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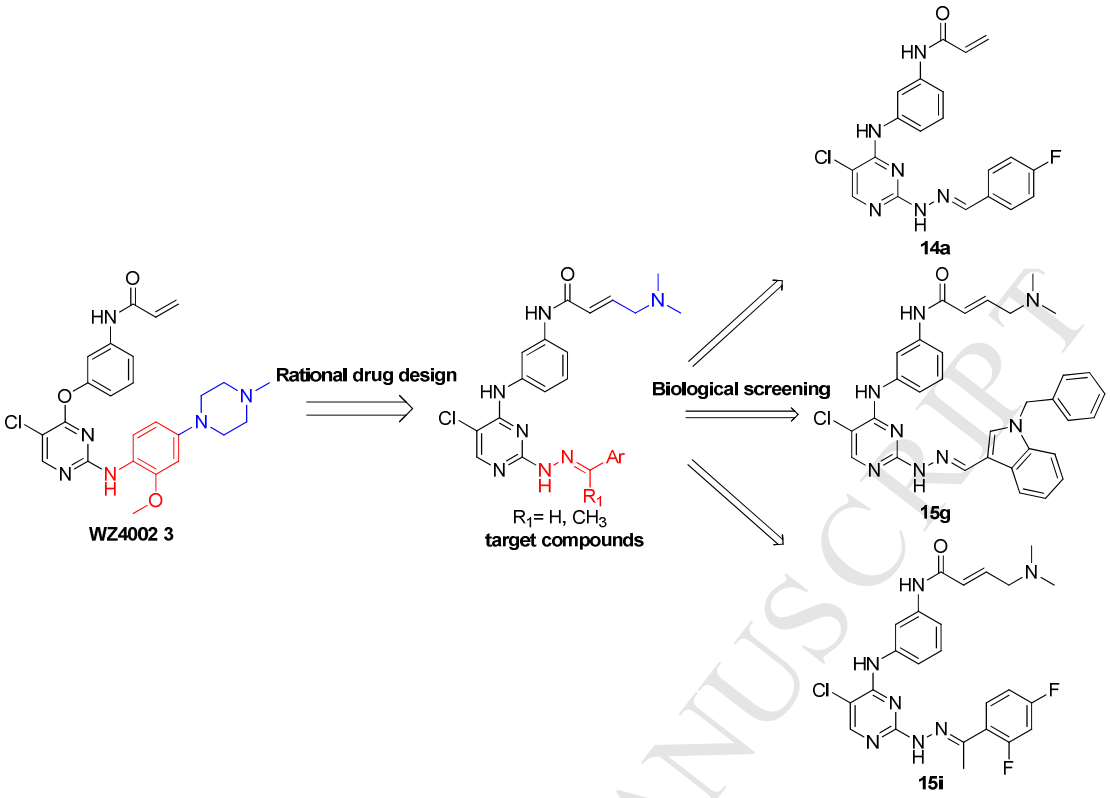

Novel hydrazone moiety-bearing aminopyrimidines as selective inhibitors of epidermal growth factor receptor T790M mutant

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Abstract

The epidermal growth factor receptor (EGFR) T790M mutant is found in approximately 50% of clinically acquired resistance to gefitinib among patients with non-small cell lung cancer (NSCLC). Here, a series of novel aminopyrimidines bearing a hydrazone moiety were identified as potent and selective EGFR inhibitors. Compounds **14a**, **15g**, and **15i** potently inhibited all EGFR mutants including EGFR T790M/L858R, EGFR T790M/delE746_A750, and EGFR T790M while they showed weak effects on the wild type (WT) EGFR. In addition, these compounds effectively suppressed proliferation of gefitinib-resistant H1975 (EGFR T790M/L858R) cells but were less potent against A549 (WT EGFR and k-Ras mutation) and HT-29 (non-special gene type) cells, showing a high safety index. Therefore, **14a**, **15g,** and **15i** might be promising candidates to overcome drug resistance mediated by the EGFR T790M mutant.

Keywords: Aminopyrimidines; Synthesis; Antitumor activity; EGFR T790M mutant

1. Introduction

The epidermal growth factor receptor (EGFR) belongs to the ErbB family of receptor tyrosine kinases and plays a crucial role in cell proliferation, survival, and differentiation *via* activation of downstream signaling pathways [1]. Overexpression or mutation of EGFR is associated with poor prognosis in a number of cancers such as lung and anal cancer [2,3]. Therefore, EGFR has become an attractive target for anticancer therapeutic intervention.

Gefitinib (Fig. 1) and erlotinib are potent reversible inhibitors of EGFR kinase and have yielded significant clinical benefits in patients with non-small cell lung cancer (NSCLC). In particular, they are more effective in patients harboring the EGFR-activating mutations such as delE746_A750 and L858R, which account for 90% of EGFR mutation in NSCLC [4–6]. However, most patients develop drug resistance after 10–14 months of gefitinib and erlotinib therapies. In approximately 50% of all resistant cases, a substitution of threonine 790 with methionine (T790M mutation) at the "gatekeeper" position of EGFR is detected, in combination with an activating mutation [7]. The T790M mutation restores the affinity of EGFR for adenosine triphosphate (ATP), which reduces the binding of ATP-competitive inhibitors to the kinase [8].

In order to overcome the resistance mechanism correlated with T790M mutation, second-generation irreversible inhibitors have been developed including afatinib, neratinib, and dacomitinib [9–11]. These compounds possess the same anilinoquinazoline template as gefitinib but contain an acrylamide functionality that can undergo a Michael addition reaction with Cys797 to achieve greater efficacy [12,13]. Unfortunately, their poor kinase selectivity against EGFR T790M over the wild-type (WT) EGFR leads to serious dose-limiting toxicity [14]. Moreover, these inhibitors have been proven inefficient in gefitinib-resistant H1975 cell models [9,15].

In recent years, numerous irreversible EGFR inhibitors designed on the basis of an aminopyrimidine scaffold have been introduced. WZ4002 (**3**), the representative compound, exhibits a significantly improved kinase selectivity against EGFR T790M, compared to quinazoline-based inhibitors, and shows potent activity in H1975 NSCLC models [16]. A modified compound, CO-1686 has just advanced to phase II clinical trials, and the results are eagerly awaited [17]. All the investigations suggest that the aminopyrimidines may be a potential scaffold for identifying inhibitors that may overcome acquired drug resistance mediated by the EGFR T790M mutant.

(Insert **Fig. 1** here)

The purpose of our study was to identify novel selective inhibitors targeting the EGFR T790M mutant based on the aminopyrimidine pharmacophore. All the irreversible aminopyrimidine compounds reported so far were designed with a modified aryl moiety linked directly to the amino group, and the aniline fragment which pointed to the outside of the active pocket, was correlated with the physicochemical properties of the compounds [16,18,19]. In this study, we reported the design, synthesis, and biological evaluation of a series of novel aminopyrimidine derivatives bearing a hydrazone moiety as selective EGFR inhibitors (Fig. 2), which demonstrated potent and selective inhibition

of EGFR expressing T790M mutations including EGFR T790M/L858R, EGFR T790M/delE746_A750, and EGFR T790M. In addition, these compounds potently suppressed the proliferation of gefitinib-resistant H1975 cells harboring the EGFR T790M/L858R.

(Insert **Fig. 2** here)

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds (**14a–14j** and **15a–15o**) containing a hydrazone moiety is shown in Scheme 1. The intermediate **6** was prepared *via* a regioselective coupling of the protected benzene-1,3-diamine **5** to the 4-position of **4** using a modified condition as previously reported [20]. Substitution of **6** with hydrazine hydrate led to the intermediate **7**, which was reacted with an appropriate aromatic aldehyde or ketone under acid conditions to generate hydrazone **8** [21,22]. The key intermediate **9** was obtained by the deprotection of **8** in the presence of trifluoroacetic acid [20]. Acylation of **9** with acryloyl chloride conveniently produced compounds **14a–14j**. On the other hand, condensation of **9** with commercial (*E*)-4-(dimethylamino)but-2-enoic acid using HATU afforded compounds **15a–15o** with good yields.

(Insert **Scheme 1** here)

The general steps for preparing the hydrazide analogs **16a** and **16b** are outlined in Scheme 2. Intermediate **11** was obtained from intermediate **6** *via* a two-step reaction using similar conditions as described for **9** and **14**. Hydrazinolysis of the ester **12** yielded the corresponding intermediate **13**, which was reacted with **11** *via* an S_N2 mechanism to generate the target compounds.

(Insert **Scheme 2** here)

The chemical structures of the target compounds were confirmed using infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS). Especially, the configuration of the imino double bonds in compounds **14a–14j** and **15a–15o** were determined using nuclear Overhauser spectroscopy (NOESY). Using compound **14b** as an example, a clear NOESY signal was observed between the proton of –NH–N= (δ 13.10, singlet) and the proton of –N=CH– (δ 8.54, singlet), which only existed in the *E*-configuration of the compound (supplementary data). The other related compounds were assigned the same *E*-configuration by analogy.

2.2. Design strategy of target compounds

Taking compound **3** as the lead, we developed a series of novel, irreversible inhibitors, which potently and selectively inhibited EGFR T790M. As part of the design strategy, a modified aryl group was introduced to the aminopyrimidine core with a more

flexible linker. We hypothesized that improved flexibility of the molecular skeleton might be beneficial to the water-solubility of compounds, despite the removal of a hydrophilic group (Fig. 2). Moreover, additional hydrogen bond donors and acceptors were expected to contribute to the interaction with EGFR. Hydrazone and hydrazide moieties have been widely applied in drug design, and as building blocks they could be found in several therapeutic agents, such as PAC-1 and Isoniazid [24,25]. Accordingly, our process started with compounds **14a**, **16a**, and **16b**, which possessed a hydrazone or hydrazide group. They were screened in two NSCLC cell lines harboring different forms of EGFR including the A549 (WT EGFR and k-Ras mutation) and gefitinib-resistant H1975 (EGFR T790M/L858R). Interestingly, the hydrazone analog (**14a**) showed a favorable activity on H1975 cells, with a half-maximal inhibitory concentration (IC_{50}) of 0.43 μM, while the hydrazide compounds (**16a** and **16b**) were less potent. Even more noteworthy was that compound **14a** exhibited much better inhibition of H1975 than A549 cells, indicating good cellular selectivity (Table 1). Therefore, detailed modifications were focused on the hydrazone analogs and a series of novel compounds (**14b–14j**) was prepared by modifying the chemical structure of **14a**. In addition, to further improve the water-solubility of these compounds, a dimethylamino group was introduced, which generated compounds **15a–15o**.

(Insert **Table 1** here)

2.3. Biological evaluation

2.3.1. Inhibitory activity against A549, H1975, and HT-29 cells

The antitumor activities of the target compounds were evaluated in A549 NSCLC (WT EGFR and k-Ras mutation) and gefitinib-resistant H1975 NSCLC (EGFR T790M/L858R) cells. In addition, these compounds were screened in HT-29 colon cancer cells, which expressed a non-special gene type, to test their toxic effects.

(Insert **Table 2.** here)

As shown in Table 2, results of a biological analysis using a standard MTT assay showed that the inhibitory activity of most compounds (**14a–14i**, **15a–15d**, and **15g–15m**) was much more potent (IC_{50}, 0.077–1.58 μM) than that of gefitinib (positive control; IC_{50}, 8.71 μM) in inhibition of H1975 cells. However, they were less potent than WZ4002 (positive control; IC_{50}, 0.058 μM). In addition, the target compounds suppressed H1975 cells more effectively than they suppressed A549 cells, which suggests that the observed potent efficacy might be attributable to selective inhibition of the mutant form of EGFR. Further, no significant toxicity was shown against HT-29 cells by all the compounds at concentrations ranging from 2.12–25.19 μM, except for compound **15m**, which demonstrates that the target compounds might possess a high safety index.

Regarding compounds **14a**, **14c**, **14e**, and **14f** the introduction of a methyl group in the hydrazone moiety (R_1) induced a weak variation in inhibition of H1975 cells, which indicates that the structure-activity relationship (SAR) in this region was limited. In contrast, the substitution pattern on the terminal aryl moiety was closely related to

potency. Compounds **14e** and **14f**, which contained an electron-withdrawing group on the benzene ring, strongly inhibited the H1975 cells (IC_{50}, 0.39 and 0.30 μM, respectively). However, decreased activity was observed for compounds **14g–14i** (IC_{50}, 0.74–1.19 μM) bearing an electron-donating group in the same region. Similar results were also observed when **15b** (IC_{50}, 0.59 μM) was compared with **15d** (IC_{50}, 1.58 μM). Shifting the F atom from the para position (**14a** IC_{50}, 0.43 μM) to the ortho position (**14b** IC_{50}, 0.29 μM) slightly improved inhibition of H1975 cells, while a significant enhancement of inhibition of A549 and HT-29 cells was also observed. The replacement of the terminal phenyl group with a pyridinyl group obviously decreased the potency, as shown with compounds **14j**, **15e**, **15n**, and **15o** (IC_{50}, 2.35–6.24 μM). A dramatically adverse result was observed with compound **15m**, which exhibited outstanding potency against H1975 cells with an IC_{50} of 0.077 μM. However, it also showed strong toxicity against HT-29 cells (IC_{50}, 0.12 μM), illustrating an off-target effect, which might limit its further development. Compound **15f** with an indol group showed a sharply reduced efficacy against H1975 cells, with an IC_{50} of 5.45 μM. Interestingly, the inhibitory activity was enhanced by 21-fold when an additional benzyl group was introduced on the *N*-position of the indol fragment (compound **15g** IC_{50}, 0.26 μM). Compound **9a** (IC_{50}, 9.92 μM) exhibited a significant loss of potency against H1975 cells compared to **14a** (IC_{50}, 0.43 μM), which demonstrated that the acrylamide fragment was essential for high activity. With an additional dimethylamino group in the acrylamide moiety, compounds (**15a–15d** and **15g–15m**) also possessed a high level of inhibitory activity, showing an attractive strategy for optimizing the physicochemical property of these compounds, if necessary.

2.3.2. Inhibitory activity against EGFR kinase

To rationalize their appealing cellular inhibition, the optimized compounds (**14a–14c**, **14e–14g**, **15a–15c**, **15f–15i**, **15m**, **and 15n**) were evaluated using EGFR kinases with different status including EGFR T790M/L858R, EGFR T790M/delE746_A750, EGFR T790M, and WT EGFR. The results obtained using a well-established mobility shift assay are summarized in Table 3, with gefitinib and WZ4002 as the positive controls.

(Insert **Table 3** here)

Most compounds (**14a**, **14b**, **14e**, **14f**, **15a–15c**, and **15g–15m**) significantly inhibited EGFR T790M/L858R with IC_{50} values ranging from 0.68–2.52 μM, which were 5.0- to 18.7-fold lower than that of gefitinib (IC_{50}, 12.72 μM). The inhibitory activities of compounds **14a**, **15a**, **15b**, **15g**, and **15i** were particularly more pronounced with IC_{50} values less than 1 μM. As shown, the IC_{50} values of the compounds against EGFR T790M/L858R were higher than that of WZ4002, but an obviously improved selectivity was observed. All the tested compounds showed low-level inhibition of WT EGFR (IC_{50}, 10.65–> 20 μM) and among them, compounds **14a**, **15a**, **15g**, and **15i** were up to 20-fold more selective against EGFR T790M/L858R than they were against WT EGFR. The biological data also illustrated that the compounds potently inhibited EGFR T790M/delE746_A750 and EGFR T790M with IC_{50} values that were lower than gefitinib. Encouragingly, compounds **14a**, **15g**, and **15i** efficiently suppressed all three kinases

expressing the T790M mutation with IC_{50} values of 0.57–1.12, 0.76–0.88, and 0.72–1.25 μM, respectively. In addition, it was noteworthy that compounds **14a**, **15g**, and **15i** possessed distinct substructures in their skeletons, highlighting a potential for further investigation of this series. On the other hand, compounds **14g**, **15f**, and **15n** exhibited disappointing activity against EGFR T790M/L858R (IC_{50}, 4.32–>20 μM), which was consistent with their weak inhibition of H1975 cells. In contrast to its excellent inhibition of H1975 (IC_{50}, 0.077 μM), compound **15m** only exhibited moderate activity against EGFR T790M/L858R (IC_{50}, 2.38 μM), which suggested that other underlying mechanisms might be involved [23].

3. Conclusion

In summary, an interesting series of aminopyrimidine derivatives bearing a hydrazone moiety have been identified as potent and selective inhibitors targeting EGFR harboring T790M mutants. Several of the compounds displayed more potent activity against EGFR mutants (EGFR T790M/L858R, EGFR T790M/delE746_A750, and EGFR T790M) than shown by gefitinib, and weak effects against WT EGFR. Compounds **14a**, **15g**, and **15i** potently inhibited all three mutant forms of EGFR with IC_{50} values in the low micromolar range. These compounds also strongly inhibited gefitinib-resistant H1975 cells expressing EGFR T790M/L858R. Importantly, they showed sharply decreased activity against A549 cells and minimal toxicity against HT-29 cells, which indicated that they might possess a favorable safety index. Our study provided a new strategy for the discovery of selective EGFR inhibitors to overcome clinical drug resistance mediated by T790M mutation. Compounds **14a**, **15g**, and **15i** exhibited the most impressive activity in the biological evaluation and have emerged as promising candidates for further development. Extensive investigations of the pharmacokinetics and *in vivo* activity of **14a**, **15g**, and **15i** are in progress, and the results will be reported in due course.

4. Experimental section

4.1. Chemistry

Reagents and solvents were obtained from commercial sources and used without further purification. The purity of the synthesized compounds was measured by high performance liquid chromatography (HPLC, Agilent, USA). Flash chromatography was performed using silica gel (300–400 mesh). All reactions were monitored by TLC on silica gel plates. Melting points were determined on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland). The ^{1}H (1D and 2D) and ^{13}C NMR were recorded on Bruker ARX-400 or ARX-600 spectrometer (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Mass spectra (MS) were measured in ESI mode on an Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA). Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). The IR spectra were recorded by means of the KBr pellet technique on a Bruker FTS 135 spectrometer.

*4.1.1. Tert-butyl 3-(2,5-dichloropyrimidin-4-ylamino)phenylcarbamate (**6**)*

To a solution of intermediate **5** (19.98g, 0.096 mol) in DMF (200 mL) were added K_2CO_3 (19.90g, 0.144 mol) and compound **4** (17.46g, 0.096 mol). The reaction was stirred at 50 °C for 3 h. After being cool to room temperature, the reaction mixture was poured into water (1000 mL). The precipitate was filtered and dried to give a crude solid. Intermediate **6** was generated as a pale-yellow solid (24.76g, 72%) using column chromatography with petroleum ether and ethyl acetate (20:1 to 9:1, v/v) as the mobile phase. mp 190.8–192.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 1H, NHCO), 9.44 (s, 1H, NH), 8.36 (s, 1H, ArH), 7.70 (s, 1H, ArH), 7.21–7.28 (m, 2H, ArH), 7.17 (dt, *J* = 7.1, 2.0 Hz, 1H, ArH), 1.48 (s, 9H, CH_3). ESI-MS m/z: 355.1 $[M+H]^+$.

*4.1.2. Tert-butyl 3-(5-chloro-2-hydrazinylpyrimidin-4-ylamino)phenylcarbamate (**7**)*

To a solution of **6** (5g, 0.014 mol) in MeCN (100 mL) was added 80% $NH_2NH_2 \cdot H_2O$ (2.5 mL). The mixture was heated to reflux and stirred for 3 h, then being cooled to room temperature. The precipitate was filtered and dried to give **7** as a gray solid (4.2g, 86%). mp 165.0–166.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.30 (s, 1H, –NH–N), 8.55 (s, 1H, NHCO), 8.30 (s, 1H, NH), 7.99 (s, 1H, ArH), 7.92 (s, 1H, ArH), 7.20–7.23 (m, 1H, ArH), 7.14 (d, *J* = 5.8 Hz, 2H, ArH), 4.23 (s, 2H, –NH–N$\underline{H_2}$), 1.48 (s, 9H, CH_3). ESI-MS m/z: 351.0 $[M+H]^+$.

*4.1.3. General procedure for preparation of compounds (**8a–8o**)*

A mixture of **7** (0.8g, 2.3 mmol), appropriate aromatic aldehyde or ketone (2.5mmol) and a catalytic amount of glacial acetic acid was refluxed in *i*-PrOH for 6–8 h when TLC showed the completion of the reaction. After being cooled to room temperature, the precipitate was filtered, washed with Et_2O and dried to afford compounds **8a–8o**, respectively, which were used without further purification.

*4.1.3.1. (E)-Tert-butyl 3-(5-chloro-2-(2-(4-fluorobenzylidene)hydrazinyl)pyrimidin-4-ylamino)phenylcarbamate (**8a**).* A yellow solid. Yield: 71%. mp 188.3–190.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (s, 1H, –NH–N), 9.35 (s, 1H, NHCO), 9.15 (s, 1H, NH), 8.16 (s, 1H, –N=CH), 8.09 (s, 1H, ArH), 7.83 (s, 1H, ArH), 7.75 (dd, *J* = 7.8, 6.0 Hz, 2H, ArH), 7.51 (s, 1H, ArH), 7.22–7.28 (m, 3H, ArH), 7.18 (t, *J* = 8.0 Hz, 1H, ArH), 1.42 (s, 9H, CH_3). ESI-MS m/z: 457.1 $[M+H]^+$.

*4.1.3.2. (E)-Tert-butyl 3-(5-chloro-2-(2-(2-fluorobenzylidene)hydrazinyl)pyrimidin-4-ylamino)phenylcarbamate (**8b**).* A yellow solid. Yield: 43%. mp 190.4–193.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 1H, –NH–N), 9.92 (s, 1H, NHCO), 9.48 (s, 1H, NH), 8.41 (s, 1H, ArH), 8.29 (s, 1H, –N=CH), 8.22 (s, 1H, ArH), 7.77 (s, 1H, ArH), 7.49 (dd, *J* = 14.0, 6.4 Hz, 1H, ArH), 7.26–7.38 (m, 5H, ArH), 1.47 (s, 9H, CH_3). ESI-MS m/z: 457.0 $[M+H]^+$.

*4.1.3.3. (E)-Tert-butyl 3-(5-chloro-2-(2-(2,4-difluorobenzylidene)hydrazinyl)pyrimidin-4-ylamino)phenylcarbamate (**8c**).* A wheat solid. Yield: 52%. mp 211.9–214.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (s, 1H, –NH–N), 10.19 (s, 1H, NHCO), 9.51 (s, 1H, NH), 8.33–8.37 (m, 3H, ArH, –N=CH), 7.75 (s, 1H, ArH), 7.34–7.40 (m, 1H, ArH), 7.32 (d, *J* = 4.9 Hz, 2H, ArH), 7.26 (td, *J* = 8.6, 2.1 Hz, 2H, ArH), 1.47 (s, 9H, CH_3). ESI-MS m/z:

475.1 $[M+H]^+$.

*4.1.3.4. (E)-Tert-butyl 3-(5-chloro-2-(2-(4-methoxybenzylidene)hydrazinyl)pyrimidin-4-yl amino)phenylcarbamate (**8d**).* A pale-yellow solid. Yield: 60%. mp 166.6–169.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H, –NH–N), 9.48 (s, 1H, NHCO), 8.27 (s, 1H, NH), 8.13 (s, 1H, –N=CH), 7.78–7.81 (m, 3H, ArH), 7.69 (s, 1H, ArH), 7.30 (d, *J* = 4.5 Hz, 2H, ArH), 7.02 (m, 3H, ArH), 3.80 (s, 3H, OCH_3), 1.46 (s, 9H, CH_3). ESI-MS m/z: 469.1 $[M+H]^+$.

*4.1.3.5. (E)-Tert-butyl 3-(5-chloro-2-(2-(pyridin-4-ylmethylene)hydrazinyl)pyrimidin-4-yl amino)phenylcarbamate (**8e**).* A pale-yellow solid. Yield: 67%. mp 211.9–213.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.54 (s, 1H, –NH–N), 9.37 (s, 1H, NHCO), 9.02 (s, 1H, NH), 8.62 (d, *J* = 6.0 Hz, 2H, ArH), 8.20 (s, 1H, –N=CH), 8.07 (s, 1H, ArH), 7.90 (s, 1H, ArH), 7.65 (d, *J* = 6.1 Hz, 2H, ArH), 7.59 (d, *J* = 7.3 Hz, 1H, ArH), 7.27 (t, *J* = 8.1 Hz, 1H, ArH), 7.17 (d, *J* = 7.9 Hz, 1H, ArH), 1.42 (s, 9H, CH_3). ESI-MS m/z: 440.0 $[M+H]^+$.

*4.1.3.6. (E)-Tert-butyl 3-(2-(2-((1H-indol-3-yl)methylene)hydrazinyl)-5-chloropyrimidin-4 -ylamino)phenylcarbamate (**8f**).* A yellow solid. Yield: 45%. ESI-MS m/z: 478.1 $[M+H]^+$.

*4.1.3.7. (E)-Tert-butyl 3-(2-(2-((1-benzyl-1H-indol-3-yl)methylene)hydrazinyl)-5-chloropy rimidin-4-ylamino)phenylcarbamate (**8g**).* A yellow solid. Yield: 62%. ESI-MS m/z: 568.2 $[M+H]^+$.

*4.1.3.8. (E)-Tert-butyl 3-(5-chloro-2-(2-(1-(4-fluorophenyl)ethylidene)hydrazinyl)pyrimidi n-4-ylamino)phenylcarbamate (**8h**).* A pale-yellow solid. Yield: 72%. mp 172.6–174.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.53 (s, 1H, –NH–N), 10.13 (s, 1H, NHCO), 9.54 (s, 1H, NH), 8.29 (s, 1H, ArH), 8.09 (dd, *J* = 7.8, 5.9 Hz, 2H, ArH), 7.84 (s, 1H, ArH), 7.26–7.33 (m, 5H, ArH), 2.40 (s, 3H, CH_3), 1.47 (s, 9H, CH_3). ESI-MS m/z: 471.1 $[M+H]^+$.

*4.1.3.9. (E)-Tert-butyl 3-(5-chloro-2-(2-(1-(2,4-difluorophenyl)ethylidene)hydrazinyl)pyri midin-4-ylamino)phenylcarbamate (**8i**).* A pale-yellow solid. Yield: 69%. mp 163.4–166.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.62 (s, 1H, –NH–N), 10.10 (s, 1H, NHCO), 9.56 (s, 1H, NH), 8.26 (s, 1H, ArH), 7.97 (dd, *J* = 15.9, 8.7 Hz, 1H, ArH), 7.88 (s, 1H, ArH), 7.28–7.39 (m, 4H, ArH), 7.20 (td, *J* = 8.5, 2.4 Hz, 1H, ArH), 2.39 (s, 3H, CH_3), 1.47 (s, 9H, CH_3). ESI-MS m/z: 489.1 $[M+H]^+$.

*4.1.3.10. (E)-Tert-butyl 3-(5-chloro-2-(2-(1-p-tolylethylidene)hydrazinyl)pyrimidin-4-yla mino)phenylcarbamate (**8j**).* A pale-yellow solid. Yield: 53%. mp 187.9–189.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.44 (s, 1H, –NH–N), 10.15 (s, 1H, NHCO), 9.51 (s, 1H, NH), 8.26 (s, 1H, ArH), 7.91 (d, *J* = 8.0 Hz, 2H, ArH), 7.78 (s, 1H, ArH), 7.32–7.34 (m, 3H, ArH), 7.27 (d, *J* = 8.2 Hz, 2H, ArH), 2.36 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 1.48 (s, 9H, CH_3). ESI-MS m/z: 467.2 $[M+H]^+$.

*4.1.3.11. (E)-Tert-butyl 3-(5-chloro-2-(2-(1-(4-methoxyphenyl)ethylidene)hydrazinyl)pyri midin-4-ylamino)phenylcarbamate (**8k**).* A pale-yellow solid. Yield: 61%. mp

179.2–182.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H, –NH–N), 9.36 (s, 1H, NHCO), 9.10 (s, 1H, NH), 8.17 (s, 1H, ArH), 7.89 (s, 1H, ArH), 7.83 (d, *J* = 8.2 Hz, 2H, ArH), 7.72 (s, 1H, ArH), 7.25 (t, *J* = 8.1 Hz, 1H, ArH), 7.16 (d, *J* = 7.9 Hz, 1H, ArH), 6.97 (d, *J* = 8.8 Hz, 2H, ArH), 3.81 (s, 3H, OCH_3), 2.27(s, 3H, CH_3), 1.45 (s, 9H, CH_3). ESI-MS m/z: 483.3 $[M+H]^+$.

*4.1.3.12. (E)-Tert-butyl 3-(5-chloro-2-(2-(1-(3-methoxyphenyl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenylcarbamate (**8l**).* A pale-yellow solid. Yield: 58%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.56 (s, 1H, –NH–N), 10.16 (s, 1H, NHCO), 9.55 (s, 1H, NH), 8.27 (s, 1H, ArH), 7.84 (s, 1H, ArH), 7.54–7.57 (m, 2H, ArH), 7.30–7.39 (m, 4H, ArH), 7.04 (dd, *J* = 8.2, 2.1 Hz, 1H, ArH), 3.83 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3), 1.48 (s, 9H, CH_3). ESI-MS m/z: 483.2 $[M+H]^+$.

*4.1.3.13. (E)-Tert-butyl 3-(5-chloro-2-(2-(1-(pyridin-2-yl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenylcarbamate (**8m**).* A yellow solid. Yield: 60%. mp 195.9–196.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H, –NH–N), 9.54 (s, 1H, NHCO), 9.47 (s, 1H, NH), 8.59 (d, *J* = 4.4 Hz, 1H, ArH), 8.36 (d, *J* = 7.7 Hz, 1H, ArH), 8.27 (s, 1H, ArH), 7.94 (s, 1H, ArH), 7.85–7.90 (m, 1H, ArH), 7.55 (d, *J* = 5.6 Hz, 1H, ArH), 7.41 (dd, *J* = 6.6, 5.5 Hz, 1H, ArH), 7.24–7.30 (m, 2H, ArH), 2.43 (s, 3H, CH_3), 1.45 (s, 9H, CH_3). ESI-MS m/z: 454.1 $[M+H]^+$.

*4.1.3.14. (E)-Tert-butyl 3-(5-chloro-2-(2-(1-(pyridin-3-yl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenylcarbamate (**8n**).* A yellow solid. Yield: 77%. mp 166.6–169.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H, –NH–N), 10.06 (s, 1H, NHCO), 9.55 (s, 1H, NH), 9.35 (s, 1H, ArH), 8.72–8.75 (m, 1H, ArH), 8.67 (d, *J* = 7.9 Hz, 1H, ArH), 8.32 (s, 1H, ArH), 7.89 (s, 1H, ArH), 7.71 (dd, *J* = 7.4, 5.5 Hz, 1H, ArH), 7.40 (s, 1H, ArH), 7.33 (d, *J* = 6.4 Hz, 2H), 2.46 (s, 3H, CH_3), 1.47 (s, 9H, CH_3). ESI-MS m/z: 454.1 $[M+H]^+$.

*4.1.3.15. (E)-Tert-butyl 3-(5-chloro-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenylcarbamate (**8o**).* An orange solid. Yield: 52%. mp 174.8–176.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H, –NH–N), 9.45 (s, 1H, NHCO), 9.27 (s, 1H, NH), 8.74 (d, *J* = 6.4 Hz, 2H, ArH), 8.27 (s, 1H, ArH), 8.06 (d, *J* = 6.3 Hz, 2H, ArH), 7.96 (s, 1H, ArH), 7.58 (d, *J* = 7.7 Hz, 1H, ArH), 7.29 (t, *J* = 8.1 Hz, 1H, ArH), 7.20 (d, *J* = 8.2 Hz, 1H, ArH), 2.35 (s, 3H, CH_3), 1.41 (s, 9H, CH_3). ESI-MS m/z: 454.2 $[M+H]^+$.

*4.1.4. General procedure for preparation of compounds (**9a–9o**)*

TFA (20 mmol) was added to a solution of **8** (2 mmol) in CH_2Cl_2 (50 mL) at room temperature. The reaction mixture was stirred for 2–7 h, and was monitored by TLC. The mixture was extracted with water (2 × 100 mL), the combined aqueous layer was neutralized with aqueous K_2CO_3 to pH 7–8. The precipitate was filtered and dried to generate the compounds **9a–9o**, respectively.

*4.1.4.1. (E)-N^1-(5-Chloro-2-(2-(4-fluorobenzylidene)hydrazinyl)pyrimidin-4-yl)benzene-1,3-diamine (**9a**).* A white solid. Yield: 66%. mp 230.1–233.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (s, 1H,–NH–N), 8.45 (s, 1H, NH), 8.08 (s, 1H, –N=CH), 8.07 (s, 1H, ArH), 7.71 (dd, *J* = 8.7, 5.7 Hz, 2H, ArH), 7.17–7.25 (m, 3H, ArH), 6.99 (t, *J* = 8.0 Hz,

2H, ArH), 6.34 (dd, *J* = 7.8, 1.4 Hz, 1H, ArH), 4.97 (s, 2H, NH_2). ESI-MS m/z: 356.9 $[M+H]^+$.

*4.1.4.2. (E)-N^1-(5-Chloro-2-(2-(2-fluorobenzylidene)hydrazinyl)pyrimidin-4-yl)benzene-1, 3-diamine (**9b**).* A white solid. Yield: 46%. mp 236.6.9–239.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 1H, –NH–N), 8.55 (s, 1H, NH), 8.31 (s, 1H, –N=CH), 8.13 (d, *J* = 1.9 Hz, 1H, ArH), 7.99 (t, *J* = 7.6 Hz, 1H, ArH), 7.40 (dd, *J* = 13.7, 6.4 Hz, 1H, ArH), 7.19–7.28 (m, 3H, ArH), 7.01 (td, *J* = 8.0, 2.1 Hz, 2H, ArH), 6.37 (d, *J* = 7.8 Hz, 1H, ArH), 5.09 (s, 2H). ESI-MS m/z: 356.9 $[M+H]^+$.

*4.1.4.3. (E)-N^1-(5-Chloro-2-(2-(2,4-difluorobenzylidene)hydrazinyl)pyrimidin-4-yl)benzene-1,3-diamine (**9c**).* A gray solid. Yield: 65%. mp 243.6–245.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.19 (s, 1H, –NH–N), 8.53 (s, 1H, NH), 8.24 (s, 1H, –N=CH), 8.11 (s, 1H, ArH), 8.01 (dd, *J* = 15.7, 8.4 Hz, 1H, ArH), 7.29–7.34 (m, 1H, ArH), 7.17 (td, *J* = 8.8, 2.1 Hz, 2H, ArH), 7.00 (dd, *J* = 10.7, 5.1 Hz, 2H, ArH), 6.36 (dd, *J* = 7.9, 1.3 Hz, 1H, ArH), 5.02 (s, 2H, NH_2). ESI-MS m/z: 375.0 $[M+H]^+$.

*4.1.4.4. (E)-N^1-(5-Chloro-2-(2-(4-methoxybenzylidene)hydrazinyl)pyrimidin-4-yl)benzene-1,3-diamine (**9d**).* A white solid. Yield: 51%. mp 229.4–232.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H, –NH–N), 8.42 (s, 1H, NH), 8.06 (s, 1H, –N=CH), 8.03 (s, 1H, ArH), 7.79 (d, *J* = 8.8 Hz, 1H, ArH), 7.60 (d, *J* = 8.7 Hz, 2H, ArH), 7.21 (s, 1H, ArH), 7.04 (d, *J* = 8.8 Hz, 1H), 6.98 (d, *J* = 6.3 Hz, 2H), 6.34 (dd, *J* = 7.9, 1.3 Hz, 1H, ArH), 5.00 (s, 2H, NH_2), 3.78 (s, 3H, OCH_3). ESI-MS m/z: 369.0 $[M+H]^+$.

*4.1.4.5. (E)-N^1-(5-Chloro-2-(2-(pyridin-4-ylmethylene)hydrazinyl)pyrimidin-4-yl)benzene-1,3-diamine (**9e**).* A white solid. Yield: 76%. mp 235.7–237.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H, –NH–N), 8.59 (s, 1H, NH), 8.58 (s, 2H, ArH), 8.15 (s, 1H, –N=CH), 8.06 (s, 1H, ArH), 7.61 (d, *J* = 5.9 Hz, 2H, ArH), 7.17 (d, *J* = 6.2 Hz, 1H, ArH), 7.00–7.05 (m, 2H, ArH), 6.39 (dd, *J* = 7.9, 1.2 Hz, 1H, ArH), 5.16 (s, 2H, NH_2). ESI-MS m/z: 340.0 $[M+H]^+$.

*4.1.4.6. (E)-N^1-(2-(2-((1H-Indol-3-yl)methylene)hydrazinyl)-5-chloropyrimidin-4-yl)benzene-1,3-diamine (**9f**).* A white solid. Yield: 39%. mp 186.5–187.1 °C.ESI-MS m/z: 378.1 $[M+H]^+$.

*4.1.4.7. (E)-N^1-(2-(2-((1-Benzyl-1H-indol-3-yl)methylene)hydrazinyl)-5-chloropyrimidin-4-yl)benzene-1,3-diamine (**9g**).* A yellow solid. Yield: 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H, –NH–N), 8.77 (s, 1H, NH), 8.35 (d, *J* = 9.2 Hz, 2H, ArH), 8.10 (s, 1H, –N=CH), 7.89 (s, 1H, ArH), 7.50 (d, *J* = 8.3 Hz, 2H, ArH), 7.13–7.34 (m, 9H, ArH), 7.06 (t, *J* = 7.9 Hz, 1H, ArH), 6.84 (s, 1H, ArH), 6.43 (dd, *J* = 7.3, 0.6 Hz, 1H, ArH), 5.44 (s, 2H, NH_2). ESI-MS m/z: 468.1 $[M+H]^+$.

*4.1.4.8. (E)-N^1-(5-Chloro-2-(2-(1-(4-fluorophenyl)ethylidene)hydrazinyl)pyrimidin-4-yl)benzene-1,3-diamine (**9h**).* A gray solid. Yield: 71%. mp 177.8–178.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H, –NH–N), 8.50 (s, 1H, NH), 8.13 (s, 1H, ArH), 7.92 (dd, *J* = 8.3, 5.8 Hz, 2H, ArH), 7.31 (d, *J* = 7.6 Hz, 1H, ArH), 7.24 (t, *J* = 8.8 Hz, 2H, ArH),

6.99–7.05 (dd, *J* = 14.6, 6.4 Hz, 2H, ArH), 6.37 (d, *J* = 7.6 Hz, 1H, ArH), 5.18 (s, 2H, NH_2), 2.28 (s, 3H, CH_3). ESI-MS m/z: 370.9 $[M+H]^+$.

*4.1.4.9. (E)-N^1-(5-Chloro-2-(2-(1-(2,4-difluorophenyl)ethylidene)hydrazinyl)pyrimidin-4-yl)benzene-1,3-diamine (**9i**).* A gray solid. Yield: 66%. mp 148.4–150.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H, –NH–N), 8.47 (s, 1H, NH), 8.13 (s, 1H, ArH), 7.80 (dd, *J* = 15.7, 8.5 Hz, 1H, ArH), 7.25–7.30 (m, 1H, ArH), 7.22 (d, *J* = 7.7 Hz, 1H, ArH), 7.12–7.18 (m, 1H, ArH), 7.07 (s, 1H, ArH), 6.96 (t, *J* = 7.9 Hz, 1H, ArH), 6.33 (d, *J* = 7.5 Hz, 1H, ArH), 5.01 (s, 2H, NH_2), 2.29 (s, 3H, CH_3). ESI-MS m/z: 389.0 $[M+H]^+$.

*4.1.4.10. (E)-N^1-(5-Chloro-2-(2-(1-p-tolylethylidene)hydrazinyl)pyrimidin-4-yl)benzene-1,3-diamine (**9j**).* A white solid. Yield: 73%. mp 171.1–172.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H, –NH–N), 8.95 (s, 1H, NH), 8.16 (s, 1H, ArH), 7.82 (d, *J* = 8.2 Hz, 2H, ArH), 7.32 (s, 1H, ArH), 7.24 (d, *J* = 8.1 Hz, 2H, ArH), 7.06 (t, *J* = 8.0 Hz, 1H, ArH), 6.99 (s, 1H, ArH), 6.44 (dd, *J* = 7.9, 1.3 Hz, 1H, ArH), 2.34 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), (The active hydrogen of NH_2 was disappeared). ESI-MS m/z: 367.2 $[M+H]^+$.

*4.1.4.11. (E)-N^1-(5-Chloro-2-(2-(1-(4-methoxyphenyl)ethylidene)hydrazinyl)pyrimidin-4-yl)benzene-1,3-diamine (**9k**).* A gray solid. Yield: 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (s, 1H, –NH–N), 9.13 (s, 1H, NH), 8.17 (s, 1H, ArH), 7.87–7.90 (m, 3H, ArH), 7.07 (d, *J* = 8.0 Hz, 1H, ArH), 7.02–7.05 (m, 1H, ArH), 6.98 (d, *J* = 9.0 Hz, 2H, ArH), 6.45 (d, *J* = 7.5 Hz, 1H, ArH), 3.81 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3), (The active hydrogen of NH_2 was disappeared).

*4.1.4.12. (E)-N^1-(5-Chloro-2-(2-(1-(3-methoxyphenyl)ethylidene)hydrazinyl)pyrimidin-4-yl)benzene-1,3-diamine (**9l**).* A gray solid. Yield: 59%. mp 205.9–208.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H, –NH–N), 8.41 (s, 1H, NH), 8.13 (s, 1H, ArH), 7.46 (dd, *J* = 14.3, 7.6 Hz, 2H, ArH), 7.34 (dd, *J* = 14.8, 6.6 Hz, 2H, ArH), 6.99–7.02 (m, 2H, ArH), 6.94 (d, *J* = 7.3 Hz, 1H, ArH), 6.33 (d, *J* = 7.9 Hz, 1H, ArH), 4.95 (s, 2H, NH_2), 3.79 (s, 3H, OCH_3), 2.28 (s, 3H, CH_3). ESI-MS m/z: 383.0 $[M+H]^+$.

*4.1.4.13. (E)-N^1-(5-Chloro-2-(2-(1-(pyridin-2-yl)ethylidene)hydrazinyl)pyrimidin-4-yl)benzene-1,3-diamine (**9m**).* A gray solid. Yield: 70%. mp 104.5–107.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H, –NH–N), 8.55 (d, *J* = 4.2 Hz, 1H, ArH), 8.53 (s, 1H, NH), 8.22 (d, *J* = 8.1 Hz, 1H, ArH), 8.17 (s, 1H, ArH), 7.83 (td, *J* = 8.0, 1.7 Hz, 1H, ArH), 7.32–7.35 (m, 2H, ArH), 7.04–7.05 (m, 1H, ArH), 7.01 (d, *J* = 8.0 Hz, 1H, ArH), 6.37 (dd, *J* = 7.9, 1.3 Hz, 1H, ArH), 5.02 (s, 2H, NH_2), 2.37 (s, 3H, CH_3). ESI-MS m/z: 353.9 $[M+H]^+$.

*4.1.4.14. (E)-N^1-(5-Chloro-2-(2-(1-(pyridin-3-yl)ethylidene)hydrazinyl)pyrimidin-4-yl)benzene-1,3-diamine (**9n**).* A white solid. Yield: 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (s, 1H, –NH–N), 9.06 (d, *J* = 1.8 Hz, 1H, ArH), 8.54 (dd, *J* = 4.7, 1.3 Hz, 1H, ArH), 8.50 (s, 1H, NH), 8.21–8.23 (m, 1H, ArH), 8.15 (s, 1H, ArH), 7.42–7.45 (m, 2H, ArH), 7.00–7.04 (m, 2H, ArH), 6.35 (dd, *J* = 7.9, 1.2 Hz, 1H, ArH), 5.01 (s, 2H, NH_2), 2.32 (s, 3H, CH_3). ESI-MS m/z: 353.9 $[M+H]^+$.

4.1.4.15. (E)-N^1-(5-Chloro-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)pyrimidin-4-yl)benzene-1,3-diamine (**9o**). A gray solid. Yield: 75%. mp 209.1–210.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H, –NH–N), 8.72 (s, 1H, NH), 8.63 (d, *J* = 5.7 Hz, 2H, ArH), 8.20 (s, 1H, ArH), 7.86 (d, *J* = 5.9 Hz, 2H, ArH), 7.33 (d, *J* = 7.9 Hz, 1H, ArH), 7.06 (dd, *J* = 10.5, 5.1 Hz, 2H, ArH), 6.45 (d, *J* = 7.6 Hz, 1H, ArH), 2.31 (s, 3H, CH_3), (The active hydrogen of NH_2 was disappeared). ESI-MS m/z: 353.9 $[M+H]^+$.

*4.1.5. N^1-(2,5-Dichloropyrimidin-4-yl)benzene-1,3-diamine (**10**)*

TFA (15.96g, 0.14 mmol) was added to a solution of **6** (4.96g, 14 mmol) in CH_2Cl_2 (200 mL). The reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated under vacuum, which was then dissolved in water. The solution was alkalized with aqueous K_2CO_3, the precipitate was filtered and dried to give compound **10** as a pale-yellow solid (2.67g, 75%). ESI-MS m/z: 354.9 $[M+H]^+$.

*4.1.6. N-(3-(2,5-Dichloropyrimidin-4-ylamino)phenyl)acrylamide (**11**)*

To a solution of **10** (2g, 7.9 mmol) and DIPEA (1.38 mL, 7.9 mmol) in CH_2Cl_2 (50 mL) at 0 °C, acryloyl chloride (0.64 mL, 7.9 mmol) was added dropwise. The mixture was stirred for 1 h, then extracted with water (2 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4, and evaporated to give the crude residue. Intermediate **11** was obtained as a pale-yellow solid (1.9g, 78%) after purification by silica gel column chromatography (petroleum ether:ethyl acetate = 2:1, v/v). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H, NHCO), 9.59 (s, 1H, NH), 8.39 (s, 1H, ArH), 7.91 (br, 1H, ArH), 7.47 (d, *J* = 8.0 Hz, 1H, ArH), 7.34 (t, *J* = 8.0 Hz, 1H, ArH), 7.28 (dd, *J* = 8.3, 1.4 Hz, 1H, ArH), 6.47 (dd, *J* = 17.0, 10.1 Hz, 1H, –C<u>H</u>=CH_2), 6.27 (dd, *J* = 17.0, 2.0 Hz, 1H, –CH=C<u>H</u>$_2$), 5.77 (dd, *J* = 10.1, 2.0 Hz, 1H, –CH=C<u>H</u>$_2$). ESI-MS m/z: 308.8 $[M+H]^+$.

*4.1.7. General procedure for preparation of compounds (**13a–13b**)*

Compound **12** (0.013 mol) and 80% $NH_2NH_2 \cdot H_2O$ (5 mL) were added to EtOH (10 mL), the mixture was stirred under reflux for 5 h. After being cooled to room temperature, the precipitate was obtained by filtration, and was dried to give the title compounds, respectively.

*4.1.7.1. 4-Fluorobenzohydrazide (**13a**).* A white solid. Yield: 75%. mp 161.9–164.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78 (s, 1H, –NH–N), 7.83–7.93 (m, 2H, ArH), 7.22–7.33 (m, 2H, ArH), 4.48 (s, 2H, –NH–N<u>H</u>$_2$). ESI-MS m/z: 155.1 $[M+H]^+$.

*4.1.7.2. 4-Methoxybenzohydrazide (**13b**).* A white solid. Yield: 69%. mp 137.9–139.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.60 (s, 1H, –NH–N), 7.75–7.86 (m, 2H, ArH), 6.92–7.03 (m, 2H, ArH), 4.42 (s, 2H, –NH–N<u>H</u>$_2$), 3.80 (s, 3H, OCH_3). ESI-MS m/z: 167.2 $[M+H]^+$.

*4.1.8. General procedure for preparation of compounds (**14a–14j**)*

Acryloyl chloride (1 mmol) was added dropwise to a solution of intermediate **9** (1 mmol) and DIPEA (1 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction was stirred for 1 h. The target compounds **14a–14j** were afforded after purification by silica gel column chromatography.

*4.1.8.1. (E)-N-(3-(5-Chloro-2-(2-(4-fluorobenzylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)acrylamide (**14a**).* Flash column chromatography was performed using (petroleum ether:ethyl acetate, 5:1 to 2:1 v/v). HPLC purity: 98.2%. A white solid. Yield: 32%. mp 225.8–227.2 °C. IR (KBr, cm^{-1}): 3413.7 (NH), 3264.8 (RC=CH), 1657.9 (CO). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H, –NH–N), 10.34 (s, 1H, NHCO), 9.06 (s, 1H, NH), 8.17 (s, 1H, –N=CH), 8.13 (br, 2H, ArH), 7.72 (dd, *J* = 8.6, 5.7 Hz, 2H, ArH), 7.58 (d, *J* = 53.2 Hz, 2H, ArH), 7.28 (dt, *J* = 17.7, 8.5 Hz, 3H, ArH), 6.53 (dd, *J* = 16.8, 10.1 Hz, 1H, –C<u>H</u>=CH_2), 6.26 (dd, *J* = 17.0, 1.9 Hz, 1H, –CH=C<u>H_2</u>), 5.74 (dd, *J* = 10.3, 1.6 Hz, 1H, –CH=C<u>H_2</u>). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.18, 163.64, 161.74, 156.54, 139.56, 139.41, 132.54, 132.20, 128.97 (2C), 128.88, 128.80, 127.09, 118.27, 116.28 (2C), 116.06, 115.69, 114.44, 105.24. ESI-MS m/z: 411.2 $[M+H]^+$. Anal. calcd. for $C_{20}H_{16}ClFN_6O$ (%): C, 58.47; H, 3.93; N, 20.46; found (%): C, 58.52; H, 3.88; N, 20.37.

*4.1.8.2. (E)-N-(3-(5-Chloro-2-(2-(2-fluorobenzylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)acrylamide (**14b**).* Flash column chromatography was performed using (petroleum ether:ethyl acetate, 10:1 to 4:1 v/v). HPLC purity: 98.6%. A white solid. Yield: 43%. mp 222.7–223.8 °C. IR (KBr, cm^{-1}): 3392.9 (NH), 3217.4 (RC=CH), 1667.2 (CO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.10 (s, 1H, –NH–N), 10.67 (s, 1H, NHCO), 10.27 (s, 1H, NH), 8.54 (s, 1H, –N=CH), 8.32 (d, *J* = 55.7 Hz, 3H, ArH), 7.75 (d, *J* = 5.9 Hz, 1H, ArH), 7.52 (dd, *J* = 13.3, 5.7 Hz, 1H, ArH), 7.39 (t, *J* = 7.9 Hz, 1H, ArH), 7.32 (dd, *J* = 16.3, 8.2 Hz, 3H, ArH), 6.63 (dd, *J* = 16.9, 10.2 Hz, 1H, –C<u>H</u>=CH_2), 6.28 (d, *J* = 16.9 Hz, 1H, –CH=C<u>H_2</u>), 5.76 (d, *J* = 10.3 Hz, 1H, –CH=C<u>H_2</u>). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.80, 162.47, 159.98, 157.93, 140.01, 137.70, 132.93, 132.40, 129.38, 127.38, 127.32, 125.26, 125.23, 121.67, 119.78, 117.41, 116.54, 116.33, 115.75, 106.51. ESI-MS m/z: 411.1 $[M+H]^+$. Anal. calcd. for $C_{20}H_{16}ClFN_6O$ (%): C, 58.47; H, 3.93; N, 20.46; found (%): C, 58.42; H, 3.97; N, 20.37.

*4.1.8.3. (E)-N-(3-(5-Chloro-2-(2-(2,4-difluorobenzylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)acrylamide (**14c**).* Flash column chromatography was performed using (petroleum ether:ethyl acetate, 10:1 to 4:1 v/v). HPLC purity: 99.0%. A white solid. Yield: 52%. mp 263.6–265.2 °C. IR (KBr, cm^{-1}): 3428.1 (NH), 3208.7 (RC=CH), 2953.0 (CH_3), 1675.3 (CO). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 1H, –NH–N), 10.16 (s, 1H, NHCO), 8.94 (s, 1H, NH), 8.23 (s, 1H, –N=CH), 8.16 (s, 1H, ArH), 7.99 (s, 1H, ArH), 7.92 (dd, *J* = 15.5, 8.6 Hz, 1H, ArH), 7.73 (d, *J* = 6.5 Hz, 1H, ArH), 7.44 (d, *J* = 7.7 Hz, 1H, ArH), 7.31–7.35 (m, 2H, ArH), 7.13 (td, *J* = 8.8, 2.1 Hz, 1H, ArH), 6.46 (dd, *J* = 16.9, 10.1 Hz, 1H, –C<u>H</u>=CH_2), 6.25 (dd, *J* = 17.0, 1.9 Hz, 1H, –CH=C<u>H_2</u>), 5.75 (m, 1H, –CH=C<u>H_2</u>). ESI-MS m/z: 429.1 $[M+H]^+$. Anal. calcd. for $C_{20}H_{15}ClF_2N_6O$ (%): C, 56.02; H, 3.53; N, 19.60; found (%): C, 55.93; H, 3.60; N, 19.72.

*4.1.8.4. (E)-N-(3-(2-(2-((1-Benzyl-1H-indol-3-yl)methylene)hydrazinyl)-5-chloropyrimidin-4-ylamino)phenyl)acrylamide (**14d**).* Flash column chromatography was performed using (petroleum ether:ethyl acetate, 4:1 to 3:2 v/v). HPLC purity: 98.3%. A pale-yellow solid. Yield: 30%. mp 163.3–165.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H, –NH–N), 10.14 (s, 1H, NH), 8.74 (s, 1H, NH), 8.28 (s, 1H, –N=CH), 8.26 (d, *J* = 7.8 Hz, 1H, ArH), 8.10 (s, 1H, ArH), 7.86 (s, 1H, ArH), 7.81 (s, 1H, ArH), 7.46 (dd, *J* = 8.2, 3.3

Hz, 2H, ArH), 7.21–7.33 (m, 7H, ArH), 7.15–7.19 (m, 1H, ArH), 7.07 (t, *J* = 7.4 Hz, 1H, ArH), 6.42 (dd, *J* = 16.9, 10.3 Hz, 1H, –C<u>H</u>=CH_2), 6.22 (dd, *J* = 16.9, 2.0 Hz, 1H, –CH=C<u>H_2</u>), 5.70 (dd, *J* = 10.2, 1.6 Hz, 1H, , –CH=C<u>H_2</u>), 5.41 (s, 2H, CH_2). ESI-MS m/z: 522.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{24}ClN_7O$ (%): C, 66.73; H, 4.63; N, 18.78; found (%): C, 66.65; H, 4.51; N, 18.86.

*4.1.8.5. (E)-N-(3-(5-Chloro-2-(2-(1-(4-fluorophenyl)ethylidene)hydrazinyl)pyrimidin-4-yl amino)phenyl)acrylamide (**14e**).* Flash column chromatography was performed using (petroleum ether:ethyl acetate, 10:3 to 5:3 v/v). HPLC purity: 97.9%. A white solid. Yield: 65%. mp 225.8–228.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H, –NH–N), 10.28 (s, 1H, NHCO), 9.30 (s, 1H, NH), 8.22 (s, 1H, ArH), 8.10 (s, 1H, ArH), 7.93 (dd, *J* = 7.3, 6.0 Hz, 2H, ArH), 7.71 (s, 1H, ArH), 7.48 (d, *J* = 8.4 Hz, 1H, ArH), 7.32–7.36 (m, 1H, ArH), 7.21–7.25 (m, 2H, ArH), 6.47 (dd, *J* = 16.9, 10.1 Hz, 1H, –C<u>H</u>=CH_2), 6.25 (dd, *J* = 16.9, 1.6 Hz, 1H, –CH=C<u>H_2</u>), 5.74 (dd, *J* = 10.2, 1.3 Hz, 1H, –CH=C<u>H_2</u>), 2.32 (s, 3H, CH_3). ESI-MS m/z: 425.3 $[M+H]^+$. Anal. calcd. for $C_{21}H_{18}ClFN_6O$ (%): C, 59.37; H, 4.27; N, 19.78; found (%): C, 59.26; H, 4.25; N, 19.83.

*4.1.8.6. (E)-N-(3-(5-Chloro-2-(2-(1-(2,4-difluorophenyl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)acrylamide (**14f**).* Flash column chromatography was performed using (petroleum ether:ethyl acetate, 10:1 to 3:1 v/v). HPLC purity: 98.6%. A white solid. Yield: 36%. mp 236.2–238.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H, –NH–N), 10.02 (s, 1H, NHCO), 8.90 (s, 1H, NH), 8.18 (s, 1H, ArH), 8.10 (s, 1H, ArH), 7.78 (d, *J* = 7.7 Hz, 1H, ArH), 7.71 (td, *J* = 8.9, 7.0 Hz, 1H, ArH), 7.39 (dd, *J* = 8.4, 0.8 Hz, 1H, ArH), 7.24–7.31 (m, 2H, ArH), 7.09 (td, *J* = 8.6, 2.7 Hz, 1H, ArH), 6.45 (dd, *J* = 17.0, 10.1 Hz, 1H, –C<u>H</u>=CH_2), 6.25 (dd, *J* = 17.0, 2.0 Hz, 1H, –CH=C<u>H_2</u>), 5.74 (dd, *J* = 10.1, 2.0 Hz, 1H, –CH=C<u>H_2</u>), 2.28 (s, 3H, CH_3). ESI-MS m/z: 443.5 $[M+H]^+$. Anal. calcd. for $C_{21}H_{17}ClF_2N_6O$ (%): C, 56.96; H, 3.87; N, 18.98; found (%): C, 60.07; H, 3.80; N, 19.11.

*4.1.8.7. (E)-N-(3-(5-Chloro-2-(2-(1-p-tolylethylidene)hydrazinyl)pyrimidin-4-ylamino)ph enyl)acrylamide (**14g**).* Flash column chromatography was performed using (petroleum ether:ethyl acetate, 2:1 to 5:3 v/v). HPLC purity: 99.1%. A white solid. Yield: 61%. mp 188.8–189.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H, –NH–N), 9.94 (s, 1H, NHCO), 8.92 (s, 1H, NH), 8.17 (s, 1H, ArH), 8.07 (s, 1H, ArH), 7.92 (s, 1H, ArH), 7.74 (d, *J* = 7.8 Hz, 2H, ArH), 7.45 (dd, *J* = 8.1, 1.3 Hz, 1H, ArH), 7.31 (t, *J* = 7.8 Hz, 1H, ArH), 7.19 (d, *J* = 7.9 Hz, 2H, ArH), 6.45 (dd, *J* = 16.9, 10.1 Hz, 1H, –C<u>H</u>=CH_2), 6.26 (dd, *J* = 16.9, 1.9 Hz, 1H, –CH=C<u>H_2</u>), 5.73 (dd, *J* = 10.3, 1.7 Hz, 1H, –CH=C<u>H_2</u>), 2.32 (s, 3H, CH_3), 2.26 (s, 3H, CH_3). ESI-MS m/z: 421.5 $[M+H]^+$. Anal. calcd. for $C_{22}H_{21}ClN_6O$ (%): C, 62.78; H, 5.03; N, 19.97; found (%): C, 62.65; H, 4.96; N, 19.82.

*4.1.8.8. (E)-N-(3-(5-Chloro-2-(2-(1-(4-methoxyphenyl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)acrylamide (**14h**).* Flash column chromatography was performed using (petroleum ether:ethyl acetate, 5:1 to 3:2 v/v). HPLC purity: 98.7%. A white solid. Yield: 55%. mp 217.0–219.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.55 (s, 1H, –NH–N), 10.36 (s, 1H, NHCO), 10.34 (s, 1H, NH), 8.29 (s, 1H, ArH), 7.96–7.80 (m, 3H, ArH), 7.58 (d, *J* = 7.6 Hz, 1H, ArH), 7.37–7.44 (m, 2H, ArH), 7.00 (d, *J* = 9.0 Hz, 2H, ArH),

6.50 (dd, *J* = 17.0, 10.1 Hz, 1H, –C<u>H</u>=CH_2), 6.28 (dd, *J* = 16.9, 1.8 Hz, 1H, –CH=C<u>H_2</u>), 5.78 (dd, *J* = 10.1, 1.8 Hz, 1H, –CH=C<u>H_2</u>), 3.82 (s, 3H, OCH_3), 2.37 (s, 3H, CH_3). ESI-MS m/z: 437.2 $[M+H]^+$. Anal. calcd. for $C_{22}H_{21}ClN_6O_2$ (%): C, 60.48; H, 4.85; N, 19.24; found (%): C, 60.53; H, 4.76; N, 19.07.

*4.1.8.9. (E)-N-(3-(5-Chloro-2-(2-(1-(3-methoxyphenyl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)acrylamide (**14i**).* Flash column chromatography was performed using (petroleum ether:ethyl acetate, 4:1 to 3:2 v/v). HPLC purity: 99.3%. A white solid. Yield: 29%. mp 198.2–200.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.59 (s, 1H, –NH–N), 10.35 (s, 2H, NHCO, NH), 8.29 (s, 1H, ArH), 7.96 (s, 1H, ArH), 7.53–7.56 (m, 3H, ArH), 7.36–7.44 (m, 3H, ArH), 7.06 (dd, *J* = 8.0, 1.9 Hz, 1H, ArH), 6.48 (dd, *J* = 17.0, 10.1 Hz, 1H, –C<u>H</u>=CH_2), 6.28 (dd, *J* = 17.0, 1.9 Hz, 1H, –CH=C<u>H_2</u>), 5.79 (dd, *J* = 10.1, 1.9 Hz, 1H, –CH=C<u>H_2</u>), 3.82 (s, 3H, OCH_3), 2.39 (s, 3H, CH_3). ESI-MS m/z: 437.2 $[M+H]^+$. Anal. calcd. for $C_{22}H_{21}ClN_6O_2$ (%): C, 60.48; H, 4.85; N, 19.24; found (%): C, 60.42; H, 4.73; N, 19.32.

*4.1.8.10. (E)-N-(3-(5-Chloro-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)acrylamide (**14j**).* Flash column chromatography was performed using (petroleum ether:ethyl acetate, 1:1 to 1:3 v/v). HPLC purity: 98.6%. A yellow solid. Yield: 51%. mp 152.1–154.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.31 (s, 1H, –NH–N), 10.22 (s, 1H, NHCO), 8.99 (s, 1H, NH), 8.56 (d, *J* = 5.5 Hz, 2H, ArH), 8.22 (s, 1H, ArH), 8.13 (s, 1H, ArH), 7.82 (d, *J* = 7.7 Hz, 1H, ArH), 7.73 (d, *J* = 5.5 Hz, 2H, ArH), 7.43 (d, *J* = 7.6 Hz, 1H, ArH), 7.34 (t, *J* = 8.0 Hz, 1H, ArH), 6.45 (dd, *J* = 16.8, 10.1 Hz, 1H, –C<u>H</u>=CH_2), 6.24 (dd, *J* = 16.9, 0.5 Hz, 1H, –CH=C<u>H_2</u>), 5.72 (dd, *J* = 10.7, 0.6 Hz, 1H, –CH=C<u>H_2</u>), 2.29 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.59, 158.48, 156.47, 155.19, 150.03 (2C), 146.30, 143.15, 139.65, 139.39, 132.41, 128.88, 127.22, 120.27 (2C), 118.23, 115.40, 114.30, 105.87, 13.00. ESI-MS m/z: 408.1 $[M+H]^+$. Anal. calcd. for $C_{20}H_{18}ClN_7O$ (%): C, 58.90; H, 4.45; N, 24.04; found (%): C, 58.82; H, 4.37; N, 24.15.

*4.1.9. General procedure for preparation of compounds (**15a–15o**)*

A solution of appropriate **9** (0.8 mmol), (*E*)-4-(dimethylamino)but-2-enoic acid (0.8 mmol), DIPEA (2 mmol) and HATU (0.8 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 3–9 h. The solution was washed with aqueous K_2CO_3 (2 × 5 mL), then brine (5 mL), and evaporated to give the crude residue. The target compounds **15a–15o** were obtained after purification by silica gel column chromatography.

*4.1.9.1. (E)-N-(3-(5-Chloro-2-((E)-2-(4-fluorobenzylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (**15a**).* Flash column chromatography was performed using (CH_2Cl_2:MeOH, 30:1 to 20:1 v/v). HPLC purity: 98.2%. A white solid. Yield: 42%. mp 218.7–220.1 °C. IR (KBr, cm^{-1}): 3413.6 (NH), 3243.6 (RC=CH), 2940.3, 2857.0 (CH_2, CH_3), 2776.1 (N–CH_3), 1674.1 (CO). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.05 (s, 1H, –NH–N), 10.06 (s, 1H, NHCO), 8.85 (s, 1H, NH), 8.12 (s, 1H, –N=CH), 8.06 (s, 1H, ArH), 8.00 (s, 1H, ArH), 7.64–7.67 (m, 3H, ArH), 7.41 (d, *J* = 8.3 Hz, 1H, ArH), 7.29 (t, *J* = 8.1 Hz, 1H, ArH), 7.19 (t, *J* = 8.8 Hz, 2H, ArH), 6.70 (dt, *J* = 15.3, 5.9 Hz, 1H, –CH=C<u>H</u>–CH_2), 6.28 (d, *J* = 15.4 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.01 (d, *J* = 5.4 Hz,

2H, –CH=CH–C<u>H</u>$_2$), 2.13 (s, 6H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.63, 158.12, 156.38, 155.37, 141.76, 140.12, 139.63, 139.53, 132.46, 128.86, 128.57 (2C), 126.50, 118.09, 116.22 (2C), 116.00, 115.52, 114.47, 105.02, 60.20, 45.58 (2C). ESI-MS m/z: 468.1 $[M+H]^+$. Anal. calcd. for $C_{23}H_{23}ClFN_7O$ (%): C, 59.04; H, 4.95; N, 20.95; found (%): C, 58.95; H, 4.77; N, 21.02.

*4.1.9.2. (E)-N-(3-(5-Chloro-2-((E)-2-(2-fluorobenzylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (**15b**).* Flash column chromatography was performed using (CH_2Cl_2:MeOH, 50:1 to 25:1 v/v). HPLC purity: 98.5%. A white solid. Yield: 67%. mp 194.4–196.2 °C. IR (KBr, cm^{-1}): 3422.6 (NH), 2925.8, 2852.2 (CH_2, CH_3), 2779.5 (N–CH_3), 1674.5 (CO). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 1H, –NH–N), 10.16 (s, 1H, NHCO), 8.91 (s, 1H, NH), 8.30 (s, 1H, –N=CH), 8.17 (s, 1H, ArH), 8.00 (s, 1H, ArH), 7.91 (t, *J* = 7.5 Hz, 1H, ArH), 7.73 (s, 1H, ArH), 7.45 (d, *J* = 7.6 Hz, 1H, ArH), 7.38 (dd, *J* = 13.3, 6.7 Hz, 1H, ArH), 7.31 (t, *J* = 8.0 Hz, 1H, ArH), 7.23 (dd, *J* = 16.2, 8.4 Hz, 2H, ArH), 6.73 (m, 1H, –CH=C<u>H</u>–CH_2), 6.35 (d, *J* = 15.4 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.29 (d, *J* = 5.2 Hz, 2H, –CH=CH–C<u>H</u>$_2$), 2.37 (s, 6H, CH_3). ESI-MS m/z: 468.1 $[M+H]^+$. Anal. calcd. for $C_{23}H_{23}ClFN_7O$ (%): C, 59.04; H, 4.95; N, 20.95; found (%): C, 59.10; H, 4.86; N, 20.99.

*4.1.9.3. (E)-N-(3-(5-Chloro-2-((E)-2-(2,4-difluorobenzylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (**15c**).* Flash column chromatography was performed using (CH_2Cl_2:MeOH, 50:1 to 22:1 v/v). HPLC purity: 99.3%. A white solid. Yield: 55%. mp 189.1–191.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 11.45 (s, 1H, –NH–N), 10.55 (s, 1H, NHCO), 8.92 (s, 1H, NH), 8.30 (s, 1H, –N=CH), 8.19 (s, 1H, ArH), 8.17 (s, 1H, ArH), 7.92 (dd, *J* = 15.5, 8.5 Hz, 1H, ArH), 7.67 (s, 1H, ArH), 7.54 (d, *J* = 7.3 Hz, 1H, ArH), 7.28–7.34 (m, 2H, ArH), 7.15 (td, *J* = 8.7, 2.0 Hz, 1H, ArH), 6.77 (m, 1H, –CH=C<u>H</u>–CH_2), 6.56 (d, *J* = 15.1 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.82 (d, *J* = 6.1 Hz, 2H, –CH=CH–C<u>H</u>$_2$), 2.69 (s, 6H, CH_3). ESI-MS m/z: 486.1 $[M+H]^+$. Anal. calcd. for $C_{23}H_{22}ClF_2N_7O$ (%): C, 56.85; H, 4.56; N, 20.18; found (%): C, 56.80; H, 4.61; N, 20.23.

*4.1.9.4. (E)-N-(3-(5-Chloro-2-((E)-2-(4-methoxybenzylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (**15d**).* Flash column chromatography was performed using (CH_2Cl_2:MeOH, 35:1 to 20:1 v/v). HPLC purity: 97.2%. A white solid. Yield: 53%. mp 145.1–147.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (s, 1H, –NH–N), 10.08 (s, 1H, NHCO), 8.80 (s, 1H, NH), 8.10 (s, 1H, –N=CH), 8.01 (s, 1H, ArH), 7.99 (s, 1H, ArH), 7.70 (s, 1H, ArH), 7.55 (d, *J* = 8.8 Hz, 2H, ArH), 7.42 (d, *J* = 7.7 Hz, 1H, ArH), 7.28 (t, *J* = 8.1 Hz, 1H, ArH), 6.93 (d, *J* = 8.8 Hz, 2H, ArH), 6.70 (dt, *J* = 15.4, 5.9 Hz, 1H, –CH=C<u>H</u>–CH_2), 6.28 (d, *J* = 15.4 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.77 (s, 3H, OCH_3), 3.02 (d, *J* = 5.6 Hz, 2H, –CH=CH–C<u>H</u>$_2$), 2.13 (s, 6H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.63, 160.36, 158.17, 156.29, 155.35, 141.60, 141.33, 139.71, 139.57, 128.85, 128.44, 128.10 (2C), 126.61, 117.95, 115.38, 114.66 (2C), 114.30, 104.61, 60.20, 55.67, 45.57 (2C). ESI-MS m/z: 480.1 $[M+H]^+$. Anal. calcd. for $C_{24}H_{26}ClN_7O_2$ (%): C, 60.06; H, 5.46; N, 20.43; found (%): C, 59.89; H, 5.50; N, 20.28.

4.1.9.5. (E)-N-(3-(5-Chloro-2-((E)-2-(pyridin-4-ylmethylene)hydrazinyl)pyrimidin-4-ylam

*ino)phenyl)-4-(dimethylamino)but-2-enamide (**15e**).* Flash column chromatography was performed using (CH_2Cl_2:MeOH, 30:1 to 15:1 v/v). HPLC purity: 98.8%. A white solid. Yield: 32%. mp 200.5–202.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.45 (s, 1H, –NH–N), 10.17 (s, 1H, NHCO), 8.97 (s, 1H, NH), 8.54 (d, *J* = 5.2 Hz, 1H, ArH), 8.28 (d, *J* = 3.1 Hz, 1H, ArH), 8.19 (s, 1H, –N=CH), 8.04–8.07 (m, 2H, ArH), 7.66 (d, *J* = 7.7 Hz, 1H, ArH), 7.54 (d, *J* = 5.2 Hz, 1H, ArH), 7.45 (d, *J* = 8.0 Hz, 1H, ArH), 7.32 (t, *J* = 8.3 Hz, 1H, ArH), 7.11 (dd, *J* = 8.0, 4.0 Hz, 1H, ArH), 6.71 (m, 1H, –CH=C<u>H</u>–CH_2), 6.29 (d, *J* = 15.1 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.02 (d, *J* = 3.6 Hz, 2H, –CH=CH–C<u>H_2</u>), 2.14 (s, 6H, CH_3). ESI-MS m/z: 451.1 $[M+H]^+$. Anal. calcd. for $C_{22}H_{23}ClN_8O$ (%): C, 58.60; H, 5.14; N, 24.85; found (%): C, 58.48; H, 5.22; N, 24.72.

*4.1.9.6. (E)-N-(3-(2-((E)-2-((1H-Indol-3-yl)methylene)hydrazinyl)-5-chloropyrimidin-4-yl amino)phenyl)-4-(dimethylamino)but-2-enamide (**15f**).* Flash column chromatography was performed using (CH_2Cl_2:MeOH, 30:1 to 15:1 v/v). HPLC purity: 98.0%. A yellow solid. Yield: 29%. mp 127.2–129.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ11.82 (s, 1H, NH), 10.52 (s, 1H, –NH–N), 10.31 (s, 1H, NHCO), 9.76 (s, 1H, NH), 8.45 (s, 1H, ArH), 8.41 (d, *J* = 7.2 Hz, 1H, ArH), 8.26 (s, 1H, –N=CH), 7.92 (d, *J* = 8.1 Hz, 2H, ArH), 7.59 (d, *J* = 7.6 Hz, 1H, ArH), 7.45 (dd, *J* = 14.1, 7.6 Hz, 2H, ArH), 7.35 (s, 1H, ArH), 7.23 (dd, *J* = 16.6, 8.6 Hz, 2H, ArH), 6.76 (m, 1H, –CH=C<u>H</u>–CH_2), 6.52 (d, *J* = 15.4 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.23 (d, *J* = 5.6 Hz, 2H, –CH=CH–C<u>H_2</u>), 2.81 (s, *J* = 3.5 Hz, 6H, CH_3). ESI-MS m/z: 489.1 $[M+H]^+$. Anal. calcd. for $C_{25}H_{25}ClN_8O$ (%): C, 61.41; H, 5.15; N, 22.92; found (%): C, 61.46; H, 5.10; N, 22.90.

*4.1.9.7. (E)-N-(3-(2-((E)-2-((1-Benzyl-1H-indol-3-yl)methylene)hydrazinyl)-5-chloropyri midin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (**15g**).* Flash column chromatography was performed using (CH_2Cl_2:MeOH, 100:1 to 25:1 v/v). HPLC purity: 98.1%. A pale-yellow solid. Yield: 25%. mp 196.9–199.8 °C. IR (KBr, cm^{-1}): 3422.4 (NH), 3237.4 (RC=CH), 2924.9, 2852.7 (CH_2, CH_3), 2780.8 (N–CH_3), 1673.9 (CO). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H, –NH–N), 10.05 (s, 1H, NHCO), 8.73 (s, 1H, NH), 8.28 (s, 1H, –N=CH), 8.26 (d, *J* = 7.9 Hz, 1H, ArH), 8.10 (s, 1H, ArH), 7.85 (s, 1H, ArH), 7.81 (s, 1H, NH), 7.45 (d, *J* = 8.2 Hz, 2H, ArH), 7.28–7.31 (m, 3H, ArH), 7.22–7.25 (m, 4H, ArH), 7.15–7.19 (m, 1H, ArH), 7.07 (t, *J* = 7.5 Hz, 1H, ArH), 6.69 (dt, *J* = 15.3, 5.9 Hz, 1H, –CH=C<u>H</u>–CH_2), 6.25 (d, *J* = 15.3 Hz, 1H, –C<u>H</u>=CH–CH_2), 5.41 (s, 2H, CH_2), 3.01 (d, *J* = 5.4 Hz, 2H, –CH=CH–C<u>H_2</u>), 2.13 (s, 6H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.67, 158.31, 156.45, 155.34, 141.59, 139.85, 139.75, 139.03, 139.02, 138.18, 137.29, 132.26, 129.06 (2C), 128.96, 127.96, 127.61 (2C), 126.57, 125.40, 123.09, 122.89, 120.95, 118.03, 115.02, 112.63, 110.81, 103.88, 60.17, 49.71, 45.58 (2C). ESI-MS m/z: 579.4 $[M+H]^+$. Anal. calcd. for $C_{32}H_{31}ClN_8O$ (%): C, 66.37; H, 5.40; N, 19.35; found (%): C, 66.32; H, 5.38; N, 19.51.

*4.1.9.8. (E)-N-(3-(5-Chloro-2-((E)-2-(1-(4-fluorophenyl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (**15h**).* Flash column chromatography was performed using (CH_2Cl_2:MeOH, 60:1 to 20:1 v/v). HPLC purity: 98.5%. A white solid. Yield: 63%. mp 151.9–153.0 °C. IR (KBr, cm^{-1}): 3405.7 (NH), 2923.0, 2852.5 (CH_2, CH_3), 2784.9 (N–CH_3), 1680.0 (CO). ^{1}H NMR (400 MHz,

DMSO-d_6) δ 10.47 (s, 1H, –NH–N), 10.03 (s, 1H, NHCO), 8.89 (s, 1H, NH), 8.18 (s, 2H, ArH), 7.82–7.88 (m, 3H, ArH), 7.47 (d, *J* = 7.9 Hz, 1H, ArH), 7.32 (t, *J* = 8.1 Hz, 1H, ArH), 7.20 (t, *J* = 8.8 Hz, 2H, ArH), 6.76 (dt, *J* = 14.3, 7.0 Hz, 1H, –CH=C<u>H</u>–CH_2), 6.49 (d, *J* = 15.3 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.84 (d, *J* = 6.5 Hz, 2H, –CH=CH–C<u>H_2</u>), 2.70 (s, 6H, CH_3), 2.30 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.95, 162.48, 161.52, 158.75, 156.29, 155.21, 145.22, 139.80, 139.22, 135.78, 132.22, 128.92, 128.18 (2C), 118.18, 115.55, 115.30 (2C), 114.03, 105.18, 57.37, 42.56 (2C), 13.78. ESI-MS m/z: 482.1 $[M+H]^+$. Anal. calcd. for $C_{24}H_{25}ClFN_7O$ (%): C, 59.81; H, 5.23; N, 20.34; found (%): C, 59.77; H, 5.28; N, 20.35.

4.1.9.9. (E)-N-(3-(5-Chloro-2-((E)-2-(1-(2,4-difluorophenyl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide ***(15i)***. Flash column chromatography was performed using (CH_2Cl_2:MeOH, 50:1 to 30:1 v/v). HPLC purity: 99.1%. A white solid. Yield: 63%. mp 162.3–163.6 °C. IR (KBr, cm^{-1}): 3415.8 (NH), 2943.2, 2821.4 (CH_2, CH_3), 2778.4 (N–CH_3), 1675.3 (CO). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H, –NH–N), 10.03 (s, 1H, NHCO), 8.89 (s, 1H, NH), 8.18 (s, 1H, ArH), 8.11 (s, 1H, –N=CH, ArH), 7.68–7.76 (m, 2H, ArH), 7.39 (d, *J* = 7.8 Hz, 1H, ArH), 7.27 (ddd, *J* = 17.9, 12.8, 5.3 Hz, 2H, ArH), 7.09 (dt, *J* = 8.4, 2.0 Hz, 1H, ArH), 6.73 (dt, *J* = 15.3, 6.2 Hz, 1H, –CH=C<u>H</u>–CH_2), 6.33 (d, *J* = 15.1 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.22 (s, 2H, –CH=CH–C<u>H_2</u>), 2.29 (s, 3H, CH_3), 2.28 (s, 6H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δδ 163.31, 161.32, 161.30, 159.40, 158.71, 156.30, 155.25, 143.38, 139.69, 139.41, 131.24, 128.89, 117.88, 115.16, 113.90, 112.09, 105.53, 105.09, 104.83, 99.99, 59.41, 44.77 (2C), 17.19. ESI-MS m/z: 500.3 $[M+H]^+$. Anal. calcd. for $C_{24}H_{24}ClF_2N_7O$ (%): C, 57.66; H, 4.84; N, 19,61; found (%): C, 57.58; H, 4.90; N, 19.56.

4.1.9.10. (E)-N-(3-(5-Chloro-2-((E)-2-(1-p-tolylethylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide ***(15j)***. Flash column chromatography was performed using (CH_2Cl_2:MeOH, 50:1 to 30:1 v/v). HPLC purity: 98.7%. A white solid. Yield: 63%. mp 135.3–138.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (s, 1H, –NH–N), 9.95 (s, 1H, NHCO), 8.85 (s, 1H, NH), 8.17 (s, 2H, ArH), 7.89 (d, *J* = 7.3 Hz, 1H, ArH), 7.73 (d, *J* = 8.1 Hz, 2H, ArH), 7.49 (d, *J* = 7.9 Hz, 1H, ArH), 7.30 (t, *J* = 8.1 Hz, 1H, ArH), 7.19 (d, *J* = 8.0 Hz, 2H, ArH), 6.76 (dt, *J* = 14.8, 6.7 Hz, 1H, –CH=C<u>H</u>–CH_2), 6.47 (d, *J* = 15.3 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.12 (d, *J* = 7.2 Hz, 2H, –CH=CH–C<u>H_2</u>), 2.56 (s, 6H, CH_3), 2.32 (s, 3H, CH_3), 2.28 (s, 3H, CH_3). ESI-MS m/z: 478.2 $[M+H]^+$. Anal. calcd. for $C_{25}H_{28}ClN_7O$ (%): C, 62.82; H, 5.90; N, 20.51; found (%): C, 62.75; H, 5.92; N, 20.52.

4.1.9.11. (E)-N-(3-(5-Chloro-2-((E)-2-(1-(4-methoxyphenyl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide ***(15k)***. Flash column chromatography was performed using (CH_2Cl_2:MeOH, 50:1 to 30:1 v/v). HPLC purity: 98.6%. A white solid. Yield: 67%. mp 131.1–132.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33 (s, 1H, –NH–N), 9.87 (s, 1H, NHCO), 8.87 (s, 1H, NH), 8.16 (s, 1H, ArH), 8.11 (d, *J* = 2.4 Hz, 1H, ArH), 7.87 (br, 1H, ArH), 7.77 (d, *J* = 7.7 Hz, 2H, ArH), 7.46 (d, *J* = 7.3 Hz, 1H, ArH), 7.30–7.33 (m, 1H, ArH), 6.92 (d, *J* = 7.9 Hz, 2H, ArH), 6.73 (m, 1H, –CH=C<u>H</u>–CH_2), 6.43 (d, *J* = 15.3 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.79 (s, 3H, OCH_3), 3.69 (d, *J*

= 6.3 Hz, 2H, –CH=CH–C<u>H</u>$_2$), 2.60 (s, 6H, CH_3), 2.25 (s, 3H, CH_3). ESI-MS m/z: 494.5 $[M+H]^+$. Anal. calcd. for $C_{25}H_{28}ClN_7O_2$ (%): C, 60.78; H, 5.71; N, 19.85; found (%): C, 60.71; H, 5.76; N, 19.83.

*4.1.9.12. (E)-N-(3-(5-Chloro-2-((E)-2-(1-(3-methoxyphenyl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (**15l**).* Flash column chromatography was performed using (CH_2Cl_2:MeOH, 100:1 to 35:1 v/v). HPLC purity: 98.3%. A white solid. Yield: 45%. mp 88.2–90.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18 (s, 1H, –NH–N), 9.96 (s, 1H, NHCO), 8.88 (s, 1H, NH), 8.19 (s, 1H, ArH), 8.01 (m, 2H, ArH), 7.45 (d, *J* = 7.8 Hz, 1H, ArH), 7.40 (d, *J* = 7.9 Hz, 1H, ArH), 7.30–7.33 (m, 3H, ArH), 6.93 (dd, *J* = 8.2, 1.7 Hz, 1H, ArH), 6.73 (dt, *J* = 14.1, 6.0 Hz, 1H, –CH=C<u>H</u>–CH_2), 6.34 (d, *J* = 15.1 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.77 (s, 3H, OCH_3), 3.42 (d, *J* = 6.1 Hz, 2H, –CH=CH–C<u>H</u>$_2$), 2.37 (s, 6H, CH_3), 2.28 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.16, 162.54, 159.72, 158.78, 156.30, 155.25, 146.05, 140.72, 139.87, 139.43, 129.66, 129.32, 128.91, 118.68, 117.81, 115.06, 113.77, 113.65, 112.32, 105.25, 59.07, 55.63 (2C), 44.41, 13.81. ESI-MS m/z: 494.3 $[M+H]^+$. Anal. calcd. for $C_{25}H_{28}ClN_7O_2$ (%): C, 60.78; H, 5.71; N, 19.85; found (%): C, 60.79; H, 5.82; N, 19.80.

*4.1.9.13. (E)-N-(3-(5-Chloro-2-((E)-2-(1-(pyridin-2-yl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (**15m**).* Flash column chromatography was performed using (CH_2Cl_2:MeOH, 30:1 to 12:1 v/v). HPLC purity: 97.9%. A yellow solid. Yield: 36%. mp 186.5–188.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H, –NH–N), 9.71 (s, 1H, NHCO), 9.17 (s, 1H, NH), 8.56 (d, *J* = 4.6 Hz, 1H, ArH), 8.25 (s, 1H, ArH), 8.12–8.20 (m, 2H, ArH), 7.76–7.80 (m, 2H, ArH), 7.47 (dd, *J* = 7.9, 0.7 Hz, 1H, ArH), 7.33–7.38 (m, 2H, ArH), 6.72 (m, 1H, –CH=C<u>H</u>–CH_2), 6.44 (d, *J* = 15.2 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.92 (d, *J* = 6.9 Hz, 2H, –CH=CH–C<u>H</u>$_2$), 2.77 (s, 6H, CH_3), 2.39 (s, 3H, CH_3). ESI-MS m/z: 465.3 $[M+H]^+$. Anal. calcd. for $C_{23}H_{25}ClN_8O$ (%): C, 59.41; H, 5.42; N, 24.10; found (%): C, 59.37; H, 5.43; N, 23.99.

*4.1.9.14. (E)-N-(3-(5-Chloro-2-((E)-2-(1-(pyridin-3-yl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (**15n**).* Flash column chromatography was performed using (CH_2Cl_2:MeOH, 50:1 to 15:1 v/v). HPLC purity: 99.0%. A yellow solid. Yield: 52%. mp 206.7–207.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H, –NH–N), 10.20 (s, 1H, NHCO), 9.03 (d, *J* = 1.3 Hz, 1H, NH), 8.93 (s, 1H, ArH), 8.53 (d, *J* = 4.5 Hz, 1H, ArH), 8.21 (s, 1H, ArH), 8.15–8.17 (m, 2H, ArH), 7.93 (d, *J* = 6.0 Hz, 1H, ArH), 7.44 (d, *J* = 7.6 Hz, 1H, ArH), 7.40 (dd, *J* = 8.0, 4.8 Hz, 1H, ArH), 7.32 (t, *J* = 8.1 Hz, 1H, ArH), 6.74 (dt, *J* = 14.3, 7.0 Hz, 1H, –CH=C<u>H</u>–CH_2), 6.46 (d, *J* = 15.4 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.77 (d, *J* = 5.4 Hz, 2H, –CH=CH–C<u>H</u>$_2$), 2.66 (s, 6H, CH_3), 2.34 (s, 3H, CH_3). ESI-MS m/z: 465.3 $[M+H]^+$. Anal. calcd. for $C_{23}H_{25}ClN_8O$ (%): C, 59.41; H, 5.42; N, 24.10; found (%): C, 59.45; H, 5.37; N, 24.16.

*4.1.9.15. (E)-N-(3-(5-Chloro-2-((E)-2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (**15o**).* Flash column chromatography was performed using (CH_2Cl_2:MeOH, 35:1 to 13:1 v/v). HPLC purity: 98.5%. A yellow solid. Yield: 26%. mp 237.3–238.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H,

–NH–N), 10.29 (s, 1H, NHCO), 8.94 (s, 1H, NH), 8.55 (d, *J* = 5.0 Hz, 2H, ArH), 8.22 (s, 1H, ArH), 8.19 (s, 1H, ArH), 7.72–7.76 (m, 3H, ArH), 7.46 (d, *J* = 7.6 Hz, 1H, ArH), 7.31 (t, *J* = 8.1 Hz, 1H, ArH), 6.71 (dt, *J* = 14.8, 5.7 Hz, 1H, –CH=C<u>H</u>–CH_2), 6.33 (d, *J* = 15.2 Hz, 1H, –C<u>H</u>=CH–CH_2), 3.06 (d, *J* = 5.1 Hz, 2H, –CH=CH–C<u>H_2</u>), 2.30 (s, 3H, CH_3), 2.16 (s, 6H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.45, 162.56, 158.53, 156.40, 155.26, 150.16 (2C), 146.19, 143.19, 139.62, 139.60, 128.82, 127.71, 120.23 (2C), 117.94, 115.27, 114.10, 105.86, 59.62, 44.95 (2C), 13.11. ESI-MS m/z: 465.2 $[M+H]^+$. Anal. calcd. for $C_{23}H_{25}ClN_8O$ (%): C, 59.41; H, 5.42; N, 24.10; found (%): C, 59.38; H, 5.46; N, 24.16.

*4.1.10. General procedure for preparation of compounds (**16a–16b**)*

A mixture of **11** (0.5 mmol), **13** (0.5 mmol) and TFA (0.6 mmol) in *i*-PrOH (5 mL) was stirred under reflux for 3 h. The reaction mixture was cooled to room temperature, and the precipitate was filtered to give a crude product, which was recrystallized from *i*-PrOH to generate the target compounds.

*4.1.10.1. N-(3-(5-Chloro-2-(2-(4-fluorobenzoyl)hydrazinyl)pyrimidin-4-ylamino)phenyl)acrylamide (**16a**).* Flash column chromatography was performed using (petroleum ether:ethyl acetate, 5:1 to 3:2 v/v)). HPLC purity: 98.7%. A white solid. Yield: 39%. mp 197.9–200.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H, NH), 10.06 (s, 1H, NH), 8.97 (s, 1H, NHCO), 8.82 (s, 1H, NH), 8.09 (s, 1H, ArH), 7.87–7.93 (m, 3H, ArH), 7.46 (s, 1H, ArH), 7.30 (br, 3H, ArH), 6.63 (s, 1H, ArH), 6.45 (dd, *J* = 14.7, 10.5 Hz, 1H, –C<u>H</u>=CH_2), 6.25 (d, *J* = 16.6 Hz, 1H, –CH=C<u>H_2</u>), 5.74 (d, *J* = 10.1 Hz, 1H, –CH=C<u>H_2</u>). ESI-MS m/z: 427.0 $[M+H]^+$. Anal. calcd. for $C_{20}H_{16}ClFN_6O_2$ (%): C, 56.28; H, 3.78; N, 19.69; found (%): C, 56.31; H, 3.71; N, 19.82.

*4.1.10.2. N-(3-(5-Chloro-2-(2-(4-methoxybenzoyl)hydrazinyl)pyrimidin-4-ylamino)phenyl)acrylamide (**16b**).* Flash column chromatography was performed using (petroleum ether:ethyl acetate, 6:1 to 1:1 v/v)). HPLC purity: 99.3%. A white solid. Yield: 72%. mp 213.6–215.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.79 (s, 1H, NH), 10.67 (s, 1H, NH), 10.49 (s, 1H, NH), 10.35 (s, 1H, NHCO), 8.29 (s, 1H, ArH), 8.03 (s, 1H, ArH), 7.93 (d, *J* = 8.3 Hz, 2H, ArH), 7.62 (d, *J* = 7.4 Hz, 1H, ArH), 7.41 (t, *J* = 7.7 Hz, 1H, ArH), 7.34 (d, *J* = 7.5 Hz, 1H, ArH), 7.08 (d, *J* = 8.3 Hz, 2H, ArH), 6.53 (dd, *J* = 17.0, 10.1 Hz, 1H, –C<u>H</u>=CH_2), 6.28 (d, *J* = 17.2 Hz, 1H, –CH=C<u>H_2</u>), 5.78 (d, *J* = 10.6 Hz, 1H, –CH=C<u>H_2</u>), 3.83 (s, 3H, OCH_3). ESI-MS m/z: 439.5 $[M+H]^+$. Anal. calcd. for $C_{21}H_{19}ClN_6O_3$ (%): C, 57.47; H, 4.36; N, 19.15; found (%): C, 57.80; H, 4.29; N, 19.12.

4.2. Pharmacology

4.2.1. In vitro enzymatic activity assay

The experiments were carried out by a well-established mobility shift assay, and EGFR kinases (EGFR T790M/L858R, EGFR T790M/delE746_A750, EGFR T790M and WT EGFR) were purchased from Invitrogen. The kinase base buffer was consist of 50 mM HEPES (pH 7.5), 0.0015% Brij35 and 2 mM DTT, while the stop buffer contained a mixture of 100 mM HEPES (pH 7.5), 0.015% Brij-35, 0.2% Coating Reagent and 50 mM

EDTA.

Initially, the tested compounds were diluted to 50-fold of the final desired highest concentration in reaction by 100% DMSO. Subsequently, 30 μL of the solution was transferred to 60 μL of 100% DMSO in the next well and so forth for a total of 5 concentrations. No compound and no enzyme controls were prepared by adding 100 μL DMSO to two empty wells in the same 96-well plate. Then, 10 μL of compound was transferred to a new 96-well plate, which was marked as the intermediate plate. Additional 90 μL of kinase buffer was added to each well of intermediate plate. The mixture in intermediate plate was shaked for 10 min. The assay plate was prepared after transferring 5 μL of each well from the 96-well intermediate plate to a 384-well plate in duplicates. The prepared enzyme solution (appropriate EGFR in kinase base buffer) was added to the assay plate, which was then incubated at room temperature for 10 min, followed by the addition 10 μL of prepared peptide solution (FAM-labeled peptide and ATP in kinase base buffer). The mixture was incubated at 28 °C for another 1 hour, then 25 μL of stop buffer was added. The convertion data was copied from Caliper program, and the values were converted to inhibition values. Percent inhibition = (max-conversion)/(max-min) × 100.

4.2.2. MTT assay

The target compounds were screened in A549, H1975 and HT-29 cells by a standard MTT assay. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS).

Approximate 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The tested compounds were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 μg/mL, and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with an ELISA reader. All of the compounds were tested three times in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of at least three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgements

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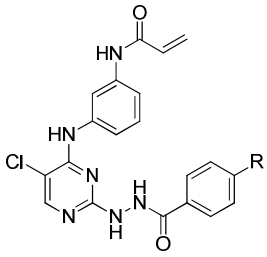
Legends

Figure 1. Representative EGFR inhibitors.
Figure 2. Design strategy of target compounds.

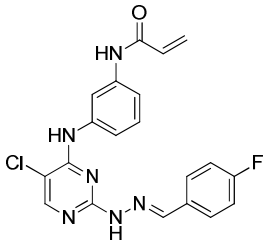
Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 50 °C, 3 h; (b) $NH_2NH_2 \cdot H_2O$, MeCN, reflux, 3 h; (c) appropriate aromatic aldehyde or ketone, *i*-PrOH, reflux, 6–8 h; (d) TFA, DCM, 25 °C, 2–7 h; (e) Acryloyl chloride, DIPEA, DCM, 25 °C, 1 h or (*E*)-4-(dimethylamino)but-2-enoic acid, HATU, DIPEA, DCM, 25 °C, 3–9 h.
Scheme 2. Reagents and conditions: (a) TFA, DCM, 25 °C, 1 h; (b) Acryloyl chloride, DIPEA, DCM, 25 °C, 1 h; (c) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 5 h; (d) TFA, *i*-PrOH, reflux, 3 h.

Table 1. Cellular antiproliferative activities of compounds **14a**, **16a** and **16b**
Table 2. Cellular antiproliferative activities of the hydrazone analogs
Table 3. *In vitro* enzymatic inhibition of compounds on different forms of EGFR

Table 1. Cellular antiproliferative activities of compounds **14a**, **16a** and **16b**



16a and 16b
16a: R = F
16b: R = OCH_3

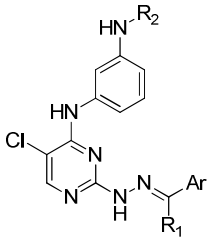


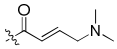
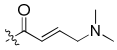
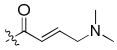
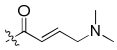
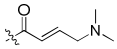
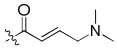
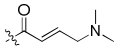
14a

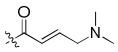
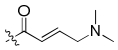
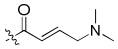
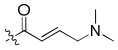
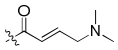
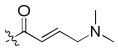
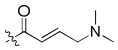
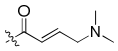
Compd	IC_{50} (μM)[a]	
	A549	H1975
14a	10.44 ± 1.36	0.43 ± 0.17
16a	31.00 ± 3.13	5.31 ± 0.96
16b	25.99 ± 2.25	8.46 ± 0.77

[a]The biological data are generated from at least three independent experiments.

Table 2. Cellular antiproliferative activities of the hydrazone analogs



Compd	Ar	R_1	R_2	IC_{50} (μM)[a]		
				A549	H1975	HT-29
9a	4-F-Ph	H	H	13.68 ± 2.59	9.92 ± 0.76	12.32 ± 1.03
14a	4-F-Ph	H		10.44 ± 1.36	0.43 ± 0.17	6.34 ± 1.59
14b	2-F-Ph	H		6.06 ± 0.54	0.29 ± 0.07	3.50 ± 0.69
14c	2,4-$(F)_2$-Ph	H		11.25 ± 1.77	0.38 ± 0.06	4.76 ± 0.66
14d		H		>100	1.35 ± 0.39	11.03 ± 2.33
14e	4-F-Ph	Me		8.20 ± 0.38	0.39 ± 0.11	5.05 ± 0.71
14f	2,4-$(F)_2$-Ph	Me		1.63 ± 0.25	0.30 ± 0.12	4.36 ± 1.24
14g	4-CH_3-Ph	Me		5.36 ± 1.68	0.74 ± 0.15	3.21 ± 0.67
14h	4-CH_3O-Ph	Me		7.71 ± 0.72	1.06 ± 0.37	4.56 ± 1.17
14i	3-CH_3O-Ph	Me		10.33 ± 2.24	1.19 ± 0.25	8.99 ± 1.22
14j	4-pyridyl	Me		28.71 ± 1.69	3.69 ± 1.07	12.80 ± 0.96
15a	2-F-Ph	H		8.94 ± 1.26	0.44 ± 0.08	3.66 ± 0.68
15b	4-F-Ph	H		6.77 ± 0.35	0.59 ± 0.16	3.30 ± 0.77
15c	2,4-$(F)_2$-Ph	H		2.17 ± 0.23	0.17 ± 0.05	2.92 ± 0.08
15d	4-CH_3O-Ph	H		6.24 ± 0.67	1.58 ± 0.29	4.82 ± 1.23
15e	pyridin-4-yl	H		47.68 ± 3.54	4.13 ± 0.35	25.19 ± 2.59
15f	indol-3-yl	H		28.68 ± 0.67	5.45 ± 0.37	9.68 ± 1.35
15g		H		2.91 ± 0.16	0.26 ± 0.12	2.12 ± 0.37

15h	4-F-Ph	Me		8.96 ± 1.56	1.16 ± 0.26	10.2 ± 1.09
15i	2,4-$(F)_2$-Ph	Me		5.57 ± 0.76	0.48 ± 0.05	3.46 ± 0.32
15j	4-CH_3-Ph	Me		10.8 ± 1.41	1.37 ± 0.27	11.8 ± 0.30
15k	4-CH_3O-Ph	Me		7.12 ± 0.33	0.99 ± 0.18	6.83 ± 0.57
15l	3-CH_3O-Ph	Me		11.38 ± 0.39	0.91 ± 0.06	3.87 ± 0.41
15m	pyridin-2-yl	Me		0.83 ± 0.12	0.077 ± 0.005	0.12 ± 0.06
15n	pyridin-3-yl	Me		17.29 ± 2.40	2.35 ± 0.15	9.66 ± 0.37
15o	pyridin-4-yl	Me		34.86 ± 1.12	6.24 ± 0.36	10.93 ± 0.59
gefitinib				9.86 ± 0.62	8.71 ± 0.60	8.19 ± 0.68
WZ4002				1.87 ± 0.37	0.058 ± 0.022	2.95 ± 0.29

[a]The biological data are generated from at least three independent experiments.

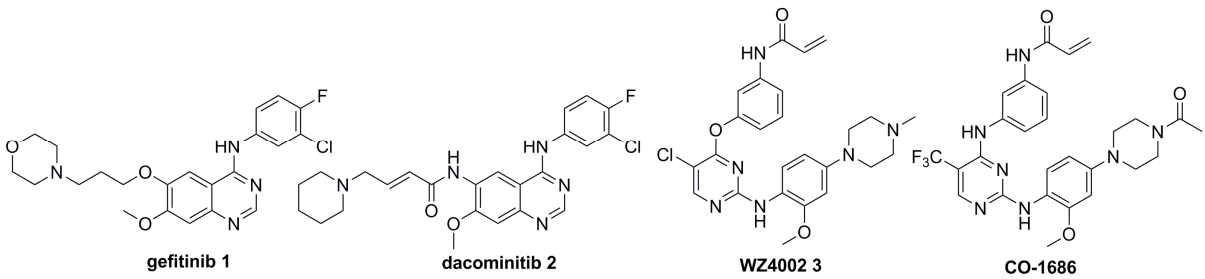
Table 3. *In vitro* enzymatic inhibition of compounds on different forms of EGFR

Compd	EGFRIC_{50} (μM)[a]				
	T790M/L858R	T790M/delE746_A750	T790M	WT	TL[b]/WT selectivity
14a	0.86	0.57	1.12	>20	>23.3
14b	1.51	1.91	4.74	>20	>13.2
14c	11.57	ND	ND	>20	>1.7
14e	1.68	1.29	2.53	>20	>11.9
14f	2.27	ND	ND	11.75	5.2
14g	5.67	ND	ND	>20	>3.5
15a	0.79	3.27	1.56	>20	>25.3
15b	0.68	2.23	1.49	10.65	15.7
15c	2.52	5.11	2.51	>20	>7.9
15f	>20	ND	ND	>20	-
15g	0.88	0.76	0.79	>20	>22.7
15h	2.35	ND	ND	15.32	6.5
15i	0.72	1.25	0.82	>20	>27.8
15m	2.38	ND	ND	>20	>8.4
15n	4.32	ND	ND	>20	>4.6
gefitinib	12.72	10.62	4.27	0.022	-
WZ4002	0.031	ND	ND	0.079	2.5

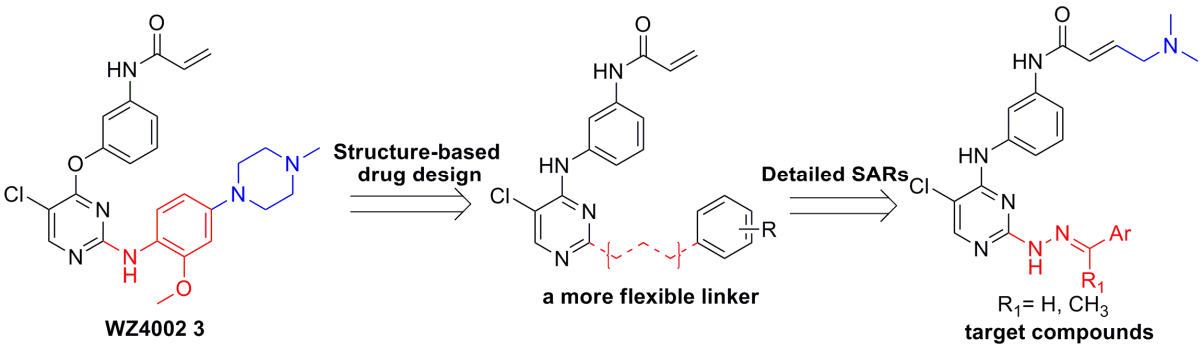
[a]The biological data are means from at least two replicated experiments.

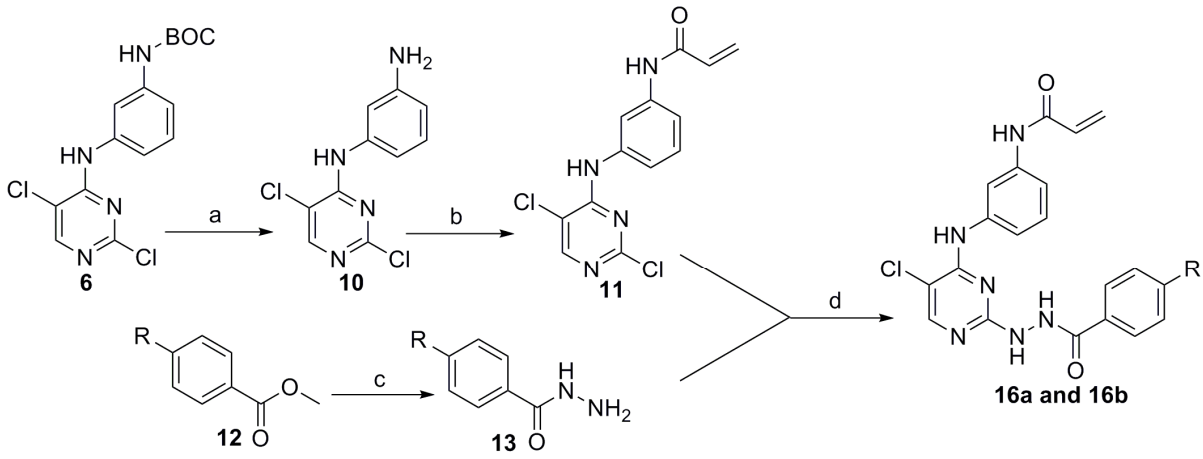
[b]TL represents EGFR T790M/L858R.

ND: not determined.

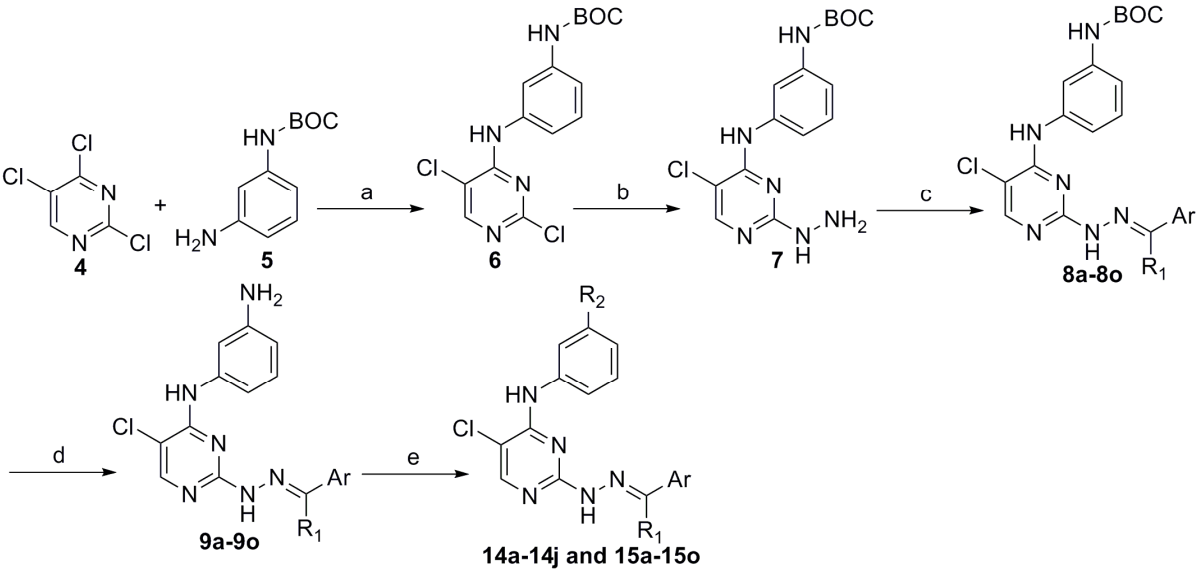


gefitinib 1 dacominitib 2 WZ4002 3 CO-1686





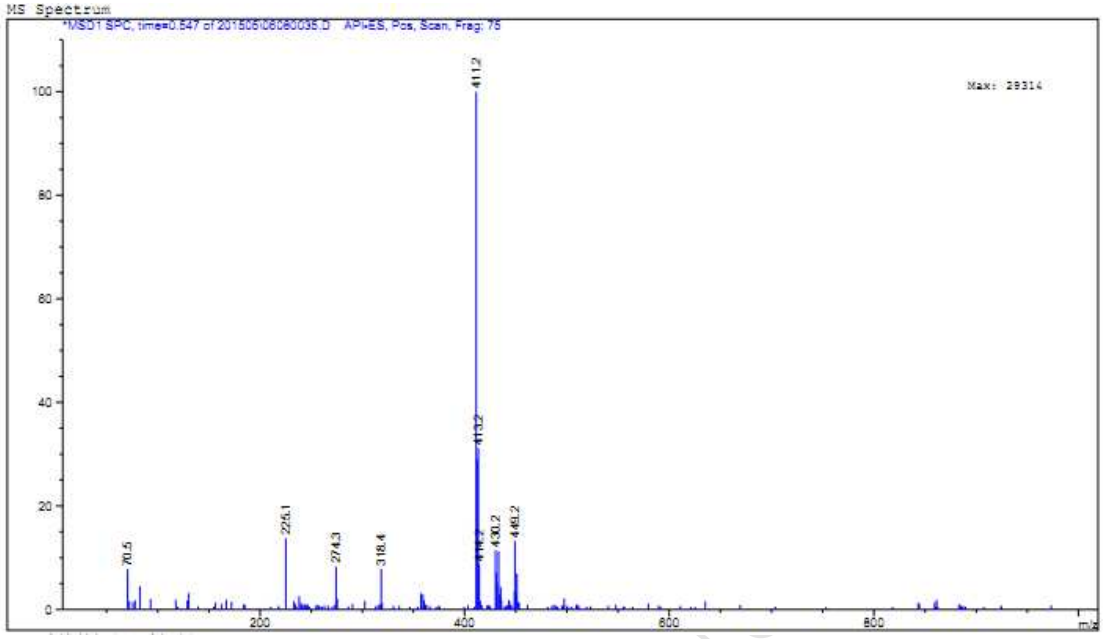
12a and 13a: R = F
12b and 13b: R = OCH_3



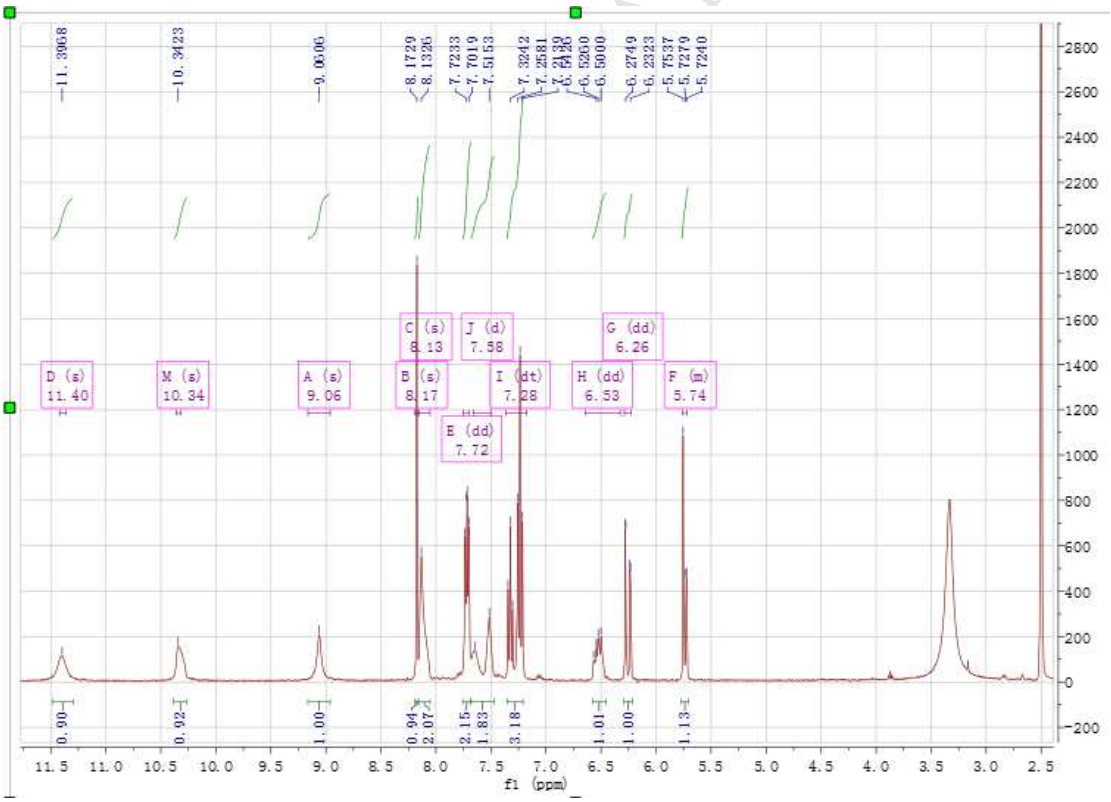
- A series of novel aminopyrimidines were identified as selective EGFR inhibitors.
- The compounds potently inhibited EGFR expressing T790M mutation.
- The compounds effectively suppressed proliferation of H1975 cells.
- Compounds **14a**, **15g** and **15i** were promising candidates for further development.

Compound 14a:

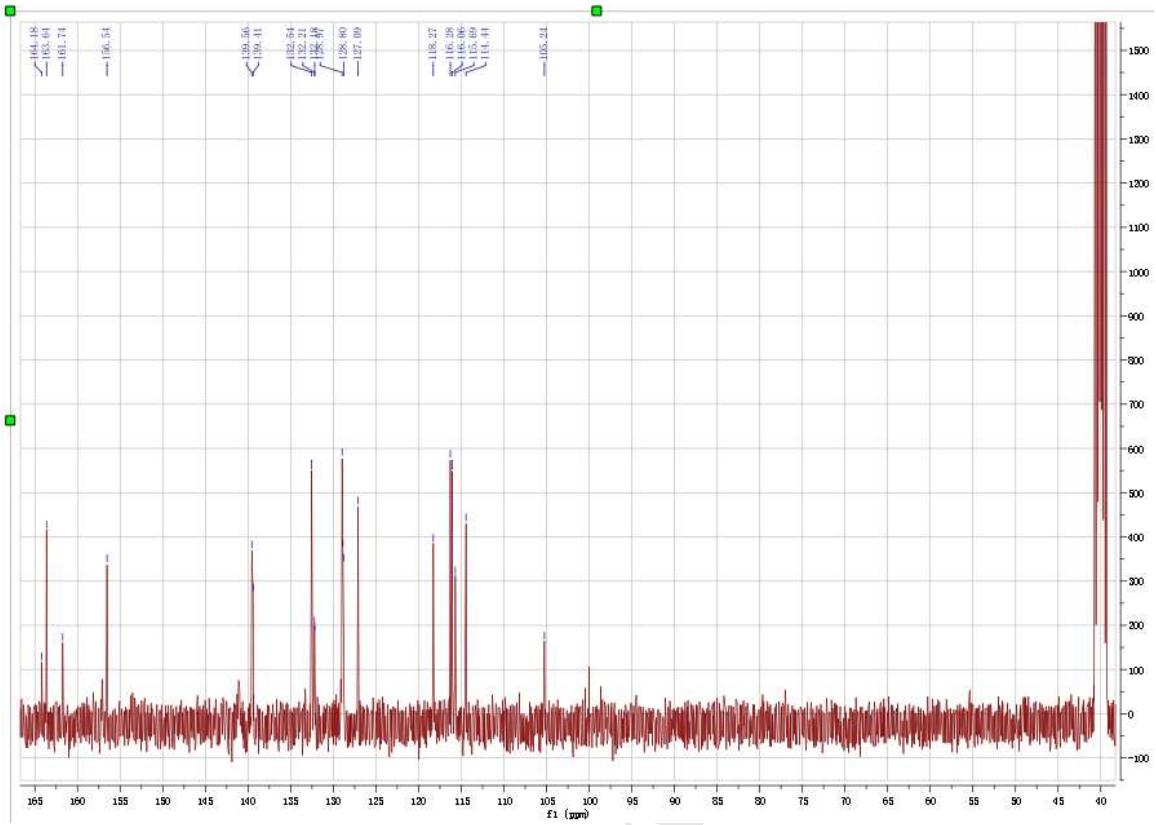
1. The MS of compound **14a**



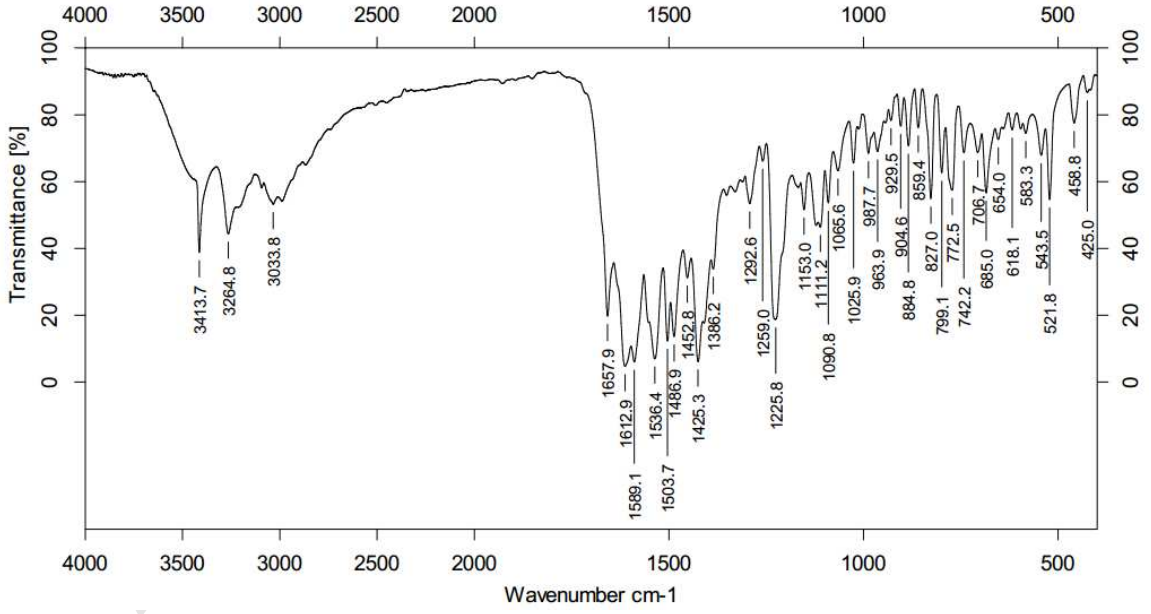
2. The ^{1}H-NMR (400 MHz, DMSO-d_6) of compound **14a**



3. The ^{13}C-NMR (101 MHz, DMSO-d_6) of compound **14a**

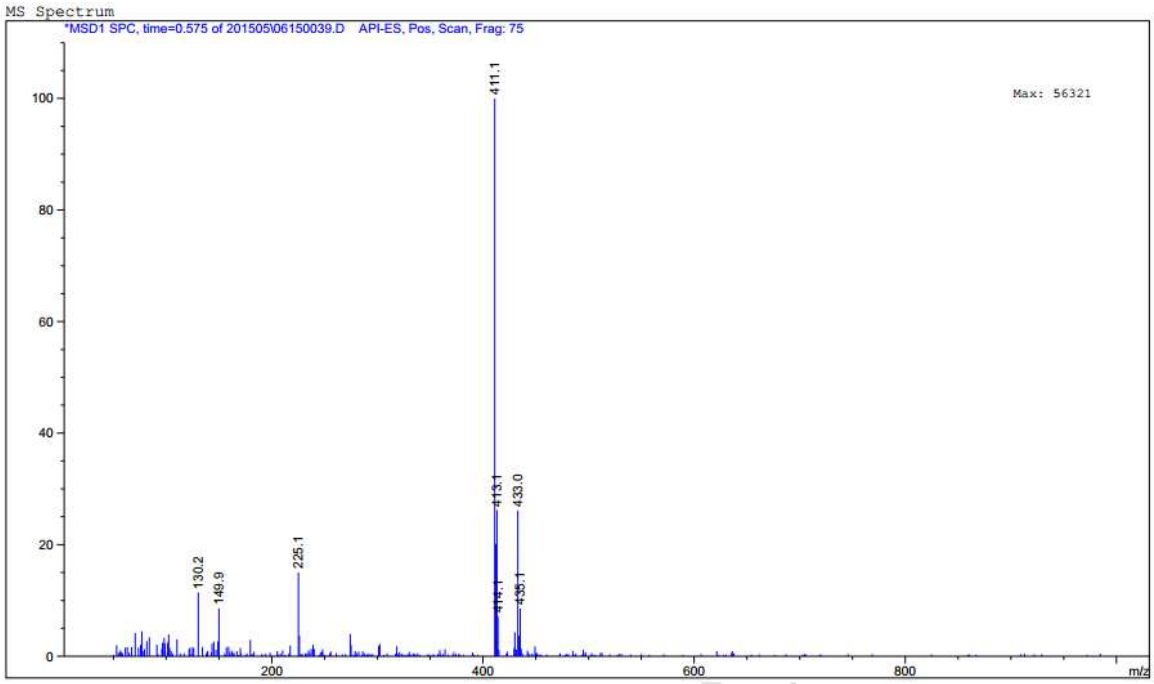


4. The IR spectra of compound **14a**

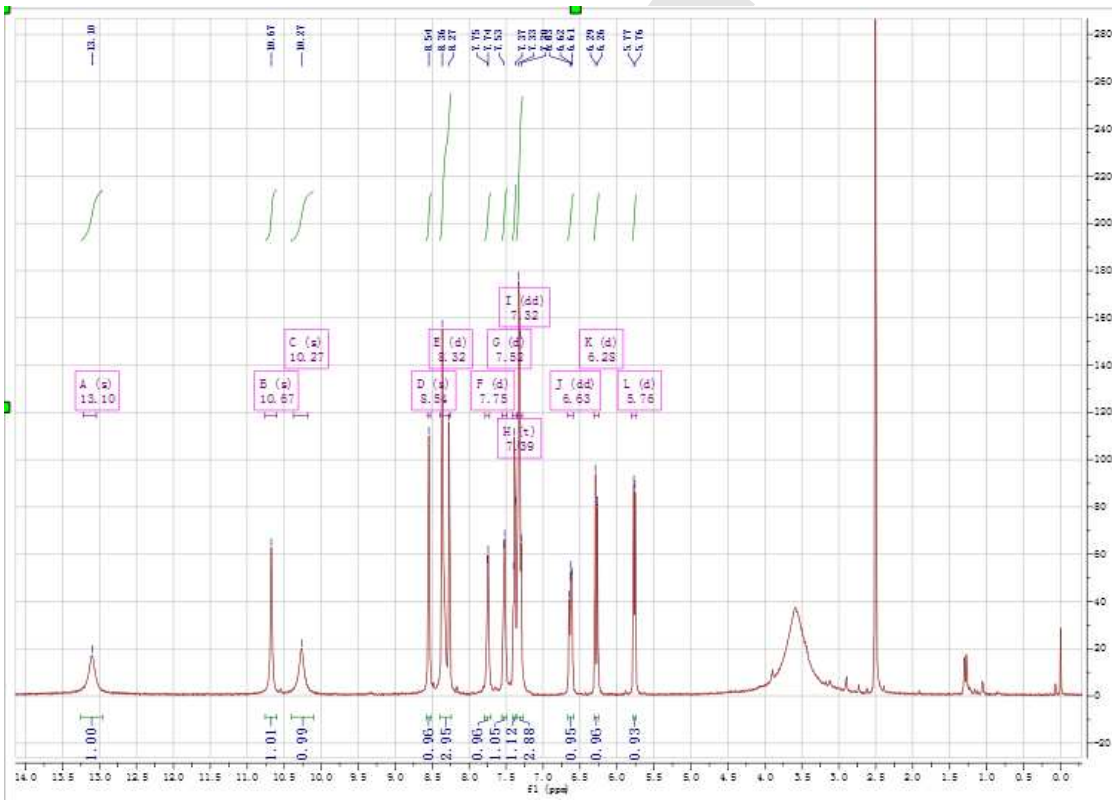


Compound 14b:

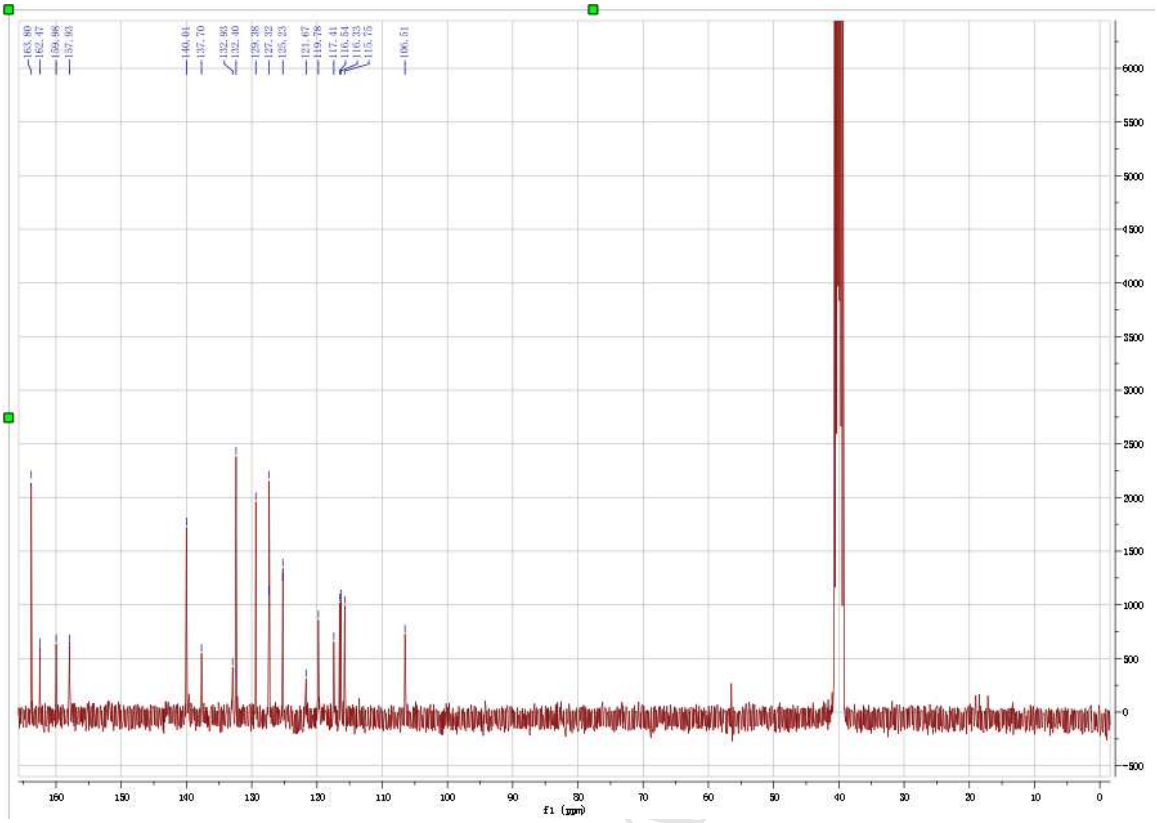
1. The MS of compound **14b**



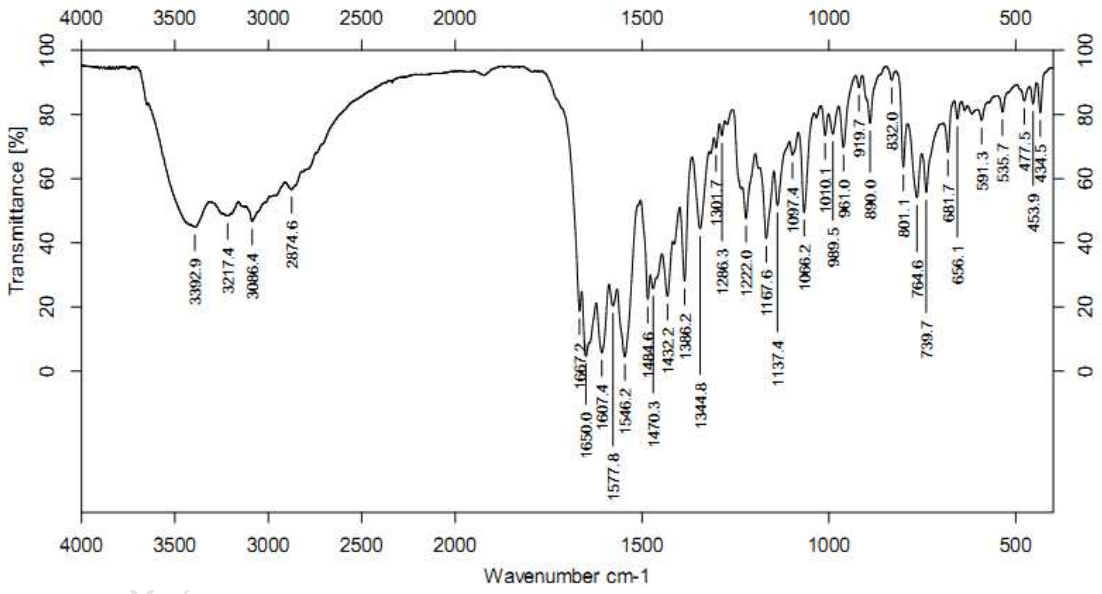
2. The ^{1}H-NMR (600 MHz, DMSO-d_6) of compound **14b**



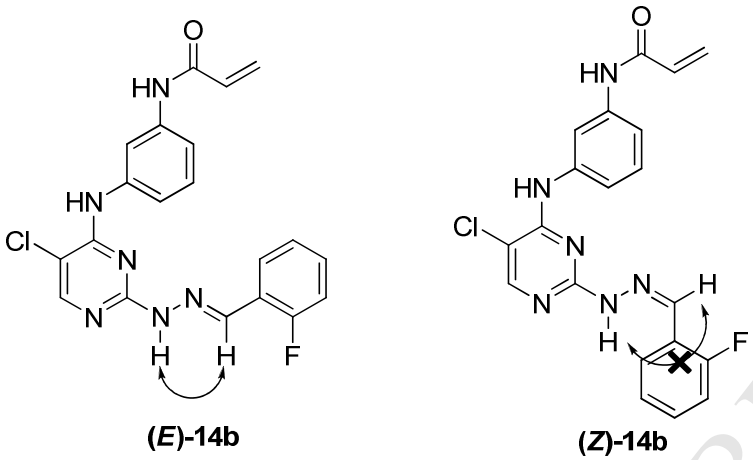
3. The ^{13}C-NMR (101 MHz, DMSO-d_6) of compound **14b**



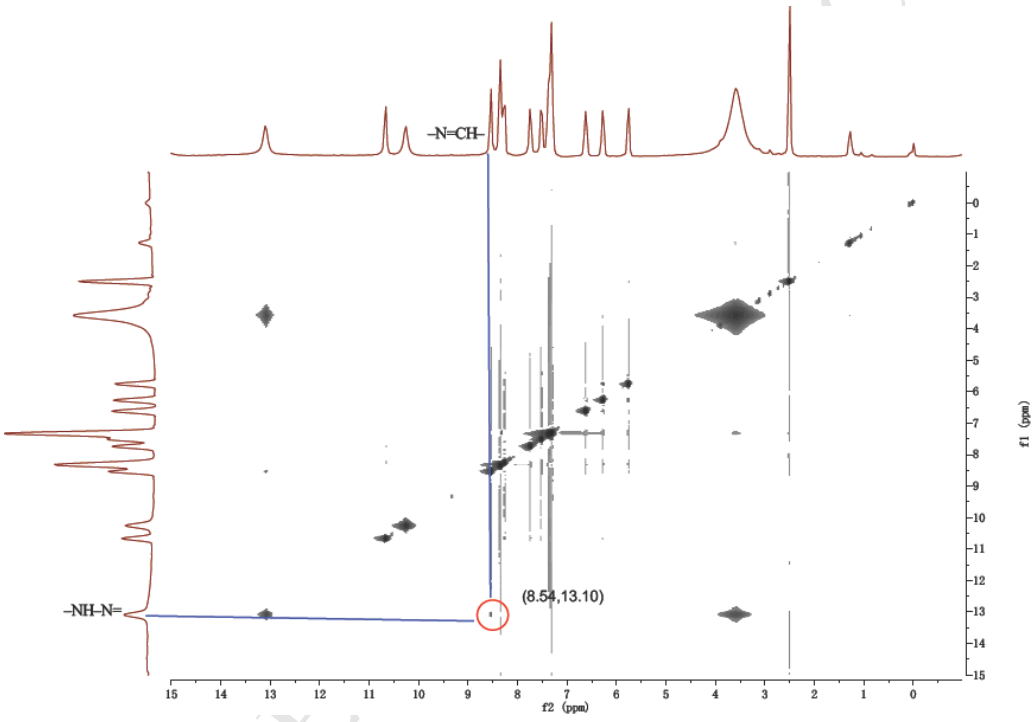
4. The IR spectra of compound **14b**



5. The structure of compound **(*E*)-14b**

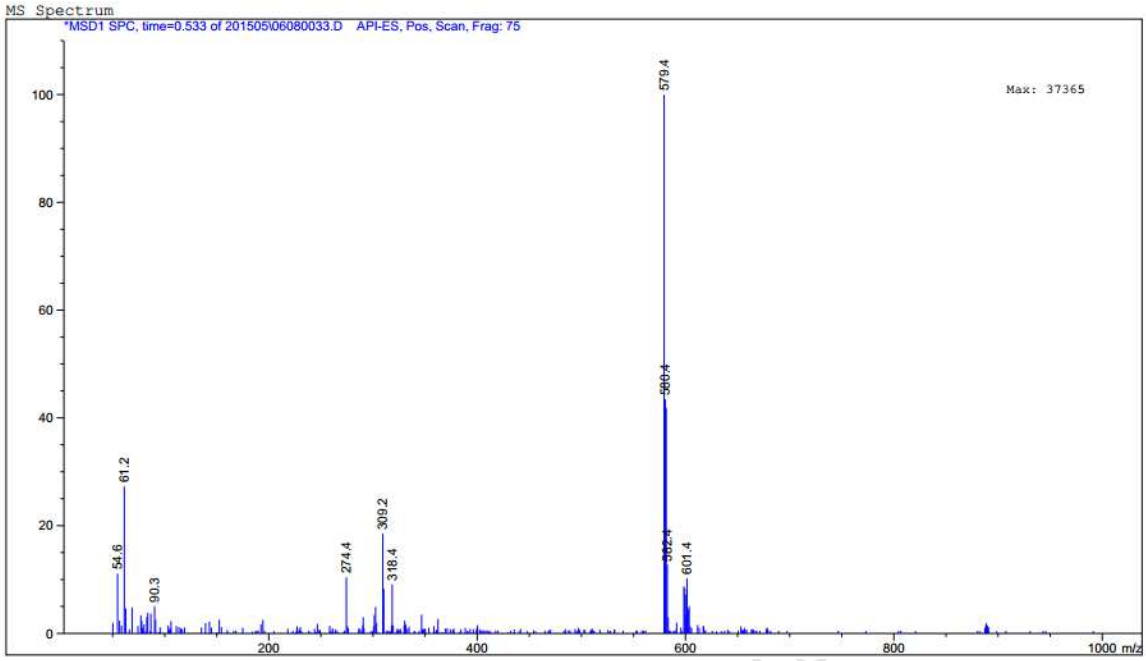


6. NOESY Spectrum (600 MHz, DMSO-d_6) of compound **(*E*)-14b**

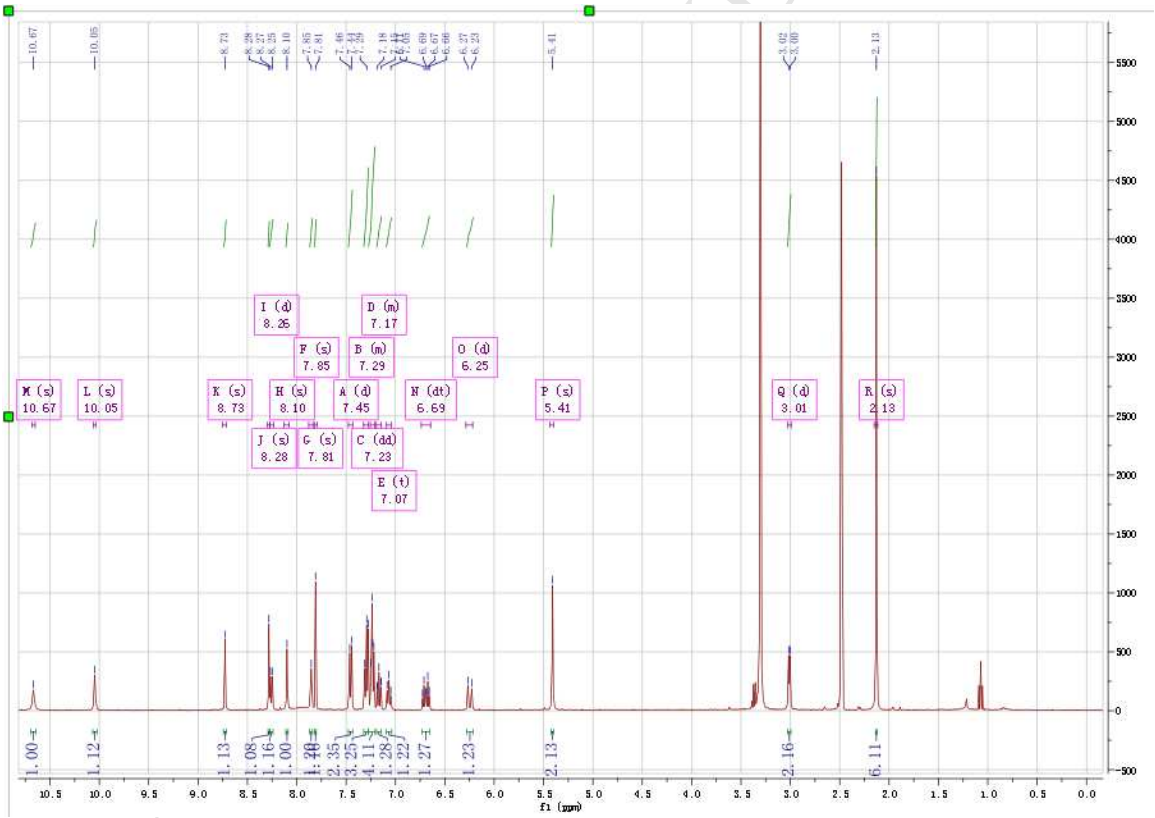


Compound **15g:**

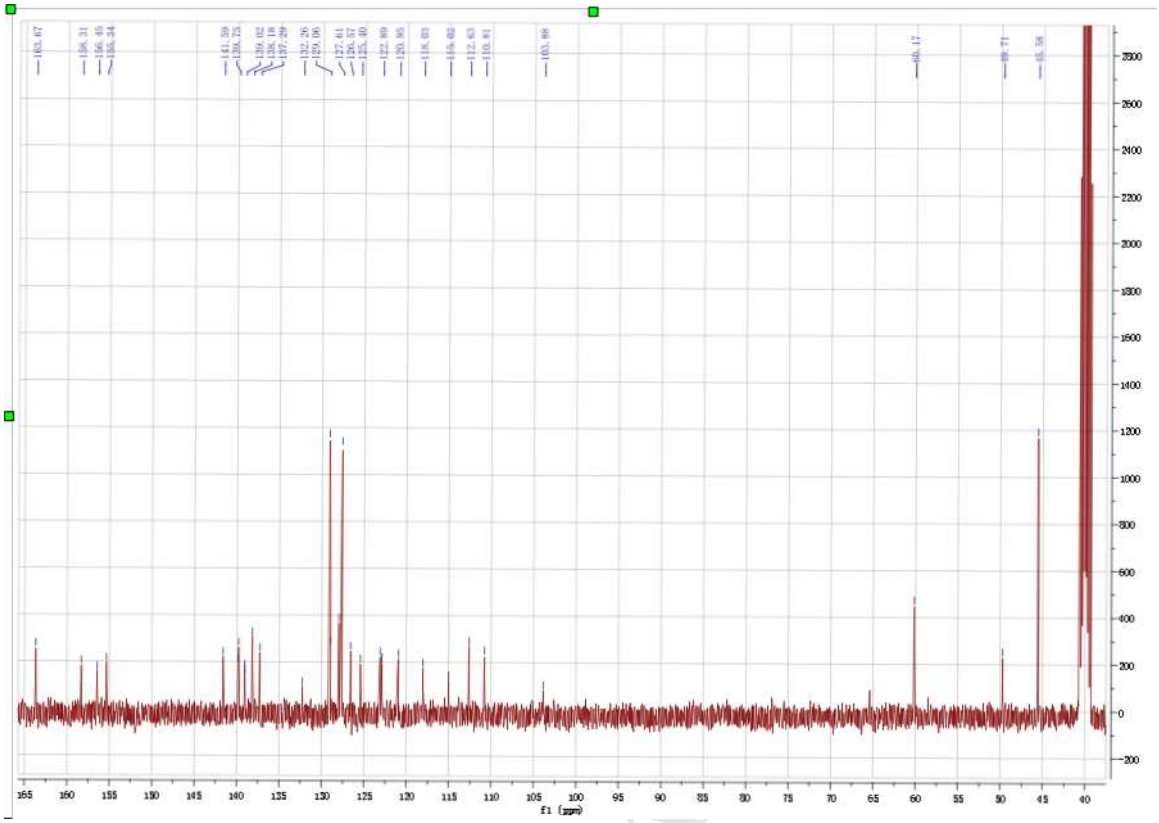
1. The MS of compound **15g**



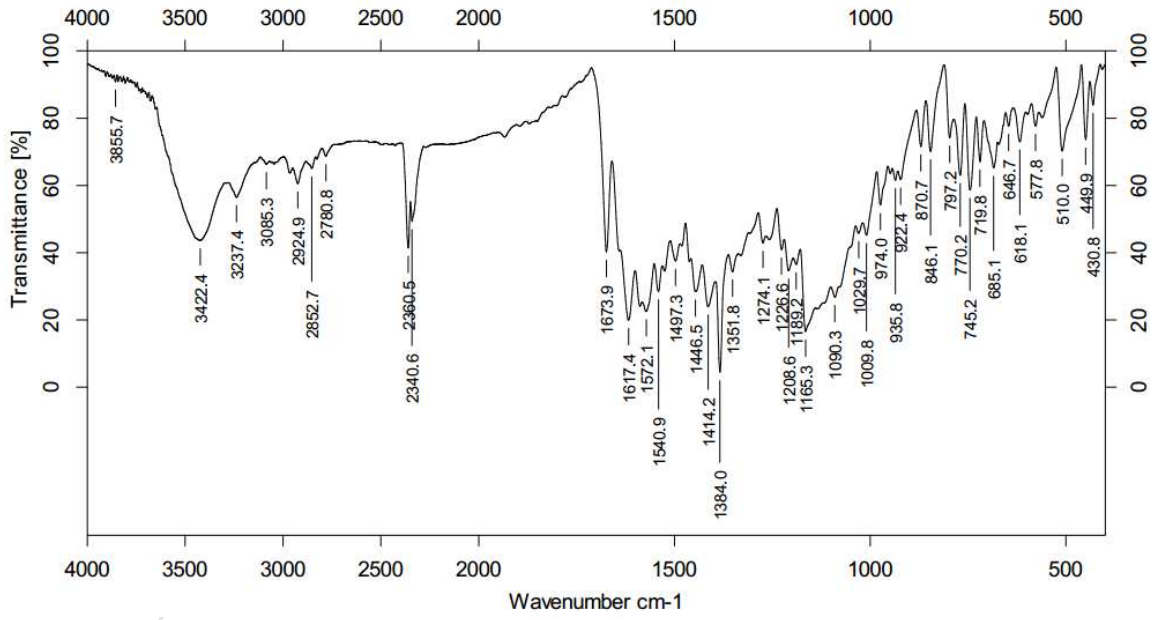
2. The ^{1}H-NMR (400 MHz, DMSO-d_6) of compound **15g**



3. The ^{13}C-NMR (101 MHz, DMSO-d_6) of compound **15g**

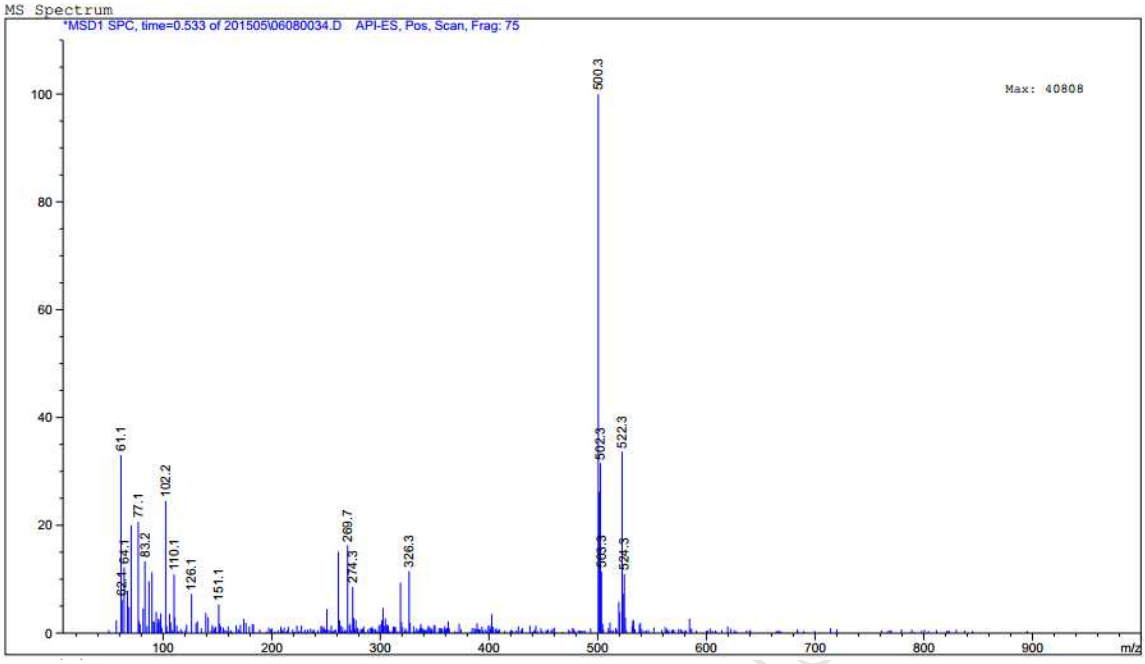


4. The IR spectra of compound **15g**

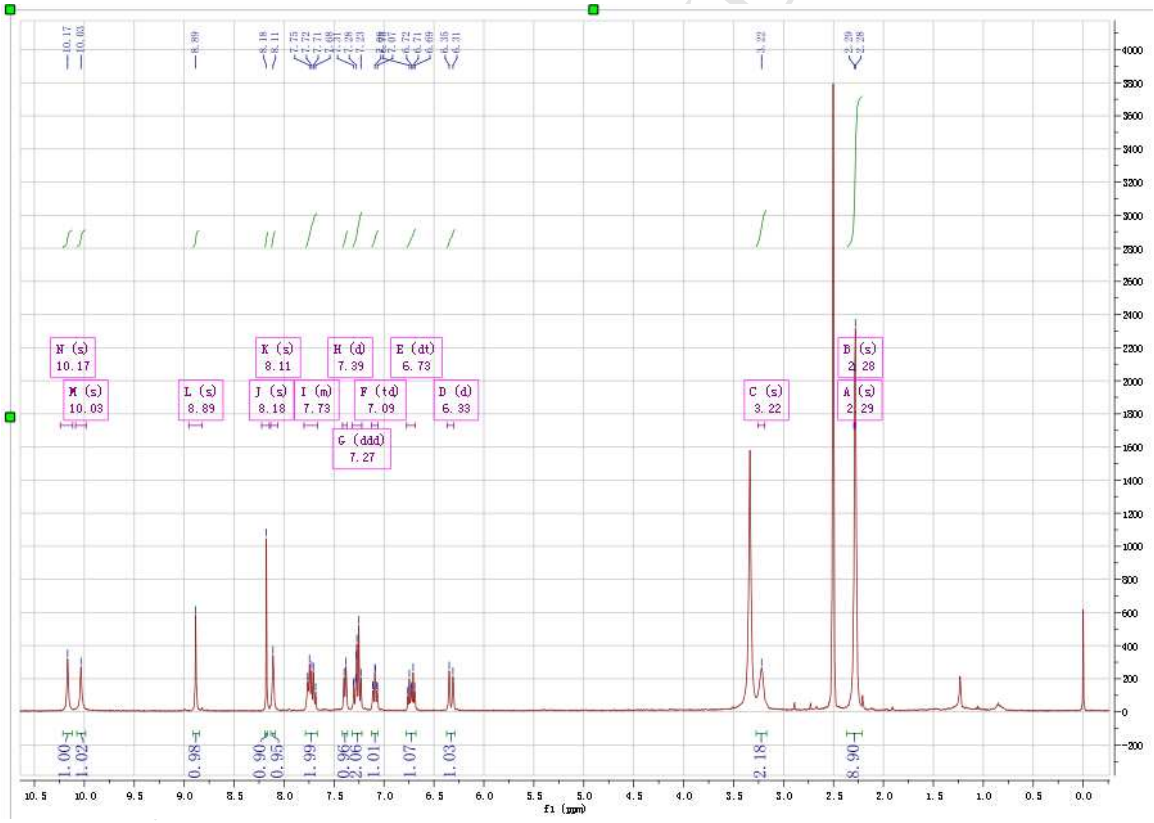


Compound **15i**:

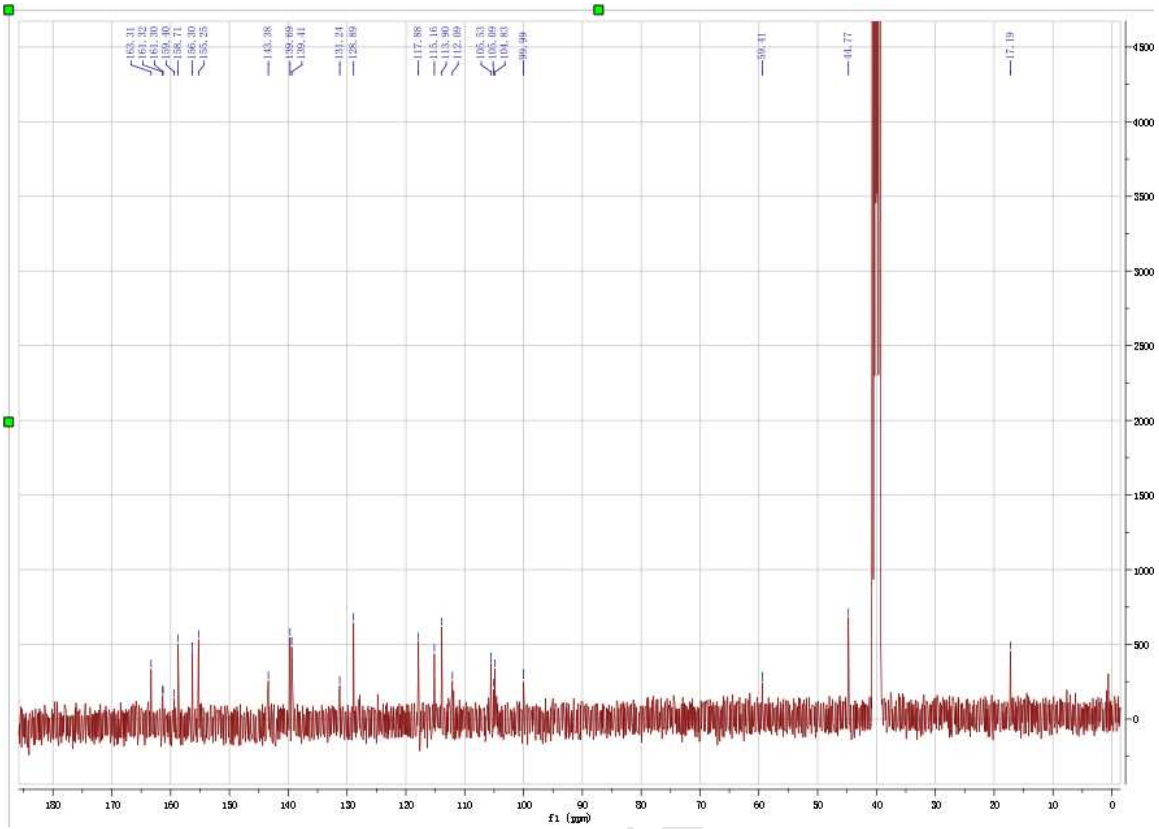
1. The MS of compound **15i**



2. The ^{1}H-NMR (400 MHz, DMSO-d_6) of compound **15i**



3. The ^{13}C-NMR (101 MHz, DMSO-d_6) of compound **15i**



4. The IR spectra of compound **15i**