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C-5 Substituted Pyrido[2,3-*d*]pyrimidin-7-ones as Highly Specific Kinase Inhibitors Targeting Clinical Resistance related EGFR^{T790M} Mutant

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Development of specific kinase inhibitors has been a long-standing challenge in chemical biology and drug discovery. We have successfully discovered a series of C-5 substituted pyrido[2,3-*d*]pyrimidin-7-ones as highly specific inhibitors against clinical resistance related EGFR^{T790M} mutant. One of the most promising compounds **9f** tightly binds with the EGFR^{T790M} mutant and strongly inhibits its enzymatic function with an IC_{50} value of 0.80 nM, and displays an extraordinary target specificity with S(35) and S(10) selectivity scores of 0.005 and 0.000, respectively, in a kinase selectivity profiling study against 456 different kinases at 100nM. The compound also selectively suppresses the proliferation of EGFR^{T790M} mutated H1975 NSCLC cells with an IC_{50} value of 2.80 nM, but is significantly less toxic to cells with wild type EGFR. Compound **9f** may serve as a promising lead compound for drug discovery overcoming the acquired resistance of NSCLC patients without adverse toxicities.

Introduction

Among the 518 kinases identified to date, epidermal growth factor receptor (EGFR, erbB1, HER1) is one of the most validated molecular targets for anticancer therapy. Several reversible or irreversible inhibitors, e.g. gefinitib (**1**), erlotinib (**2**) and gilotrif (BIBW2992, **3**), have been approved by US Food and Drug Administration (FDA) and achieved significant benefits for clinical management of non-small cell lung cancer (NSCLC) patients with activating mutation in the EGFR kinase domain (i.e. L858R and del E746-A750).[1-3] However, acquired resistance against current therapies becomes a major clinical challenge. EGFR "gatekeeper" T790M mutation (Threonine ^{790} → Methionine ^{790}) is a primary mechanism for the resistance against the reversible EGFR inhibitor therapy, [4] which accounts for approximately 60% of clinically resistant NSCLC patients. [5] The EGFR^{T790M} mutation has been demonstrated as a driving force for the resistant NSCLC cell growth, whereas is not detected in normal human tissues. Thus, specifically targeting EGFR^{T790M} represents an ideal strategy to overcome the acquired resistance of NSCLC patients without adverse toxicities. However, development of specific kinase inhibitors has been a long-standing challenge in chemical biology and drug discovery because most of the kinases share highly structural similarity in their ATP binding domains.

A number of irreversible Cys797-reacting EGFR^{T790M} inhibitors have been discovered. [6] However, most of them suffer from unacceptably low clinical Maximum-Tolerated Dose (MTD) in resistant patients because of their poor margin over wild type EGFR (EGFR^{WT}) inhibition. [7, 8] Only recently, several selective EGFR^{T790M} inhibitors were identified, [9-11] among which CO-1686 (**5**) [11] and AZD9291 (**6**) [12,17] have been demonstrated significant benefits for the EGFR^{T790M} mutated NSCLC patients in clinical investigations. Both of the compounds were granted "Breakthrough Therapy" designations by US FDA in 2014. However, side effects such as hyperglycaemia or skin rash were recently reported for a portion of CO-1686-treated patients, which may be due to its concurrent inhibition against EGFR^{WT} and the other kinases. [4] Diarrhea and skin rash were also observed as the most common all-cause adverse events for the AZD9291-treatment [18] which may be due to the EGFR^{WT} inhibition of its active metabolites.[12] It is still desirable to identify novel wild-type sparing EGFR^{T790M} inhibitors with improve selectivity profiles.

Herein, we would like to report the design, synthesis and biological evaluation of C-5 substituted pyrido[2,3-d]pyrimidin-7-ones as new selective EGFR^{T790M} inhibitors.

Results and discussion

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Conformational constraint is a tractable strategy to improve a drug's target specificity. [16] Accordingly, we designed and synthesized a series of conformationally rigid lead molecules (**9**), in which a privileged pyrido[2,3-*d*]pyrimidin-7-one core that has been widely utilized in clinically investigated or FDA approved drugs including palbociclib (**8**) [19] is adopted. An acrylamide moiety is preserved in the molecules to potentially capture an essential nucleophilic addition with Cys797 in EGFR^{T790M} (Fig. 1). [20, 21]

Compounds **9** were readily prepared by utilizing 2-chloro or 2-methylthio pyrido[2,3-*d*]pyrimidin-7(8*H*)-ones (**10**) as the starting materials (Supporting Information). Compounds **10** coupled with substituted anilines (**11**) and followed by deprotection to obtain intermediates **12**. A straightforward acryloylation of compounds **12** produced the designed compounds **9a – 9t** in good yields (Scheme 1, Supporting Information).

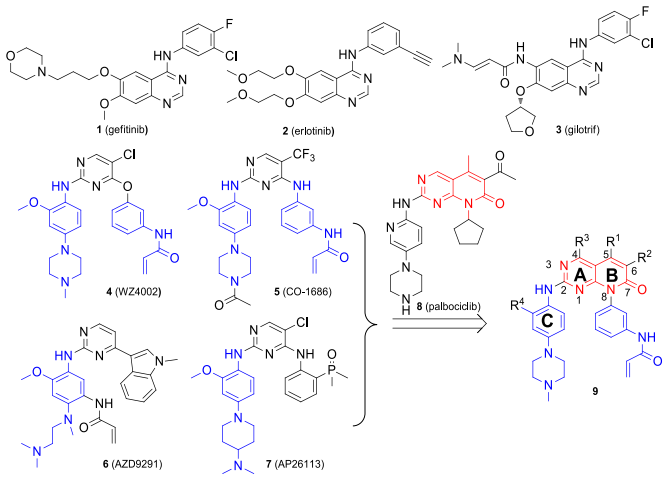
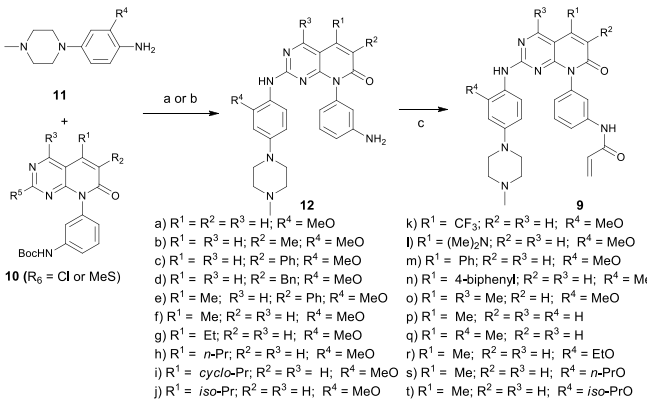


Figure 1. Design of pyrido[2,3-*d*]pyrimidin-7-ones as new druglike selective EGFR^{T790M} inhibitors *via* a scaffold-chopping strategy



Scheme 1. Syntheses of the designed inhibitors **9a – 9t**. Reagents and conditions: a) i) TFA, 2-Butanol, 100 °C; ii) TFA, DCM, rt, 60-80% (two steps). b) i) m-CPBA, DCM, 0 °C to rt; ii) 11, TFA, 2-butanol, 110 °C,; iii) TFA, DCM, rt, 30-40% (three steps). c) acryloyl chloride, DIPEA, DCM, 0 °C, 55-70%

The kinase inhibitory activities against EGFR$^{L858R/T790M}$ and EGFR^{WT} (IC_{50}) of the compounds were evaluated by using a well-established ELISA assay. [22] Five well recognized EGFR inhibitors, i.e. **1** (gefitinib), **3** (BIBW2992), WZ4002 (**4**), **5** (CO-1686) and **6** (AZD 9291), were included as positive controls. Under the experimental conditions, all of the drugs displayed similar IC_{50} values and the selectivity profiles to the reported data. [9,12,17,23] Compound **9a**, our first designed molecule, potently inhibited EGFR$^{L858R/T790M}$ with an IC_{50} value of 0.5 nM, which is 13-29 folds more potent than the 2nd or 3rd generation EGFR inhibitors (**3**, **4**, **5** and **6**) in a parallel comparison. Furthermore, the compound demonstrated an approximate 30-fold selectivity between the T790M mutant and the wild type EGFR, which is comparable to that of clinically investigated drug **5** or **6**. It also showed a preferred binding with EGFR$^{L858R/T790M}$ with a K_d value of 0.43 nM, but is 32.5 times less potent to EGFR^{WT}, in an active-site-dependent competition binding assay conducted by DiscovRx, SanDiego, USA (Table 1). [29,30]

Table 1. *In vitro* kinase inhibitory activities of compounds **9a – 9t** against EGFR$^{L858R/T790M}$ and EGFR^{WT}, and their binding affinities with the proteins.

Cpds	Kinase inhibition (IC_{50}, nM) [a]		Binding affinities (K_d, nM) [b]	
	EGFR^{WT}	EGFR$^{L858R/T790M}$	EGFR^{WT}	EGFR$^{L858R/T790M}$
9a	15.0±2.4	0.50±0.10	14.0	0.43
9b	16.0±4.9	1.3±0.3	6.2	0.88
9c	7.8±4.8	0.20±0.00	2.5	0.50
9d	1.0±0.1	0.5±0.3	1.2	0.50
9e	20.0±1.4	1.6±0.0	4.3	0.91
9f	240±24	0.80±0.10	48.	0.26
9g	114±42	6.7±0.8	140	1.8
9h	970±260	17.8±6.5	N.D.	N.D.
9i	120±12	10.0±0.1	57.0	2.2
9j	98.0±3.3	94±2.8	N.D.	N.D.
9k	>1000	39±2.7	N.D.	N.D.
9l	210±20	95±6.1	N.D.	N.D.
9m	97±18	21±4.1	N.D.	N.D.
9n	>1000.0	>1000.0	N.D.	N.D.
9o	>1000	280±10	N.D.	N.D.
9p	>1000	2.9±0.7	34.0	0.16
9q	>1000	23.5±2.4	72.0	0.27
9r	170±100	11.0±0.7	350	1.2
9s	270±60	19.0±3.6	N.D.	N.D.
9t	>1000	78±27	N.D.	N.D.
1	13±9.3	845±34	2.4 [c]	10.9 [c]
3	2.4±0.4	9.5±1.9	0.25 [c]	1.10 [c]
4	35±3. 1	6.4±1.6	46.0 [c]	N.R.
5	350±21	14.3±4.5	180 [c]	7.0 [c]
6	210±13	7.0±1.5	N.R.	N.R.

[a] EGFR activity assays were performed using an ELISA assay according to the reported protocols. [23] The compounds were incubated with the kinase reaction mixture for 1.0 hr before measurement. The data are means from 3 independent experiments. [b] Binding constant values (K_d) were determined from DiscovRx. The data are means from two independent experiments and the variation is less than 15%. [c] Reported data. [9,24-27] N. D., not determined. N.R., not reported.

Further structure-activity relationship investigation demonstrated that 6- position (R^2 in ring B) of the compound is well tolerated by various hydrophobic substituents [e.g. methyl (**9b**), phenyl (**9c**) and benzyl (**9d**)] without obviously affecting the EGFR$^{L858R/T790M}$ inhibitory activity. However, the substitutions caused significant selectivity loss between EGFR$^{L858R/T790M}$ and EGFR^{WT} in both kinase assay and binding assay evaluations. For instance, 6-benzyl analogue **9d** displayed similar IC_{50} value for EGFR$^{L858R/T790M}$ to that of **9a**, but its selectivity between EGFR^{WT} and T790M mutant was dramatically decreased to about 2.0 folds. A large substitution

at 5- position (R^1) is detrimental to the kinase inhibition. For instance, 5-ethyl (**9g**), 5-(n-)propyl (**9h**), 5-(iso-) propyl (**9j**), 5-CF3 (**9k**), 5-*N, N*-dimethylamino (**9l**), and 5-phenyl (**9m**) derivatives displayed 13-190 fold potency loss comparing with the parental compound **9a**, and 5-biphenyl derivative **9n** totally abolished its EGFR inhibitory activity. Encouragingly, 5-methyl analogue **9f** displayed an IC_{50} value of 0.8 nM against $EGFR^{L858R/T790M}$, whereas the corresponding number for $EGFR^{WT}$ is about 300 fold greater. Further binding affinity determination confirmed that **9f** selectively bound with the T790M mutant with a K_d value of 0.26 nM, but was 184.6 folds less potent to the wild type protein. Interestingly, induction of an additional 4-methyl group (**9o**, R^3 = Me) in **9f** caused a 345-fold potency decrease. Further investigation also revealed that the R^4-methoxy group in **9f** could be removed without obviously affecting the potency and selectivity (**9p**). However, when the R^4-methoxy moiety was replaced by a methyl (**9q**), ethoxyl (**9r**), (*n*-)propoxyl (**9r**) or *iso*- propoxyl group (**9t**), the resulting compounds are significantly less potent.

In view of its encouraging *in vitro* activity and selectivity profiles, compound **9f** was selected as an example for further validating its selective $EGFR^{T790M}$ inhibition by investigating the effect on activation of EGFR and downstream signals in H1975 and A431 cancer cells harbouring different forms of EGFR (Fig. 2). It is shown that **9f** dose-dependently inhibits the phosphorylation of EGFR and downstream proteins such as AKT and ERK with no obvious effect on their total proteins in H1975 NSCLC cells with $EGFR^{T790M}$ mutation. However, its effect in A431 cells is significantly less potent. For instance, compound **9f** almost induced a complete blockage of EGFR activation in H1975 cells at 10 nM, but it only showed detectible inhibition when a concentration of 100 nM was used for A431 cells. Similar to the previous observation, the positive compound 4 also displayed selective suppression EGFR signals in H1975 cells, while **9f** is apparently about 10 folds more potent.

To build a confidence on the covalent binding mode of **9f** with $EGFR^{T790M}$, an inhibitor washout assay was performed in H1975 NSCLC cells (Fig. 3). [28] The results clearly showed that activation of $EGFR^{L858R/T790M}$ and downstream ERK is constantly inhibited and no sign of $EGFR^{L858R/T790M}$ phosphorylation recovery was observed after **9f** was removed for 24 hrs, which is quite similar to compound **4**, strongly suggesting that the compound irreversibly bound with the target. The irreversible binding of **9f** with $EGFR^{L858R/T790M}$ was also confirmed by an in vitro mobility shift assay (Fig. S1, Supporting Information). [27]

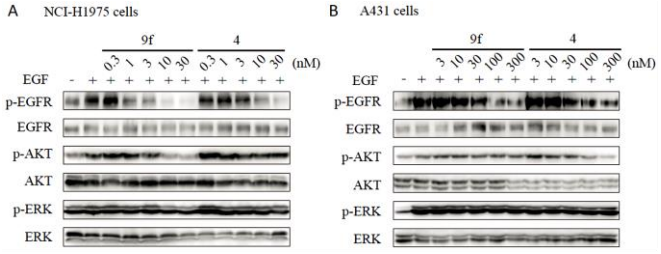


Figure 2. Compound **9f** potently and selectively inhibits the activation of EGFR signals in H1975 NSCLC cells (A) but is significantly less potent in A431 cancer cells (B). Cells were starved for 12 hrs and treated with or without inhibitors for 2 hrs at indicated concentrations, respectively. Cells were then stimulated by 50 ng/ml EGFR for 15 min and harvested for Western blot analysis.

In order to demonstrate the target specificity of **9f**, a kinase selectivity profiling study was conducted by using the DiscovRx screening platform. The results revealed that **9f** displayed an excellent specificity on $EGFR^{T790M}$ (Fig. 4, Fig. S2, Supporting Information). [29] For instance, compound **9f** showed almost 100% competition rates (99.5% inhibition, Ctrl% = 0.05) with $EGFR^{L858R/T790M}$ and $EGFR^{T790M}$, while it did not show obvious binding (inhibition rate < 65%, or Ctrl% > 35%) with almost all of the 456 kinases (including 395 non-mutated kinases) evaluated at 100 nM (which is about 130 folds of its IC_{50} value against $EGFR^{L858R/T790M}$). The S(35) and S(1) selectivity scores of **9f** are 0.005 and 0.000, respectively, supporting it as one of the most specific $EGFR^{T790M}$ inhibitors to date.

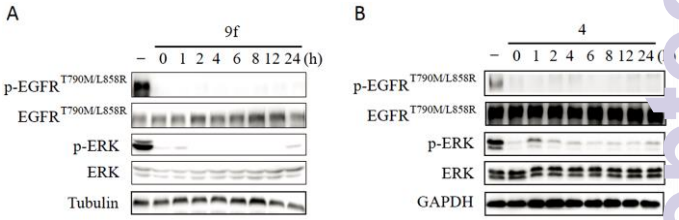


Figure 3. Inhibitor wash-out assay demonstrates the irreversible binding of **9f** with $EGFR^{L858R/T790M}$. NCI-H1975 cells were treated with or without compound **9** (1.0 μM) for 2 hrs, and then the medium with compound was removed and fresh medium was added immediately (defined as 0 hr). And then at the indicated time points, cells were harvest and proteins were extracted and subjected to Western blot analysis (A). Compound **4** was used as a positive control (B)

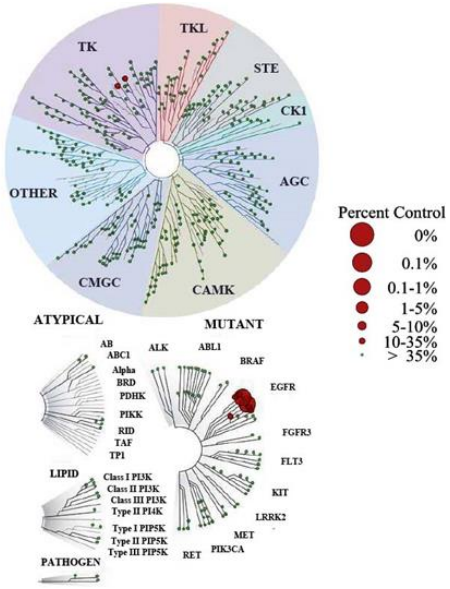


Figure 4. The KINOMEsacn tree spot map illustrating the selectivity profiles of compound **9f** *versus* a panel of 456 kinase targets (including 395 wild-type kinases). The size of the red circle is proportional to the percentage of DMSO control, where 0 and 35% of control equals 100 and 65% competition respectively. [27]

The resistance-overcoming potential of **9f** was investigated by evaluating its antiproliferative activities against H1975 NSCLC cells harbouring $EGFR^{L858R/T790M}$. Its effects on $EGFR^{WT}$ overexpressing A431 epidermoid carcinoma cells and a panel of normal cells, were also determined to monitor the potential off-target toxicity (Table S2 and S3, Supporting Information). It was shown that **9f** strongly inhibits the growth of H1975 cancer cells with an IC_{50} value of 2.8 nM, while its activity against A431 cancer cells is about 870 nM and is over 311 fold less potent than that for H1975 cells, which further validates its $EGFR^{T790M}$ selectivity over the wild type kinase. More significantly, **9f** displays over 1000-folds less cytotoxicity against a panel of normal cell lines, supporting its favourable safety index and promising potential for further development.

Conclusions

In summary, C-5 substituted pyrido[2,3-*d*]pyrimidin-7-ones were identified as highly specific $EGFR^{T790M}$ inhibitors. A representative compound **9f** selectively binds with and inhibits $EGFR^{T790M}$ with pM potencies, while not showing obvious effect on a panel of 456 kinases at 100 nM concentration, supporting it as one of the most specific kinase inhibitors to date. Moreover, the compound selectively suppresses proliferation of $EGFR^{T790M}$ mutated H1975 NSCLC cells with a low nM IC_{50} value, but is significantly less toxic to a variety of normal cell lines. Compound **9f** may serve as a promising lead compound for new drug discovery overcoming the acquired resistance of NSCLC patients without adverse toxicities.

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References

1 T. Mitsudomi, S. Morita, Y. Yatabe, S. Negoro, I. Okamoto, J. Tsurutani, T. Seto, M. Satouchi, H. Tada, T. Hirashima, K. Asami, N. Katakami, M. Takada, H. Yoshioka, K. Shibata, S. Kudoh, E. Shimizu, H. Saito, S. Toyooka, K. Nakagawa, M. Fukuoka, W. J. O. Grp, *Lancet Oncol* 2010, **11**, 121-128.

2 R. Rosell, E. Carcereny, R. Gervais, A. Vergnenegre, B. Massuti, E. Felip, R. Palmero, R. Garcia-Gomez, C. Pallares, J. M. Sanchez, R. Porta, M. Cobo, P. Garrido, F. Longo, T. Moran, A. Insa, F. De Marinis, R. Corre, I. Bover, A. Illiano, E. Dansin, J. de Castro, M. Milella, N. Reguart, G. Altavilla, U. Jimenez, M. Provencio, M. A. Moreno, J. Terrasa, J. Munoz-Langa, J. Valdivia, D. Isla, M. Domine, O. Molinier, J. Mazieres, N. Baize, R. Garcia-Campelo, G. Robinet, D. Rodriguez-Abreu, G. Lopez-Vivanco, V. Gebbia, L. Ferrera-Delgado, P. Bombaron, R. Bernabe, A. Bearz, A. Artal, E. Cortesi, C. Rolfo, M. Sanchez-Ronco, A. Drozdowskyj, C. Queralt, I. de Aguirre, J. L. Ramirez, J. J. Sanchez, M. A. Molina, M. Taron, L. Paz-Ares, G. F. Pneumocancerologie, A. I. O. Toracica, *Lancet Oncol* 2012, **13**, 239-246.

3 G. M. Keating, *Drugs* 2014, **74**, 207-221.

4 J. Remon, T. Moran, M. Majem, N. Reguart, E. Dalmau, D. Marquez-Medina, P. Lianes, *Cancer Treat Rev* 2014, **40**, 93-101.

5 a) S. Kobayashi, T. J. Boggon, T. Dayaram, P. A. Janne, O. Kocher, M. Meyerson, B. E. Johnson, M. J. Eck, D. G. Tenen, B. Halmos, *New Engl J Med* 2005, **352**, 786-792; b) L. V. Sequist, B. A. Waltman, D. Dias-Santagata, S. Digumarthy, A. B. Turke, P. Fidias, K. Bergethon, A. T. Shaw, S. Gettinger, A. K. Cosper, S. Akhavanfard, R. S. Heist, J. Temel, J. G. Christensen, J. C. Wain, T. J. Lynch, K. Vernovsky, E. J. Mark, M. Lanuti, A. J. Iafrate, M. Mino-Kenudson, J. A. Engelman, *Sci Transl Med* 2011, **3**, 75ra26.

6 a) E. L. Kwak, R. Sordella, D. W. Bell, N. Godin-Heymann, R. I. Okimoto, B. W. Brannigan, P. L. Harris, D. R. Driscoll, P. Fidias, T. J. Lynch, S. K. Rabindran, J. P. McGinnis, A. Wissner, S. V. Sharma, K. J. Isselbacher, J. Settleman, D. A. Haber, *P Natl Acad Sci USA* 2005, **102**, 7665-7670; b) A. J. Gonzales, K. E. Hook, I. W. Althaus, P. A. Ellis, E. Trachet, A. M. Delaney, P. J. Harvey, T. A. Ellis, D. M. Amato, J. M. Nelson, D. W. Fry, T. Zhu, C. M. Loi, S. A. Fakhoury, K. M. Schlosser, K. E. Sexton, R. T. Winters, J. E. Reed, A. J. Bridges, D. J. Lettiere, D. A. Baker, J. X. Yang, H. T. Lee, H. Tecle, P. W. Vincent, *Mol Cancer Ther* 2008, **7**, 1880-1889; c) D. Li, L. Ambrogio, T. Shimamura, S. Kubo, M. Takahashi, L. R. Chirieac, R. F. Padera, G. I. Shapiro, A. Baum, F. Himmelsbach, W. J. Rettig, M. Meyerson, F. Solca, H. Greulich, K. K. Wong, *Oncogene* 2008, **27**, 4702-4711; d) G. R. Oxnard, M. E. Arcila, J. Chmielecki, M. Ladanyi, V. A. Miller, W. Pao, *Clin Cancer Res* 2011, **17**, 5530-5537.

7 a) L. V. Sequist, B. Besse, T. J. Lynch, V. A. Miller, K. K. Wong, B. Gitlitz, K. Eaton, C. Zacharchuk, A. Freyman, C. Powell, Ananthakrishnan, S. Quinn, J. C. Soria, *J Clin Oncol* 2010, **28**, 3076-3083; b) P. A. Janne, K. Reckamp, M. Koczywas, D. R. Camidge, J. A. Engelman, F. Khuri, A. Rajan, S. Gadgeel, I. Taylor, J. Liang, J. O'Connell, G. Giaccone, *J Thoracic Oncol* 2009, **4**, S293-S294.

8 a) S. S. Ramalingam, F. Blackhall, M. Krzakowski, C. H. Barrios, K. Park, I. Bover, D. S. Heo, R. Rosell, D. C. Talbot, R. Frank, S. P. Letrent, A. Ruiz-Garcia, I. Taylor, J. Q. Liang, A. K. Campbell, J. O'Connell, M. Boyer, *J Clin Oncol* 2012, **30**, 3337-3344; b) L. V. Sequist, J. C.-H. Yang, N. Yamamoto, K. O'Byrne, V. Hirsh, T. Mok, S. L. Geater, S. Orlov, C.-M. Tsai, M. Boyer, *J Clin Oncol* 2013, **31**, 3327-3334.

9 W. J. Zhou, D. Ercan, L. Chen, C. H. Yun, D. N. Li, M. Capelletti, A. B. Cortot, L. Chirieac, R. E. Iacob, R. Padera, J. R. Engen, K. K. Wong, M. J. Eck, N. S. Gray, P. A. Janne, *Nature* 2009, **462**, 1070-1074.

10 A. O. Walter, R. T. T. Sjin, H. J. Haringsma, K. Ohashi, J. Sun, K. Lee, A. Dubrovskiy, M. Labenski, Z. D. Zhu, Z. G. Wang, M. Sheets, T. St Martin, R. Karp, D. van Kalken, P. Chaturvedi, D. S. Niu, M. Nacht, R. C. Petter, W. Westlin, K. Lin, S. Jaw-Tsai, M. J. Raponi, T. Van Dyke, J. Etter, Z. Weaver, W. Pao, J. Singh, A. G. Simmons, T. C. Harding, A. Allen, *Cancer Discov* 2013, **3**, 1404-1415.

11 L. V. Sequist, J.-C. Soria, S. M. Gadgeel, H. Wakelee, D. R. Camidge, A. Varga, P. Fidias, A. Wozniak, J. Neal, R. C. Doebele,

E. B. Garon, S. S. Jaw-Tsai, J. C. Stern, A. R. Allen, J. W. Goldman, *J Clin Oncol* 2013, **31**, abstr 2524.

12 D. A. Cross, S. E. Ashton, S. Ghiorghiu, C. Eberlein, C. A. Nebhan, P. J. Spitzler, J. P. Orme, M. R. Finlay, R. A. Ward, M. J. Mellor, G. Hughes, A. Rahi, V. N. Jacobs, M. Red Brewer, E. Ichihara, J. Sun, H. Jin, P. Ballard, K. Al-Kadhimi, R. Rowlinson, T. Klinowska, G. H. Richmond, M. Cantarini, D. W. Kim, M. R. Ranson, W. Pao, *Cancer Discov* 2014, **4**, 1046-1061.

13 D. R. Camidge, L. Bazhenova, R. Salgia, G. J. Weiss, C. J. Langer, A. T. Shaw, N. I. Narasimhan, D. J. Dorer, J. Zhang, S. N. Gettinger, *Eur J Cancer* 2013, **49**, S795-S795

14 a) D. Kim, D. Lee, J. Kang, K. Park, J. Han, J. Lee, I. Jang, H. Kim, J. Son, J. Kim, *J Clin Oncol* 2014, **32**, 5s; b) K.-O. Lee, M. Y. Cha, M. Kim, J. Y. Song, J.-H. Lee, Y. H. Kim, Y.-M. Lee, K. H. Suh, J. Son, *Cancer Res* 2014, **74**, abstr LB-100.

15 H. J. Lee, G. Schaefer, T. P. Heffron, L. Shao, X. F. Ye, S. Sideris, S. Malek, E. Chan, M. Merchant, H. La, S. Ubhayakar, R. L. Yauch, V. Pirazzoli, K. Politi, J. Settleman, *Cancer Discov* 2013, **3**, 168-181.

16 a) S. H. Chang, L. W. Zhang, S. L. Xu, J. F. Luo, X. Y. Lu, Z. Zhang, T. F. Xu, Y. X. Liu, Z. C. Tu, Y. Xu, X. M. Ren, M. Y. Geng, J. Ding, D. Q. Pei, K. Ding, *J Med Chem* 2012, **55**, 2711-2723; b) S. L. Xu, L. W. Zhang, S. H. Chang, J. F. Luo, X. Y. Lu, Z. C. Tu, Y. X. Liu, Z. Zhang, Y. Xu, X. M. Ren, K. Ding, *Medchemcomm* 2012, **3**, 1155-1159; c) T. F. Xu, L. W. Zhang, S. L. Xu, C. Y. Yang, J. F. Luo, F. Ding, X. Y. Lu, Y. X. Liu, Z. C. Tu, S. L. Li, D. Q. Pei, Q. Cai, H. L. Li, X. M. Ren, S. M. Wang, K. Ding, *Angew Chem Int Edit* 2013, **52**, 8387-8390; d) W. Zhou, X. F. Liu, Z. C. Tu, L. W. Zhang, X. Ku, F. Bai, Z. J. Zhao, Y. F. Xu, K. Ding, H. L. Li, *J Med Chem* 2013, **56**, 7821-7837; e) S. L. Xu, T. F. Xu, L. W. Zhang, Z. Zhang, J. F. Luo, Y. X. Liu, X. Y. Lu, Z. C. Tu, X. M. Ren, K. Ding, *J Med Chem* 2013, **56**, 8803-8813.

17 M. R. Finlay, M. Anderton, S. Ashton, P. Ballard, P. A. Bethel, M. R. Box , R. H. Bradbury, S. J. Brown, S. Butterworth , A. Campbell, C. Chorley, N. Colclough, D. A. Cross, G. S. Currie, M. Grist, L. Hassall, G. B. Hill, D. James , M. James, P. Kemmitt, T. Klinowska, G. Lamont, S. G. Lamont, N. Martin, H. L. McFarland, M. J. Mellor, J. P. Orme, D. Perkins, P. Perkins, G. Richmond, P. Smith, R. A. Ward, M. J. Waring, D. Whittaker, S. Wells, G. L. Wrigley, *J Med Chem* 2014, **57**, 8249-8267.

18 P. A. Jänne, J. C. Yang, D. W. Kim, D. Planchard, Y. Ohe, S. S. Ramalingam, M. J. Ahn, S. W. Kim, W. C. Su, L. Horn, D. Haggstrom, E. Felip, J. H. Kim, P. Frewer, M. Cantarini, K. H. Brown, P. A. Dickinson, S. Ghiorghiu, M. Ranson, *New Engl J Med* 2015, **372**, 1689-1699.

19 K. T. Flaherty, P. M. LoRusso, A. DeMichele, V. G. Abramson, R. Courtney, S. S. Randolph, M. N. Shaik, K. D. Wilner, P. J. O'Dwyer, G. K. Schwartz, *Clin Cancer Res* 2012, **18**, 568-576.

20 K. Ding, T. F. Xu, F. Ding, L. W. Zhang, X, Y, Lu, W, H, Li, J. Ding, M. Y. Geng, WO, 2014/079232 A1.

21 Soon after the authors filed the patent application, Amgen Inc. independently filed a similar patent on this class of compounds: R. Wurz, A. Tasker, S. Tadesse, L. H. Pettus, T. T. Nguyen, F.-T. Hong, B. J. Herberich, E. Harrington, J. J. Chen, J. Brown, WO, 2014/134308 A1.

22 T. Peng, J.-R. Wu, L.-J. Tong, M.-Y. Li, F. Chen, Y.-X. Leng, R. Qu, K. Han, Y. Su, Y. Chen, W.-h. Duan, H. Xie, J. Ding, *Acta pharmacologica Sinica* 2014, **35**, 916-928.

23 D. Li, L. Ambrogio, T. Shimamura, S. Kubo, M. Takahashi, L Chirieac, R. Padera, G. Shapiro, A. Baum, F. Himmelsbach, W. J Rettig, M. Meyerson, F. Solca, H. Greulich, K.-K. Wo g *Oncogene* 2008, **27**, 4702-4711.

24 M. A. Fabian, W. H. Biggs, D. K. Treiber, C. E. Atteridge, M. D. Azimioara, M. G. Benedetti, T. A. Carter, P. Ciceri, P. T. Edeen, M Floyd, J. M. Ford, M. Galvin, J. L. Gerlach, R. M. Grotzfeld, S Herrgard, D. E. Insko, M. A. Insko, A. G. Lai, J. M. Lelias, S. A Mehta, Z. V. Milanov, A. M. Velasco, L. M. Wodicka, H. K. Patel, P. P. Zarrinkar, D. J. Lockhart, *Nat Biotechnol* 2005, **23**, 329-336.

25 A. O. Walter, R. Tjin, H. Haringsma, K. K. Lin, A. Dubrovskiy, K Lee, T. S. Martin, R. Karp, Z. Zhu, D. Niu, M. Nacht, K. Suda, T Mitsudomi, R. C. Petter, W. F. Westlin, J. Singh, M. Raponi, A Allen, *Cancer Res* 2012, **72**, Abstract nr 1791.

26 M. I. Davis, J. P. Hunt, S. Herrgard, P. Ciceri, L. M. Wodicka, Pallares, M. Hocker, D. K. Treiber, P. P. Zarrinkar, *Nat Biotechnol* 2011, **29**, 1046-U1124.

27 C. H. Yun, K. E. Mengwasser, A. V. Toms, M. S. Woo, H. Greulich, K. K. Wong, M. Meyerson, M. J. Eck, *P Natl Acad Sci USA* 2008 **105**, 2070-2075.

28 H. Xie, L. Lin, L. Tong, Y. Jiang, M. Zheng, Z. Chen, X. Jiang, X Zhang, X. Ren, W. Qu, Y. Yang, H. Wan, Y. Chen, J. Zuo, H Jiang, M. Geng, J. Ding, *Plos One* 2011, **6**, e21487.

29 M. W. Karaman, S. Herrgard, T. K. Treiber, P. Gallant, C. E Atteridge, B. T. Campbell, K. W. Chan, P. Ciceri, M. I. Davis, P. T. Edeen, R. Faraoni, M. Floyd, J. P. Hunt, D. J. Lockhart, Z. V Milanov, M. J. Morrison, G. Pallares, H. K. Patel, S. Pritchard, L. M. Wodicka, P. P. Zarrinkar, *Nat. Biotechnol.* 2008, **26** 127-132.

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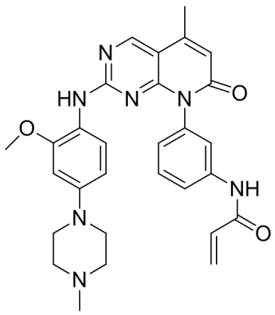
Table of Contents

C-5 Substituted Pyrido[2,3-*d*]pyrimidin-7-ones as Highly Specific Kinase Inhibitors Targeting Clinical Resistance related EGFR^{T790M} Mutant

Tianfeng Xu, Ting Peng, Xiaomei Ren, Lianwen Zhang, Lei Yu, Jinfeng Luo, Zhang Zhang, Zhengchao Tu, Linjiang Tong, Zhaoru Huang, Xiaoyun Lu, Meiyu Geng, Hua Xie, Jian Ding, and Ke Ding



EGFR$^{L858R/T790M}$:
Kinase inhibition(IC_{50}) : 0.8 nM
Protein binding (K*d*) : 0.26 nM
Target specificity (465 kinases, @ 100nM)
S(35) : 0.005
(S(10) : 0.000
Antiproliferation (IC_{50}):
H1975 cells (EGFR$^{L858R/T790M}$): 2.8 nM
Selectivity over cells without EGFR^{T790M}: 310-1000 folds

C-5 substituted pyrido[2,3-*d*]pyrimidin-7-ones were discovered as highly potent and specific inhibitors targeting clinical resistance related EGFR$^{L858R/T790M}$ mutant.