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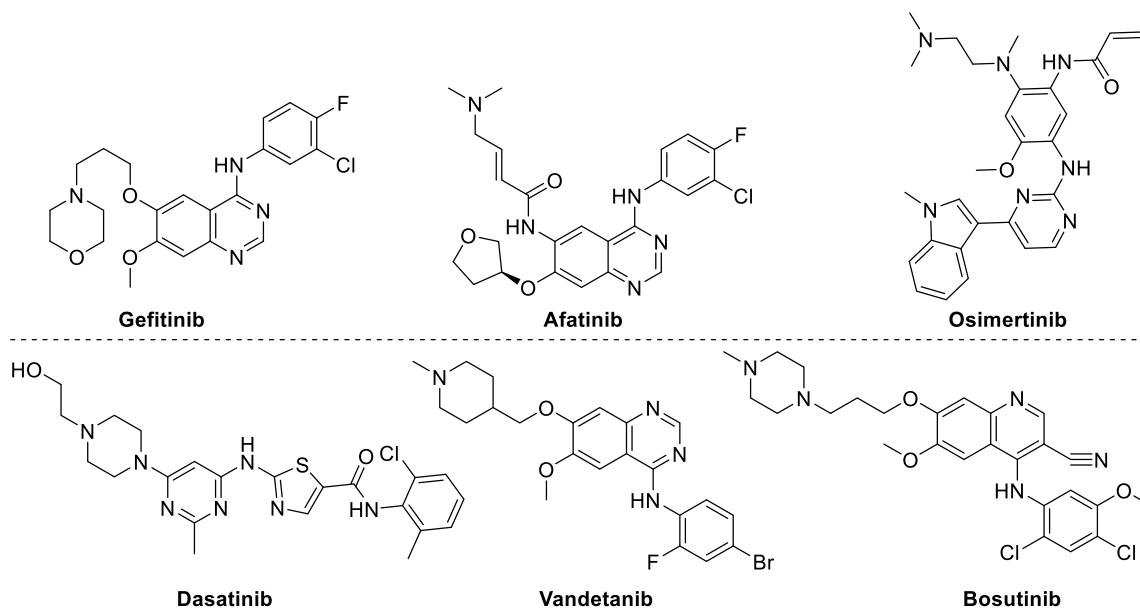
## SYNTHESIS AND BIOLOGICAL EVALUATION OF 5-METHYLPYRIMIDINE DERIVATIVES AS DUAL INHIBITORS OF EGFR AND SRC FOR CANCER TREATMENT

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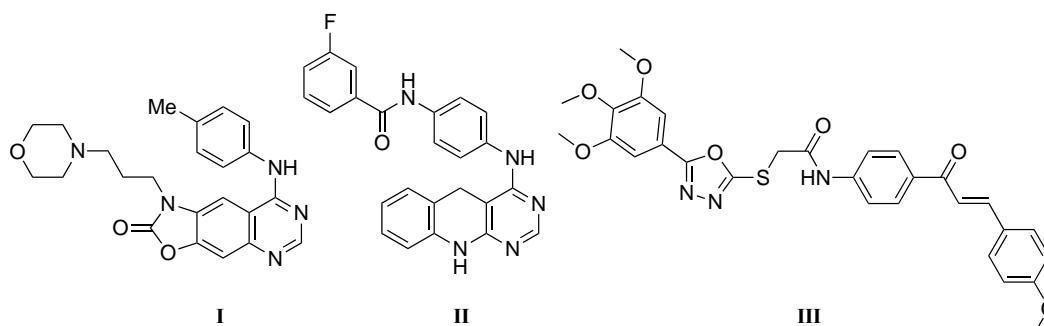
**Abstract** – In this paper, a series of new 5-methylpyrimidine derivatives was designed as dual inhibitors of EGFR and Src for cancer treatment. Twenty new compounds were synthesized and evaluated for antitumor activity with A549, HepG2, and K562 cells. Compounds **8f**, **8p**, and **8q** showed better antitumor activity than Gefitinib and Dasatinib. They were selected for testing inhibition of EGFR and Src. The IC<sub>50</sub> values of the most potent compound **8p** was reached to 1.56 μM and 0.74 μM. In addition, the ADMET properties of compounds **8f**, **8o-8q** were predicted with ADMETlab 2.0.

The epidermal growth factor receptor (EGFR) belongs to the receptor tyrosine kinase family, as it plays an important role in signal transduction pathways and tumor cell proliferation, invasion and vascularization.<sup>1</sup> About 10-50% of non-small cell lung cancer (NSCLC) patients have EGFR mutations.<sup>2</sup> Therefore, the development of NSCLC therapeutics targeting EGFR has become one of the hot spots in antitumor drug research.<sup>3</sup> As shown in **Figure 1**, many EGFR inhibitors such as Gefitinib, Afatinib, and Osimertinib have been approved in market for treatment of NSCLC.<sup>4</sup> Src is a kind of non-receptor tyrosine protein kinases, which plays a key role in many cellular regulatory processes, affecting cell adhesion, invasion, proliferation and angiogenesis.<sup>5</sup> It is estimated that in 50%-80% of NSCLC patients, Src kinase activation is at a high level.<sup>6</sup> Furthermore, the levels of Src kinase activation are closely related to the size of the tumor.<sup>7</sup> As shown in **Figure 1**, many Src inhibitors such as Dasatinib, Vandetanib, and Bosutinib have been used for treatment NSCLC in clinic.<sup>8</sup>



**Figure 1.** EGFR and Src inhibitors in market

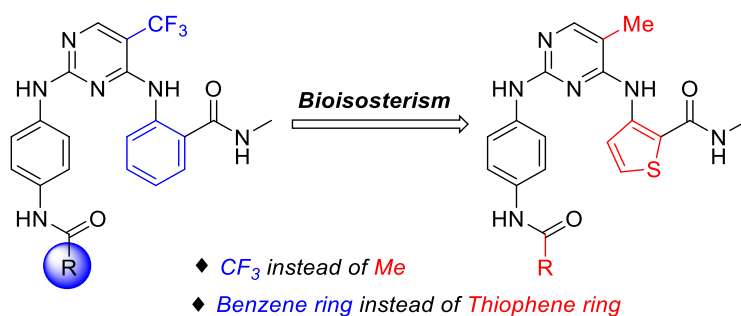
In 2018, Chen reported that Src activation mediated the binding of PI3K to EGFR, leading to AKT phosphorylation, which in turn induced tumor cell survival and signaling pathway migration.<sup>9</sup> In addition, Dasatinib (Src inhibitor) has been shown to prevent EGFR-induced ERK1/2 activation by inhibiting MAPK, thereby affecting EGFR-resistant tumor cell survival, proliferation, apoptosis and migration.<sup>10</sup> Based on EGFR/Src synergy, Src inhibitors have been used in combination with EGFR inhibitors to address EGFR inhibitor resistance in clinical practice.<sup>11</sup> For example, the combination of Dasatinib and Afatinib significantly reduced the proliferation of T790M mutation-resistant cells in NSCLC cell lines.<sup>12</sup> As shown in **Figure 2**, there were three new EGFR and Src inhibitors have been reported in recent years.<sup>13</sup>



**Figure 2.** Dual inhibitors of EGFR/Src

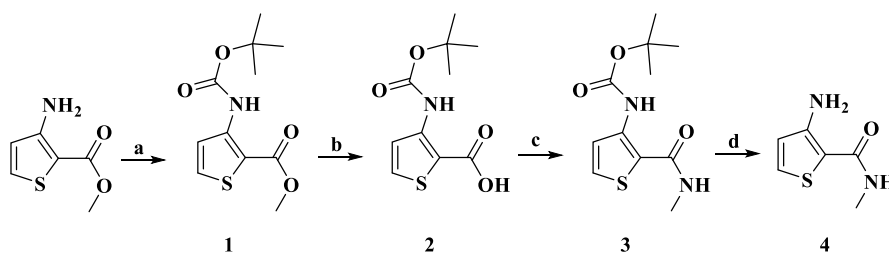
A large number of studies have shown that 2-phenylaminopyrimidine is a privileged structural moiety in the inhibitors of EGFR and Src.<sup>14</sup> And also, aminothiophene derivatives have been proved as good

bioactive backbone in many antimicrobial or antitumor reagents.<sup>15</sup> To develop new antitumor reagents for NSCLC, we are very interested in design and synthesis new EGFR/Src inhibitors. On the basis the previous reports and our long-term research in the field of kinase inhibitors,<sup>16</sup> as shown in **Figure 3**, we herein would introduce our progress in 5-methylpyrimidine derivatives as dual inhibitors of EGFR/Src. Our design strategy could be summarized that CF<sub>3</sub> group was replaced by CH<sub>3</sub> group and benzene ring was instead of thiophene ring.

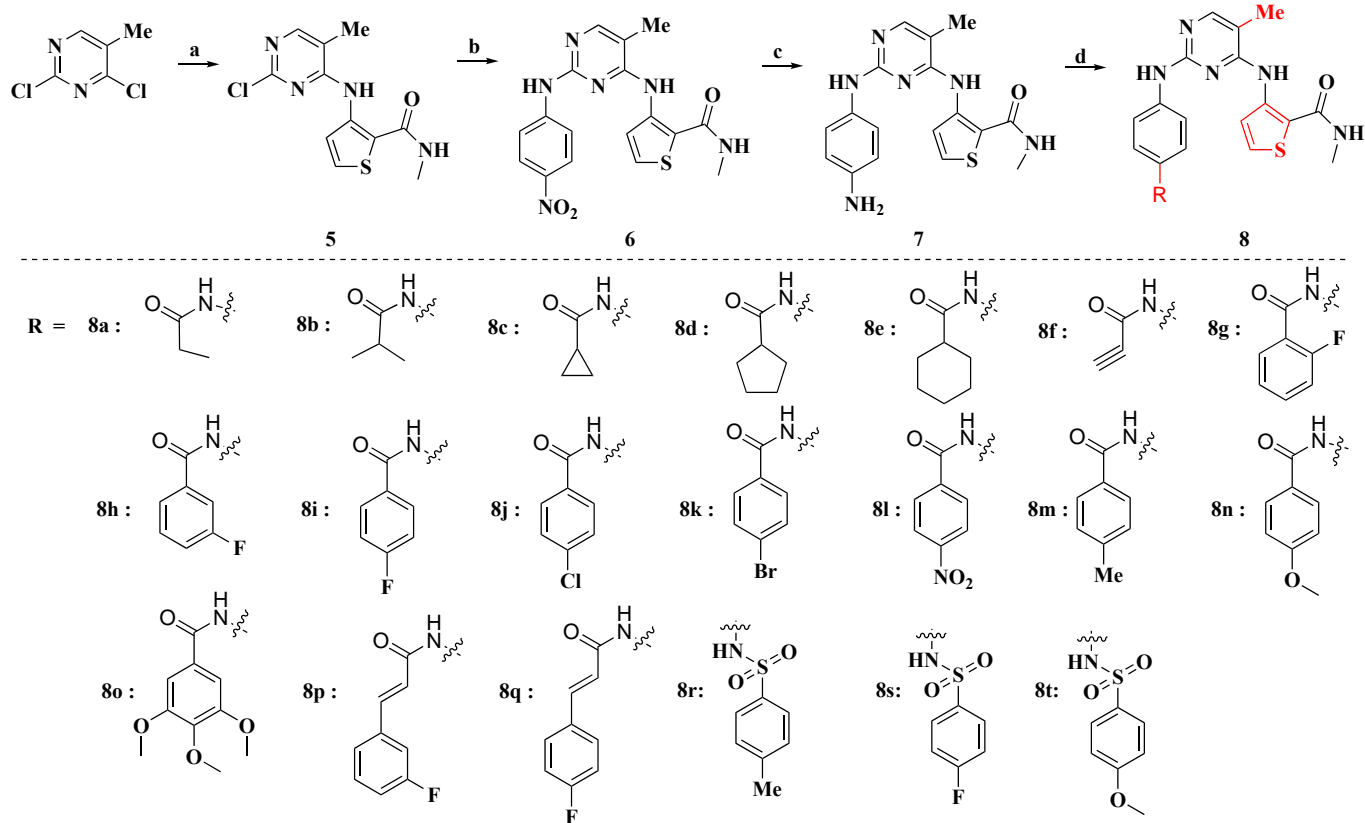


**Figure 3.** Design strategy of EGFR/Src inhibitors

As shown in **Scheme 1**, commercially available methyl 3-aminothiophene-2-carboxylate was used as starting material and its amino group was protected to give compound **1** in 50% yield. The ester group was hydrolyzed under alkaline conditions to give compound **2** with 97% yield. Then, compound **3** was obtained by dehydration condensation reaction of compound **2** with methylamine hydrochloride. Subsequently, the protecting group was removed under acidic conditions to give the key intermediate **4** in 74% yield. Next in **Scheme 2**, 2,4-dichloro-5-methylpyrimidine was nucleophilically substituted with compound **4** to give compound **5** with 90% yield. In the presence of trifluoroacetic acid, compound **6** was obtained in 90% yield from compound **5** with *p*-nitroaniline. The nitro group was reduced to amino group in the presence of hydrogen to give compound **7** in 73% yield. Finally, compound **7** was subjected to substitution reaction with the corresponding acid or sulfonyl chloride using DMF as solvent to give products **8a-8t** in 14%-87% yields.



**Scheme 1.** Synthetic route of compound **4**. Reagents and conditions: a) di-*tert*-butyl dicarbonate, DMAP, DIEA, 40 °C, 50% yield; b) THF, NaOH, 70 °C, 97% yield; c) MeNH<sub>2</sub>·HCl, HATU, DIEA, DMF, 25 °C, 96% yield; d) HCl, NaHCO<sub>3</sub>, DCM, 25 °C, 74% yield.



**Scheme 2.** Synthetic route of target compounds **8a-8t**. Reagents and conditions: a) NaH, DMF, rt, 90% yield; b) TFA, DMF, 80 °C, 75% yield; c) H<sub>2</sub>, Pd/C, rt, 73% yield; d) HATU, DIEA, DMF, rt, or TEA, DMF, 14-87% yield.

EGFR overexpressing A549 cell, HepG2 cell and Src overexpressing K562 cell were used to evaluate the antitumor activity of the target compounds.<sup>17</sup> The MTT method was used to evaluate the effects of target compounds **8a-8t** against A549, HepG2 and K562 cancer cells. Gefitinib and Dasatinib were used as positive control. The results of the target compounds tested for tumor cell growth inhibitory activity were summarized in **Table 1**. Against A549 cells and HepG2 cells, most compounds performed well activity. However, three individual compounds **8f**, **8p**, and **8q** showed better antitumor activity than Gefitinib and Dasatinib. Against K562 cells, only compounds **8f**, **8p**, and **8q** showed better activity than Gefitinib. Unfortunately, there were no compound more potent than Dasatinib. Overall, the IC<sub>50</sub> values of compounds **8f**, and **8o-8q** were less than 10 μM, and these four compounds were selected for further studies.

**Table 1.** IC<sub>50</sub> values for three cancer cell lines <sup>a</sup>

Compound	IC <sub>50</sub> (μM) <sup>a</sup>		
	A549	HepG2	K562
<b>8a</b>	>20	>20	>20
<b>8b</b>	>20	18.69 ± 2.57	>20
<b>8c</b>	>20	>20	>20
<b>8d</b>	>20	>20	>20
<b>8e</b>	>20	>20	>20
<b>8f</b>	3.11 ± 0.54	7.46 ± 0.62	4.57 ± 0.39
<b>8g</b>	13.56 ± 1.48	15.38 ± 1.73	>20
<b>8h</b>	10.95 ± 0.85	11.67 ± 1.36	14.16 ± 1.75
<b>8i</b>	12.38 ± 1.29	13.97 ± 1.62	18.04 ± 1.83
<b>8j</b>	14.77 ± 1.46	15.21 ± 1.93	>20
<b>8k</b>	14.82 ± 1.51	15.33 ± 1.81	>20
<b>8l</b>	12.56 ± 0.98	17.31 ± 1.74	18.32 ± 2.05
<b>8m</b>	15.36 ± 1.52	16.28 ± 1.94	>20
<b>8n</b>	15.44 ± 1.63	16.32 ± 1.85	>20
<b>8o</b>	7.63 ± 0.61	12.04 ± 0.75	9.55 ± 0.87
<b>8p</b>	2.29 ± 0.37	5.38 ± 0.44	3.28 ± 0.26
<b>8q</b>	2.45 ± 0.35	5.87 ± 0.52	3.47 ± 0.32
<b>8r</b>	12.07 ± 0.84	14.35 ± 1.26	>20
<b>8s</b>	11.35 ± 0.79	12.87 ± 0.94	>20
<b>8t</b>	13.56 ± 1.13	16.52 ± 1.88	>20
<b>Gefitinib</b>	5.32 ± 0.69	11.07 ± 1.25	7.41 ± 0.97
<b>Dasatinib</b>	11.41 ± 1.13	9.27 ± 1.08	0.15 ± 0.087

<sup>a</sup> The values are mean ± SD of three replicates

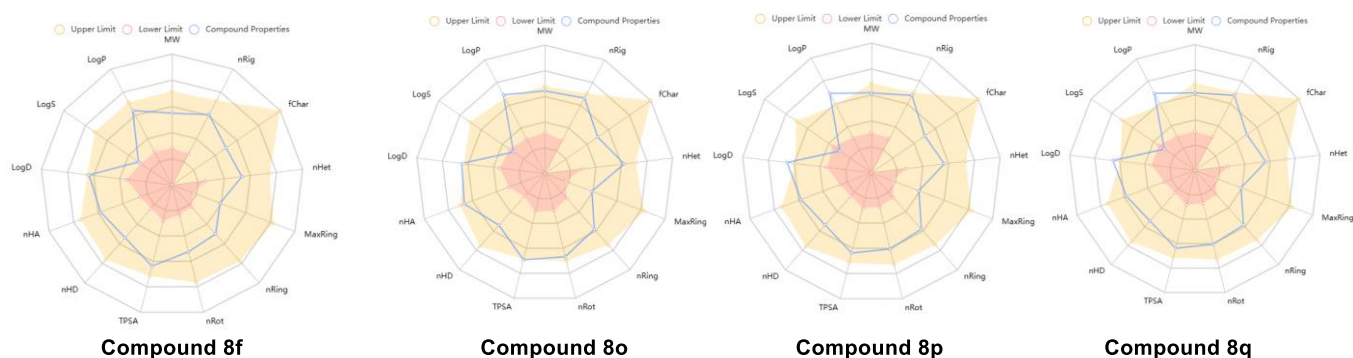
Compounds **8f** and **8o-8q** were encouraged to evaluate the inhibition for EGFR<sup>wt</sup> and Src kinase with ELISA assay as our previous reports.<sup>16</sup> As shown in **Table 2**, Gefitinib was 11 nM and Dasatinib was 7.4 nM. The two positive controls were equally consisted with the literature. Compound **8p** was the most potent and the IC<sub>50</sub> values were reached to 1.56 μM and 0.74 μM respectively. Unfortunately, the other compounds showed moderate activity against EGFR and Src. This result was the same with the activity against tumor cells. Generally, compound **8p** was a good antitumor agent for further investigations.

**Table 2.** IC<sub>50</sub> values for EGFR<sup>wt</sup> and Src <sup>a</sup>

Entry	Compound	IC <sub>50</sub> (μM)	
		EGFR <sup>wt</sup> -TK	Src
1	<b>8f</b>	5.77 ± 0.38	2.69 ± 0.19
2	<b>8o</b>	6.41 ± 0.55	3.27 ± 0.26
3	<b>8p</b>	1.56 ± 0.15	0.74 ± 0.089
4	<b>8q</b>	2.06 ± 0.73	1.85 ± 0.32
5	Gefitinib	0.011 ± 0.0057	-
6	Dasatinib	-	0.0074 ± 0.0012

<sup>a</sup> The values are mean ± SD of three replicates

Absorption, distribution, metabolism, excretion and toxicity (ADMET) of drugs is an important part of drug development.<sup>18</sup> ADMET is a key indicator of whether a small molecule compound is a drug. In the early stages of drug development, ADMET study can effectively address the safety and efficacy of drug candidates and increase the success rate of drug development.<sup>19</sup> ADMETlab 2.0, a completely web server for the predictions of ADMET properties have been available. This free website has been successfully used for developing new antitumor agents.<sup>20</sup> Compounds **8f**, **8o**, **8p**, and **8q** were employed to predict ADMET properties with this tool. As shown in **Figure 4**, the “Compound Properties” (Blue area) were lower the “Upper Limit” (Yellow area) and higher than “Lower Limit” (Red area). This indicated that compounds **8f**, **8o**, **8p**, and **8q** had good ADMET properties including LogD and LogP. The details of calculated data were put in supporting information.

**Figure 4.** Predicted ADMET properties of the target compounds

In general, a novel series of 5-methylpyrimidine derivatives were designed and synthesized for development of dual inhibitors of EGFR and Src. After evaluation three human cancer cell lines (A549, HepG2, and K562) with MTT assay, compounds **8f**, **8o-8q** performed good antiproliferative activity. Subsequently, they were selected for investigated the inhibition for EGFR and Src kinase. Furthermore, the ADMET properties of compounds **8f**, **8o-8q** were also predicted with ADMETlab 2.0. In summary, compound **8p** was worth for further studies.

## EXPERIMENTAL SECTION

Melting points were determined with X-4X digital display micro melting point analyzer (uncorrected, Shanghai Microelectronics Technology Co., Ltd.).  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectroscopic data were recorded with Bruker 400 MHz NMR spectrometer in DMSO- $d_6$  solution, which was provided by School of Pharmaceutical Sciences in Guizhou University. And the TMS was served as the internal standard. The high-resolution mass spectrometer (HRMS) was tested in TSQ 8000 and AB SCIEX X500R QTOF. Compounds **1-4** were synthesized according to the previous report in literature.<sup>21</sup>

### **3-(2-Chloro-5-methylpyrimidin-4-ylamino)thiophene-2-carboxylic acid carboxamide (5)**

2,4-Dichloro-5-trifluoromethylpyrimidine (9.32 mmol) and compound **4** (10.25 mmol) were stirred in DMF (15 mL) at room temperature. Then sodium hydride (46.6 mmol) was added at 0 °C and stirred overnight. After the reaction, the organic phase was extracted with EtOAc (3×30 mL), washed with saturated aq. NaCl (3×30 mL), dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and vacuum concentrated to obtain crude compounds. The crude product was purified by recrystallization through MeOH to give a white solid (2.38 g, 90% yield). mp 145.7-147.2 °C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  11.53 (s, 1H), 8.33 (d,  $J$  = 5.6 Hz, 1H), 8.23 (d,  $J$  = 5.6 Hz, 1H), 8.19 (s, 1H), 7.82 (d,  $J$  = 5.6 Hz, 1H), 2.79 (d,  $J$  = 4.4 Hz, 3H), 2.19 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  165.1, 158.7, 157.4, 157.0, 143.3, 129.3, 122.45, 115.2, 113.1, 26.6, 13.2; ESI-HRMS C<sub>11</sub>H<sub>11</sub>ClN<sub>4</sub>OS ([M+H]<sup>+</sup>): calcd 283.0414, found 283.0417.

### **3-[5-Methyl-2-(4-nitrophenylamino)pyrimidin-4-ylamino]thiophene-2-carboxylic acid methylamide (6)**

The mixture of compound **5** (4 mmol) and *p*-nitroaniline (4.8 mmol) was added to DMF (11 mL), and then trifluoroacetic acid (12 mmol) was added dropwise. The solution was stirred overnight in argon at 80 °C. After the reaction was complete, the mixture was cooled to room temperature, carefully added with H<sub>2</sub>O (30 mL) and extracted with EtOAc (3 x 30 mL). The organic phase was washed with saturated aq. NaHCO<sub>3</sub>, dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and vacuum concentrated. The crude product was purified by recrystallization through DCM to give a yellow solid (1.15g, 75% yield). mp 138.4-139.5 °C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  11.26 (s, 1H), 10.01 (s, 1H), 8.57 (d,  $J$  = 5.6 Hz, 1H), 8.23 (t,  $J$  = 4.4 Hz, 1H), 8.19 (d,  $J$  = 9.2 Hz, 2H), 8.11 (s, 1H), 8.05 – 7.97 (m, 2H), 7.81 (d,  $J$  = 5.6 Hz, 1H), 2.79 (d,  $J$  =

4.4 Hz, 3H), 2.16 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-}d_6$ )  $\delta$  165.3, 157.8, 157.7, 155.7, 148.2, 144.3, 140.3, 128.8, 125.5, 123.6, 117.7, 111.9, 108.8, 26.5, 13.3; ESI-HRMS  $\text{C}_{17}\text{H}_{16}\text{N}_6\text{O}_3\text{S}$  ( $[\text{M}+\text{H}]^+$ ): calcd 385.1077, found 385.1086.

**3-[2-(4-Aminophenylamino)-5-methylpyrimidin-4-ylamino]thiophene-2-carboxylic acid methylamide (7)**

A mixture of compound **6** (768.4 mg) and palladium on carbon (192.1 mg) was stirred in MeOH (7 mL). The solution was stirred at room temperature for overnight under hydrogen atmosphere. After the reaction completed, the reaction solution was filtered in celite. The filtration was concentrated under vacuum, and dryness to give a yellow solid (520 mg, 73% yield). mp 158.9-160.2 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  11.04 (s, 1H), 8.64 (s, 1H), 8.49 (d,  $J = 5.6$  Hz, 1H), 8.13 (q,  $J = 4.4$  Hz, 1H), 7.89 (s, 1H), 7.64 (d,  $J = 5.6$  Hz, 1H), 7.26 (d,  $J = 8.4$  Hz, 2H), 6.64 – 6.45 (m, 2H), 4.76 (s, 2H), 2.78 (d,  $J = 4.6$  Hz, 3H), 2.08 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-}d_6$ )  $\delta$  165.4, 159.7, 157.4, 156.4, 144.9, 144.0, 130.5, 128.3, 123.7, 122.3, 114.4, 110.8, 104.9, 26.5, 13.2; ESI-HRMS  $\text{C}_{17}\text{H}_{18}\text{N}_6\text{OS}$  ( $[\text{M}+\text{H}]^+$ ): calcd 355.1335, found 355.1337.

A mixture of compound **7** (0.42 mmol) and DIEA (0.63 mmol) was stirred in DMF (2 mL). Then add the corresponding carboxylic acid (0.42 mmol) and HATU (239.40 mg, 0.63 mmol). The mixture was stirred at dry room temperature for 12 h. After reaction, the organic phase was extracted with EtOAc (3×30 mL), washed with saturated aq. NaCl (3×30 mL), dried with  $\text{Na}_2\text{SO}_4$ , filtered and vacuum concentrated to obtain crude compounds. The crude product is purified with MeOH to obtain the products **8a** – **8q**.

**3-[5-Methyl-2-((4-propionylamino)phenylamino)pyrimidin-4-ylamino]thiophene-2-carboxylic acid methylamide (8a)**

Off white solid; 55% yield; mp 249.5-250.7 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  11.11 (s, 1H), 9.72 (s, 1H), 9.08 (s, 1H), 8.55 (d,  $J = 5.6$  Hz, 1H), 8.17 (q,  $J = 4.4$  Hz, 1H), 7.98 (d,  $J = 0.8$  Hz, 1H), 7.70 (d,  $J = 5.6$  Hz, 1H), 7.64 – 7.57 (m, 2H), 7.52 – 7.45 (m, 2H), 2.78 (d,  $J = 4.4$  Hz, 3H), 2.30 (q,  $J = 7.6$  Hz, 2H), 2.11 (d,  $J = 0.8$  Hz, 3H), 1.09 (t,  $J = 7.6$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  176.6, 170.1, 163.7, 162.3, 160.9, 149.5, 141.5, 138.3, 133.2, 128.4, 124.7, 124.5, 115.9, 110.9, 34.6, 31.3, 18.0, 15.0; ESI-HRMS  $\text{C}_{20}\text{H}_{22}\text{N}_6\text{O}_2\text{S}$  ( $[\text{M}+\text{H}]^+$ ): calcd 411.1597, found 411.1595.

**3-[2-((4-Isobutyrylamino)phenylamino)-5-methylpyrimidin-4-ylamino]thiophene-2-carboxylic acid methylamide (8b)**

White solid; 23% yield; mp 170.1-171.9 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  11.11 (s, 1H), 9.68 (s, 1H), 9.08 (s, 1H), 8.55 (d,  $J = 5.6$  Hz, 1H), 8.17 (d,  $J = 4.6$  Hz, 1H), 7.98 (s, 1H), 7.71 (d,  $J = 5.6$  Hz, 1H), 7.61 (d,  $J = 8.8$  Hz, 2H), 7.53 – 7.44 (m, 2H), 2.78 (d,  $J = 4.4$  Hz, 3H), 2.57 (p,  $J = 6.8$  Hz, 1H), 2.11 (s, 3H), 1.10 (d,  $J = 6.8$  Hz, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-}d_6$ )  $\delta$  175.1, 165.4, 158.9, 157.5, 156.2, 144.7, 136.8, 133.5, 128.5, 123.7, 120.1, 119.6, 111.2, 106.1, 35.3, 26.5, 20.1, 13.3; ESI-HRMS  $\text{C}_{21}\text{H}_{24}\text{N}_6\text{O}_2\text{S}$

([M+H]<sup>+</sup>): calcd 425.1754, found 425.1753.

**3-{2-[4-(Cyclopropanecarbonylamino)phenylamino]-5-methylpyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8c)**

Off white solid; 42% yield; mp 204.2-205.8 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.11 (s, 1H), 10.05 (s, 1H), 9.08 (s, 1H), 8.55 (d, *J* = 5.6 Hz, 1H), 8.17 (q, *J* = 4.4 Hz, 1H), 7.98 (d, *J* = 0.8 Hz, 1H), 7.70 (d, *J* = 5.6 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 2H), 7.51 – 7.44 (m, 2H), 2.78 (d, *J* = 4.4 Hz, 3H), 2.11 (s, 3H), 1.81 – 1.69 (m, 1H), 0.82 – 0.73 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 171.5, 165.4, 158.9, 157.5, 156.2, 144.7, 136.8, 133.5, 128.4, 123.7, 119.9, 119.7, 111.2, 106.1, 26.5, 14.9, 13.3, 7.4; ESI-HRMS C<sub>21</sub>H<sub>22</sub>N<sub>6</sub>O<sub>2</sub>S ([M+H]<sup>+</sup>): calcd 423.1597, found 423.1598.

**3-{2-[4-(Cyclopentanecarbonylamino)phenylamino]-5-methylpyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8d)**

Off white solid; 42% yield; mp 211.5-212.4 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.11 (s, 1H), 9.72 (s, 1H), 9.08 (s, 1H), 8.55 (d, *J* = 5.6 Hz, 1H), 8.17 (q, *J* = 4.4 Hz, 1H), 7.98 (s, 1H), 7.71 (d, *J* = 5.6 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 2H), 7.53 – 7.45 (m, 2H), 2.78 (d, *J* = 4.6 Hz, 3H), 2.76 – 2.65 (m, 1H), 2.11 (s, 3H), 1.91 – 1.79 (m, 2H), 1.79 – 1.63 (m, 4H), 1.61 – 1.52 (m, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 174.3, 165.4, 158.9, 157.5, 156.2, 144.7, 136.8, 133.6, 128.5, 123.7, 120.0, 119.6, 111.2, 106.1, 45.6, 30.6, 26.5, 26.2, 13.3; ESI-HRMS C<sub>23</sub>H<sub>26</sub>N<sub>6</sub>O<sub>2</sub>S ([M+H]<sup>+</sup>): calcd 451.1910, found 451.1913.

**3-{2-[4-(Cyclohexanecarbonylamino)phenylamino]-5-methylpyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8e)**

Off white solid; 63% yield; mp 221.7-223.4 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.10 (s, 1H), 9.66 (s, 1H), 9.07 (s, 1H), 8.55 (d, *J* = 5.6 Hz, 1H), 8.17 (q, *J* = 4.4 Hz, 1H), 7.97 (d, *J* = 0.8 Hz, 1H), 7.70 (d, *J* = 5.6 Hz, 1H), 7.59 (d, *J* = 9.2 Hz, 2H), 7.49 (d, *J* = 9.2 Hz, 2H), 2.78 (d, *J* = 4.4 Hz, 3H), 2.37 – 2.24 (m, 1H), 2.13 – 2.07 (m, 3H), 1.81 – 1.71 (m, 4H), 1.49 – 1.35 (m, 2H), 1.34 – 1.13 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 174.2, 165.4, 158.9, 157.5, 156.2, 144.7, 136.7, 133.6, 128.4, 123.7, 120.0, 119.6, 111.1, 106.1, 45.3, 29.7, 26.5, 25.9, 25.8, 13.3; ESI-HRMS C<sub>24</sub>H<sub>28</sub>N<sub>6</sub>O<sub>2</sub>S ([M+H]<sup>+</sup>): calcd 465.2067, found 465.2069.

**3-[5-Methyl-2-(4-propynoylamino)phenylamino]pyrimidin-4-ylamino]thiophene-2-carboxylic acid methylamide (8f)**

Yellow solid; 14% yield; mp 249.3-250.3 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.12 (s, 1H), 10.67 (s, 1H), 9.18 (s, 1H), 8.55 (d, *J* = 5.6 Hz, 1H), 8.17 (q, *J* = 4.4 Hz, 1H), 7.99 (d, *J* = 0.8 Hz, 1H), 7.71 (d, *J* = 5.6 Hz, 1H), 7.65 (d, *J* = 9.2 Hz, 2H), 7.50 (d, *J* = 9.2 Hz, 2H), 4.36 (s, 1H), 2.78 (d, *J* = 4.4 Hz, 3H), 2.12 (s, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.4, 158.8, 157.5, 156.1, 149.7, 144.7, 144.7, 138.0, 132.1, 128.5, 123.7, 120.7, 119.5, 111.2, 106.4, 79.1, 77.2, 26.5, 13.3; ESI-HRMS C<sub>20</sub>H<sub>18</sub>N<sub>6</sub>O<sub>2</sub>S ([M+H]<sup>+</sup>): calcd 407.1284, found 407.1281.

**3-{2-[4-(2-Fluorobenzoylamino)phenylamino]-5-methylpyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8g)**

Off white solid; 14% yield; mp 196.9-197.3 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.14 (s, 1H), 10.28 (s, 1H), 9.19 (s, 1H), 8.57 (d, *J* = 5.6 Hz, 1H), 8.18 (d, *J* = 4.8 Hz, 1H), 8.00 (d, *J* = 1.0 Hz, 1H), 7.72 (d, *J* = 5.6 Hz, 1H), 7.68 (d, *J* = 9.2 Hz, 3H), 7.63 (d, *J* = 9.2 Hz, 2H), 7.61 – 7.53 (m, 1H), 7.40 – 7.29 (m, 2H), 2.79 (d, *J* = 4.5 Hz, 3H), 2.12 (s, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.4, 162.7, 160.6, 158.9, 158.1, 157.5, 156.1, 144.7, 137.6, 132.9, 130.4, 128.5, 125.7, 125.6, 125.0, 125.0, 123.7, 120.7, 119.6, 116.7, 116.5, 111.2, 106.3, 26.5, 13.3; ESI-HRMS C<sub>24</sub>H<sub>21</sub>FN<sub>6</sub>O<sub>2</sub>S ([M+H]<sup>+</sup>): calcd 477.1503, found 477.1502.

**3-{2-[4-(3-Fluorobenzoylamino)phenylamino]-5-methylpyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8h)**

Off white solid; 53% yield; mp > 250 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.13 (s, 1H), 10.22 (s, 1H), 9.18 (s, 1H), 8.57 (d, *J* = 5.6 Hz, 1H), 8.18 (q, *J* = 4.4 Hz, 1H), 8.00 (d, *J* = 1.2 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.82 – 7.74 (m, 1H), 7.73 (d, *J* = 5.6 Hz, 1H), 7.73 – 7.63 (m, 1H), 7.64 – 7.54 (m, 4H), 7.50 – 7.39 (m, 1H), 2.79 (d, *J* = 4.4 Hz, 3H), 2.18 – 2.10 (m, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.39, 163.63, 161.20, 158.88, 157.54, 156.15, 144.69, 137.89 (d, *J* = 6.8 Hz), 137.72, 132.75, 131.00 (d, *J* = 8.0 Hz), 128.48, 124.26, 124.23, 123.69, 121.46, 119.47, 118.73 (d, *J* = 20.8 Hz), 114.84 (d, *J* = 22.8 Hz), 111.21, 106.32, 26.53, 13.28; ESI-HRMS C<sub>24</sub>H<sub>21</sub>FN<sub>6</sub>O<sub>2</sub>S ([M+H]<sup>+</sup>): calcd 477.1503, found 477.1507.

**3-{2-[4-(4-Fluorobenzoylamino)phenylamino]-5-methylpyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8i)**

Off white solid; 30% yield; mp 249.7-250.8 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.13 (s, 1H), 10.17 (s, 1H), 9.18 (s, 1H), 8.57 (d, *J* = 5.6 Hz, 1H), 8.18 (q, *J* = 4.4 Hz, 1H), 8.10 – 8.00 (m, 2H), 8.00 (s, 1H), 7.73 (d, *J* = 5.4 Hz, 1H), 7.72 – 7.62 (m, 4H), 7.42 – 7.32 (m, 2H), 2.79 (d, *J* = 4.5 Hz, 3H), 2.15 – 2.09 (m, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.4, 164.4, 163.2, 158.9, 157.6, 156.0, 144.7, 137.5, 133.0, 132.0, 131.9, 130.7 (d, *J* = 8.8 Hz), 128.5, 123.7, 121.4, 119.6, 115.7 (d, *J* = 21.6 Hz), 111.2, 106.3, 26.5, 13.3; ESI-HRMS C<sub>24</sub>H<sub>21</sub>FN<sub>6</sub>O<sub>2</sub>S ([M+H]<sup>+</sup>): calcd 477.1503, found 477.1503.

**3-{2-[4-(4-Chlorobenzoylamino)phenylamino]-5-methylpyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8j)**

Yellow solid; 22% yield; mp 213.5-214.2 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.12 (s, 1H), 10.22 (s, 1H), 9.17 (s, 1H), 8.57 (d, *J* = 5.6 Hz, 1H), 8.17 (q, *J* = 4.4 Hz, 1H), 8.03 – 7.96 (m, 3H), 7.73 (d, *J* = 5.6 Hz, 1H), 7.72 – 7.62 (m, 4H), 7.64 – 7.58 (m, 2H), 2.79 (d, *J* = 4.4 Hz, 3H), 2.12 (s, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.4, 14.4, 158.9, 157.5, 156.2, 144.7, 137.7, 136.6, 134.3, 132.8, 130.0, 128.9, 128.5, 123.7, 121.4, 119.5, 111.2, 106.3, 26.5, 13.3; ESI-HRMS C<sub>24</sub>H<sub>21</sub>ClN<sub>6</sub>O<sub>2</sub>S ([M+Na]<sup>+</sup>): calcd 493.1207, found 493.1202.

**3-{2-[4-(4-Bromobenzoylamino)phenylamino]-5-methylpyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8k)**

Off white solid; 32% yield; mp > 250 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.12 (s, 1H), 10.22 (s, 1H), 9.17 (s, 1H), 8.57 (d, *J* = 5.6 Hz, 1H), 8.18 (d, *J* = 4.4 Hz, 1H), 8.00 (s, 1H), 7.94 – 7.89 (m, 2H), 7.78 – 7.70 (m, 3H), 7.72 – 7.62 (m, 4H), 2.79 (d, *J* = 4.4 Hz, 3H), 2.12 (s, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.4, 164.5, 158.9, 157.5, 156.2, 144.7, 137.7, 134.6, 132.8, 131.8, 130.2, 128.5, 125.6, 123.7, 121.4, 119.5, 111.2, 106.3, 26.5, 13.3; ESI-HRMS C<sub>24</sub>H<sub>21</sub>BrN<sub>6</sub>O<sub>2</sub>S ([M+H]<sup>+</sup>): calcd 559.0522, found 559.0522.

**3-{5-Methyl-2-[4-(4-nitrobenzoylamino)phenylamino]pyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8l)**

Off white solid; 44% yield; mp 163.2-164.2 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.13 (s, 1H), 10.48 (s, 1H), 9.20 (s, 1H), 8.58 (d, *J* = 5.6 Hz, 1H), 8.38 (d, *J* = 8.8 Hz, 2H), 8.25 – 8.14 (m, 3H), 8.01 – 7.98 (m, 1H), 7.84 – 7.64 (m, 5H), 2.79 (d, *J* = 4.6 Hz, 3H), 2.13 (s, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.4, 163.8, 158.9, 157.5, 156.1, 149.5, 144.7, 141.3, 138.0, 132.5, 129.6, 128.5, 124.0, 123.7, 121.5, 119.4, 111.2, 106.4, 26.5, 13.3; ESI-HRMS C<sub>24</sub>H<sub>21</sub>N<sub>7</sub>O<sub>4</sub>S ([M+H]<sup>+</sup>): calcd 504.1448, found 504.1445.

**3-{5-Methyl-2-[4-(4-methylbenzoylamino)phenylamino]pyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8m)**

Off white solid; 64% yield; mp > 250 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.12 (s, 1H), 10.06 (s, 1H), 9.15 (s, 1H), 8.57 (d, *J* = 5.6 Hz, 1H), 8.18 (d, *J* = 4.4 Hz, 1H), 8.00 (d, *J* = 1.2 Hz, 1H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.73 (d, *J* = 5.6 Hz, 1H), 7.66 (s, 4H), 7.34 (d, *J* = 7.6 Hz, 2H), 2.79 (d, *J* = 4.4 Hz, 3H), 2.39 (s, 3H), 2.12 (s, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.4, 165.4, 158.9, 157.5, 156.2, 144.7, 141.8, 137.4, 133.2, 132.7, 129.3, 128.5, 128.1, 123.7, 121.4, 119.5, 111.2, 106.2, 26.5, 21.5, 13.3; ESI-HRMS C<sub>25</sub>H<sub>24</sub>N<sub>6</sub>O<sub>2</sub>S ([M+H]<sup>+</sup>): calcd 473.1754, found 473.1754.

**3-{2-[4-(4-Methoxybenzoylamino)phenylamino]-5-methylpyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8n)**

Yellow solid; 59% yield; mp > 250 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.12 (s, 1H), 9.99 (s, 1H), 9.14 (s, 1H), 8.57 (d, *J* = 5.6 Hz, 1H), 8.18 (d, *J* = 4.4 Hz, 1H), 8.02 – 7.94 (m, 3H), 7.73 (d, *J* = 5.4 Hz, 1H), 7.66 (s, 4H), 7.06 (d, *J* = 8.8 Hz, 2H), 3.85 (s, 3H), 2.79 (d, *J* = 4.8 Hz, 3H), 2.12 (s, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.4, 164.9, 162.2, 158.9, 157.5, 156.2, 144.7, 137.3, 133.3, 129.9, 128.5, 127.6, 123.7, 121.4, 119.5, 114.02, 111.2, 106.2, 55.9, 26.5, 13.2; ESI-HRMS C<sub>25</sub>H<sub>24</sub>N<sub>6</sub>O<sub>3</sub>S ([M+H]<sup>+</sup>): calcd 489.1703, found 489.1702.

**3-{5-Methyl-2-[4-(3,4,5-trimethoxybenzoylamino)phenylamino]pyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8o)**

Yellow solid; 44% yield; mp 219.5-220.3 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.13 (s, 1H), 10.04 (s, 1H), 9.17 (s, 1H), 8.57 (d, *J* = 5.6 Hz, 1H), 8.18 (q, *J* = 4.4 Hz, 1H), 8.02 – 7.99 (m, 1H), 7.75 – 7.68 (m,

3H), 7.66 – 7.59 (m, 2H), 7.30 (s, 2H), 3.88 (s, 6H), 3.74 (s, 3H), 2.79 (d,  $J = 4.4$  Hz, 3H), 2.13 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  165.4, 164.9, 158.9, 157.5, 156.2, 153.1, 144.7, 140.6, 137.6, 132.9, 130.7, 128.5, 123.7, 121.7, 119.4, 111.2, 106.3, 105.6, 60.6, 56.6, 26.5, 13.3; ESI-HRMS  $\text{C}_{27}\text{H}_{28}\text{N}_6\text{O}_5\text{S}$  ( $[\text{M}+\text{H}]^+$ ): calcd 549.1914, found 549.1915.

**3-(2-{4-[3-(3-Fluorophenyl)acryloylamino]phenylamino}-5-methylpyrimidin-4-ylamino)thiophene-2-carboxylic acid methylamide (8p)**

Yellow solid; 34% yield; mp 219.5-220.3 °C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  11.12 (s, 1H), 10.14 (s, 1H), 9.16 (s, 1H), 8.57 (d,  $J = 5.6$  Hz, 1H), 8.18 (d,  $J = 4.4$  Hz, 1H), 7.99 (d,  $J = 1.2$  Hz, 1H), 7.72 (d,  $J = 5.6$  Hz, 1H), 7.67 (d,  $J = 9.2$  Hz, 2H), 7.62 (s, 1H), 7.60 (d,  $J = 4.4$  Hz, 1H), 7.57 – 7.42 (m, 4H), 7.31 – 7.20 (m, 1H), 6.87 (d,  $J = 15.6$  Hz, 1H), 2.79 (d,  $J = 4.4$  Hz, 3H), 2.21 – 2.03 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  165.4, 164.2, 163.2, 161.7, 158.9, 157.5, 156.2, 144.7, 138.7, 138.0 (d,  $J = 8.0$  Hz), 137.4, 133.2, 131.5 (d,  $J = 8.4$  Hz), 128.5, 124.6, 124.2 (d,  $J = 2.4$  Hz), 123.7, 120.0 (d,  $J = 42.0$  Hz), 116.8 (d,  $J = 21.2$  Hz), 114.5 (d,  $J = 21.6$  Hz), 111.2, 106.3, 26.5, 13.3; ESI-HRMS  $\text{C}_{26}\text{H}_{23}\text{FN}_6\text{O}_2\text{S}$  ( $[\text{M}+\text{Na}]^+$ ): calcd 525.1479, found 525.1473.

**3-(2-{4-[3-(4-Fluorophenyl)acryloylamino]phenylamino}-5-methylpyrimidin-4-ylamino)thiophene-2-carboxylic acid methylamide (8q)**

Yellow solid; 32% yield; mp > 250 °C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  11.12 (s, 1H), 10.10 (s, 1H), 9.15 (s, 1H), 8.57 (d,  $J = 5.6$  Hz, 1H), 8.18 (d,  $J = 4.4$  Hz, 1H), 7.99 (d,  $J = 0.8$  Hz, 1H), 7.73 – 7.65 (m, 5H), 7.63 – 7.54 (m, 3H), 7.34 – 7.25 (m, 2H), 6.78 (d,  $J = 15.6$  Hz, 1H), 2.79 (d,  $J = 4.4$  Hz, 3H), 2.17 – 2.07 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  165.40, 164.49, 163.44, 162.02, 158.89, 157.53, 156.15, 144.70, 138.81, 137.25, 133.29, 132.00, 130.28 (d,  $J = 8.4$  Hz), 128.46, 123.69, 122.88, 119.93 (d,  $J = 37.2$  Hz), 116.47 (d,  $J = 21.6$  Hz), 111.18, 106.25, 26.53, 13.28; ESI-HRMS  $\text{C}_{26}\text{H}_{23}\text{FN}_6\text{O}_2\text{S}$  ( $[\text{M}+\text{Na}]^+$ ): calcd 525.1479, found 525.1476.

A mixture of compound **7** (0.42 mmol) and triethylamine (0.42 mmol) was stirred in DMF (2 mL). Then add the corresponding acyl chloride (0.42 mmol). The mixture was stirred at dry room temperature for 12 h. After reaction, the organic phase was extracted with EtOAc (3×30 mL), washed with saturated aq. NaCl (3×30 mL), dried with  $\text{Na}_2\text{SO}_4$ , filtered and vacuum concentrated to obtain crude compounds. The crude product is purified with MeOH to obtain the products **8r** – **8t**.

**3-{5-Methyl-2-[4-(toluene-4-sulfonylamino)phenylamino]pyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8r)**

Yellow solid; 28% yield; mp > 250 °C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  11.10 (s, 1H), 9.87 (s, 1H), 9.09 (s, 1H), 8.49 (d,  $J = 5.6$  Hz, 1H), 8.17 (d,  $J = 4.4$  Hz, 1H), 7.95 (d,  $J = 0.8$  Hz, 1H), 7.65 – 7.57 (m, 3H), 7.53 (d,  $J = 8.8$  Hz, 2H), 7.37 – 7.30 (m, 2H), 7.02 – 6.92 (m, 2H), 2.78 (d,  $J = 4.4$  Hz, 3H), 2.33 (s, 3H), 2.10 (d,  $J = 0.8$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  165.4, 158.8, 157.5, 156.1, 144.6, 143.4,

138.4, 137.3, 131.1, 130.0, 128.3, 127.2, 123.6, 122.4, 120.0, 111.2, 106.4, 26.5, 21.4, 13.2; ESI-HRMS  $C_{24}H_{24}N_6O_3S_2$  ( $[M+H]^+$ ): calcd 509.1424, found 509.1423.

**3-{2-[4-(4-Fluorobenzenesulfonylamino)phenylamino]-5-methylpyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8s)**

Brown solid; 26% yield; mp > 250 °C;  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  11.11 (s, 1H), 9.95 (s, 1H), 9.12 (s, 1H), 8.50 (d,  $J = 5.6$  Hz, 1H), 8.17 (d,  $J = 4.4$  Hz, 1H), 7.96 (d,  $J = 0.8$  Hz, 1H), 7.81 – 7.73 (m, 2H), 7.65 (d,  $J = 5.6$  Hz, 1H), 7.58 – 7.53 (m, 2H), 7.44 – 7.34 (m, 2H), 6.96 (d,  $J = 8.8$  Hz, 2H), 2.78 (d,  $J = 4.4$  Hz, 3H), 2.17 – 2.03 (m, 3H);  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  165.4, 163.4, 158.7, 157.5, 156.0, 144.6, 138.8, 136.4 (d,  $J = 3.2$  Hz), 130.6, 130.2 (d,  $J = 9.6$  Hz), 128.4, 123.6, 122.9, 119.9, 116.8 (d,  $J = 22.8$  Hz), 111.3, 106.5, 26.5, 13.2. ESI-HRMS  $C_{23}H_{21}N_6O_3S_2$  ( $[M+H]^+$ ): calcd 513.1173, found 513.1168.

**3-{2-[4-(4-Methoxybenzenesulfonylamino)phenylamino]-5-methylpyrimidin-4-ylamino}thiophene-2-carboxylic acid methylamide (8t)**

Yellow solid; 87% yield; mp 249.1-250.2 °C;  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  11.10 (s, 1H), 9.80 (s, 1H), 9.09 (s, 1H), 8.48 (d,  $J = 5.6$  Hz, 1H), 8.17 (q,  $J = 4.4$  Hz, 1H), 7.95 (d,  $J = 1.2$  Hz, 1H), 7.69 – 7.60 (m, 3H), 7.53 (d,  $J = 8.8$  Hz, 2H), 7.05 (d,  $J = 8.8$  Hz, 2H), 6.97 (d,  $J = 9.2$  Hz, 2H), 3.79 (s, 3H), 2.78 (d,  $J = 4.4$  Hz, 3H), 2.10 (d,  $J = 0.8$  Hz, 3H);  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  165.4, 162.7, 158.8, 157.5, 156.1, 144.6, 138.3, 131.7, 131.2, 129.4, 128.3, 123.6, 122.4, 120.0, 114.7, 111.2, 106.4, 56.0, 26.5, 13.2; ESI-HRMS  $C_{24}H_{24}N_6O_4S_2$  ( $[M+H]^+$ ): calcd 525.1373, found 525.1372.

**Cancer Cell Lines Evaluation** A549 (Human non-small cell lung cancer cell line) cells, HepG2 (Human hepatocellular carcinomas cell line) cells and K562 (Human erythroleukemic cancer cell line) cells were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. All cell lines were maintained in RPMI 1640 or DMEM complete medium. The cytotoxicity of the synthesized compounds against cell lines was determined by MTT assay described as the previous reports.

**EGFR<sup>wt</sup> and Src assay** Recombinant EGFR<sup>wt</sup> and Src were purchased from Sino Biology Inc. Antiphosphotyrosine mouse mAb was purchased from PTM Bio. The effects of compounds on the activity of EGFR<sup>wt</sup> and Src were determined by enzyme-linked immunosorbent assays (ELISAs) with recombinant EGFR<sup>wt</sup> and Src according to reported methods.

**Predicted ADMET Properties** The absorption, distribution, metabolism, elimination, toxicity (ADMET) of compounds **8f**, **8o-8q** were calculated in ADMETlab 2.0 (Website: <https://admetmesh.scbdd.com/>).

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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