ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Oxopyrido[2,3-*d*]pyrimidines as Covalent L858R/T790M Mutant Selective Epidermal Growth Factor Receptor (EGFR) Inhibitors

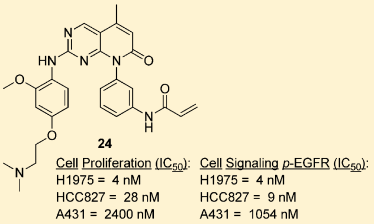
Ryan P. Wurz,*[,†] Liping H. Pettus,[†] Kate Ashton,[†] James Brown,[†] Jian Jeffrey Chen,[†] Brad Herberich,[†] Fang-Tsao Hong,[†] Essa Hu-Harrington,[†] Tom Nguyen,[†] David J. St. Jean, Jr.,[†] Seifu Tadesse,[†] David Bauer,[◆] Michele Kubryk,[◆] Jinghui Zhan,[‡] Keegan Cooke,[‡] Petia Mitchell,[‡] Kristin L. Andrews,[§] Faye Hsieh,[∥] Dean Hickman,[∥] Nataraj Kalyanaraman,[∥] Tian Wu,[⊥] Darren L. Reid,[⊥] Edward K. Lobenhofer,[○] Dina A. Andrews,[#] Nancy Everds,[#] Roberto Guzman,[#] Andrew T. Parsons,[+] Simon J. Hedley,[▽] Jason Tedrow,[▽] Oliver R. Thiel,[▽] Matthew Potter,[∞] Robert Radinsky,[‡] Pedro J. Beltran,[‡] and Andrew S. Tasker[†]

[†]Medicinal Chemistry, [‡]Oncology Research, [§]Molecular Structure, [∥]Pharmacokinetics and Drug Metabolism, [⊥]Oral Delivery − Product and Process Development, [○]Discovery Toxicology, [#]Pathology, [▽]Chemical Process R&D, Amgen Inc., One Amgen Center Drive, Thousand Oaks, California 91320-1799, United States

[◆]Medicinal Chemistry, [+]Chemical Process R&D, [∞]Analytical R&D, Amgen Inc., 360 Binney Avenue, Cambridge, Massachusetts 02142-1011, United States

Supporting Information

ABSTRACT: In nonsmall cell lung cancer (NSCLC), the threonine[790]−methionine[790] (T790M) point mutation of EGFR kinase is one of the leading causes of acquired resistance to the first generation tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib. Herein, we describe the optimization of a series of 7-oxopyrido[2,3-*d*]pyrimidinyl-derived irreversible inhibitors of EGFR kinase. This led to the discovery of compound **24** which potently inhibits gefitinib-resistant EGFR^{L858R,T790M} with 100-fold selectivity over wild-type EGFR. Compound **24** displays strong antiproliferative activity against the H1975 nonsmall cell lung cancer cell line, the first line mutant HCC827 cell line, and promising antitumor activity in an EGFR^{L858R,T790M} driven H1975 xenograft model sparing the side effects associated with the inhibition of wild-type EGFR.



KEYWORDS: *Epidermal growth factor receptor, EGFR, nonsmall cell lung cancer, EGFR T790M mutant, irreversible inhibitor, kinase inhibitor*

Aberrant signaling by the epidermal growth factor receptor (EGFR, ErbB1) kinase is a well validated target in the lung cancer setting. Several EGFR inhibitors, such as gefitinib and erlotinib (Figure 1), have achieved significant clinical benefit in the management of nonsmall cell lung cancer (NSCLC) in patients with activating point mutations in exon 21 in the EGFR kinase domain, such as L858R and in-frame deletions, such as del(E746−A750), in exon 19. Unfortunately, a secondary threonine[790]−methionine[790] mutation (T790M) in the EGFR catalytic domain is responsible for approximately 50% of the clinically acquired drug resistance in NSCLC patients.[1−3]

In order to address resistance to the first generation of reversible tyrosine kinase inhibitors (TKIs), a second generation of irreversible[4,5] EGFR inhibitors was developed.[6−9] CI-1033 (canertinib),[10] HKI-272 (neratinib),[11,12] and PF-00299804 (dacomitinib)[13] advanced into late stage clinical development and structurally resemble the first generation of EGFR inhibitors (Figure 1). Afatinib (gilotrif) was recently approved by the US FDA for treatment of late-stage NSCLC patients with actively mutated EGFR.[14] All the second generation inhibitors, however, lack significant selectivity between EGFR^{T790M} mutants and the wild-type kinase which leads to dose-limiting toxicities in these patients, including skin rash and diarrhea. Only limited clinical efficacy was observed for these inhibitors, and this may be due to the fact that the inhibitors at the tolerated therapeutic concentrations are insufficient to adequately suppress the functions of EGFR^{T790M} mutant.

The desire to identify inhibitors selective for the EGFR mutants (especially T790M) while sparing EGFR^{WT} led to a third generation of irreversible EGFR inhibitors pioneered by Zhou and co-workers with the discovery of WZ-4002 (Gatekeeper Pharmaceuticals Inc.). This generation of inhibitors demonstrated that much higher levels of selectivity for EGFR^{T790M} were possible (Figure 2).[15] Since that report, a significant number of third generation inhibitors have entered early clinical trials. Recent disclosures by Clovis Oncology and AstraZeneca revealed their clinical candidates CO-1686[16] and

Received: May 8, 2015
Accepted: July 27, 2015
Published: July 27, 2015

DOI: 10.1021/acsmedchemlett.5b00193
ACS Med. Chem. Lett. 2015, 6, 987−992

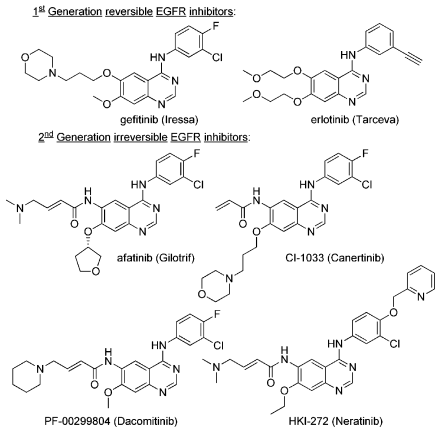


Figure 1. First and second generation EGFR inhibitors.

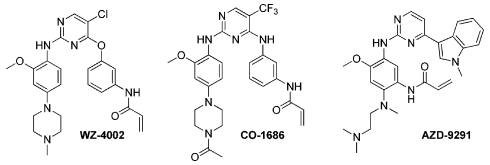


Figure 2. Third generation EGFR inhibitors.

AZD-9291[17−19] which possess selectivity for the $EGFR^{L858R,T790M}$ mutant over $EGFR^{WT}$.[20−22]

Our efforts in this area began with compound **1** which was the result of structure-guided design. Inspired by our previous research efforts on p38 MAP kinase[23,24] and the structural similarities of the ATP binding pockets of p38 and EGFR, we explored the fusion of a pyridone ring onto the scaffold of WZ-4002 (Figure 3). The carbonyl oxygen of the pyridone ring would

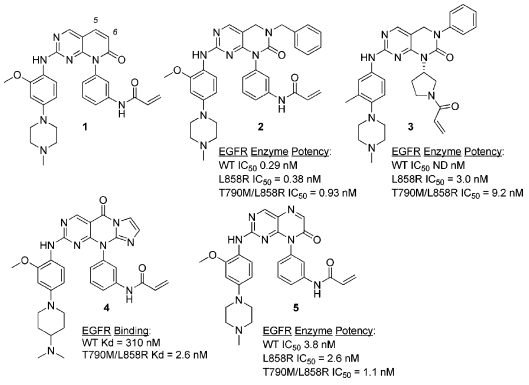
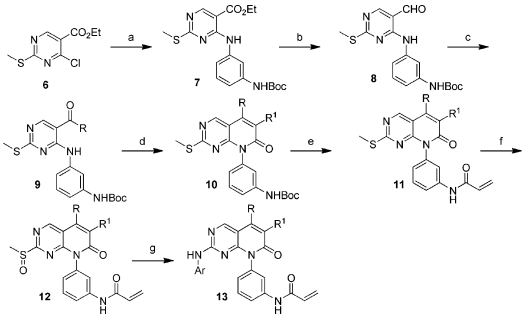


Figure 3. Bicyclic scaffolds resulting from the fusion of heterocycles onto the core of WZ-4002.

serve as a hydrogen-bond acceptor to satisfy the coordination requirements of a crystallographically resolved water molecule in the ATP binding pocket of EGFR protein and help improve inhibitor potency. Ding and co-workers have also explored similar approaches and recently communicated their efforts involving the fusion of cyclic ureas (compounds **2**−**3**),[25,26] a pyrimidino[4,5-*d*]pyrimidin-4(*1H*)-one scaffold (compound **4**),[27] and a pteridine-7(*8H*)-one scaffold (compound **5**)[28] onto the WZ-4002 core with varying degrees of selectivity for $EGFR^{L858R,T790M}$ over $EGFR^{WT}$.

We designed a synthesis of the 7-oxopyrido[2,3-*d*]pyrimidinyl core that would allow for versatile analoging (Scheme 1).[29,30]

Scheme 1. Synthesis of EGFR Inhibitor Analogs[a]



[a]Reagents and conditions: (a) K_2CO_3 (2 equiv), *m*-NH_2−C_6H_4−NHBoc, DMF, 80 °C, 86%; (b) (i) $LiAlH_4$ (2.5 equiv), THF, 0 °C; (ii) MnO_2, $CHCl_3$, rt, 66% (2 steps); (c) (i) RMgBr (3.2 equiv), THF, 0 °C−rt, 1 h; (ii) MnO_2, $CHCl_3$, 60 °C, 16 h; (d) $R^1CH_2CO_2Et$, LiHMDS, THF, −78 °C−rt; (e) (i) TFA, CH_2Cl_2, rt, 30 min; (ii) acryloyl chloride, Hunig's base, CH_2Cl_2, 0 °C−rt, 1 h; (f) *m*-CPBA (1.1 equiv), CH_2Cl_2, 0 °C, 1 h; (g) DMAC, Ar−NH_2 (2.2 equiv), 95 °C, 6 h.

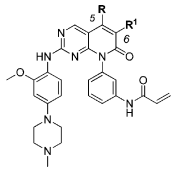
Commercially available ethyl 4-chloro-2-(methylthio)-pyrimidine-5-carboxylate (**6**) underwent an S_NAr-type displacement with mono-Boc protected 1,3-phenylenediamine to produce diaryl amine **7** in high yield. Reduction of the ethyl ester with lithium aluminum hydride afforded the corresponding alcohol, which was subsequently oxidized with MnO_2 to aldehyde **8**. Treatment of the aldehyde with the appropriate Grignard reagent followed by oxidation of the resulting secondary alcohol to ketone **9** provided a substrate for an aldol-like cyclocondensation reaction with ester enolates to afford the 7-oxopyridopyrimidinyl scaffold (**10**).[31] In this manner, analogs with the desired substitution patterns at the 5- and 6-positions of the pyridone ring could be prepared. The Boc-protecting group was removed with TFA, and the acrylamide warhead was introduced using acryloyl chloride to provide intermediate **11**. Oxidation of the methyl(thio)pyrimidinyl motif to the sulfoxide with *m*-CPBA furnished sulfoxide (**12**) which underwent S_NAr displacement with the desired aniline to afford the final product **13**.

An NCI-H1975 adenocarcinoma cell line bearing $EGFR^{L858R,T790M}$ point mutations and an HCC827 adenocarcinoma cell line bearing the deletion mutation ($EGFR^{\Delta 746-750}$) were used for potency optimization while a A431 epidermoid carcinoma cell line bearing overexpressed $EGFR^{WT}$ was used as a counterscreen, allowing an early assessment of selectivity over $EGFR^{WT}$.

With a synthetic route in hand to facilitate exploration of a structure−activity relationship for the 7-oxopyrido[2,3-*d*]-pyrimidinyl analogs, compound **1** was prepared (via Scheme 1, *omitting step c*). Compound **1** displayed single digit nanomolar activity in both of the EGFR mutant cell lines with good selectivity against the A431 cell line (Table 1). This compound was further profiled in a biochemical cell signaling assay by meso scale discovery (MSD)[32] and showed reduced selectivity (ca. 17-fold) against the A431 cell line (Table 3). Given the large differences in affinities for ATP[33] between the different mutant forms and $EGFR^{WT}$, we believed it would be beneficial to further improve upon selectivity over $EGFR^{WT}$ in order to give a better window of tolerability. These large differences in ATP affinity also influenced our decision to use cell-based assays to direct SAR efforts instead of the traditional enzyme-based assays.

DOI: 10.1021/acsmedchemlett.5b00193
ACS Med. Chem. Lett. 2015, 6, 987−992

Table 1. Antiproliferation Activities for Compounds Arising from Modifications to the Pyridone Ring[a]



compd	R	R^1	H1975 IC_{50} (nM)	HCC827 IC_{50} (nM)	A431 IC_{50} (nM)
AZD9291	N/A	N/A	26	6	849
1	H	H	8	4	5229
14	Me	H	4	12	1200
15	Me	OMe	10	41	1387
16	H	OMe	273	61	201
17	H	Bn	4	18	250

[a]Data represent an average of at least two separate determinations. Standard deviations are reported in the Supporting Information and vary by less than ±50%.

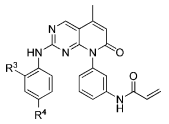
Modeling studies docking 7-oxopyrido[2,3-*d*]pyrimidinyl analogs into the reported crystal structure of WZ-4002 in $EGFR^{T790M}$ (PDB code: 3IKA) suggested that introduction of small hydrophobic groups into the gatekeeper pocket would potentially benefit selectivity. The threonine present in $EGFR^{WT}$ is more sterically congested and polar in nature while the $EGFR^{T790M}$ bears a methionine residue which is hydrophobic and, given the lack of a β-branch, more accommodating of small aliphatic substitutions. Given these differences in the gatekeeper pocket between the T790M mutant and $EGFR^{WT}$ protein, we investigated a brief but focused series of substitution patterns in the 5- and 6-positions on the 7-oxopyrido[2,3-*d*]pyrimidinyl ring.

Introduction of a methyl group in the 5-position of the pyridone ring (**14**) led to a compound which was roughly equipotent to compound **1** in the H1975 (H1975 IC_{50} = 4 nM) and HCC827 cell lines (HCC827 IC_{50} = 12 nM) and also >100-fold selective against the wild-type expressing cell line (A431 IC_{50} = 1200 nM). This potency and selectivity profile was also confirmed by the MSD assay in which the potency in the A431 cell line remained ca. 100-fold (A431 IC_{50} = 386 nM, Table 3). Introduction of a methoxy group to the 6-position (compound **15**) resulted in a very similar profile with slight erosion in potency in the H1975 cell line (H1975 IC_{50} = 10 nM) and a small loss in selectivity. Removal of the 5-methyl group (compound **16**) led to a significant loss in potency and a complete loss in selectivity for the $EGFR^{L858R,T790M}$ mutant over $EGFR^{WT}$. Introduction of a benzyl group in the 6-position (compound **17**) was found to adversely influence selectivity over $EGFR^{WT}$.

Compound **14** was then evaluated for its pharmacodynamic (PD) effect in female athymic nude mice. These mice were implanted with matrigel plugs containing H1975 tumor cells (5×10^6 cells) and dosed orally with **14** at 30 mg/kg. At 4 h post dosing, tumor cell plugs and plasma were harvested. Total p-EGFR levels in the plug were measured with a quantitative MSD assay resulting in 90% inhibition of p-EGFR (Table 2). Compound **14** was also efficacious in an H1975 murine xenograft model resulting in tumor stasis at 30 mg/kg QD.[34] However, in an exploratory 4-day rat toxicology study, hepatobiliary toxicity (moderate to marked increased serum biomarkers of hepatobiliary perturbation/injury with histologic evidence of minimal to mild portal inflammation and hepatocellular necrosis) was observed at low exposure multiples. Due to the observed toxicity, we decided to explore structure–activity relationships (SAR) for replacements for the *N*-methylpiperazine motif on the left-hand side of the inhibitor while maintaining the 5-methyl substitution on the pyridone ring. It quickly became apparent that small modifications to the left-hand side had a significant impact on the properties of the inhibitor. For example, removal of the *ortho*-methoxy group on the aniline resulted in compound **18** which maintained potency in the proliferation assays. However, this compound had a greatly diminished pharmacodynamic (PD) effect as compared to **14** presumably due to a precipitous drop in exposure. *N*-Acylation of the piperazinyl group (compound **19**) resulted in loss in inhibitor potency as did removal of the methyl group (compound **20**).

An *N*-methylpiperidine (compound **21**) was a replacement that was tolerated in terms of potency in the mutant cell lines but resulted in a loss in selectivity for the $EGFR^{L858R,T790M}$ mutant over $EGFR^{WT}$. A morphilino-group (compound **22**) resulted in complete loss of potency in the proliferation assay due to a signficant loss in permeability. Ring opening of the *N*-methylpiperazine to a *N,N,N′*-trimethylethylenediamine (compound **23**) resulted in a compound that was virtually equipotent to **14** and maintained a robust PD effect. Compound **23** was

Table 2. Antiproliferation Activities for Compounds Arising from Modifications to the Left-Hand Side[a]

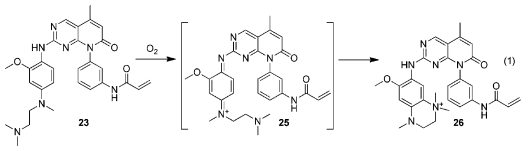


Cmpd	R^3	R^4	H1975 IC50 (nM)	HCC827 IC50 (nM)	A431 IC50 (nM)	PD[b] % Inhib
AZD 9291	N/A	N/A	26	6	849	92
14	OMe		4	12	1200	90
18	H		4	7	>3000	5
19	OMe		30	58	>5000	NT
20	OMe		68	211	>1000	NT
21	OMe		4	33	681	NT
22	OMe		>10,000	Undef.	NT	NT
23	OMe		8	22	>1000	80
24	OMe		4	28	2400	90

[a]Data represents an average of at least two separate determinations. Standard deviations are reported in the Supporting Information and vary by less than ±50%. [b]Athymic nude mice were implanted with matrigel plugs containing 5×10^6 H1975 cells. Animals (n = 3) were treated with control or compound (30 mg/kg po dose). Plug and plasma samples were collected 4 h post sample administration. Results denotes % p-EGFR/PRAS40 vs control. NT = not tested.

DOI: 10.1021/acsmedchemlett.5b00193
ACS Med. Chem. Lett. 2015, 6, 987–992

found to be slightly less efficacious in an H1975 xenograft model than **14** (90% TGI at 30 mg/kg QD). However, in an exploratory 4-day rat toxicology study this analogue was devoid of hepatobiliary toxicity. Efforts to prepare this compound on larger scale for additional toxicology studies led to the realization of a potential stability liability for this compound. A variable amount of the quaternary ammonium salt byproduct (**26**) was observed during isolation of the final API. Mechanistically, this decomposition product presumably results from oxidation of the electron-rich aniline ring to aza-quinone intermediate **25** followed by 1,4-nucleophilic addition to restore aromaticity (eq 1).



This mechanistic proposal led to the design of an analog with a slightly less electron-rich aniline. Compound **23**, the corresponding ether linked analogue (of compound **24**), was prepared and found to have slightly improved potency and greatly improved stability. Compound **24** also maintained the robust PD effect as observed with compound **23**. Its selectivity was also confirmed in the MSD assay with >100-fold selectivity over $EGFR^{WT}$ (Table 3). Hepatobiliary toxicity was also not observed when this compound was tested in a 4-day rat toxicology study.

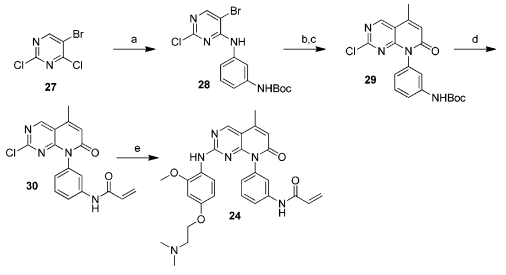
Table 3. Cell Signaling: Inhibition of p-EGFR by MSD[a]

compd	H1975 IC_{50} (nM)	HCC827 IC_{50} (nM)	A431 IC_{50} (nM)
AZD9291	15	6	348
1	3	3	50
14	4	12	386
23	6	12	521
24	4	9	1054

[a]Data represent an average of at least two separate determinations. Standard deviations are reported in the Supporting Information and vary by less than ±50%.

As demand for larger quantities of material grew to facilitate additional studies, a more efficient synthetic route was developed (Scheme 2). This alternative route used commercially available

Scheme 2. Second Generation Synthesis of Compound 24[a]



[a]Reagents and conditions: (a) K_2CO_3 (1.3 equiv), *m*-NH_2–C_6H_4–NHBoc, DMF, rt, 16 h, 98%; (b) $PdCl_2(PhCN)_2$, P(*o*-tolyl)$_3$, crotonic acid, DIEA, PhMe, 60 °C; (c) Ac_2O, 60 °C, 1 h, 60% (2 steps); (d) (i) 4 N HCl, 1,4-dioxane, 50 °C (ii) 10N NaOH, acryloyl chloride, 92% (2 steps); (e) Ar-NH_2·HCl (1.1 equiv), 2,2,2-trifluoroethanol, reflux, 16 h, 51%.

5-bromo-2,4-dichloropyrimidine (**27**) and an S_NAr displacement with the mono-Boc protected 1,3-phenylenediamine to furnish **28**. Heck coupling with crotonic acid followed by intramolecular dehydration of the resulting adduct with Ac_2O afforded the 7-oxopyrido[2,3-*d*]pyrimidine core **29** in modest yields. Subsequent Boc-deprotection using HCl, followed by neutralization with NaOH and treatment with acryloyl chloride, yielded the compound **30**. Anilines could be introduced via S_NAr displacement of the chloride in refluxing 2,2,2-trifluoroethanol. In the case of compound **24**, it is noteworthy that the use of the monohydrochloride salt of the aniline is critical for the success of this reaction to prevent displacement of the chloride by the more electron-rich dimethylamine pendant of this reactant.

Modeling of compound **24** in the $EGFR^{T790M}$ protein suggested a number of key interactions with the mutant EGFR protein that likely contributed to this compound's potency.[35] The N–H of the aminopyrimidine engages the linker residue in a pair of hydrogen bonding interactions (2.9, 3.2 Å) with the Met793 residue of the linker region of the EGFR protein in the ATP binding pocket. The crystallographically resolved water molecule between catalytic Lys745/Asp855 residues has an H-bond satisfied by the pyridone carbonyl. The 5-methyl group is projected back into the gatekeeper pocket engaging the Met790 residue with van der Waals contacts. This is sterically and electrostatically unfavorable in the case of $EGFR^{WT}$ protein where the gatekeeper residue is a threonine. The acrylamide motif is projected with the right trajectory onto the floor of the ATP binding pocket to allow for a Michael addition with the Cys797 residue.

The pharmacokinetic (PK) properties of several compounds were assessed (Table 4). These were found to have clearance in

Table 4. Pharmacokinetic Properties of Selected Compounds[a]

	iv[b]			po	
compd	CL ((L/h)/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	$AUC_{(0\text{-inf.})}$ (μM·h)	*F* (%)
1	8.9	19	1.4	0.18	6.6[c]
14	10.6	7.8	3.0	0.18	9.5[c]
23	7.7	20	2.6	0.16	21[d]
24	9.0	22	4.5	0.66	10[e]

[a]Pharmacokinetic parameters following administration in male Sprague–Dawley rats; mean values from three animals per dosing route. [b]Dosed at 1 mg/kg as a solution in DMSO. [c]po doses were 10 mg/kg in 1% Pluronic F68, 2% hydroxypropyl methylcellulose (HPMC), 15% hydroxypropyl β-cyclodextrin (HPBCD), 82% water/methanesulfonic acid pH 4.5. [d]po doses were 10 mg/kg in 20% hydroxypropyl β-cyclodextrin (HPBCD), 80% water/methanesulfonic acid pH 4.0. [e]po doses were 10 mg/kg in 1% Pluronic F68, 2% hydroxypropyl methylcellulose (HPMC), 97% water/methanesulfonic acid pH 4.0.

excess of liver blood flow in rats and low oral bioavailability. This was believed to be related to the high turnover of the acrylamide motif in the presence of glutathione. Although these compounds had poor pharmacokinetic properties in rats,[36,37] we believed it would be possible to obtain a sustained pharmacodynamic (PD) effect due to the covalent nature of the interaction. Compound **24** was then evaluated in a time course PD experiment upon oral dosing at 30 mg/kg.[38] At 2, 4, 8, 12, and 24 h time points post dosing, tumor cell plugs and plasma were harvested. Compound **24** showed a >50% inhibition of phosphorylation of EGFR for

DOI: 10.1021/acsmedchemlett.5b00193
ACS Med. Chem. Lett. 2015, 6, 987–992

>12 h. Compound **24** reached the maximal concentration of 0.10 μM at 2 h, and systemic exposure ($AUC_{0\text{-}inf}$) was 0.33 μM·h.

In a mouse H1975 xenograft model, compound **24** was administered orally at 30 mg/kg daily (QD) beginning on day 6. This resulted in 90% tumor growth inhibition ($AUC_{0\text{-}t}$ = 0.56 μM·h). It is noteworthy that no changes in body weights of all treatment groups were observed.[39]

The selectivity of compound **24** against a panel of 100 protein and lipid kinases was examined in the ScanMAX KINOMEscan panel.[40] In this panel, the competitive binding of **24** at 1 μM was measured as a percentage of control (POC). Competitive binding (POC < 30%) by **24** was observed for only two kinases, BTK (17% POC) and TTK (4% POC). As a follow-up, the K_d values were measured on a number of kinases including BLK (320 nM), BTK (290 nM), $EGFR^{WT}$ (220 nM), $EGFR^{T790M}$ (0.96 nM), ERBB2 (220 nM), ERBB4 (130 nM), GAK (430 nM), ITK (140 nM), JAK1 (JH2 domain-pseudokinase, 2800 nM), JAK3 (30 nM), TTK (81 nM), and TXK (410 nM). Compound **24** possessed moderate permeability and high efflux (10×10^{-6} cm/s and ER = >37 and >37, respectively in human and rat LLC-PK1 cell line transfected with a MDR1 gene), but exhibited low plasma protein binding (Fu = 0.25, 0.27, 0.54, and 0.46 in mouse, rat, human and dog, respectively).

In summary, a novel class of T790M mutant-selective EGFR inhibitors was designed. Compound **24** was highly selective against a panel of 100 kinases and had excellent cellular potency in proliferation assays. Compound **24** was orally efficacious in an H1975 plug PD assay in mice resulting in a >50% inhibition of p-EGFR for >12 h. In an H1975 xenograft model, oral dosing of compound **24** at 30 mg/kg daily for 2 weeks led to significant tumor growth inhibition with no observed loss in body weight. In an exploratory 4-day rat toxicology study, no hepatobiliary toxicity was observed. Additional data regarding compound **24** will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00193.

Experimental procedure for the preparation of compound **24**; NMR data for all compounds; Description of assays; ScanMAX KINOMEscan panel data for compound **24** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: 805-313-5400. Fax: 805-480-1337. E-mail: rwurz@amgen.com.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

AUC, area under the plasma concentration−time curve; CL, clearance; EC_{50}, molar concentration that produces half maximal response; *F*, bioavailability; MRT, mean residence time; V_{ss}, volume of distribution in steady state

■ REFERENCES

(1) Sharma, S. V.; Bell, D. W.; Settleman, J.; Haber, D. A. Epidermal growth factor receptor mutations in lung cancer. *Nat. Rev. Cancer* **2007**, *7*, 169−181.

(2) Ohashi, K.; Maruvka, Y. E.; Michor, F.; Pao, W. Epidermal growth factor receptor tyrosine kinase inhibitor-resistant disease. *J. Clin. Oncol.* **2013**, *31*, 1070−1080.

(3) Chong, C. R.; Jänne, P. A. The quest to overcome resistance to EGFR-targeted therapies in cancer. *Nat. Med.* **2013**, *19*, 1389−1400.

(4) Barf, T.; Kaptein, A. Irreversible protein kinase inhibitors: balancing the benefits and risks. *J. Med. Chem.* **2012**, *55*, 6243−6262.

(5) Potashman, M. H.; Duggan, M. E. Covalent modifiers: an orthogonal approach to drug design. *J. Med. Chem.* **2009**, *52*, 1231−1246.

(6) Ou, S.-H. Second-generation irreversible epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs): A better mousetrap? A review of the clinical evidence. *Crit. Rev. Onc./Hematol.* **2012**, *83*, 407−421.

(7) Schwartz, P. A.; Kuzmic, P.; Solowiej, J.; Bergqvist, S.; Bolanos, B.; Almaden, C.; Nagata, A.; Ryan, K.; Feng, J.; Dalvie, D.; Kath, J. C.; Xu, M.; Wani, R.; Murray, B. W. Covalent EGFR inhibitor analysis reveals importance of reversible interactions to potency and mechanisms of drug resistance. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 173−178.

(8) Singh, J.; Evans, E.; Hagel, M.; Labinski, M.; Dubrovskiy, A.; Nacht, M.; Petter, R. C.; Prasad, A.; Sheets, M.; St. Martin, T.; Sjin, R. T. T.; Westlin, W.; Zhu, Z. Superiority of a novel EGFR targeted covalent inhibitor over its reversible counterpart in overcoming drug resistance. *MedChemComm* **2012**, *3*, 780−783.

(9) For an example of second generation inhibitors, see: Xia, G.; Chen, W.; Zhang, J.; Shao, J.; Zhang, Y.; Huang, W.; Zhang, L.; Qi, W.; Sun, X.; Li, B.; Xiang, Z.; Ma, C.; Xu, J.; Deng, H.; Li, Y.; Li, P.; Miao, H.; Han, J.; Liu, Y.; Shen, J.; Yu, Y. A chemical tuned strategy to develop novel irreversible EGFR-TK inhibitors with improved safety and pharmacokinetic profiles. *J. Med. Chem.* **2014**, *57*, 9889−9900.

(10) Smaill, J. B.; Rewcastle, G. W.; Loo, J. A.; Greis, K. D.; Chan, O. H.; Reyner, E. L.; Lipka, E.; Showalter, H. D. H.; Vincent, P. W.; Elliott, W. L.; Denny, W. A. Tyrosine kinase inhibitors. 17. Irreversible inhibitors of the epidermal growth factor receptor: 4-(phenylamino)-quinazoline- and 4-(phenylamino)pyrido[3,2-*d*]pyrimidine-6-acrylamides bearing additional solubilizing functions. *J. Med. Chem.* **2000**, *43*, 1380−1397.

(11) Sequist, L. V.; Besse, B.; Lynch, T. J.; Miller, V. A.; Wong, K. K.; Gitlitz, B.; Eaton, K.; Zacharchuk, C.; Freyman, A.; Powell, C.; Ananthakrishnan, R.; Quinn, S.; Soria, J.-C. Neratinib, an irreversible pan-ErbB receptor tyrosine kinase inhibitor: results of a phase II trial in patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.* **2010**, *28*, 3076−3083.

(12) Rabindran, S. K.; Discafani, C. M.; Rosfjord, E. C.; Baxter, M.; Floyd, M. B.; Golas, J.; Hallett, W. A.; Johnson, B. D.; Nilakantan, R.; Overbeek, E.; Reich, M. F.; Shen, R.; Shi, X.; Tsou, H. R.; Wang, Y. F.; Wissner, A. Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the Her-2 tyrosine kinase. *Cancer Res.* **2004**, *64*, 3958−3965.

(13) Reckamp, K. L.; Giaccone, G.; Camidge, D. R.; Gadgeel, S. M.; Khuri, F. R.; Engelman, J. A.; Koczywas, M.; Rajan, A.; Campbell, A. K.; Gernhardt, D.; Ruiz-Garcia, A.; Letrent, S.; Liang, J.; Taylor, I.; O'Connell, J. P.; Jänne, P. A. A Phase 2 trial of dacomitinib (PF-00299804), an oral, irreversible pan-HER (human epidermal growth factor receptor) inhibitor, in patients with advanced non-small cell lung cancer after failure of prior chemotherapy and erlotinib. *Cancer* **2014**, *120*, 1145−1154.

(14) Dungo, R. T.; Keating, G. M. Afatinib: first global approval. *Drugs* **2013**, *73*, 1503−1515.

(15) Zhou, W.; Ercan, D.; Chen, L.; Yun, C.-H.; Li, D.; Capelletti, M.; Cortot, A. B.; Chirieac, L.; Iacob, R. E.; Padera, R.; Engen, J. R.; Wong, K.-K.; Eck, M. J.; Gray, N. S.; Jänne, P. A. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. *Nature* **2009**, *462*, 1070−1074.

(16) Walter, A. O.; Sjin, R. T. T.; Haringsma, H. J.; Ohashi, K.; Sun, J.; Lee, K.; Dubrovskiy, A.; Labenski, M.; Zhu, Z.; Wang, Z.; Sheets, M.; St. Martin, T.; Karp, R.; van Kalken, D.; Chaturvedi, P.; Niu, D.; Nacht, M.; Petter, R. C.; Westlin, W.; Lin, K.; Jaw-Tsai, S.; Raponi, M.; Van Dyke, T.; Etter, J.; Weaver, Z.; Pao, W.; Singh, J.; Simmons, A. D.; Harding, T. C.; Allen, A. Discovery of a mutant-selective covalent inhibitor of EGFR

DOI: 10.1021/acsmedchemlett.5b00193
ACS Med. Chem. Lett. 2015, 6, 987−992

that overcomes T790M mediated resistance in NSCLC. *Cancer Discovery* **2013**, *3*, 1404−1415.
(17) 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.; Brewer, M. R.; 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*, 1046−1061.
(18) Finlay, M. R. V.; Anderton, M.; Ashton, S.; Ballard, P.; Bethel, P. A.; Box, M. R.; Bradbury, R. H.; Brown, S. J.; Butterworth, S.; Campbell, A.; Chorley, C.; Colclough, N.; Cross, D. A. E.; Currie, G. S.; Grist, M.; Hassall, L.; Hill, G. B.; James, D.; James, M.; Kemmitt, P.; Klinowska, T.; Lamont, G.; Lamont, S. G.; Martin, N.; McFarland, H. L.; Mellor, M. J.; Orme, J. P.; Perkins, D.; Perkins, P.; Richmond, G.; Smith, P.; Ward, R. A.; Waring, M. J.; Whittaker, D.; Wells, S.; Wrigley, G. L. Discovery of a potent and selective EGFR inhibitor (AZD9291) of both sensitizing and T790M resistance mutations that spares wild type form of the receptor. *J. Med. Chem.* **2014**, *57*, 8249−8267.
(19) For an account on the research leading up to the discovery of AZD-9291, see: Ward, R. A.; Anderton, M. J.; Ashton, S.; Bethel, P. A.; Box, M.; Butterworth, S.; Colclough, N.; Chorley, C. G.; Chuaqui, C.; Cross, D. A. E.; Dakin, L. A.; Debreczeni, J. E.; Eberlein, C.; Finlay, M. R. V.; Hill, G. B.; Grist, M.; Klinowska, T. C. M.; Lane, C.; Martin, S.; Orme, J. P.; Smith, P.; Wang, F.; Waring, M. J. Structure- and reactivity-based development of covalent inhibitors of the activating and gatekeeper mutant forms of the epidermal growth factor receptor (EGFR). *J. Med. Chem.* **2013**, *56*, 7025−7048.
(20) It should be noted that there has also been an effort on the development of reversible $EGFR^{T790M}$ selective inhibitors; see for example: Lee, H.-J.; Schaefer, G.; Heffron, T. P.; Shao, L.; Ye, X.; Sideris, S.; Malek, S.; Chan, E.; Merchant, M.; La, H.; Ubhayakar, S.; Yauch, R. L.; Pirazzoli, V.; Politi, K.; Settleman, J. Noncovalent Wild-type-sparing inhibitors of EGFR T790M. *Cancer Discovery* **2013**, *3*, 168−181.
(21) Sogabe, S.; Kawakita, Y.; Igaki, S.; Iwata, H.; Miki, H.; Cary, D. R.; Takagi, T.; Takagi, S.; Ohta, Y.; Ishikawa, T. Structure-based approach for the discovery of pyrrolo[3,2-*d*]pyrimidine-based EGFR T790M/L858R mutant inhibitors. *ACS Med. Chem. Lett.* **2013**, *4*, 201−205.
(22) Hanan, E. J.; Eigenbrot, C.; Bryan, M. C.; Burdick, D. J.; Chan, B. K.; Chen, Y.; Dotson, J.; Heald, R. A.; Jackson, P. S.; La, H.; Lainchbury, M. D.; Malek, S.; Purkey, H. E.; Schaefer, G.; Schmidt, S.; Seward, E. M.; Sideris, S.; Tam, C.; Wang, S.; Yeap, S. K.; Yen, I.; Yin, J.; Yu, C.; Zilberleyb, I.; Heffron, T. P. Discovery of selective and noncovalent diaminopyrimidine-based inhibitors of epidermal growth factor receptor containing the T790M resistance mutation. *J. Med. Chem.* **2014**, *57*, 10176−10191.
(23) Pettus, L. H.; Wurz, R. P.; Xu, S.; Herberich, B.; Henkle, B.; Liu, Q.; McBride, H. J.; Mu, S.; Plant, M. H.; Saris, C. J. M.; Sherman, L.; Wong, L. M.; Chmait, S.; Lee, M. R.; Mohr, C.; Hsieh, F.; Tasker, A. S. Discovery and evaluation of 7-alkyl-1,5-bis-aryl-pyrazolopyridinones as highly potent, selective, and orally efficacious inhibitors of p38α mitogen-activated protein kinase. *J. Med. Chem.* **2010**, *53*, 2973−2985.
(24) Wurz, R. P.; Pettus, L. H.; Xu, S.; Henkle, B.; Sherman, L.; Plant, M.; Miner, K.; McBride, H.; Wong, L. M.; Saris, C. J. M.; Lee, M. R.; Chmait, S.; Mohr, C.; Hsieh, F.; Tasker, A. S. Part 1: Structure-activity relationship (SAR) investigations of fused pyrazoles as potent, selective and orally available inhibitors of p38α mitogen-activated protein kinase. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4724−4728.
(25) Chang, S.; Zhang, L.; Xu, S.; Luo, J.; Lu, X.; Zhang, Z.; Xu, T.; Liu, Y.; Tu, Z.; Xu, Y.; Ren, X.; Geng, M.; Ding, J.; Pei, D.; Ding, K. Design, synthesis, and biological evaluation of novel conformationally constrained inhibitors targeting epidermal growth factor receptor $threonine^{790} \rightarrow methionine^{790}$ mutant. *J. Med. Chem.* **2012**, *55*, 2711−2723.
(26) Xu, S.; Xu, T.; Zhang, L.; Zhang, Z.; Luo, J.; Liu, Y.; Lu, X.; Tu, Z.; Ren, X.; Ding, K. Design, synthesis and biological evaluation of 2-oxo-3,4-dihydropyrimido[4,5-*d*]pyrimidinyl derivatives as new irreversible epidermal growth factor receptor inhibitors with improved pharmacokinetic properties. *J. Med. Chem.* **2013**, *56*, 8803−8813.
(27) Xu, T.; Zhang, L.; Xu, S.; Yang, C.-Y.; Luo, J.; Ding, F.; Lu, X.; Liu, Y.; Tu, Z.; Li, S.; Pei, D.; Cai, Q.; Li, H.; Ren, X.; Wang, S.; Ding, K. Pyrimido[4,5-*d*]pyrimidin-4(*1H*)-one derivatives as selective inhibitors of EGFR $threonine^{790}$ to $methionine^{790}$ (T790M) mutants. *Angew. Chem., Int. Ed.* **2013**, *52*, 8387−8390.
(28) Zhou, W.; Liu, X.; Tu, Z.; Zhang, L.; Ku, X.; Bai, F.; Zhao, Z.; Xu, Y.; Ding, K.; Li, H. Discovery of pteridin-7(*8H*)-one-based irreversible inhibitors targeting the epidermal growth factor receptor (EGFR) kinase T790M/L858R mutant. *J. Med. Chem.* **2013**, *56*, 7821−7837.
(29) Wurz, R.; Tasker, A.; Tadesse, S.; Pettus, L. H.; Nguyen, T. T.; Hong, F.-T.; Herberich, B. J.; Harrington, E.; Chen, J. J.; Brown, J. Substituted 7-oxo-pyrido[2,3-*d*]pyrimidines and their use for the treatment of EGFR/ERBB2 related disorders. Patent application: WO 2014/134308, 2014.
(30) For related work see: Ding, K.; Xu, T.; Ding, F.; Zhang, L.; Lu, X.; Li, W.; Ding, J.; Geng, M. 7-Oxo-pyridopyrimidine derivatives, pharmaceutical compositions and uses thereof. Patent application: WO 2014/079232, 2014.
(31) Apsunde, T.; Wurz, R. P. Pyridin-2-one synthesis using ester enolates and aryl aminoaldehydes and ketones. *J. Org. Chem.* **2014**, *79*, 3260−3266.
(32) See for example: Gowan, S. M.; Hardcastle, A.; Hallsworth, A. E.; Valenti, M. R.; Hunter, L.-J. K.; de Haven Brandon, A. K.; Garrett, M. D.; Raynaud, F.; Workman, P.; Aherne, W.; Eccles, S. A. Application of meso scale technology for the measurement of phosphoproteins in human tumor xenografts. *Assay Drug Dev. Technol.* **2007**, *5*, 391−401.
(33) The $KM_{[ATP]}$ values for $EGFR^{WT}$, $EGFR^{T790M}$, $EGFR^{L858R}$, and $EGFR^{T790M/L858R}$ are 5.2, 5.9, 148, and 8.4 μM, respectively; see: 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. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 2070−2075.
(34) Data not shown.
(35) See Figure 3 in Supporting Information.
(36) For a discussion on preclinical ADME properties of 10 clinically late stage or marketed covalent inhibitors, see: Moghaddam, M. F.; Tang, Y.; O'Brien, Z.; Richardson, S. J.; Bacolod, M.; Chaturedi, P.; Apuy, J.; Kulkarni, A. A proposed screening paradigm for discovery of covalent inhibitor drugs. *Drug Metab. Lett.* **2014**, *8*, 19−30.
(37) For a discussion on reactivity of acrylamides with glutathione, see: Naven, R. T.; Kantesaria, S.; Nadanaciva, S.; Schroeter, T.; Leach, K. L. High throughput glutathione and Nrf2 assays to assess chemical and biological reactivity of cysteine-reactive compounds. *Toxicol. Res.* **2013**, *2*, 235−244.
(38) See Figure 1 in Supporting Information for results.
(39) See Figure 2 in Supporting Information for results.
(40) See Supporting Information for details.

DOI: 10.1021/acsmedchemlett.5b00193
ACS Med. Chem. Lett. 2015, 6, 987−992