^{TM2}	parental cells	PC9	PC9-EGFR^{TM2}	H1299[b]
18k	0.258 ± 0.167	0.141 ± 0.081	1.691 ± 0.253	1.192 ± 0.727	0.603 ± 0.294	2.115 ± 0.302
brigatinib (**9**)	0.286 ± 0.176	0.155 ± 0.046	4.191 ± 1.614	0.829 ± 0.148	1.110 ± 0.028	1.049 ± 0.160
AZD9291 (**5**)	2.893 ± 0.601	2.885 ± 0.689	4.427 ± 1.162	0.011 ± 0.005	3.210 ± 0.853	>10.0

[a]BaF3-EGFR^{TM1}, BaF3-EGFR^{TM2}, and PC9-EGFR^{TM2} cell lines were built by Jian Ding's laboratory, Shanghai Institute of Materia Medica, Chinese Academy of Sciences. PC9-EGFR^{TM2} cell line was constructed by introducing the T790M/C797S mutant into PC9 (EGFR^{19Del}). Cellular assay was determined using the CCK8 assay. [b]H1299 (EGFR^{WT}) cellular assay was determined using sulforhodamine B (SRB) assay. The data were shown as mean ± SD from at least three independent experiments. TM1, L858R/T790M/C797S; TM2, 19Del/T790M/C797S.

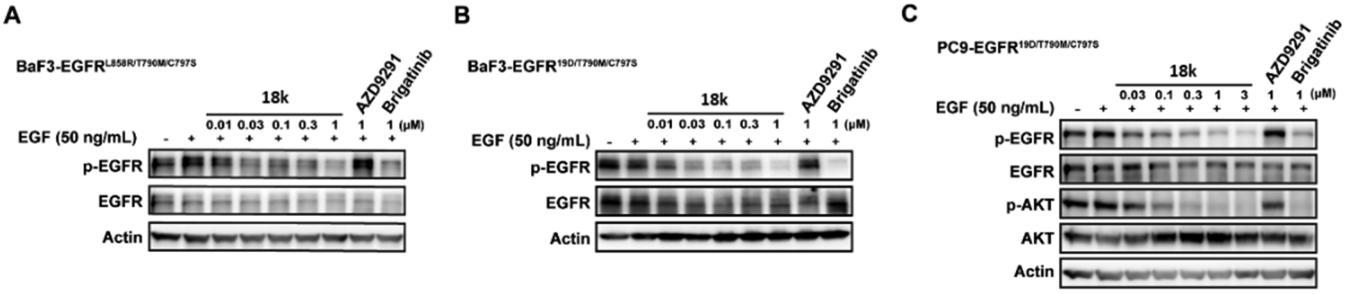


Figure 5. Target inhibition potency of **18k** in EGFR^{C797S} mutant BaF3 cells and PC9 cells. Cells were treated with indicated compounds for 2 h, and the activation of EGFR and downstream signaling molecule were evaluated using Western Blot analysis.

Table 3. Mean PK Parameters of 18f and 18k after po or iv Administration in SD Rats[a]

	18f		18k	
parameters	po[b]	iv[c]	po[b]	iv[c]
$T_{1/2}$ (h)	2.84 ± 0.11	3.73 ± 1.36	2.16 ± 0.2	2.67 ± 0.45
T_{max} (h)	1.50 ± 0.87		2.67 ± 1.15	
C_{max} (ng/mL)	470 ± 155	345 ± 52	868 ± 181	288 ± 16
$AUC_{(0-t)}$ (h·ng/mL)	2553 ± 704	862 ± 88	4552 ± 1154	929 ± 91
$AUC_{(0-\infty)}$ (h·ng/mL)	2563 ± 709	898 ± 57	5219 ± 1536	1060 ± 144
CL (mL/min/kg)		9.30 ± 0.57		7.97 ± 1.17
$MRT_{(0-\infty)}$ (h)	4.79 ± 0.49	4.08 ± 1.24	4.37 ± 0.62	3.65 ± 0.56
*V*ss (mL/kg)		2275 ± 710		1720 ± 40
F (%)	49.3 ± 11.8		81.7 ± 20.7	

[a]*n* = Three animals per study. [b]Dosed at 3 mg/kg. [c]Dosed at 0.5 mg/kg.

1).[29] Beginning with the commercially available 2-iodoaniline (**19**) and dimethylphosphine oxide, intermediate **20** was obtained via a palladium-catalyzed coupling reaction. Then a two-step, sequential S_NAr reaction was conducted to give the target compounds **18a**−**18k**. Biological evaluation showed that compound **18a** indeed exhibited a 16-fold improved EGFR$^{L858R/T790M/C797S}$ (EGFR^{TM1}) inhibitory potency with an IC_{50} value of 2.4 nM compared with **9** (Table 1). In addition , the switch of the substituent position also resulted in EGFR^{WT} and EGFR$^{L858R/T790M}$ (EGFR^{DM}) potency improvement by factors of 5.1 and 21.2, respectively. Notably, compound **18a** exhibited an improved wild-type sparing selectivity with an EGFR^{WT}/EGFR^{TM1} ratio of 21.3, while the corresponding value for **9** was 6.8. Further investigation also revealed that the 3′-position could be substituted by a variety of substituents, e.g., −CH_3 (**18b**), −OCF_3 (**18c**), −CF_3 (**18d**), −CF_2H (**18e**), and −Cl (**18f**), to demonstrate wild-type sparing strong inhibition against the resistant EGFR^{TM1} and EGFR^{DM} kinases. Particularly, compounds **18b** and **18f** exhibited the strongest EGFR^{TM1} inhibition with IC_{50} values of 0.7 and 0.6 nM, respectively. The compounds could also potently suppress EGFR^{DM} kinase with IC_{50} values of 2.7 and 1.8 nM, but their activities against EGFR^{WT} were 23- and 21-fold less potent, respectively. Additionally, results showed that a replacement of 5-Cl of the pyrimidine motif in **18f** by a Br group (**18g**) barely led to an obvious effect on the activity and selectivity, while the 5-CF_3 substituted analogue (**18h**) totally abolished the EGFR inhibitory potency. The investigation also suggested the aniline group at the pyrimidine 2-position could be replaced by a pyridinyl moiety (**18i**) without causing a significant potency change to the EGFR triple mutated kinase (IC_{50} = 2.1 nM). Further investigation revealed that 3′,5′-dicholor analogue **18j** was 55-fold less potent than **18f** against EGFR^{TM1} triple mutant, while the 2′,5′-dicholor compound **18k** exhibited identical EGFR^{TM1} inhibition to that of **18f**, with an IC_{50} value of 0.7 nM. Further evaluation suggested that **18k** potently suppressed the EGFR$^{19Del/T790M/C797S}$ mutated kinase, which is a clinically more common resistant mutation, with an IC_{50} value of 3.6 (±1.3) nM. In addition, **18k** was also highly potent against EGFR$^{L858R/T790M}$ with an IC_{50} value of 0.7 nM, but its potency against EGFR^{WT} was 94.1-fold less potent.

The X-ray cocrystal structure of EGFR$^{T790M/C797S}$ with **18f** was solved (PDB 7ER2, Figure 3a). Similar to our prediction, the results showed that the molecule anchored in the hinge

https://doi.org/10.1021/acsmedchemlett.1c00555
ACS Med. Chem. Lett. 2022, 13, 196−202

region of the ATP-binding pocket with bidentate hydrogen bond interactions between Met^{793} and 2-aminopyrimidine motif. Interestingly, structural alignment of EGFR-**18f** complex and EGFR-brigatinib complex (PDB 7AEM) showed that the backbone amido linkages between Thr^{854} and Asp^{855} pointed in opposite directions (Figure 3b). Therefore, instead of forming a hydrogen bond directly with side chain OH of Thr^{854} as exhibited in Figure 2a, the oxygen atom on the DMPO group of **18f** fostered water-molecule-mediated hydrogen bond interactions with the backbone carbonyl of Thr^{854} and additionally the side chain NH_3 of Lys^{745}, inducing a stable conformation and improving binding affinity toward the protein. In addition, the 3′-Cl in **18f** located in an appropriate cavity with no steric clash with any residues, providing a rational explanation for the improved EGFR inhibitory potency. Additionally, the C5-chlorine atom on the pyrimidine scaffold was directed toward the "gatekeeper" residue Met^{790} with a distance of 3.2 Å, indicating that this position was unable to accommodate large hydrophobic groups such as CF_3, which explains the poor potency of **18h**.

Having demonstrated the selectivity of **18k** for its direct target EGFR, we next examined its inhibitory activities against a small kinome panel at a concentration of 100 nM. The results in Figure 4 showed that **18k** exhibited >30× selectivity in all 28 tyrosine kinases examined (see the Supporting Information for raw inhibition rate). Strikingly, **18k** markedly attenuated the inhibitory effect on ALK, the original target of brigatinib, indicating its improved kinase selectivity.

Furthermore, the antiproliferative activity of **18k** was evaluated in BaF3 cells stably expressing $EGFR^{L858R/T790M/C797S}$ (BaF3-$EGFR^{TM1}$) and $EGFR^{19Del/T790M/C797S}$ (BaF3-$EGFR^{TM2}$) mutants (Table 2). It was shown that **18k** exhibited moderate growth inhibition against the cell models with IC_{50} values of 0.258 and 0.141 μM, respectively, which were comparable to those of drug **9**, whereas its cytotoxic effect on BaF3 parental cells was a bit more potent with an IC_{50} value of 1.69 μM. Compound **18k** also exhibited a 0.60 μM IC_{50} value against the proliferation of self-constructed osimertinib-resistant PC9 NSCLC cells harboring $EGFR^{19Del/T790M/C797S}$ mutation (PC9-$EGFR^{TM2}$). Selectivity was observed against PC9 ($EGFR^{19Del}$) and H1299 ($EGFR^{WT}$) cell lines, which was within the kinase selectivity margin. Further immunoblotting analysis suggested that the compound dose-dependently suppressed the phosphorylation of EGFR in BaF3-$EGFR^{TM1}$, BaF3-$EGFR^{TM2}$, as well as PC9-$EGFR^{TM2}$ cells, supporting its cellular inhibitory potency on the resistance related EGFR mutations (Figure 5).

The pharmacokinetic (PK) parameters of **18f** and **18k** were also assessed in Sprague−Dawley (SD) rats (Table 3). Both compounds exhibited good PK properties with high oral bioavailability, decent exposure, and acceptable half-life. For instance, **18k** exhibited an oral bioavailability value of 81.7%, an AUC (area under curve) value of 5219 h·ng/mL, and a $T_{1/2}$ value of 2.67 h, which were optimal for oral administration for the following in vivo efficacy investigation.

In summary, a straightforward structure optimization of brigatinib was conducted to yield compound **18k** as a new potent and selective $EGFR^{T790M/C797S}$ inhibitor. The compound strongly suppressed the $EGFR^{L858R/T790M/C797S}$ and $EGFR^{19Del/T790M/C797S}$ kinases with sub-nanomolar IC_{50} values but was approximately 94-fold less potent against the wild type EGFR. A cocrystal structure of $EGFR^{T790M/C797S}$ with a close derivative **18f** was also solved to provide understanding of the binding mode of the inhibitor. Moreover, compound **18k** exhibited cellular activities in all three cell models, BaF3-$EGFR^{TM1}$, BaF3-$EGFR^{TM2}$, and PC9-$EGFR^{TM2}$, comparable to that of brigatinib. Compound **18k** is orally bioavailable and demonstrates highly desirable PK properties, making it a promising lead compound for further structural optimization.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00555.

Chemistry, biological assay, X-ray crystallography data, and copies of NMR spectra and HPLC purity data (PDF)

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https://doi.org/10.1021/acsmedchemlett.1c00555
ACS Med. Chem. Lett. 2022, 13, 196−202

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors appreciate the financial support from National Natural Science Foundation of China (21807045, 82173650, 81820108029, 81874284, 22037003, 22077050, and 81903638), Guangdong Province (2021A1515011239 and 2018B030337001), the National Science & Technology Major Project "Key New Drug Creation and Manufacturing Program", China (2019ZX09301157-004), and the "Personalized Medicines-Molecular Signature-based Drug Discovery and Development", Strategic Priority Research Program of the Chinese Academy of Sciences (XDA12020112).

■ ABBREVIATIONS

EGFR, epidermal growth factor receptor; NSCLC, nonsmall cell lung cancer; 19Del, Exon 19 deletion; MTD, maximal tolerated dose; WT, wild type; NMPA, National Medical Products Administration of China; FDA, U.S. Food and Drug Administration; NDA, New Drug Application; DMPO, dimethylphosphine oxide group; TM1, L858R/T790M/C797S; TM2, 19Del/T790M/C797S; DM, L858R/T790M; PK, pharmacokinetic; AUC, area under curve; SD rats, Sprague−Dawley rats; ALK, anaplastic lymphoma kinase; CCK8, cell counting kit 8; SRB, sulforhodamine.

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https://doi.org/10.1021/acsmedchemlett.1c00555
ACS Med. Chem. Lett. 2022, 13, 196−202