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# Design, synthesis and SAR study of 2-aminopyrimidines with diverse Michael addition acceptors for chemically tuning the potency against EGFR<sup>L858R/T790M</sup>

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## ABSTRACT:

The covalent binding nature of irreversible kinase inhibitors potentially increases the severity of “off-target” toxicity. Based on our continual strategy of chemically tuning the Michael addition acceptors, herein, we further explore the relationship among the electronic nature of Michael addition acceptors and EGFR<sup>T790M</sup> mutation selectivity as well as “off-target” toxicity balance. By perturbing the electronic nature of acrylamide moiety, compound **8a** with a chloro-group at the  $\alpha$ -position of the Michael addition acceptor was identified. It was found that **8a** retained the excellent EGFR<sup>L858R/T790M</sup> potency ( $IC_{50} = 3.9$  nM) and exhibited good anti-proliferative activities against the gefitinib-resistant NCI-H1975 cells ( $IC_{50} = 0.75$   $\mu$ M). Moreover, **8a** displayed a significant EGFR<sup>WT</sup> selectivity and much weaker inhibitory activity against non-EGFR dependent SW620 cell and COS7. Preliminary study showed that **8a** could arrest NCI-H1975 cells in G0/G1 phase. This work provides a promising chemical tuned strategy for balancing the mutant-EGFR potency and selectivity as well as “off-target” toxicity. **KEYWORDS:** NSCLC; EGFR; resistant mutants; chemical tuned; Michael addition acceptors

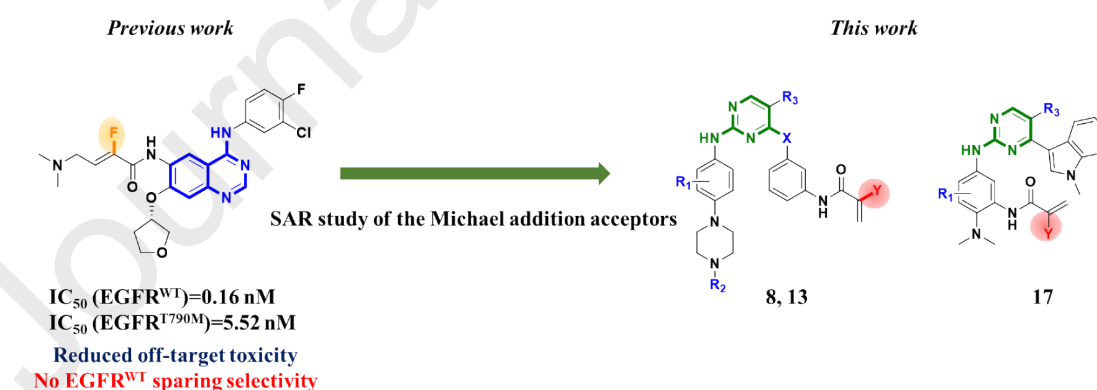
## 1. INTRODUCTION

Overexpression or abnormal activation of the epidermal growth factor receptor (EGFR) is one of the major drivers of non-small cell lung cancer (NSCLC), which accounts for 85% of lung cancer. Approximately 50% of Asian patients and 11-16% of patients in Western countries with NSCLC harbor mutations in EGFR. [1-2] The majority of genetic EGFR alterations are deletions within exon 19 (60%) and L858R point mutation (30%). [3] These sensitizing EGFR mutations pave the way for the development and therapeutic application of EGFR TKIs. The first-generation inhibitors gefitinib, [4-5] erlotinib [6] and icotinib [7] as well as the second-generation inhibitors afatinib [8] were approved for the treatment of patients with the most frequent EGFR mutations (L858R and exon 19 deletions). However, patients acquired resistances and suffered a relapse within 18 months of TKIs treatment. Biopsies of resistant tumors revealed a single point mutation

of the gatekeeper residue (T790M) as the major resistant mechanism.<sup>[9]</sup> Due to the unacceptable dose-limiting toxicity associated with quinazoline core against the wild-type (WT) EGFR inhibition, even irreversible inhibitor afatinib did not exhibit improved clinical efficacy for the NSCLC patients who harbor T790M mutation.<sup>[10]</sup> Hence, third-generation inhibitors, such as rociletinib<sup>[11]</sup>, osimertinib,<sup>[12]</sup> olmutinib<sup>[13]</sup> and almonertinib (NCT03849768), structurally based on 2-aminopyrimidine were developed to overcome the above “on-target” toxicity. In contrast to the classical quinazoline core, the aminopyrimidine scaffold demonstrated excellent selectivity sparing EGFR<sup>WT</sup> and good activity against the T790M resistance mutation.<sup>[14-15]</sup> Also these EGFR inhibitors are developed with irreversible Cys797-targeting moieties.<sup>[16]</sup> This strategy would enable the drugs to bind the target covalently. The off-rate is negligible compared to that of a non-covalent drug and therefore these drugs would have a prolonged therapeutic effect and circumvent drug resistance.<sup>[17]</sup>

However, it is far from perfect that the covalent binding nature of irreversible inhibitors potentially increases the severity of “off-target” toxicity.<sup>[18]</sup> The intrinsic irreversible chemistry of reactive acrylamide moiety is more likely to form permanent covalent adducts<sup>[19]</sup> with off-target proteins, especially those off-target kinases with a homologous cysteine or unrelated targets with hyper-reactive cysteine.<sup>[20]</sup> How to exploit novel covalent inhibitors for overcoming the issue of drug resistance and reducing the risk of nonspecific covalent binding is still challenging.

Our previous work has demonstrated that fluoro-substituted olefins **could** retain the mutant-EGFR **inhibition** and increase the safety index of irreversible inhibitors. (**Figure 1**)<sup>[21]</sup> But the previous hit did not show excellent EGFR<sup>WT</sup> selectivity. To our continued efforts, novel 2-aminopyrimidine analogues bearing  $\alpha$ -position substituted acrylamides, including F, Cl, Ph, CH<sub>3</sub>, were designed and synthesized in this work. We aimed to investigate the relationship among the electronic nature of the acrylamide and mutant-EGFR selectivity as well as the “off-target” toxicity.



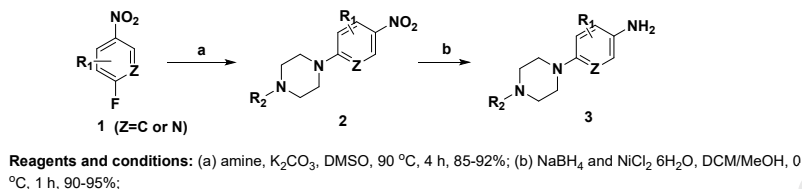
**Figure 1.** Strategy on chemical tuning the electronic nature of the Michael addition acceptors

## 2. RESULTS AND DISCUSSION

### 2.1 CHEMISTRY

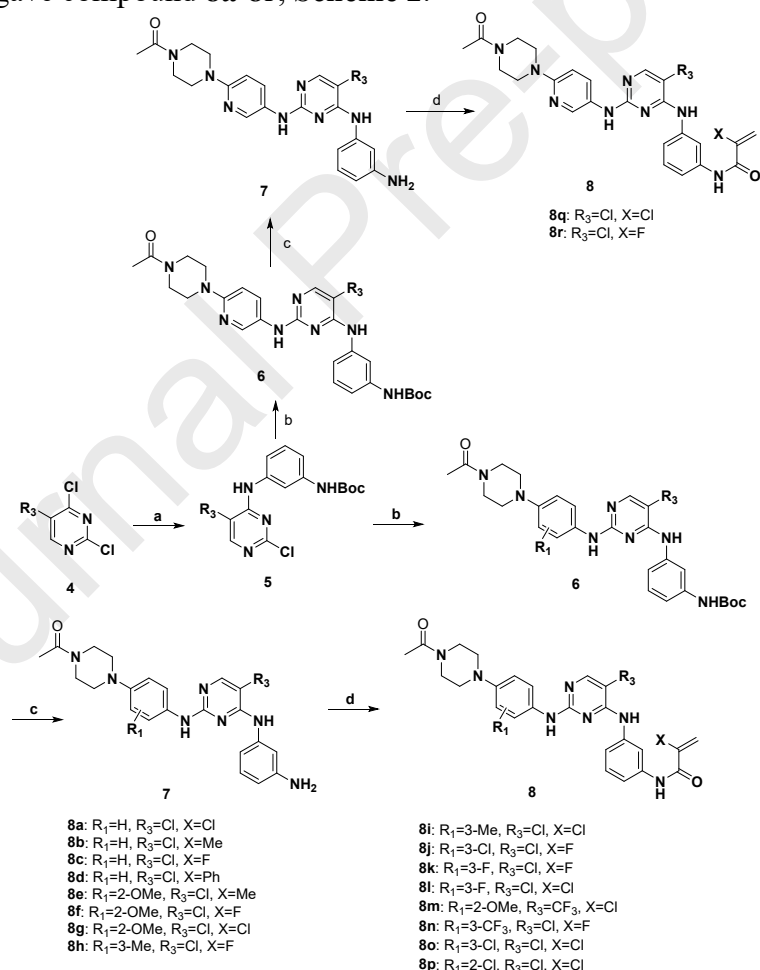
To investigate the effect of the electronic nature of the acrylamide moiety against EGFR mutants, a series of molecules were synthesized as shown in **Scheme 2-4**. The

key intermediate **3** were prepared following the procedure in **Scheme 1** as previously reported. [22] The substitution of 1-fluoro-4-nitrobenzene or 2-fluoro-5-nitropyridine **1** either with 1-methylpiperazine or N-acetyl-piperazine afforded the corresponding products **2**. The compound **3** was easily obtained by reducing the nitro group of **2** via NaBH<sub>4</sub> and NiCl<sub>2</sub>·6H<sub>2</sub>O.



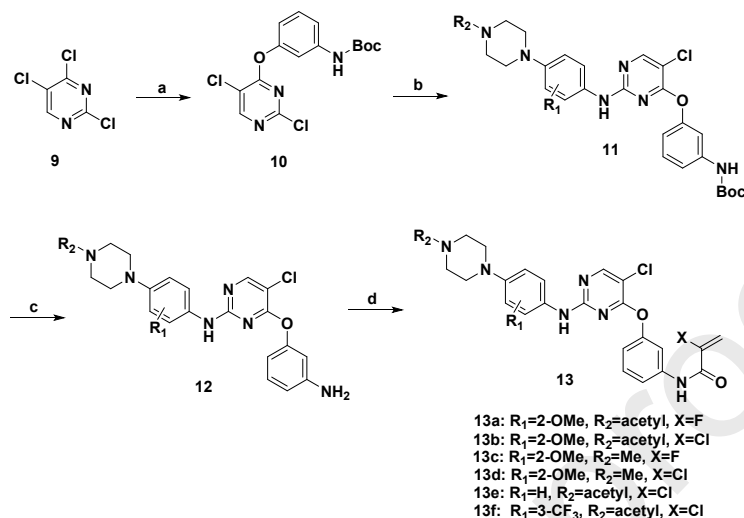
**Scheme 1.** Synthesis of the intermediates **3**.

*Tert*-butyl (3-aminophenyl) carbamate was region-selectively coupled to the 4-position of 2,4-dichloropyrimidine analogues **4** to afford 2-chloro-4-anilopyrimidines **5** at room temperature. [23] Coupling reaction of **3** with **5** was accomplished in the presence of 1N HCl under reflux, offering anilinyrimidine **6**. [24] The protection group Boc were removed by TFA to produce **7**. Acylation of amino group of **7** using diverse acrylic acid gave compound **8a-8r**, **Scheme 2**.



**Scheme 2.** Synthesis of the compound **8**.

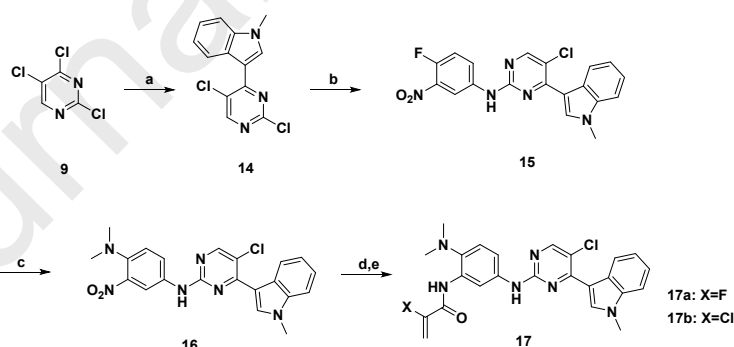
Similarly, [25] in **Scheme 3**, the region-selective coupling of **9** with *tert*-butyl (3-hydroxyphenyl) carbamate gave the 4-position etherification product **10** at room temperature. And the 2-chloro group was coupled with **3** to deliver **11** in the presence of 1N HCl under reflux. The Boc group of **11** was removed by TFA to produce arylamine **12**. Acylation of amino group of **12** using diverse acrylic acid gave **13a-13f**.



**Reagents and conditions:**(a) *tert*-butyl (3-hydroxyphenyl)carbamate, DIPEA, *n*-BuOH, 50 °C, 4 h, 72%; (b) **3**, 1N HCl, *n*-BuOH, reflux, 4 h, 50-60%; (c) TFA, DCM, 0 °C, 1 h, 90-95%; (d) acrylic acid, (COCl)<sub>2</sub>, TEA, DCM, 0 °C-rt, 4 h, 40-55%.

### Scheme 3. Synthesis of the compound **13**

To access indole derivatives **14**, commercial 2, 4, 5-trichloropyrimidine was used. As previously reported, the introduction of the *N*-methylindole was achieved *via* AlCl<sub>3</sub> [26] (**Scheme 4**) and subsequent S<sub>N</sub>AR with 4-fluoro-3-nitroaniline to give **15**. The fluoro group of **15** was substituted with dimethylamine to afford **16**. The nitro-group of **16** was reduced followed with acylation, delivering **17a-17b**.



**Reagents and conditions:**(a) AlCl<sub>3</sub>, DCE, 80 °C, 2 h, 89%; (b) aniline, 1N HCl, *n*-BuOH, reflux, 4 h, 85%; (c) K<sub>2</sub>CO<sub>3</sub>, (CH<sub>3</sub>)<sub>2</sub>NH, DMF, 100 °C, 2 h, 93%; (d) NaBH<sub>4</sub> and NiCl<sub>2</sub> 6H<sub>2</sub>O, DCM/MeOH, 0 °C, 1 h, 92%; (e) acrylic acid, (COCl)<sub>2</sub>, TEA, DCM, 0 °C-rt, 4 h, 40-55%.

### Scheme 4. Synthesis of the compound **17**

## 2.2 BIOLOGICAL EVALUATION

### 2.2.1 Cellular and Kinase Inhibitory Activity

The antiproliferative effects of **8a-8r**, **13a-13f** and **17a-17b** against gefitinib-sensitive A431 bearing EGFR<sup>WT</sup> and gefitinib-resistant NCI-H1975 bearing EGFR<sup>L858R/T790M</sup> were evaluated using Afatinib and Rociletinib as positive controls. We

first investigated the electron nature of Michael addition receptors. As shown in **Table 1**, modifying the acrylamide moiety with chloro (**8a**, X=Cl, 0.75  $\mu\text{M}$ ) and fluoro (**8c**, X=F, 0.34  $\mu\text{M}$ ) were able to retain the potencies against gefitinib-resistant NCI-H1975 cells. While replacing the  $\alpha$ -position of acrylamides with methyl (**8b**, X=Me, 5.57  $\mu\text{M}$ ) or phenyl (**8d**, X=Ph, 5.79  $\mu\text{M}$ ) almost decreased the potencies by 10 folds. These **seemed** the strong electron-withdrawing substituents on Michael addition acceptors would be more favor for the mutant-EGFR inhibition. Next, we examined the impacts of  $R_1$  on the left-hand side of the NH-substituted phenyl ring. The hydrogen atom in **8a** and **8c** were replaced with 2-methoxyl (**8g** and **8f**), 3-methyl (**8i** and **8h**), 3-fluoro (**8l** and **8k**), 3-chloro (**8o** and **8j**), 2-chloro (**8p**) or 3-trifluoromethyl (**8n**) groups, respectively. The results implied that **8i** and **8h** ( $R_1=3\text{-Me}$ ) exhibited the most potent mutant-EGFR inhibition with the  $\text{IC}_{50}$  values of 0.18 and 0.21  $\mu\text{M}$ , respectively. Further variants at the same position, 3-fluoro (**8l** and **8k**), 3-chloro (**8o** and **8j**), and 3-trifluoromethyl (**8n**) did not improve the inhibitory activities. While replacing the phenyl ring with pyridinyl ring (**8q** and **8r**) completely abolished the activities. When the  $R_2$  was substituted with methyl group instead of acetyl group, the resultant compounds significantly decreased the activity by 2-4 folds. (**13a** vs **13c**, **13b** vs **13d**) Further linkers between the pyrimidine ring and 4-phenyl ring at the right-hand side were also tested (**Table 1**). It **was** found that replacing the  $-\text{NH}-$  with  $-\text{O}-$  did not improve the mutant inhibition. (**8f** vs **13a**, **8g** vs **13b**, **8a** vs **13e**) Final, the substitution position of Michael addition acceptor was investigated. When the acrylamide moiety was migrated to the left-hand side of pyrimidine core and the aniline ring at the right-hand side was removed and replaced with an *N*-methylindole, the corresponding compounds **17a** and **17b** almost lost the inhibitory activity against gefitinib-resistant NCI-H1975 cells. Importantly, selectivity indexes of **inhibition** against gefitinib-sensitive A431 and gefitinib-resistance NCI-H1975 were investigated. Compared to Afatinib (SI [ $\text{IC}_{50}(\text{A431}) / \text{IC}_{50}(\text{NCI-H1975})$ ] = 1.5) and Rociletinib (SI [ $\text{IC}_{50}(\text{A431}) / \text{IC}_{50}(\text{NCI-H1975})$ ] = 11.8), some selected compounds displayed good excellent selectivity with a SI up to 25.6-fold.

Moreover, the “off-target” toxicity of selected compounds were also evaluated. The colon cancer cell SW620, which is not dependent on EGFR signaling for cell growth, is used to rule out the potential activity due to non-EGFR. Compared to the inhibitory activity against H1975 ( $\text{IC}_{50} = 0.18\text{-}0.75 \mu\text{M}$ ), **8a**, **8c**, **8h**, **8i** and **8m** displayed much weaker inhibition against SW620 with  $\text{IC}_{50}$  value up to 29.15  $\mu\text{M}$ . Besides, the monkey fibroblast cell COS7 was selected as non-tumor cell model to test the cytotoxicity. As shown in **Table 1**, the  $\text{IC}_{50}$  values of **8a**, **8o**, **8i** and **8p** ranged from 5.40-14.87  $\mu\text{M}$ , indicating the lower cytotoxicity comparing with those against NCI-H1975.

**Table 1.** Anti-proliferative activities and selectivity of compound **8**, **13** and **17** against cancer cells harboring different EGFR status and normal cell

No.	$\text{IC}_{50} / \mu\text{M}^a$		SI(A431/H1975) <sup>d</sup>	$\text{IC}_{50} / \mu\text{M}^a$	
	A431 <sup>b</sup>	H1975 <sup>c</sup>		SW620 <sup>e</sup>	COS7 <sup>f</sup>
<b>8a</b>	3.14±0.24	0.75±0.02	4.2	29.15±0.01	10.44±0.66
<b>8b</b>	10.60±0.54	5.57±0.32	1.9	/	/

<b>8c</b>	6.50±0.35	0.34±0.12	19.1	3.61±1.26	/
<b>8d</b>	6.16±0.52	5.79±0.31	1.1	/	/
<b>8e</b>	2.37±0.12	3.82±0.16	0.6	/	/
<b>8f</b>	1.58±0.13	1.82±0.28	0.9	/	/
<b>8g</b>	4.44±0.98	3.12±0.19	1.4	/	/
<b>8h</b>	5.37±0.13	0.21±0.04	25.6	2.33±0.07	/
<b>8i</b>	1.30±0.08	0.18±0.05	7.2	2.07±0.17	5.40±0.04
<b>8j</b>	7.13±1.21	4.18±0.57	1.7	/	/
<b>8k</b>	7.09±0.49	6.56±0.43	1.1	/	/
<b>8l</b>	1.14±0.14	0.42±0.42	2.7	/	/
<b>8m</b>	3.68±0.33	0.20±0.02	18.4	3.99±0.31	/
<b>8n</b>	2.86±0.33	9.88±0.14	0.3	/	/
<b>8o</b>	0.14±0.06	1.02±0.17	0.1	2.34±0.46	7.22±0.56
<b>8p</b>	1.83±0.11	1.72±0.23	1.1	3.02±1.08	14.87±1.32
<b>8q</b>	0.87±0.38	1.64±0.40	0.5	2.41±0.84	/
<b>8r</b>	18.09±0.97	20.54±0.24	0.9	/	/
<b>13a</b>	>50	7.03±0.18	/	/	/
<b>13b</b>	1.89±0.19	3.32±0.51	0.8	/	/
<b>13c</b>	10.98±0.13	16.72±0.64	0.7	/	/
<b>13d</b>	1.06±0.20	13.57±0.25	0.1	/	/
<b>13e</b>	0.62±0.14	1.15±0.13	0.5	4.60±0.40	/
<b>13f</b>	1.47±0.16	2.50±0.18	0.6	/	/
<b>17a</b>	>50	>50	/	/	/
<b>17b</b>	2.20±0.24	16.88±1.29	0.1	/	/
<b>Afatinib</b>	1.40±0.01	0.92±0.01	1.5	2.13±0.25	/
<b>Rociletinib</b>	6.01±0.10	0.51±0.01	11.8	10.50±0.50	7.39±1.11

<sup>a</sup>The inhibitory effects of individual compounds on the proliferation of cancer cell lines were determined by the MTT assay. The data reported are the mean values from two independent experiments. <sup>b</sup> A431 is human epithelial carcinoma cell line (EGFR<sup>WT</sup>). <sup>c</sup> H1975 is a human non-small-cell lung cancer cell line (EGFR<sup>L858R/T790M</sup>). <sup>d</sup> ratio of the IC<sub>50</sub> values of [A431] vs [H1975]. <sup>e</sup> SW620 is human colon carcinoma cell line (EGFR<sup>negative</sup>). <sup>f</sup> COS7 is monkey fibroblast cell (used as normal cell).

Encouraged by the good anti-proliferative activity against gefitinib-resistant cell NCI-H1975, the kinase inhibition against EGFRs including wild-type and L858R/T790M mutant were measured. As presented in **Table 2**, compounds (**8a**, **8i**, **8o** and **8q**) bearing a  $\alpha$ -chloroacrylamide group showed more excellent inhibitory activities against double-mutant kinase EGFR<sup>L858R/T790M</sup> with the IC<sub>50</sub> values ranging from 2.6-7.5 nM. The different activity between chloro-substituted **8a** and fluoro-substituted **8c** could be explained by the calculated charge distribution. (Figure S1 in supporting information) Moreover, the compounds exhibited a better selectivity between the wild-type and double-mutant EGFRs. For example, the IC<sub>50</sub> value of **8a** against EGFR<sup>WT</sup> was 70 nM, which was 17.9-fold of that against EGFR<sup>L858R/T790M</sup> (3.9 nM). Similarly, the IC<sub>50</sub> value of **8i** against EGFR<sup>WT</sup> was 51 nM, which was 19.6-fold

of that against EGFR<sup>L858R/T790M</sup> (2.6 nM). These data demonstrated that the incorporation of chloro group on acrylamide group could retain the inhibitory activity against EGFR<sup>L858R/T790M</sup> while with a good EGFR<sup>WT</sup> selectivity.

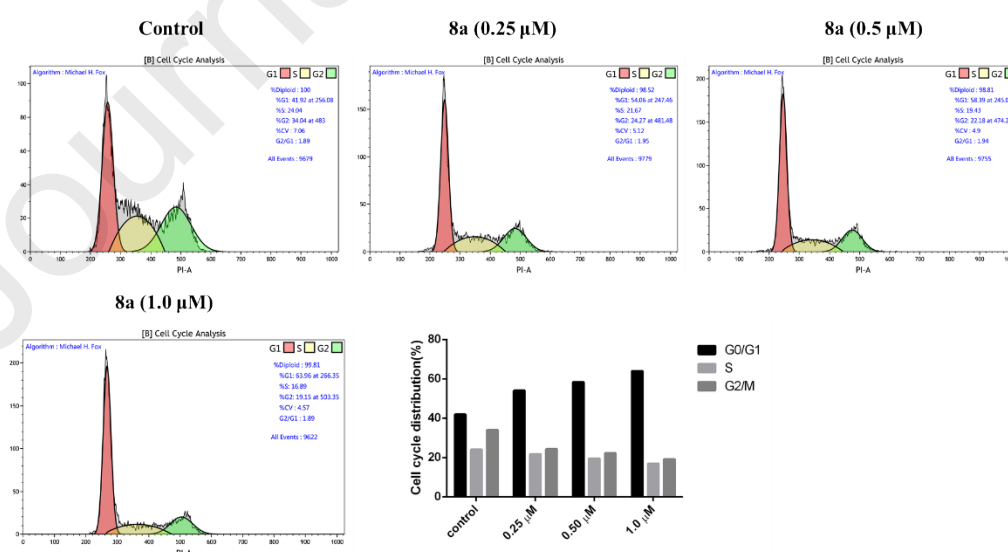
**Table 2.** Inhibitory activity of selected compounds against different types of EGFRs

No.	EGFR IC <sub>50</sub> /nM <sup>a</sup>		SI(WT/DM) <sup>b</sup>
	WT	DM(L858R/T790M)	
<b>8a</b>	70	3.9	17.9
<b>8c</b>	>1000	213	/
<b>8h</b>	>1000	174	/
<b>8i</b>	51	2.6	19.6
<b>8m</b>	257	16	16.1
<b>8o</b>	53	3.2	16.6
<b>8p</b>	301	47	6.4
<b>8q</b>	130	7.5	17.3
<b>13e</b>	130	13	10.0
<b>Afatinib</b>	1.2	21	0.06
<b>Rociletinib</b>	76	1.8	42.2

<sup>a</sup> EGFR activity assays were performed using HTRF method. The data reported are the mean values from duplicated wells. <sup>b</sup> ratio of the IC<sub>50</sub> values of [WT] vs [DM]

### 2.2.2 8a Induces Cell Cycle Arrest in NCI-H1975

In order to better verify the effect of compound **8a** on cell cycle, flow cytometry was performed on NCI-H1975 cells treated with various concentrations of **8a**. As shown in Figure 2, the percentage of cells in the G0/G1 phase increased from 41.92% to 58.39%, and those in the G2/M phase decreased from 34.04% to 22.18% after treatment with **8a** at the concentrations from 0.25  $\mu$ M to 1.0  $\mu$ M for 24 h. Accordingly, **8a** arrested NCI-H1975 cells in G0/G1 phase.



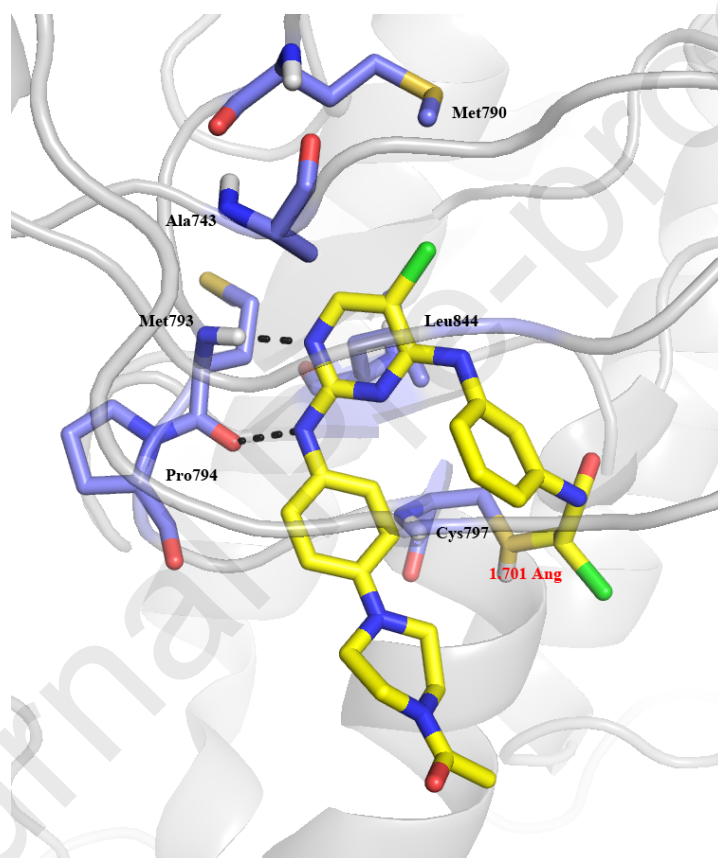
**Figure 2.** Effects of **8a** on H1975 cells cycle arrest detected by flow cytometry assay. Cells were treated with different concentrations of **8a** for 24 h, collected and fixed with



70% ethanol at 4 °C overnight. Then, the cells were stained by the mixture containing propidium iodide for 30 min at 37 °C, and the cell cycle was analyzed by a flow cytometer.

### 2.3 Molecular Docking Study of **8a**

**8a** was initially docked to the EGFR binding pocket based on the co-crystal structure of the Rociletinib-EGFR<sup>T790M</sup> (PDB ID: 5XDK) and the resulting simulated complex of **8a**-EGFR<sup>T790M</sup> was shown in **Figure 3**. The docking study revealed that **8a** occupied the binding pocket of kinase in a similar pattern to Rociletinib. The aminopyrimidine moiety of **8a** binds to Met793 residue at the **hinge region** through two strong hydrogen bonding interactions. The warhead, chloro-substituted acrylamide moiety, oriented to the thiol group of Cys797 with a measured distance of 1.70 Å, indicating the possibility of forming a covalent bond.



**Figure 3.** Putative binding mode of **8a** within the active pocket of the EGFR [T790M]

### 3. CONCLUSION

On the basis of our previous work, herein we further **explored** the relationship among electronic nature of Michael addition acceptor and EGFR<sup>T790M</sup> mutation selectivity as well as “off-target” toxicity balance. The cellular-based anti-proliferative inhibition and kinase inhibitory assay demonstrated that the chemical perturbation of acrylamide with **chloro-** group at  $\alpha$ -position could retain the good inhibitory activity against gefitinib-resistant NCI-H1975 cells with EGFR<sup>L858R/T790M</sup>. This **led** to the identification of **8a**, which was found to effectively inhibit NCI-H1975 ( $IC_{50} = 0.75 \mu\text{M}$ ) and EGFR<sup>L858R/T790M</sup> ( $IC_{50} = 3.9 \text{ nM}$ ) as well as display excellent wild-type and mutant EGFRs selectivity. Moreover, **8a** exhibited a weaker inhibition against EGFR-independent

colon cancer cell line SW620 and non-tumor monkey fibroblast cell COS7 with  $IC_{50}$  values up to 10  $\mu$ M. In all, this work provided a promising chemical tuned strategy for balancing the mutant-EGFR potency and selectivity as well as “off-target” toxicity.

## 4. EXPERIMENTAL SECTIONS

### 4.1 GENERAL METHODS

Unless otherwise noted, all solvents and chemicals were used as purchased without further purification. All reported yields are isolated yields after column chromatography.  $^1H$  NMR spectra were recorded on a Bruker DRX-500 [Bruker Biospin, Germany]. Chemical shifts are reported in ppm relative to the residual solvent peak ( $CDCl_3$ , TMS: 0.00). Multiplicity was indicated as follows: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublet); dt (triplet of doublet); td (doublet of triplet); brs (broad singlet) etc. Intermediates were purified by column chromatography on silica gel (200-300 mesh). HRMS of all biologically evaluated compounds was confirmed on a Agilent 1290 HPLC-6224 Time of Flight Mass Spectrometer using Phenomenex Luna 5l C18, 100 Å, 150-4.60 mm 5 micron column at a flow rate of 0.5 mL/min using linear gradients buffer B in A (B:  $CH_3OH$  containing 0.1% formic acid, A:  $H_2O$  containing 0.1% formic acid). Mobile phase B was increased linearly from 5% to 95% over 7 min and 95% over the next 2 min, after which the column was equilibrated to 5% for 1 min.

### 4.2 SYNTHESIS OF INTERMEDIATES

#### Synthesis and Characterization of Compounds 2a, 3a, 5a, 6a, 7a, 10, 14, 15 and 16

**1-(4-(4-nitrophenyl) piperazin-1-yl) ethan-1-one (2a).** [27] Organic solid. Yield=92%.  $^1H$  NMR (500 MHz,  $DMSO-d_6$ )  $\delta$  8.08-8.02 (m, 2H), 7.05-6.99 (m, 2H), 3.44 (t,  $J = 5.1$  Hz, 4H), 2.42 (t,  $J = 5.1$  Hz, 4H), 2.22 (s, 3H).

**1-(4-(4-aminophenyl) piperazin-1-yl) ethan-1-one (3a).** To a solution of 2a (1.0 mmol) and  $NiCl_2 \cdot 6H_2O$  (2.0 mmol) in 10 mL of  $MeOH/DCM$  (V/V=1:4) was added  $NaBH_4$  (4.0 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min then at room temperature for 30 min. The mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The crude product was purified by flash column chromatography on silica gel. Brown solid in 90% yield.  $^1H$  NMR (500 MHz,  $DMSO-d_6$ )  $\delta$  6.71 (d,  $J = 8.0$  Hz, 2H), 6.50 (d,  $J = 8.0$  Hz, 2H), 4.70 (s, 2H), 3.55-3.51 (m, 4H), 2.85 (dt,  $J = 33.0, 5.0$  Hz, 4H), 2.02 (s, 3H).

**tert-butyl (3-((2,5-dichloropyrimidin-4-yl) amino) phenyl) carbamate (5a).** [28] Yellow solid. Yield: 72%.  $^1H$  NMR (500 MHz,  $DMSO-d_6$ )  $\delta$  9.54 (s, 1H), 9.46 (s, 1H), 8.37 (s, 1H), 7.71 (t,  $J = 2.0$  Hz, 1H), 7.28-7.15 (m, 3H), 1.48 (s, 9H).

**tert-butyl (3-((2-((4-(4-acetylpiperazin-1-yl)-2-methoxyphenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl) carbamate (6a).** [29] Yellow solid. Yield: 52%.  $^1H$  NMR (500 MHz,  $DMSO-d_6$ )  $\delta$  8.41 (s, 1H), 8.01 (s, 1H), 7.70-7.68 (m, 2H), 6.95-6.92 (m, 2H), 6.77-6.75 (m, 1H), 6.65 (d,  $J = 2.5$  Hz, 1H), 6.44 (dd,  $J = 9.0, 2.5$  Hz, 1H), 6.33-6.31 (m, 1H), 4.94 (s, 2H), 3.79 (s, 3H), 3.60-3.56 (m, 4H), 3.07 (dt,  $J = 29.0, 5.0$  Hz, 4H), 2.05 (s, 3H).

**1-(4-(4-((3-aminophenyl) amino)-5-chloropyrimidin-2-yl) amino)-3-methoxyphenyl) piperazin-1-yl) ethanone (7a).** To a solution of 6a (1.0 mmol) in  $DCM$  (5 mL) was added TFA (10.0 mmol) at ice-bath. The mixture was stirred at room

temperature for 1 h. The reaction solution was neutralized with saturated aqueous NaHCO<sub>3</sub>, and extracted with DCM (3 X 15 mL). The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuo to obtain a brown solid in 90% yield. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 8.51 (s, 1H), 8.10 (s, 1H), 7.82-7.72 (m, 2H), 7.04-7.00 (m, 2H), 6.84 (d, *J* = 7.9 Hz, 1H), 6.74 (d, *J* = 2.4 Hz, 1H), 6.54-6.52 (m, 1H), 6.41-6.40 (m, 1H), 5.04 (s, 2H), 3.88 (s, 3H), 3.69-3.65 (m, 4H), 3.20-3.12 (m, 4H), 2.14 (s, 3H).

**tert-butyl (3-((2,5-dichloropyrimidin-4-yl) oxy) phenyl) carbamate (10).** <sup>[30]</sup> Yellow solid. Yield: 72%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 9.64 (s, 1H), 8.83 (s, 1H), 7.47 (t, *J* = 2.0 Hz, 1H), 7.38-7.32 (m, 2H), 6.91-6.89 (m, 1H), 1.48 (s, 9H).

**3-(2-chloropyrimidin-4-yl)-1-methyl-1H-indole (14)** A suspension of 2,4-dichloropyrimidine (1.0 mmol) and aluminum chloride (1.0 mmol) in DME was stirred at ambient temperature for 5 min. To this was added *N*-methylindole (1.0 mmol), and the mixture was heated to 80 °C for 2 h. The cool reaction mixture was added dropwise to vigorously stirring water over 5 min. Upon complete addition the mixture was stirred for 30 min, filtered and the solid washed with water. The crude product was purified by flash silica chromatography, eluting with DCM. Pure fractions were evaporated to dryness to afford **14** as a yellow solid. Yield: 89%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 8.80 (s, 1H), 8.73 (s, 1H), 8.56 (d, *J* = 7.6 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.38-7.34 (m, 1H), 7.34-7.30 (m, 1H), 3.96 (s, 3H). HRMS (ESI) calcd. for C<sub>13</sub>H<sub>10</sub>Cl<sub>2</sub>N<sub>3</sub> [M+H]<sup>+</sup> = 278.0246, found 278.0247.

**5-chloro-N-(4-fluoro-3-nitrophenyl)-4-(1-methyl-1H-indol-3-yl) pyrimidin-2-amine (15)** 1N HCl (1.2 equiv) was added in one portion to 3-(2-chloropyrimidin-4-yl)-1-methylindole (1.0 mmol) and 4-fluoro-5-nitroaniline (1.0 equiv) in *n*-BuOH. The resulting mixture was stirred at 120 °C for 4 h. The mixture was cooled to room temperature. The precipitate was collected by filtration, washed with *n*-BuOH, and dried under vacuum to afford **15** as a yellow solid. Yield: 85%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 10.11 (s, 1H), 8.70-8.68 (m, 1H), 8.62-8.60 (m, 2H), 8.55 (s, 1H), 8.11-8.08 (m, 1H), 7.58-7.52 (m, 2H), 7.34-7.30 (m, 1H), 7.22-7.19 (m, 1H), 3.94 (s, 3H). HRMS (ESI) calcd. for C<sub>19</sub>H<sub>14</sub>ClFN<sub>5</sub>O<sub>2</sub> [M+H]<sup>+</sup> = 398.0815, found 398.0816.

**N4-(5-chloro-4-(1-methyl-1H-indol-3-yl) pyrimidin-2-yl)-N1, N1-dimethyl-2-nitrobenzene-1, 4-diamine (16)** To a solution of *N*-(4-fluoro-5-nitrophenyl)-4-(1-methylindol-3-yl)pyrimidin-2-amine (1.0 mmol) and K<sub>2</sub>CO<sub>3</sub> (2.0 equiv) in DMF was added *N,N*-dimethylamine (1.0 equiv). The mixture was heated at 100 °C for 2 h. Then the reaction was evaporated and purified by silica chromatography to afford red solid. Yield: 92.6%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 9.76 (s, 1H), 8.58 (s, 2H), 8.48 (s, 1H), 8.32 (d, *J* = 2.3 Hz, 1H), 7.81 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.56 (d, *J* = 8.2 Hz, 1H), 7.30 (t, *J* = 7.5 Hz, 1H), 7.25 (d, *J* = 9.1 Hz, 1H), 7.17 (t, *J* = 7.3 Hz, 1H), 3.93 (s, 3H), 2.77 (s, 6H). HRMS (ESI) calcd. for C<sub>21</sub>H<sub>20</sub>ClN<sub>6</sub>O<sub>2</sub> [M+H]<sup>+</sup> = 423.1331, found 423.1336.

#### 4.3 Synthesis of final compounds

To a solution of acrylic acid (4.0 mmol, 4.0 equiv) in anhydrous DCM (8 mL) was added 3 drops of DMF at 0 °C and oxalyl chloride (3.47 mmol, 3.47 equiv) in DCM (2 mL) was added dropwise. Then the mixture was stirred at 0-10 °C for 20 min and at 25 °C for 2h, then the temperature of reaction mixture is adjusted to 45 °C for 5 min. Next,

the mixture was cooled to 0 °C. A solution of the aniline **7**, **12** or **16** (1.0 mmol) and Et<sub>3</sub>N (5.0 mmol, 5.0 equiv) in anhydrous DCM-DMF (6 mL/1 mL) was added dropwise to the above mixture. The mixture was stirred at room temperature for another 3-4 h. The mixture was quenched with saturated Na<sub>2</sub>CO<sub>3</sub> solution, extracted with EtOAc (3 X 20 mL) and dried over anhydrous sodium sulfate. The combined organic layer was evaporated to dryness under reduced pressure. The residue was purified through silica gel to give pure product.

#### Characterization of the final compounds

##### **N-(3-((2-((4-(4-acetylpiperazin-1-yl) phenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-chloroacrylamide (8a)**

Light yellow solid, m.p.: 123-125 °C. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 10.22 (s, 1H), 9.15 (s, 1H), 8.90 (s, 1H), 8.10 (s, 1H), 7.93 (s, 1H), 7.47 (t, *J* = 9.5 Hz, 3H), 7.42-7.26 (m, 2H), 6.75 (d, *J* = 8.5 Hz, 2H), 6.42 (d, *J* = 2.5 Hz, 1H), 6.10 (d, *J* = 3.0 Hz, 1H), 3.55 (dd, *J* = 10.0, 8.0 Hz, 4H), 3.06-2.86 (m, 4H), 2.03 (s, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 169.2, 158.8, 158.0, 155.6, 154.4, 147.0, 138.8, 137.5, 132.8, 132.2, 129.5, 124.1, 121.6, 118.1, 117.5, 115.8, 113.2, 104.8, 50.5, 50.1, 46.4, 41.5, 21.5. HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>25</sub>H<sub>25</sub>Cl<sub>2</sub>N<sub>7</sub>O<sub>2</sub>: 526.1520, found: 526.1521.

##### **N-(3-((2-((4-(4-acetylpiperazin-1-yl) phenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl) methacrylamide (8b)**

Light yellow solid, m. p.: 153-155 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.04 (m, 2H), 7.54 (s, 1H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.41-7.39 (m, 1H), 7.30 (d, *J* = 6.0 Hz, 2H), 7.10 (s, 1H), 7.06 (s, 1H), 6.86 (d, *J* = 8.5 Hz, 2H), 5.78 (s, 1H), 5.47 (s, 1H), 3.78-3.76 (m, 2H), 3.62-3.60 (m, 2H), 3.10-3.07 (m, 4H), 2.14 (s, 3H), 2.05 (s, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 169.2, 166.7, 158.1, 155.6, 154.5, 146.9, 140.8, 138.7, 138.5, 132.9, 129.4, 121.5, 120.3, 117.5, 117.3, 115.5, 113.0, 104.9, 50.5, 50.2, 46.4, 41.5, 21.5, 18.9. HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>26</sub>H<sub>28</sub>ClN<sub>7</sub>O<sub>2</sub>: 506.2066, found: 506.2072.

##### **N-(3-((2-((4-(4-acetylpiperazin-1-yl) phenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-fluoroacrylamide (8c)**

White solid, m.p.: 115-117 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.09 (s, 1H), 8.05 (s, 1H), 7.95 (s, 1H), 7.43-7.40 (m, 3H), 7.35-7.32 (m, 2H), 7.11 (s, 1H), 7.04 (s, 1H), 6.87 (d, *J* = 8.0 Hz, 2H), 5.84 (d, *J* = 48.5 Hz, 1H), 5.27 (d, *J* = 14.5 Hz, 1H), 3.77 (m, 2H), 3.61 (m, 2H), 3.08-3.10 (m, 4H), 2.14 (s, 3H). HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>25</sub>H<sub>25</sub>ClFN<sub>7</sub>O<sub>2</sub>: 510.1815, found: 510.1814.

##### **N-(3-((2-((4-(4-acetylpiperazin-1-yl) phenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-phenylacrylamide (8d)**

White solid, m.p.: 107-108 °C. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 10.28 (s, 1H), 9.11 (s, 1H), 8.89 (s, 1H), 8.09 (s, 1H), 7.97 (s, 1H), 7.48-7.47 (m, 5H), 7.37-7.35 (m, 5H), 6.76 (d, *J* = 7.0 Hz, 2H), 5.95 (s, 1H), 5.73 (s, 1H), 3.52 (m, 4H), 2.98-2.92 (m, 4H), 2.02 (s, 3H). <sup>13</sup>C NMR (125MHz, CDCl<sub>3</sub>) δ 169.1, 165.4, 158.0, 155.7, 154.2, 147.0, 145.1, 138.7, 138.3, 136.6, 132.9, 129.4, 129.1, 129.0, 128.6, 128.4, 128.2, 123.6, 121.5, 117.8, 117.6, 115.5, 113.1, 104.8, 50.6, 50.2, 46.4, 41.5, 21.5. HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>31</sub>H<sub>30</sub>ClN<sub>7</sub>O<sub>2</sub>: 568.2222, found: 568.2212

##### **N-(3-((2-((4-(4-acetylpiperazin-1-yl)-2-methoxyphenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl) methacrylamide (8e)**

White solid, m.p.: 117-119 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.14 (d, *J* = 9.0 Hz, 1H), 8.06 (s, 1H), 7.94 (s, 1H), 7.56 (s, 1H), 7.43-7.40 (m, 3H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.09 (s, 1H), 6.52 (d, *J* = 2.5 Hz, 1H), 6.41 (d, *J* = 8.5 Hz, 1H), 5.79 (s, 1H), 5.47 (s, 1H), 3.87 (s, 3H), 3.78-3.76 (m, 2H), 3.62-3.60 (m, 2H), 3.10-3.06 (m, 4H), 2.15 (s, 3H), 2.05 (s, 3H). HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>27</sub>H<sub>30</sub>ClN<sub>7</sub>O<sub>3</sub>: 536.2171, found: 536.2174.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-2-methoxyphenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-fluoroacrylamide (8f)**

White solid, m.p. 223-223 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.11 (d, *J* = 8.5 Hz, 1H), 8.07 (s, 1H), 8.00 (s, 1H), 7.96 (d, *J* = 4.0 Hz, 1H), 7.44 (t, *J* = 7.0 Hz, 2H), 7.37-7.34 (m, 2H), 7.10 (s, 1H), 6.54 (d, *J* = 2.0 Hz, 1H), 6.42 (d, *J* = 8.5 Hz, 1H), 5.85 (dd, *J* = 48.0, 3.5 Hz, 1H), 5.26 (dd, *J* = 15.0, 3.5 Hz, 1H), 3.88 (s, 3H), 3.79-3.77 (m, 2H), 3.63-3.61 (m, 2H), 3.11-3.06 (m, 4H), 2.15 (s, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 169.1, 157.8, 157.3, 156.3 (d, *J* = 301.3 Hz), 155.6, 154.6, 149.4, 147.0, 138.9, 137.2, 129.6, 123.0, 120.2, 118.3, 115.8, 113.3, 108.5, 104.7, 101.4, 100.3 (d, *J* = 15.0 Hz), 55.8, 50.8, 50.6, 46.5, 41.6, 21.5. HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>26</sub>H<sub>27</sub>ClFN<sub>7</sub>O<sub>3</sub>: 540.1921, found: 540.1928.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-2-methoxyphenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-chloroacrylamide (8g)**

Yellow solid, m.p.: 192-193 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.36 (s, 1H), 8.12 (d, *J* = 8.5 Hz, 1H), 8.06 (s, 1H), 8.02-8.00 (m, 1H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.11 (s, 1H), 6.74 (d, *J* = 1.5 Hz, 1H), 6.53 (d, *J* = 2.0 Hz, 1H), 6.42 (d, *J* = 8.5 Hz, 1H), 5.93 (d, *J* = 1.5 Hz, 1H), 3.88 (s, 3H), 3.78 (t, *J* = 4.5 Hz, 2H), 3.62 (t, *J* = 5.0 Hz, 2H), 3.11-3.07 (m, 4H), 2.15 (s, 3H). HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>26</sub>H<sub>27</sub>Cl<sub>2</sub>N<sub>7</sub>O<sub>3</sub>: 556.1625, found: 556.1632.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-3-methylphenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-fluoroacrylamide (8h)**

White solid, m.p.: 96-97 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.09 (s, 1H), 8.07 (s, 1H), 7.95 (s, 1H), 7.49 (d, *J* = 7.5 Hz, 1H), 7.35-7.32 (m, 4H), 7.11 (s, 1H), 6.96 (s, 1H), 6.92 (d, *J* = 9.0 Hz, 1H), 5.85 (dd, *J* = 48.0, 3.5 Hz, 1H), 5.28 (dd, *J* = 15.0, 3.5 Hz, 1H), 3.75-3.71 (m, 2H), 3.60-3.58 (m, 2H), 2.87-2.81 (m, 4H), 2.28 (s, 3H), 2.15 (s, 3H). HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>26</sub>H<sub>27</sub>ClFN<sub>7</sub>O<sub>2</sub>: 524.1972, found: 524.1978.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-3-methylphenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-chloroacrylamide (8i)**

White solid, m.p.: 110-111 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.36 (s, 1H), 8.06 (m, 2H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.35-7.32 (m, 3H), 7.28 (s, 1H), 7.12 (s, 1H), 6.99 (s, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.74 (d, *J* = 1.0 Hz, 1H), 5.93 (d, *J* = 1.0 Hz, 1H), 3.75-3.71 (m, 2H), 3.60-3.58 (m, 2H), 2.87-2.81 (m, 4H), 2.27 (s, 3H), 2.15 (s, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 169.3, 158.8, 158.0, 155.6, 154.5, 146.3, 138.9, 137.5, 135.3, 133.6, 132.1, 129.6, 124.2, 122.8, 119.7, 118.3, 118.2, 115.7, 113.2, 105.0, 52.4, 52.0, 47.1, 42.2, 21.6, 18.0. HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>26</sub>H<sub>27</sub>Cl<sub>2</sub>N<sub>7</sub>O<sub>2</sub>: 540.1676, found: 540.1682.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-3-chlorophenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-fluoroacrylamide (8j)**

White solid, m.p.: 176-177 °C; <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 10.31 (s, 1H), 9.44 (s, 1H), 8.38 (s, 1H), 7.86 (s, 1H), 7.37 (m, 2H), 7.14 (m, 3H), 5.73 (d, *J* = 47.5 Hz, 1H), 5.52-5.43 (m, 2H), 5.33 (d, *J* = 14.0 Hz, 1H), 3.60 (s, 4H), 2.97 (d, *J* = 31.5 Hz, 4H), 2.05 (s, 3H). HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>25</sub>H<sub>24</sub>Cl<sub>2</sub>FN<sub>7</sub>O<sub>2</sub>: 544.1425, found: 544.1429.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-3-fluorophenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-fluoroacrylamide (8k)**

Yellow solid, m.p.: 106-107 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.15 (s, 1H), 8.07 (d, *J* = 2.0 Hz, 1H), 8.03 (s, 1H), 7.58 (d, *J* = 14.5 Hz, 1H), 7.41-7.35 (m, 2H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.18 (s, 1H), 7.13 (s, 1H), 7.03 (d, *J* = 8.5 Hz, 1H), 6.85-6.82 (m, 1H), 5.84 (d, *J* = 48.0 Hz, 1H), 5.27 (d, *J* = 15.0 Hz, 1H), 3.78 (m, 2H), 3.62 (m, 2H), 2.99 (d, *J* = 17.5 Hz, 4H), 2.14 (d, *J* = 2.0 Hz, 3H). HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>25</sub>H<sub>24</sub>ClF<sub>2</sub>N<sub>7</sub>O<sub>2</sub>: 528.1721, found: 528.1725.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-3-fluorophenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-chloroacrylamide (8l)**

Yellow solid, m.p.: 100-101 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.40 (s, 1H), 8.14 (s, 1H), 8.08 (s, 1H), 7.60 (dd, *J* = 14.5, 2.5 Hz, 1H), 7.44 (d, *J* = 9.0 Hz, 1H), 7.37 (t, *J* = 8.0 Hz, 1H), 7.13 (m, 1H), 7.08 (m, 1H), 7.02 (d, *J* = 8.5 Hz, 1H), 6.85-6.82 (m, 1H), 6.74 (d, *J* = 1.5 Hz, 1H), 5.93 (d, *J* = 1.0 Hz, 1H), 3.78 (t, *J* = 5.0 Hz, 2H), 3.62 (t, *J* = 5.0 Hz, 2H), 3.01 (t, *J* = 5.0 Hz, 2H), 2.98 (t, *J* = 5.0 Hz, 2H), 2.14 (s, 3H). HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>25</sub>H<sub>24</sub>Cl<sub>2</sub>FN<sub>7</sub>O<sub>2</sub>: 544.1425, found: 544.1431.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-2-methoxyphenyl) amino)-5-(trifluoromethyl) pyrimidin-4-yl) amino) phenyl)-2-chloroacrylamide (8m)**

Yellow solid, m.p.: 113-114 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.40 (s, 1H), 8.14 (s, 1H), 8.08 (s, 1H), 7.60 (dd, *J* = 14.5, 2.5 Hz, 1H), 7.44 (d, *J* = 9.0 Hz, 1H), 7.37 (t, *J* = 8.0 Hz, 1H), 7.13 (m, 1H), 7.08 (m, 1H), 7.02 (d, *J* = 8.5 Hz, 1H), 6.85-6.82 (m, 1H), 6.74 (d, *J* = 1.5 Hz, 1H), 5.93 (d, *J* = 1.0 Hz, 1H), 3.78 (t, *J* = 5.0 Hz, 2H), 3.62 (t, *J* = 5.0 Hz, 2H), 3.01 (t, *J* = 5.0 Hz, 2H), 2.98 (t, *J* = 5.0 Hz, 2H), 2.14 (s, 3H), 1.65 (s, 3H). HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>27</sub>H<sub>27</sub>ClF<sub>3</sub>N<sub>7</sub>O<sub>3</sub>: 590.1889, found: 590.1990.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-3-(trifluoromethyl) phenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-fluoroacrylamide (8n)**

White solid, m.p.: 112-113 °C. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 10.32 (s, 1H), 9.57 (s, 1H), 9.02 (s, 1H), 8.19 (s, 1H), 8.03-7.99 (m, 2H), 7.81 (s, 1H), 7.55 (d, *J* = 6.5 Hz, 1H), 7.35-7.33 (m, 2H), 7.25 (d, *J* = 8.0 Hz, 1H), 5.69 (dd, *J* = 48.0, 3.0 Hz, 1H), 5.42 (dd, *J* = 15.5, 3.0 Hz, 1H), 3.51 (m, 4H), 2.75-2.70 (m, 4H), 2.04 (s, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 169.3, 157.5 (q, *J* = 30 Hz), 157.3, 156.1 (d, *J* = 267.5 Hz), 155.8, 153.9, 146.0, 138.5, 137.3, 137.1, 129.7, 128.1 (q, *J* = 28.75 Hz), 124.8, 124.2 (q, *J* = 161.3 Hz), 123.5, 118.2, 118.1 (q, *J* = 7.0 Hz), 116.0, 113.5, 106.0, 100.4 (d, *J* = 15 Hz), 53.9, 53.2, 47.1, 42.1, 21.6. HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>26</sub>H<sub>24</sub>ClF<sub>4</sub>N<sub>7</sub>O<sub>2</sub>: 578.1689, found: 578.1680.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-3-chlorophenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-chloroacrylamide (8o)**

White solid, m.p.: 140-141 °C. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 10.20 (s, 1H), 9.41 (s, 1H), 8.98 (s, 1H), 8.16 (s, 1H), 7.92 (s, 1H), 7.74 (s, 1H), 7.53-7.51 (m, 2H), 7.35

(d,  $J = 5.0$  Hz, 2H), 6.91 (d,  $J = 8.5$  Hz, 1H), 6.40 (d,  $J = 2.0$  Hz, 1H), 6.08 (d,  $J = 2.5$  Hz, 1H), 3.56-3.54 (m, 4H), 2.86-2.84 (m, 2H), 2.80-2.78 (m, 2H), 2.03 (s, 3H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  169.3, 158.8, 157.6, 155.7, 154.4, 143.6, 138.6, 137.6, 136.0, 132.1, 129.8, 129.3, 124.2, 121.7, 120.6, 118.8, 118.3, 116.0, 113.4, 105.6, 51.9, 51.4, 46.8, 41.9, 21.6. HRMS (m/z):  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{25}\text{H}_{24}\text{Cl}_3\text{N}_7\text{O}_2$ : 560.1130, found: 560.1139.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-2-chlorophenyl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-chloroacrylamide (8p)**

White solid, m.p.: 166-167 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-d}_6$ )  $\delta$  10.13 (s, 1H), 8.82 (s, 1H), 8.37 (s, 1H), 8.06 (s, 1H), 7.91 (s, 1H), 7.48 (d,  $J = 9.0$  Hz, 1H), 7.43 (d,  $J = 7.5$  Hz, 1H), 7.34 (d,  $J = 8.5$  Hz, 1H), 7.18 (t,  $J = 8.0$  Hz, 1H), 7.01 (d,  $J = 2.5$  Hz, 1H), 6.81 (dd,  $J = 9.0, 2.5$  Hz, 1H), 6.43 (d,  $J = 2.5$  Hz, 1H), 6.10 (d,  $J = 2.5$  Hz, 1H), 3.57-3.56 (m, 4H), 3.15-3.06 (m, 4H), 2.05 (s, 3H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  169.1, 158.7, 157.7, 155.6, 154.6, 147.1, 138.8, 137.5, 132.1, 129.6, 129.3, 124.6, 124.2, 122.7, 118.1, 117.5, 115.9, 115.8, 113.2, 105.6, 50.0, 49.7, 46.2, 41.4, 21.5. HRMS (m/z):  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{25}\text{H}_{24}\text{Cl}_3\text{N}_7\text{O}_2$ : 560.1130, found: 560.1143.

**N-(3-((2-((6-(4-acetylpiperazin-1-yl) pyridin-3-yl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-chloroacrylamide (8q)**

White solid, m.p.: 115-116 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-d}_6$ )  $\delta$  10.18 (s, 1H), 9.14 (s, 1H), 8.90 (s, 1H), 8.27 (s, 1H), 8.10 (s, 1H), 7.92 (m, 1H), 7.84 (dd,  $J = 9.0, 2.5$  Hz, 1H), 7.46 (d,  $J = 7.5$  Hz, 1H), 7.37 (s, 1H), 7.31 (t,  $J = 8.0$  Hz, 1H), 6.66 (d,  $J = 8.0$  Hz, 1H), 6.41 (d,  $J = 2.5$  Hz, 1H), 6.10 (d,  $J = 2.5$  Hz, 1H), 3.54-3.51 (m, 4H), 3.41-3.39 (m, 2H), 3.34-3.31 (m, 2H), 2.04 (s, 3H). HRMS (m/z):  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{24}\text{H}_{24}\text{Cl}_2\text{N}_8\text{O}_2$ : 527.1472, found: 527.1480.

**N-(3-((2-((6-(4-acetylpiperazin-1-yl) pyridin-3-yl) amino)-5-chloropyrimidin-4-yl) amino) phenyl)-2-fluoroacrylamide (8r)**

Yellow solid, m.p.: 135-136 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.27 (d,  $J = 2.5$  Hz, 1H), 8.14 (s, 1H), 8.04 (s, 1H), 7.97 (d,  $J = 2.5$  Hz, 1H), 7.82 (dd,  $J = 9.0, 2.5$  Hz, 1H), 7.36-7.30 (m, 3H), 7.11 (s, 1H), 6.89 (s, 1H), 6.63 (d,  $J = 9.0$  Hz, 1H), 5.84 (dd,  $J = 47.5, 3.5$  Hz, 1H), 5.27 (dd,  $J = 15.5, 3.5$  Hz, 1H), 3.78-3.74 (m, 2H), 3.9-3.56 (m, 4H), 3.45-3.43 (m, 2H), 2.15 (s, 3H). HRMS (m/z):  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{24}\text{H}_{24}\text{ClF}_2\text{N}_8\text{O}_2$ : 511.1768, found: 511.1760.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-2-methoxyphenyl) amino)-5-chloropyrimidin-4-yl) oxy) phenyl)-2-fluoroacrylamide (13a)**

White solid, m.p.: 94-95 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.25 (s, 1H), 8.03 (d,  $J = 4.5$  Hz, 1H), 7.66 (d,  $J = 7.0$  Hz, 2H), 7.49-7.45 (m, 3H), 7.05 (dd,  $J = 8.0, 1.5$  Hz, 1H), 6.46 (d,  $J = 2.0$  Hz, 1H), 6.19 (s, 1H), 5.83 (dd,  $J = 48.0, 3.5$  Hz, 1H), 5.27 (dd,  $J = 15.0, 3.5$  Hz, 1H), 3.76 (t,  $J = 5.0$  Hz, 2H), 3.61 (t,  $J = 5.0$  Hz, 2H), 3.06-3.02 (m, 4H), 2.14 (s, 3H), 1.64 (s, 3H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  169.1, 164.6, 157.9, 157.5, 157.5, 157.3, 156.0 (d,  $J = 270$  Hz), 153.0, 148.8, 146.8, 137.9, 130.2, 122.7, 119.2, 117.5, 114.4, 108.6, 101.1, 100.5 (d,  $J = 15.0$  Hz), 55.8, 50.8, 50.7, 46.4, 41.6, 21.5. HRMS (m/z):  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{26}\text{H}_{26}\text{ClF}_2\text{N}_6\text{O}_4$ : 541.1761, found: 541.1763.

**N-(3-((2-((4-(4-acetylpiperazin-1-yl)-2-methoxyphenyl) amino)-5-chloropyrimidin-4-yl) oxy) phenyl)-2-chloroacrylamide (13b)**

White solid, m.p.: 98-99 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.47 (s, 1H), 8.24 (s, 1H), 7.66 (d, *J* = 7.5 Hz, 1H), 7.51-7.47 (m, 3H), 7.05 (dd, *J* = 8.0, 1.5 Hz, 1H), 6.72 (d, *J* = 1.5 Hz, 1H), 6.46 (d, *J* = 2.5 Hz, 1H), 6.20 (s, 1H), 5.93 (d, *J* = 1.0 Hz, 1H), 3.82 (s, 3H), 3.76 (d, *J* = 5.0 Hz, 2H), 3.62-3.60 (m, 2H), 3.07-3.01 (m, 4H), 2.14 (s, 3H). <sup>13</sup>C NMR (125MHz, CDCl<sub>3</sub>) δ 169.1, 164.6, 158.7, 157.9, 157.5, 153.0, 148.7, 146.8, 138.2, 132.0, 130.1, 124.3, 122.6, 122.1, 119.2, 117.5, 114.4, 108.6, 105.8, 101.0, 55.8, 50.8, 50.6, 46.4, 41.5, 21.5. HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>26</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>6</sub>O<sub>4</sub>: 557.1465, found: 557.1467.

**N-(3-((5-chloro-2-((2-methoxy-4-(4-methylpiperazin-1-yl) phenyl) amino) pyrimidin-4-yl) oxy) phenyl)-2-fluoroacrylamide (13c)**

White solid, m.p.: 174-175 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.23 (s, 1H), 8.02 (s, 1H), 7.63 (d, *J* = 7.0 Hz, 1H), 7.51 (s, 1H), 7.46 (t, *J* = 8.0 Hz, 2H), 7.05 (d, *J* = 7.5 Hz, 1H), 6.45 (s, 1H), 6.20 (s, 1H), 5.83 (dd, *J* = 48.0, 3.0 Hz, 1H), 5.26 (dd, *J* = 15.0, 3.0 Hz, 1H), 3.81 (s, 3H), 3.11 (m, 3H), 2.58 (m, 4H), 2.36 (s, 3H). HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>25</sub>H<sub>26</sub>ClFN<sub>6</sub>O<sub>3</sub>: 513.1812, found: 513.1819.

**2-chloro-N-(3-((5-chloro-2-((2-methoxy-4-(4-methylpiperazin-1-yl) phenyl) amino) pyrimidin-4-yl) oxy) phenyl)acrylamide (13d)**

White solid, m.p.: 106-107 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.43 (s, 1H), 8.23 (s, 1H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.53 (t, *J* = 2.0 Hz, 1H), 7.46 (t, *J* = 8.5 Hz, 2H), 7.05 (dd, *J* = 8.0, 1.5 Hz, 1H), 6.73 (d, *J* = 1.5 Hz, 1H), 6.45 (d, *J* = 2.0 Hz, 1H), 6.21 (s, 1H), 5.92 (d, *J* = 1.5 Hz, 1H), 3.82 (s, 3H), 3.13-3.11 (m, 4H), 2.60-2.58 (m, 4H), 2.37 (s, 3H). <sup>13</sup>C NMR (125MHz, CDCl<sub>3</sub>) δ 164.5, 158.7, 157.9, 157.6, 153.4, 153.0, 148.8, 147.0, 138.1, 132.0, 130.1, 124.3, 121.9, 119.3, 119.2, 117.4, 114.4, 108.1, 100.4, 55.7, 55.1, 49.9, 46.0. HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>25</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>6</sub>O<sub>3</sub>: 529.1516, found: 529.1519.

**N-(3-(((4-(4-acetylpiperazin-1-yl) phenyl) amino)-5-chloropyrimidin-4-yl) oxy) phenyl)-2-chloroacrylamide (13e)**

White solid, m.p.: 185-186 °C. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 10.38 (s, 1H), 9.56 (s, 1H), 8.42 (s, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.62 (d, *J* = 2.0 Hz, 1H), 7.48 (t, *J* = 8.5 Hz, 1H), 7.25 (m, 2H), 7.07 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.69 (m, 2H), 6.43 (d, *J* = 3.0 Hz, 1H), 6.12 (d, *J* = 2.5 Hz, 1H), 3.56-3.53 (m, 4H), 2.99-2.93 (m, 4H), 2.03 (s, 3H). <sup>13</sup>C NMR (125MHz, CDCl<sub>3</sub>) δ 169.1, 164.5, 158.8, 157.9, 157.7, 152.9, 146.9, 138.1, 132.4, 131.9, 130.0, 124.4, 120.5, 119.1, 117.5, 117.4, 114.4, 106.2, 50.5, 50.1, 46.4, 41.5, 21.5. HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>25</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>6</sub>O<sub>3</sub>: 527.1360, found: 527.1363.

**N-(3-(((4-(4-acetylpiperazin-1-yl)-3-(trifluoromethyl) phenyl) amino)-5-chloropyrimidin-4-yl) oxy) phenyl)-2-chloroacrylamide (13f)**

White solid, m.p.: 145-146 °C. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 10.39 (s, 1H), 9.96 (s, 1H), 8.53 (s, 1H), 7.73 (m, 1H), 7.70-7.66 (m, 2H), 7.65-7.64 (m, 1H), 7.47 (t, *J* = 8.0 Hz, 1H), 7.22-7.21 (m, 1H), 7.08-7.07 (m, 1H), 6.43 (d, *J* = 3.0 Hz, 1H), 6.11 (d, *J* = 2.5 Hz, 1H), 3.51-3.50 (m, 4H), 2.75-2.73 (m, 2H), 2.70-2.68 (m, 2H), 2.04 (s, 3H). HRMS (m/z): [M+H]<sup>+</sup> calcd for C<sub>26</sub>H<sub>23</sub>Cl<sub>2</sub>F<sub>3</sub>N<sub>6</sub>O<sub>3</sub>: 595.1234, found: 595.1240.

**N-(5-((5-chloro-4-(1-methyl-1H-indol-3-yl) pyrimidin-2-yl) amino)-2-(dimethylamino) phenyl)-2-fluoroacrylamide (17a)**

White solid, m.p.: 204-205 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 9.53 (s, 1H), 8.54 (d, *J* = 8.0 Hz, 1H), 8.51 (d, *J* = 2.5 Hz, 1H), 8.36 (s, 1H), 8.29 (s, 1H), 7.70 (dd, *J*<sub>1</sub> = 8.5 Hz,



$J_2 = 2.5$  Hz, 1H), 7.39-7.38 (m, 1H), 7.33 (t,  $J = 7.0$  Hz, 1H), 7.24-7.20 (m, 1H), 7.22-7.20 (m, 2H), 5.81 (dd,  $J = 47.5, 3.0$  Hz, 1H), 5.24 (dd,  $J = 15.0, 3.0$  Hz, 1H), 3.90 (s, 3H), 2.67 (s, 6H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  158.7, 158.2, 157.4, 157.3 (d,  $J = 28.75$  Hz), 156.8 (d,  $J = 270$  Hz), 138.5, 137.0, 136.8, 134.4, 132.8, 127.2, 123.6, 123.0, 121.4, 120.8, 116.9, 116.4, 111.3, 111.2, 109.6, 99.6 (d,  $J = 16.25$  Hz), 45.3, 33.6. HRMS (m/z):  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{24}\text{H}_{22}\text{ClFN}_6\text{O}$ : 465.1600; found, 465.1605.

**2-chloro-N-(5-((5-chloro-4-(1-methyl-1H-indol-3-yl) pyrimidin-2-yl) amino)-2-(dimethylamino) phenyl) acrylamide (17b)**

Yellow solid, M.p: 101-102 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  10.01 (s, 1H), 8.55 (d,  $J = 8.0$  Hz, 1H), 8.50 (d,  $J = 2.5$  Hz, 1H), 8.37 (s, 1H), 8.28 (s, 1H), 7.70 (dd,  $J = 8.5, 2.5$  Hz, 1H), 7.38 (d,  $J = 8.0$  Hz, 1H), 7.34-7.31 (m, 1H), 7.26-7.23 (m, 1H), 7.20 (d,  $J = 9.0$  Hz, 1H), 6.71 (d,  $J = 1.5$  Hz, 1H), 5.91 (d,  $J = 1.5$  Hz, 1H), 3.89 (s, 3H), 2.69 (s, 6H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  158.7, 158.6, 158.2, 157.4, 138.6, 137.0, 136.7, 134.4, 133.2, 133.1, 127.2, 123.6, 123.3, 123.0, 121.4, 120.7, 116.9, 116.3, 111.2, 111.0, 109.5, 45.2, 33.6, 29.8. HRMS (m/z):  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{24}\text{H}_{22}\text{Cl}_2\text{N}_6\text{O}$ : 481.1305; found, 481.1311.

#### 4.4 BIOLOGICAL EVALUATION

##### Cell proliferation assay

The human epidermal carcinoma cell line A431 and human non-small cell lung cancer cell line NCI-H1975 were used to evaluate the potency of synthesized analogues in cell-based level. The human colon carcinoma cell line SW620 and monkey fibroblast cell COS7 were used as control cell lines to evaluate the cytotoxicity. A431 and SW620 was cultured with RPMI 1640 (GIBCO). COS7 and NCI-H1975 was cultured with Dulbecco's Modified Eagle's Medium (GIBCO). Both mediums were supplemented with penicillin, streptomycin and 10% fetal bovine serum. A431 and NCI-H1975 cells were seeded in density of 2000 cells/well and 2500 cells/well, respectively, in 96-well plates (Corning) for 24 hours. Duplicate wells were treated with test or reference compounds for 48 hours at various concentrations or DMSO (Sigma) as control. Plates were incubated at 37 °C in 5%  $\text{CO}_2$  atmosphere. Cell proliferation was determined by using MTT assay. The  $\text{IC}_{50}$  was calculated using GraphPad Prism 4.0.

##### Kinase inhibition assay

The assays were performed in vitro using Homogeneous time-resolved fluorescence (HTRF) method (Cisbio). EGFR was purchased from Sigma. The kinases and substrates were incubated first with synthesized analogues for 5 minutes in enzymatic buffer (for EGFR<sup>WT</sup> and EGFR<sup>L858R/T790M</sup>). Then ATP (Sigma) was added into the reaction mixture to start the enzyme reaction. The ATP concentrations used in each enzyme reaction were 1.65  $\mu\text{M}$ , equivalent to the  $K_m$  of ATP for the corresponding enzyme in this assay condition. The assays were conducted at room temperature for 30 minutes and stopped by detection reagents which contain EDTA. The detection step lasted for 1 hour. The  $\text{IC}_{50}$  was calculated using GraphPad Prism 4.0.

##### Cell cycle analysis

NCI-H1975 cells in 6-well plates were treated with either DMSO or compound **8a** at indicated concentrations and cultured at 37 °C and 5%  $\text{CO}_2$ . After 2 d, the cells were trypsinized, washed in cold PBS, and centrifuged. Cell pellets were re-suspended with

RNase and stained with PI. Samples were read in a flow cytometry using CytoFLEX LX (BECKMAN COULTER).

### Docking study

The X-ray structure (PDB 5XDK) was downloaded from the Protein Data Bank (PDB, <http://www.pdb.org>) as a template. The molecular modeling simulations were performed using Sybyl-X 1.3 software package. [31] The initial structure of **8a** was generated by Sketch module in Sybyl-X 1.3. The geometries of the compound were subsequently optimized using the Tripos force field with Gasteiger-Hückel charges. The produced conformation of **8a** was then inserted into the binding pocket of EGFR<sup>T790M</sup> to replace the Rociletinib for the initial structural model of **8a** binding to EGFR<sup>T790M</sup>. Molecular docking studies of **8a** with the EGFR<sup>T790M</sup> binding pocket was performed with FlexiDock module in Sybyl-X 1.3 while all of the parameters were default. [32] The docked complexes of inhibitor-enzyme were selected according to the criteria of interacting energy combined with geometrical matching quality.

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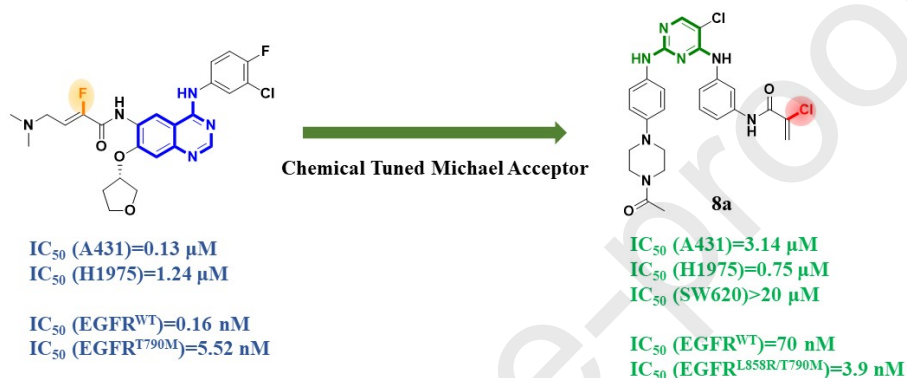
#### Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

- The electronic nature of warhead acrylamide moiety was investigated.

- **8a** retained the excellent EGFR<sup>L858R/T790M</sup> potency and good anti-proliferative activities against the NCI-H1975 cells.
- **8a** also displayed a significant EGFR<sup>WT</sup> selectivity and weaker inhibition against non-EGFR dependent SW620 and COS7 cell.
- Preliminary study showed that **8a** could arrest NCI-H1975 cells in G0/G1 phase.



- Improved EGFR<sup>L858R/T790M</sup> inhibition *via* chloroacrylamide incorporation
- Significant WT EGFR sparing selectivity (>16 fold)
- Mutant specific potency and less off-target toxicity