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# Design, synthesis and antitumor activity evaluation of trifluoromethyl-substituted pyrimidine derivatives

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ABSTRACT

In order to find efficient new antitumor drugs, a series of novel trifluoromethyl-substituted pyrimidine derivatives were designed and synthesized, and the bioactivity against four human tumor cells (PC-3, MGC-803, MCF-7 and H1975) was evaluated by MTT assay. Compound **17v** displayed potent anti-proliferative activity on H1975 ($IC_{50}$ = 2.27 μM), which was better than the positive control 5-FU ($IC_{50}$ = 9.37 μM). Further biological evaluation studies showed that compound **17v** induced apoptosis of H1975 cells and arrested the cell cycle at G2/M phase. Furthermore, compound **17v** induced H1975 cells apoptosis through increasing the expression of pro-apoptotic proteins Bax and p53 and down-regulating the anti-apoptotic protein Bcl-2. In addition, compound **17v** was able to be tightly embedded in the active pocket of EGFR. In summary, these results demonstrated that compound **17v** has a potential as a lead compound for further investigation.

Although researchers have made a lot of efforts, cancer is still the second leading cause of mortality after cardiovascular diseases[1]. As a new cancer treatment modality, cancer immunotherapy has made breakthrough progress in the treatment of numerous cancers[2,3]. Cancer immunotherapy stimulates host immune responses to destroy local and metastatic cancer cells[4]. Even though it brings promising results, chemotherapy still plays an important role in the field. Chemotherapy, one of three acknowledged cancer treatments, is frequently used in clinical cancer therapy[5]. However, due to the existence of defects such as poor selectivity and serious side effects, it is imperative to develop drugs with better antitumor activity and lower toxicity[6,7]. Pyrimidine is a nitrogen-containing heterocyclic compound with a wide range of pharmacological effects, especially in antitumor applications.[8,9]. For example, anticancer drugs such as the reversible ALK/EGFR inhibitor Brigatinib (**1**)[10] and the BTK inhibitor Vecabrutinib (**2**)[11] (Fig. 1).

In recent years, N-(3-aminophenyl)acrylamide as an active fragment has played a great role in antitumor activity. Compound **3** displayed high activity against $EGFR^{T790M/L858R}$ kinase ($IC_{50}$ = 0.71 nM) and repressed H1975 cell replication harboring $EGFR^{T790M}$ mutations at a concentration of 0.037 μM[12]. Li et al[13] reported a series of 2,5-diaminopyrimidine derivatives, among them, the most potent compound (**4**) showed potent anti-proliferative activities toward multiple B-cell lymphoma cell lines, and significantly inhibited BTK enzyme with $IC_{50}$ value of 5 nM. In addition, introducing a trifluoromethyl group into an organic molecular structure can greatly improve their physicochemical, and biological properties, such as enhanced binding selectivity, higher lipophilicity, and increased metabolic stability[14]. Compared with wild-type EGFR ($IC_{50}$ = 465 nM), compound **5** showed higher inhibitory selectivity to mutant $EGFR^{L858R/T790M}$ ($IC_{50}$ = 10.4 nM). Moreover, the potent antiproliferative activity against H1975 cells ($IC_{50}$ = 15 nM), suggested that compound **5** is a potentially useful chemical entity for the treatment of NSCLC[15]. Wang et al[16] reported a series of 3,5-disubstituted and 3,5,7-trisubstituted quinolines. Compound **6** showed the most potent enzyme activity with $IC_{50}$ of<1.0 nM. In addition, it markedly inhibited the proliferation in both NIH-3 T3-TPR-Met and BaF3-TPR-Met cell models with $IC_{50}$ of 2.0 μM and 0.39 μM.

Based on the studies of pyrimidine derivatives, we found that some compounds with better bioactivity have a sulfhydryl group at the 2-position of the pyrimidine ring or a trifluoromethyl group at the 6-position[17–20]. Therefore, the 2-mercapto-6-trifluoromethyl-pyrimidine was selected as the scaffold to study. We used the principle of molecular

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hybridization to introduce acrylamide and trifluoromethyl group into the pyrimidine skeleton to form a series of pyrimidine derivatives and evaluate their bioactivity in vitro.

The synthetic route to **16a-z** was depicted in Scheme 1. The key intermediate **10** and **15a-z** were synthesized according to previously reported procedure[21–23]. In the presence of triethylamine,

3-nitroaniline (**7**) and acryloyl chloride (**8**) were reacted in acetonitrile to form the intermediate **9**, followed by a reduction reaction in the presence of iron powder to give the key intermediate **10**. Then, reaction of ethyl 4,4,4-trifluoro-3-oxobutanoate (**11**) with thiourea (**12**) in ethanol under reflux afforded pyrimidine **13**. Compound **13** reacted with diverse benzyl chlorides in the presence of KOH to offer compounds **14a-z**, which were dissolved in 1,4-dioxane, and then heated with phosphorus oxychloride to obtain the key intermediate **15a-z**. The intermediates **15a-z** and **10** were heated at 80℃ in N, *N*-dimethylformamide (DMF) for 10 h to form the target products

**16a-z**. After that, **15d** was reacted with heterocyclics and anilines to obtain compounds **17a-z**. The structures of target compounds **16a-z** and **17a-z** were confirmed by $^{1}$H NMR, $^{13}$C NMR and high-resolution mass spectrometry (HR-MS).

The bioactivities of all the target compounds against PC-3 (human prostate cancer cell line), MGC-803 (human gastric cancer cell line), MCF-7 (human breast cancer cell line) and H1975 (non-small cell - lung cancer cell line) were evaluated by MTT assay and 5-Fluorouracil (5-FU) as the positive control. The results were summarized in Tables 1 − 2.

To investigate the effect of the acrylamide group on bioactivity, compounds **16a-z** were synthesized initially, and their bioactivities were shown in Table 1. Compared with compound **16u**, which had no substituent on benzene ring, the introduction of electron-donating groups at $R^1$ did not increase the bioactivity. Conversely, introducing an electron-withdrawing group remarkably increased the bioactivity. It was worth noting that increasing the electron withdrawing ability of substituent significantly improved the bioactivity of most compounds. In addition, the position of substituent also played a great role on the bioactivity, *para*-substitution had better bioactivity than *meta*-substitution, while *ortho*-substitution had worse bioactivity. Interestingly, replacement of the benzene ring by thiazole or aliphatic ring (**16v-z)** led to no obvious change in bioactivity. Particularly, compound **16d** with 4-$CF_3$ substitution exhibited a better inhibitory effect than the positive control 5-Fu.

Next, through further structure – activity relationship (SAR) studies, we found that different heterocyclics and anilines substitutions at $R^2$ were also important for the bioactivity. Here, we replaced the N-(3-aminophenyl)acrylamide of the best compound **16d** in the previous series with different heterocycles and anilines to synthesize the target compounds **17a-z**. The bioactivities of these compounds were shown in Table 2. As compared to the compound **16d**, the removal of the acrylamide group led to a decrease in the bioactivity of compound **17o**. Acrylamide can form a covalent bond with the cell target, which might be the reason for the loss of activity after removal. Interestingly, a similar phenomenon could be observed when acrylamide was substituted by other substituents. As shown in Table 2, most heterocyclic and piperazine substitutions attached to the 4-position of the pyrimidine skeleton also decreased the bioactivity. Particularly, compound **17v** exhibited the best bioactivity against H1975 ($IC_{50}$ = 2.27 μM) than the positive control 5-Fu ($IC_{50}$ = 9.37 μM) and compound **16d** ($IC_{50}$ = 4.77 μM).

Compounds **16d** and **17v** were further examined for possible cytotoxicity against GES-1 (normal human gastric epithelial cell line). As shown in Table 3, compared with 5-FU, we found that compounds **16d** and **17v** exhibited weak cytotoxicity against GES-1. The results indicated that compounds **16d** and **17v** had higher selectivity between the selected cancer cell line (H1975) and a normal cell line (GES-1).

Based on the above findings, in order to investigate the mechanism of these series of compounds, compound **17v** and H1975 cell line were selected for further study. Cancer cell colony formation can be used as an indirect assessment method of tumor transformation[24]. Therefore, we firstly explored the effect on cell colony formation. The results displayed that **17v** was able to inhibit the colony formation of H1975 cells dose-dependently (Fig.2A) Moreover, as the concentration of **17v** increased, the colonies by the compound-added group became smaller and fewer, compared with the control group. Obviously, the inhibition rate of compound **17v** was achieved 90% at 4 μM. Cell cycle arrest is a marker closely related to cell proliferation inhibition[25], the effect of compound **17v** on cell cycle progression was investigated. As shown in Fig. 2B, with

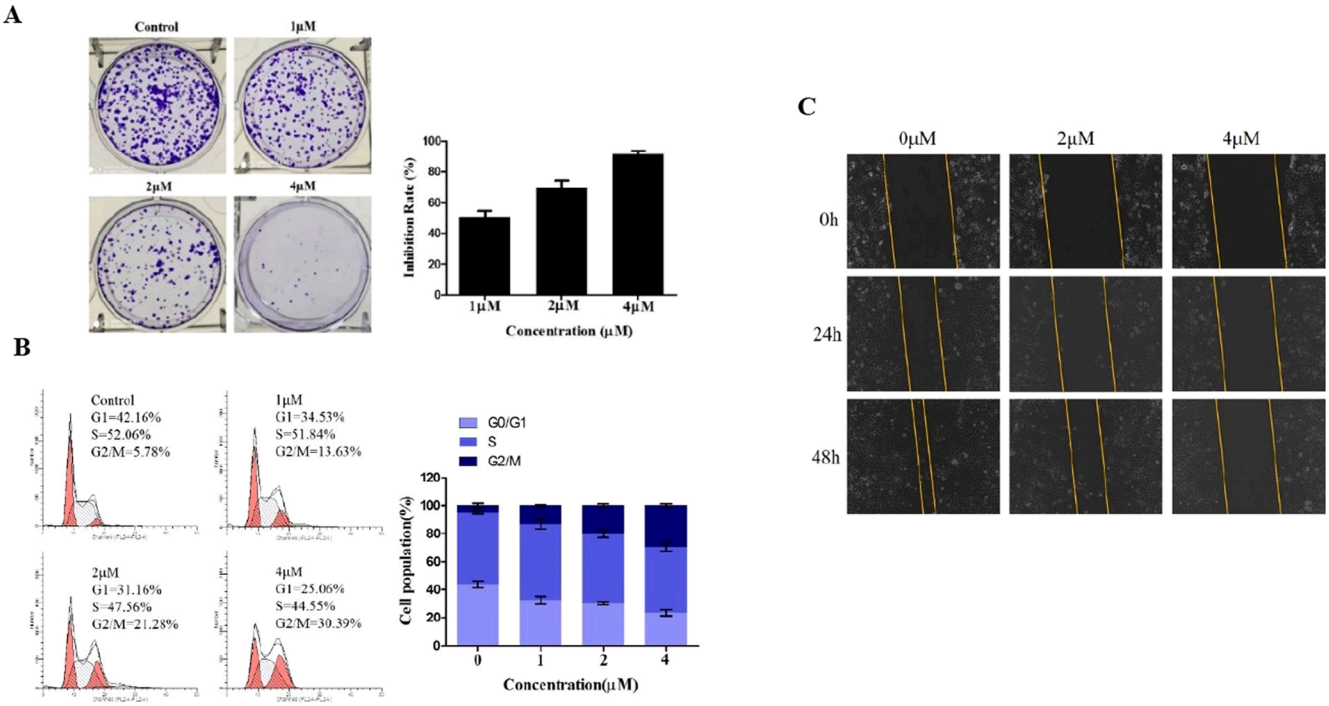
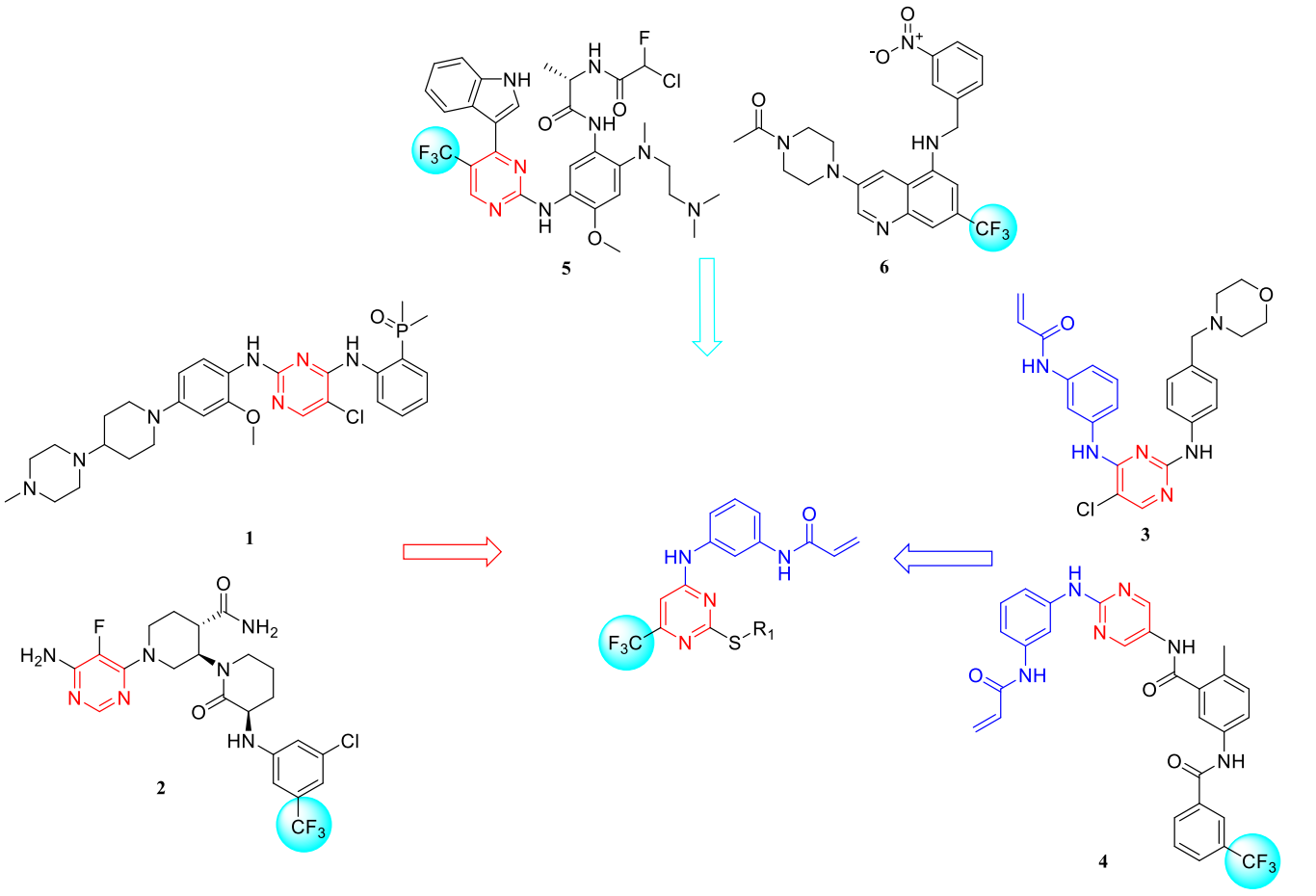



**Figure 1.** Design strategy of trifluoromethyl-substituted pyrimidine derivatives.



**Scheme 1.** Synthesis of the Target Pyrimidine-Based Derivatives. Reagents and conditions: (a) acetonitrile, $Et_3N$, 0°C, 2 h; (b) absolute methanol, iron powder, ammonium chloride, 65°C, 2 h; (c) absolute ethanol, KOH, reflux, 6 h; (d) Acetone-$H_2O$, diverse benzyl chlorides, KOH, 80°C, 1.5 h; (e) 1,4-Dioxane, $POCl_3$, 90°C, 1 h; (f) N-(3-aminophenyl)acrylamide, DMF, absolute $K_2CO_3$, 80°C, 10 h; (g) DMF, absolute $K_2CO_3$, 80°C, 6 h.

the increasing concentration of compound **17v** (0, 1, 2, and 4 μM), the percentage of H1975 cells in G2/M phase increased dramatically (from 5.78% to 30.39%), which evidently indicated that compound **17v** could assert H1975 cell at G2/M phase.

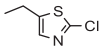
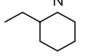
Next, in consideration of the higher bioactivity of **17v** against H1975 cells, we investigated whether compound **17v** could effectively inhibit the migration and invasion of H1975 cells. As shown in Fig. 2C, compound **17v** significantly and dose-dependently decreased the scratch-repaired capacity of the cells, which indicated that compound **17v** inhibited the migration of H1975 cells in a time-dependent and concentration-dependent manner.

To determine whether the inhibitory effect of **17v** on cell proliferation were accompanied by enhanced cancer cell apoptosis, DAPI staining was performed after treating H1975 cells of compound **17v** with variable level (0, 2, and 4 μM) for 48 h. As shown in Fig. 3A, control groups of H1975 cells showed intact nucleus; while after treatment of compound **17v**, the nucleus shrinked, dense particles appeared, the nucleus were broken into fragments and the number of change in apoptotic morphology increased significantly. These findings indicated that compound **17v** could induce apoptosis in H1975 cells. In addition, Annexin V-FITC/PI double staining flow cytometry method was also used. As shown in Fig. 3B, in H1975 cells, compound **17v** increased the percentage of apoptotic cells from 3.86% of the control to 5.04%, 11.13% and 16.68% (including the early and late apoptosis) at 1, 2 and 4 μM for 48 h, respectively, while the percentage of apoptotic cells increased to 7.70%, 17.31% and 29.08% at the same concentrations for 72 h. These results indicated that compound **17v** could induce apoptosis in H1975 cells. Finally, in order to further evaluate the mechanism of compound **17v** on H1975 cells, western blot analysis was used to explore the expression of apoptosis-related proteins. As shown in Fig. 3C, the expression of pro-apoptotic proteins Bax and p53 was significantly increased, while anti-apoptotic protein Bcl-2 was down-regulated. In summary, these results indicated that compound **17v** induced H1975 cells apoptosis through the activation of intrinsic apoptotic pathway.

At present, many third-generation EGFR inhibitors are based on pyrimidine[26,27], so we decided to insert the compounds **16d** and **17v** into the EGFR pocket to explore the interaction between compounds and EGFR. By using MOE 2020 for molecular docking, we found that compounds **16d** and **17v** were able to be tightly embedded in the pocket of EGFR (PDB code: 5GNK), which was retrieved from the protein database (http://www.rcsb.org/pdb) for the docking calculations. As shown in Fig. 4, as anticipated, compound **16d** formed a covalent bond between its acryloyl group and the Cys797 reside in the EGFR protein. Moreover, compound **16d** formed a hydrogen bond with residue Met793 (2.38 Å). Interestingly, compound **17v** used a completely different binding mode from **16d**. Compound **17v** interacted with Val726 (2.92 Å) by π-H interaction. At the same time, the 2-mercapto-1,3,4-thiadiazole group of compound **17v** formed hydrogen bonds with Arg841 (3.68 Å), Asn842 (3.32 Å), Phe723 (2.52 Å) and Gly724 (2.44 Å), respectively. These findings indicated that compounds **16d** and **17v** may have inhibitory effects on EGFR, while the detail mechanisms need to be further examined in our future study.

In conclusion, a series of novel 2,4-substituted pyrimidine derivatives were synthesized, and the anti-proliferative activities of the target compounds against PC-3, MGC-803, MCF-7, and H1975 cells lines

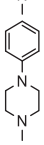
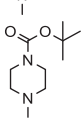
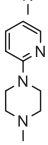
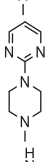
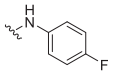
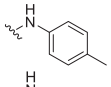
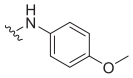
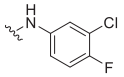
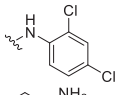
**Table 1**
Anti-proliferative activities [$IC_{50}$/(μmol•$L^{-1}$)] of **16a-z** against four cancer cell lines[a].

| Compounds | $R^1$ | $IC_{50}$(μM) PC-3 | MGC-803 | MCF-7 | H1975 |
|---|---|---|---|---|---|
| **16a** | 4-$CH_3$-Bn | 17.81 ± 1.25 | 18.47 ± 1.26 | 25.71 ± 1.14 | 14.98 ± 0.77 |
| **16b** | 3-$CH_3$-Bn | 9.05 ± 0.95 | 12.56 ± 1.09 | 26.71 ± 1.42 | 13.23 ± 1.12 |
| **16c** | 2-$CH_3$-Bn | 15.05 ± 1.17 | 18.19 ± 1.08 | 33.65 ± 1.52 | 14.63 ± 1.16 |
| **16d** | 4-$CF_3$-Bn | 5.65 ± 0.98 | 9.80 ± 0.99 | 7.08 ± 0.85 | 4.77 ± 0.44 |
| **16e** | 3-$CF_3$-Bn | 5.92 ± 0.77 | 18.68 ± 1.27 | 12.41 ± 1.09 | 5.07 ± 0.70 |
| **16f** | 2-$CF_3$-Bn | 7.96 ± 0.91 | 26.61 ± 1.42 | 17.18 ± 1.23 | 6.55 ± 0.55 |
| **16g** | 4-$OCH_3$-Bn | 15.82 ± 0.76 | 15.07 ± 0.71 | 7.59 ± 0.88 | 10.24 ± 1.01 |
| **16h** | 3-$OCH_3$-Bn | 11.53 ± 1.06 | 32.44 ± 1.51 | 8.91 ± 0.95 | 9.45 ± 0.97 |
| **16i** | 2-$OCH_3$-Bn | 9.96 ± 0.99 | 32.71 ± 1.51 | 22.03 ± 1.34 | 6.50 ± 0.81 |
| **16j** | 4-F-Bn | 9.22 ± 0.96 | 9.25 ± 0.96 | 14.93 ± 0.69 | 10.14 ± 1.11 |
| **16k** | 3-F-Bn | 8.57 ± 0.89 | 7.14 ± 0.85 | 23.03 ± 1.36 | 13.73 ± 1.03 |
| **16l** | 2-F-Bn | 8.67 ± 0.93 | 13.38 ± 1.12 | 31.14 ± 1.19 | 15.42 ± 0.73 |
| **16m** | 4-Cl-Bn | 9.99 ± 1.02 | 29.32 ± 1.46 | 15.77 ± 1.10 | 11.69 ± 0.43 |
| **16n** | 3-Cl-Bn | 10.52 ± 1.02 | 35.21 ± 1.54 | 10.13 ± 1.06 | 14.79 ± 0.57 |
| **16o** | 2-Cl-Bn | 13.56 ± 1.13 | 28.45 ± 1.45 | 11.81 ± 1.07 | 13.14 ± 0.71 |
| **16p** | 4-Br-Bn | 6.32 ± 0.81 | 29.41 ± 1.46 | 11.70 ± 0.67 | 6.44 ± 0.92 |
| **16q** | 3-Br-Bn | 7.44 ± 0.87 | 22.81 ± 1.35 | 14.27 ± 1.15 | 7.44 ± 0.65 |
| **16r** | 2-Br-Bn | 8.17 ± 0.91 | 30.86 ± 1.49 | 20.15 ± 1.30 | 9.62 ± 0.75 |
| **16s** | 2.4-diCl-Bn | 6.66 ± 0.82 | 18.58 ± 1.26 | 6.25 ± 0.79 | 15.31 ± 0.72 |
| **16t** | 2.6-diCl-Bn | 14.55 ± 1.16 | 34.68 ± 1.54 | 9.66 ± 0.98 | 9.03 ± 0.95 |
| **16u** | Bn | 14.16 ± 1.15 | 22.29 ± 1.34 | 19.06 ± 1.09 | 21.94 ± 0.84 |
| **16v** |  | 11.53 ± 1.06 | 38.32 ± 1.58 | 10.72 ± 1.03 | 10.89 ± 0.83 |
| **16w** |  | 12.48 ± 1.09 | 34.12 ± 1.53 | 11.24 ± 1.05 | 17.99 ± 0.90 |
| **16x** |  | 8.96 ± 0.96 | 28.34 ± 1.45 | 17.67 ± 1.24 | 16.29 ± 0.51 |
| **16y** |  | 12.87 ± 1.11 | 37.24 ± 1.57 | 18.32 ± 1.26 | 17.59 ± 0.74 |
| **16z** |  | 12.63 ± 1.10 | 38.66 ± 1.58 | 14.16 ± 1.15 | 19.18 ± 0.62 |
| **5-FU**[b] | – | 6.23 ± 0.67 | 9.05 ± 0.96 | 8.77 ± 0.56 | 9.37 ± 0.39 |

[a] Antitumor activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% ($IC_{50}$). Data are presented as the mean ± SDs of three independent experiments
[b] Positive control.

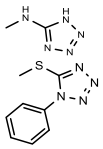
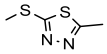
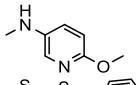
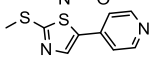
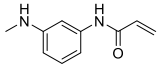
were evaluated. Among them, compound **17v** showed the best bioactivity against H1975 cells. Further mechanism research showed that compound **17v** significantly inhibited the migration and colony formation of H1975 cells and induced cell cycle arrest at G2/M phase. Moreover, DAPI staining, apoptosis and western blot experiments indicated that compound **17v** induced H1975 cells apoptosis through the activation of intrinsic apoptotic pathway. In addition, molecular docking revealed that compound **17v** could bind well to the pocket of EGFR. Taken together, these results suggested that compound **17v** might be a valuable lead compound for antitumor agents.

**Table 2**
Anti-proliferative activities [$IC_{50}$ / (μmol•$L^{-1}$)] of **17a-z** against four cancer cell lines[a].

| Compounds | $R^2$ | $IC_{50}$(μM) PC-3 | MGC-803 | MCF-7 | H1975 |
|---|---|---|---|---|---|
| **17a** |  | 15.88 ± 1.20 | 5.87 ± 0.27 | 10.73 ± 1.03 | 6.21 ± 0.79 |
| **17b** |  | 40.84 ± 1.89 | >50 | >50 | >50 |
| **17c** |  | 46.82 ± 1.83 | 44.03 ± 1.64 | >50 | >50 |
| **17d** |  | 34.67 ± 1.54 | 15.82 ± 1.19 | 30.16 ± 1.48 | 23.91 ± 1.37 |
| **17e** |  | 27.21 ± 1.43 | 25.61 ± 1.25 | 21.98 ± 1.34 | 14.97 ± 1.17 |
| **17f** |  | 42.74 ± 1.91 | 35.12 ± 1.34 | >50 | >50 |
| **17g** |  | 40.61 ± 1.60 | 22.15 ± 1.87 | >50 | >50 |
| **17h** |  | >50 | 23.54 ± 1.02 | >50 | 17.38 ± 0.86 |
| **17i** |  | >50 | 35.24 ± 1.03 | >50 | 18.34 ± 0.91 |
| **17j** |  | 14.13 ± 1.87 | 19.23 ± 1.45 | >50 | 17.45 ± 1.77 |
| **17k** |  | 16.46 ± 1.98 | 32.33 ± 1.47 | >50 | 20.56 ± 1.51 |
| **17l** |  | 35.34 ± 1.54 | 8.70 ± 0.94 | 22.23 ± 1.34 | 13.75 ± 1.13 |
| **17m** |  | 34.97 ± 1.68 | 41.44 ± 1.54 | >50 | 22.09 ± 1.78 |
| **17n** |  | 29.59 ± 1.45 | 35.45 ± 1.55 | >50 | 33.54 ± 1.76 |
| **17o** |  | 23.15 ± 1.36 | 8.97 ± 0.95 | 13.54 ± 1.13 | 23.56 ± 1.12 |
| **17p** |  | 19.51 ± 1.29 | 15.54 ± 1.19 | 10.87 ± 1.03 | 9.92 ± 0.99 |
| **17q** |  | 11.96 ± 1.07 | 21.26 ± 1.32 | 14.47 ± 1.16 | 5.84 ± 0.76 |
| **17r** |  | >50 | 28.58 ± 1.45 | >50 | 14.03 ± 1.14 |
| **17s** |  | 25.21 ± 1.40 | >50 | 43.22 ± 1.63 | 23.94 ± 1.37 |
| **17t** | | >50 | >50 | >50 | >50 |

(*continued on next page*)

**Table 2** (*continued*)

| Compounds | $R^2$ | $IC_{50}$(μM) PC-3 | MGC-803 | MCF-7 | H1975 |
|---|---|---|---|---|---|
| **17u** |  | 45.26 ± 1.65 | 12.41 ± 1.09 | 39.48 ± 0.35 | 24.32 ± 1.38 |
| **17v** |  | 7.88 ± 1.03 | 10.85 ± 1.03 | 8.81 ± 1.27 | 2.27 ± 0.35 |
| **17w** |  | >50 | 11.44 ± 1.05 | >50 | 3.06 ± 0.48 |
| **17x** |  | 40.59 ± 1.60 | 24.47 ± 1.38 | 44.93 ± 1.65 | 24.17 ± 1.38 |
| **17y** |  | 23.83 ± 1.37 | 12.22 ± 0.08 | 36.91 ± 1.56 | 6.32 ± 0.80 |
| **17z** |  | 7.35 ± 0.86 | 5.77 ± 0.76 | 10.09 ± 1.04 | 16.74 ± 1.22 |
| **16d** |  | 5.65 ± 0.98 | 9.80 ± 0.99 | 7.08 ± 0.85 | 4.77 ± 0.44 |
| **5-FU**[b] | – | 6.23 ± 0.67 | 9.05 ± 0.96 | 8.77 ± 0.56 | 9.37 ± 0.39 |

[a] Antitumor activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% ($IC_{50}$). Data are presented as the mean ± SDs of three independent experiments
[b] Positive control

**Table 3**
Anti-proliferative activities [$IC_{50}$ / (μmol•$L^{-1}$)] of **16d** and **17v** against human normal cell line[a].

| Compounds | $IC_{50}$(μM) H1975 | GES-1 | SI[c] |
|---|---|---|---|
| **16d** | 4.77 ± 0.44 | 31.47 ± 0.79 | 6.60 |
| **17v** | 2.27 ± 0.35 | 37.42 ± 0.86 | 16.48 |
| **5-FU**[b] | 9.37 ± 0.39 | 19.27 ± 0.58 | 2.06 |

[a] Cells were treated with different concentrations of the compounds for 72 h. $IC_{50}$ values are the Mean ± SD of three separate experiments.
[b] Positive control. $SI^c = IC_{50}$(GES-1)/$IC_{50}$(H1975)

## Experimental

### *General procedure*

Reagents and solvents were purchased from commercial sources and were used without further purification. All reactions were monitored by thin-layer chromatography (TLC) with detection by UV light (254 nm). $^1$H and $^{13}$C NMR spectra were respectively determined with a 400 and 101 MHz spectrometer. High-resolution mass spectra of target compounds were recorded on a Waters Q-T Micromass spectrometer using electrospray ionization. The melting points were measured on an X-5 micromelting apparatus.

### *General procedure for the synthesis of compound **9***

Compound **9** was synthesized according to the literature[21]. 3-nitroaniline (7.24 mmol, 1.00 eq) was dissolved in 20 mL acetonitrile, then triethylamine (10.86 mmol, 1.50 eq) and acryloyl chloride (10.86 mmol, 1.50 eq) were slowly added in an ice bath. The reaction mixture was stirred at room temperature for 2 h, and then it was added to ice water (100 mL) with constant stirring. After 5 min, the precipitate was filtered under reduced pressure and washed with water to yield product **9** (brown solid, yield 82%).

### *General procedure for the synthesis of compound **10***

Compound **10** was synthesized according to the literature[21]. Compound **9** (5.20 mmol, 1.00 eq), iron powder (36.43 mmol, 7.00 eq) and ammonium chloride (15.61 mmol, 3.00 eq) in 30 mL of absolute methanol were heated at 65 ℃ for 2 h. The reaction mixture was filtered under reduced pressure, water was added to the filtrate, and the mixture was extracted with ethyl acetate, and then purified by column chromatography to get the target compound **10** (yellow solid, yield 63%).

### *General procedure for the synthesis of compound **13***

Compound **13** was synthesized according to the literature[22]. Potassium hydroxide (32.59 mmol, 1.50 eq) was dissolved in 50 mL absolute ethanol, and then thiourea (26.07 mmol, 1.20 eq) and ethyl 4,4,4-trifluoroacetoacetate (21.73 mmol, 1.00 eq) were added. The reaction mixture was stirred and heated under reflux for 6 h. Upon completion, the precipitated product was filtered off and washed with ethanol to afford the product **13** (white solid, yield 79%).

### *General procedure for the synthesis of compounds **14a-z***

Compounds **14a-z** were synthesized according to the literature[23]. Potassium hydroxide (7.11 mmol, 1.50 eq) and compound **13** (5.69 mmol, 1.20 eq) in 25 mL of water was stirred at room temperature for 5 min. Next, diverse benzyl chlorides (1.00 eq) were dissolved in 3 mL of acetone and added to the above mixture. The reaction mixtures were heated to 80 °C for 1.5 h. Upon completion, the precipitated products were filtered off and washed with water to afford the products **14a-z** (yield 68–90%).

### *General procedure for the synthesis of compounds **15a-z***

Compounds **15a-z** were synthesized according to the literature[23]. Compounds **14a-z** (2.10 mmol 1.00 eq) were dissolved in 15 mL 1,4-Dioxane, then excessive $POCl_3$ (2–3 mL) was slowly added. The reaction mixtures were heated to 90 °C for 1 h, cooled to room temperature, and then the mixtures were added to ice water (100 mL) while stirring. The precipitates were filtered under reduced pressure and washed with water to yield compounds **15a-z** (yield 57–76%).

### *General procedure for the synthesis of compounds **16a-z***

Compound **10** (787.62 μmol, 1.20 eq) and absolute $K_2CO_3$ (984.53 μmol, 1.50 eq) were dissolved in 5 mL DMF and then compounds **15a-z** (1.00 eq) were added, next, the reaction mixtures were heated to 80 °C for 10 h. When the reactions were completed, extracted with water and ethyl acetate, and then purified by column chromatography to get the target compounds **16a-z**. All of the target compounds have never been reported in published articles.

*N-(3-((2-((4-methylbenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide **(16a)*** white solid, yield 61%, mp 176.4–177.8℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.24 (s, 1H), 10.21 (s, 1H), 8.13 (s, 1H), 7.42 (d, $J$ = 6.6 Hz, 1H), 7.36–7.28 (m, 2H), 7.25 (d, $J$ = 8.0 Hz, 2H), 7.07 (d, $J$ = 7.9 Hz, 2H), 6.87 (s, 1H), 6.44 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.26 (dd, $J$ = 17.0, 1.9 Hz, 1H), 5.76 (dd, $J$ = 10.1, 1.9 Hz, 1H), 4.35 (s, 2H), 2.25 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.71, 163.17, 160.25, 151.91, 139.47, 138.62, 136.19, 134.31, 131.76, 129.14, 128.86, 126.98, 124.71, 121.98, 119.25, 115.98, 114.93, 111.80, 34.01, 20.61. HR-MS (ESI): Calcd for $C_{22}H_{20}F_3N_4OS$ $[M+H]^+$: 445.1310, found: 445.1307.

*N-(3-((2-((3-methylbenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide **(16b)*** white solid, yield 57%, mp 164.6–165.8℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.24 (s, 1H), 10.21 (s, 1H), 8.15 (s, 1H), 7.42 (d, $J$ = 6.6 Hz, 1H), 7.34–7.28 (m, 2H), 7.16 (t, $J$ = 6.7 Hz, 3H), 7.03 (d, $J$ = 4.0 Hz, 1H), 6.87 (s, 1H), 6.45 (dd, $J$ = 17.0,

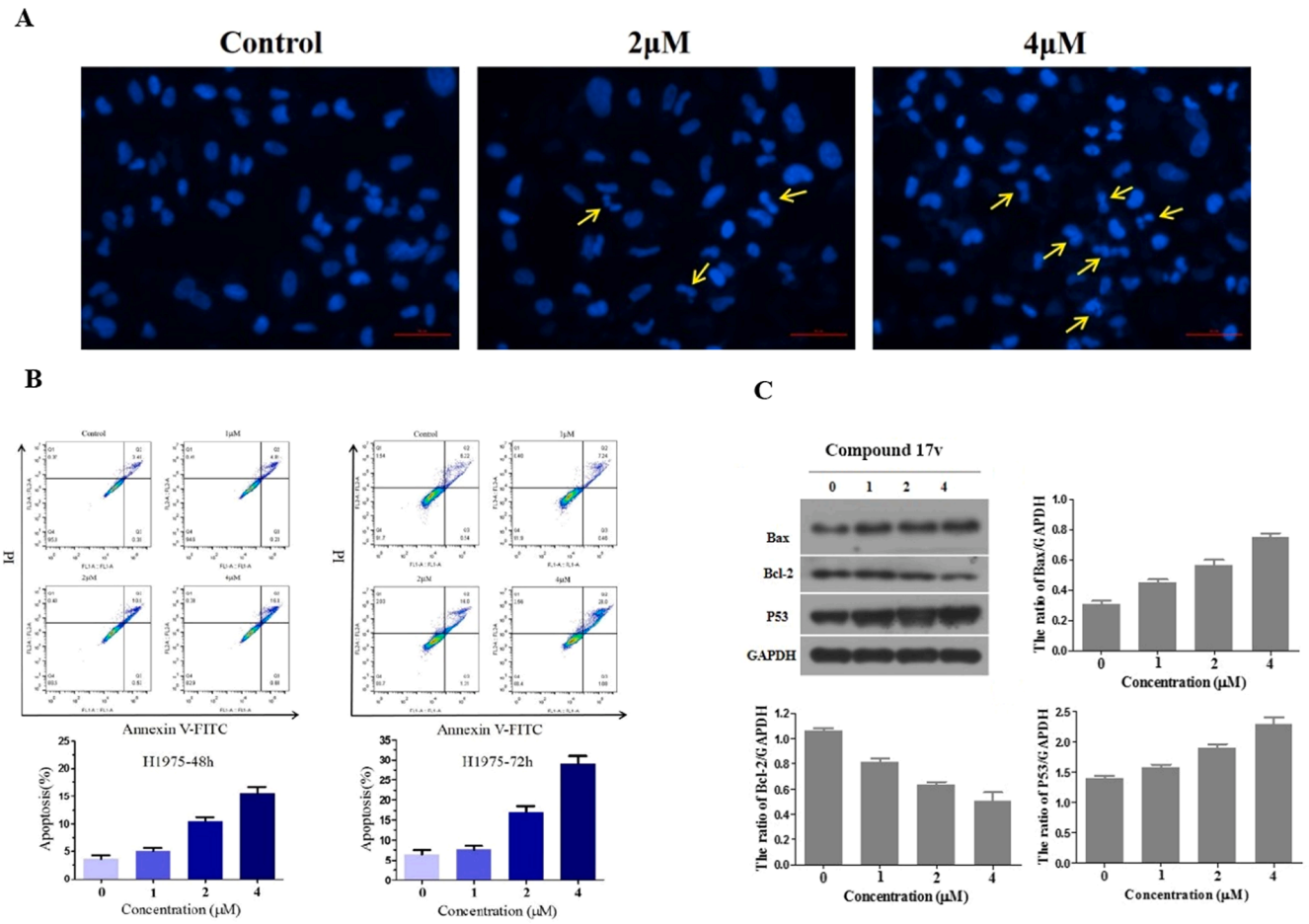



**Figure 2.** Compound **17v** inhibited the proliferation of H1975 cells. (A) H1975 cell colonies treated with compound **17v** at different concentrations for 9 days, and the quantitative analysis of colony formation inhibition rate. (B) Effect of compound **17v** on cell cycle in H1975 cells, and the quantitative analysis of cell cycle distribution of H1975 cells for 24 h. (C) Scratch assay of H1975 cells with different concentrations of compound **17v**. The dates were represented as the Mean ± SD. All experiments were performed at least three times.

10.1 Hz, 1H), 6.27 (dd, $J = 17.0$, 2.0 Hz, 1H), 5.76 (dd, $J = 10.1$, 2.0 Hz, 1H), 4.35 (s, 2H), 2.21 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.65, 163.18, 160.24, 152.26, 139.49, 138.60, 137.39, 137.32, 131.77, 129.70, 129.12, 128.18, 127.62, 126.95, 125.97, 121.98, 119.26, 116.03, 114.92, 111.84, 34.20, 20.81. HR-MS (ESI): Calcd for $C_{22}H_{20}F_3N_4OS$ $[M + H]^+$: 445.1310, found: 445.1311.

*N-(3-((2-((2-methylbenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16c)*** off-white solid, yield 62%, mp 162.2–163.3℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.26 (s, 1H), 10.21 (s, 1H), 8.16 (s, 1H), 7.42 (d, $J = 7.2$ Hz, 1H), 7.37–7.31 (m, 1H), 7.28 (dd, $J = 12.5$, 7.9 Hz, 2H), 7.21–7.13 (m, 2H), 7.09 (td, $J = 7.2$, 1.6 Hz, 1H), 6.89 (s, 1H), 6.44 (dd, $J = 17.0$, 10.1 Hz, 1H), 6.24 (dd, $J = 17.0$, 2.0 Hz, 1H), 5.74 (dd, $J = 10.1$, 2.0 Hz, 1H), 4.42 (s, 2H), 2.36 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.72, 163.17, 160.26, 152.26, 139.47, 138.64, 136.57, 134.72, 131.74, 130.16, 129.74, 129.12, 127.42, 126.93, 125.89, 121.98, 119.25, 115.91, 114.91, 111.78, 32.67, 18.78. HR-MS (ESI): Calcd for $C_{22}H_{20}F_3N_4OS$ $[M + H]^+$: 445.1310, found: 445.1310.

*N-(3-((6-(trifluoromethyl)-2-((4-(trifluoromethyl)benzyl)thio) pyrimidin-4-yl)amino)phenyl)acrylamide* ***(16d)*** white solid, yield 52%, mp 166.1–167.3℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.26 (s, 1H), 10.22 (s, 1H), 8.13 (s, 1H), 7.60 (q, $J = 8.5$ Hz, 4H), 7.38 (s, 1H), 7.35–7.26 (m, 2H), 6.88 (s, 1H), 6.46 (dd, $J = 17.0$, 10.1 Hz, 1H), 6.27 (dd, $J = 17.0$, 2.0 Hz, 1H), 5.76 (dd, $J = 10.1$, 2.0 Hz, 1H), 4.48 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.14, 163.20, 160.35, 152.25, 142.91, 139.50, 138.52, 131.74, 129.15, 127.73, 127.41, 126.97, 125.04, 122.83, 121.94, 119.21, 116.18, 115.09, 112.01, 33.56. Calcd for $C_{22}H_{17}F_6N_4OS$ $[M + H]^+$: 499.1027, found: 499.1028.

*N-(3-((6-(trifluoromethyl)-2-((3-(trifluoromethyl)benzyl)thio) pyrimidin-4-yl)amino)phenyl)acrylamide* ***(16e)*** white solid, yield 59%, mp 179.1–180.2℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.26 (s, 1H), 10.21 (s, 1H), 8.15 (s, 1H), 7.78 (s, 1H), 7.69 (d, $J = 7.6$ Hz, 1H), 7.58 (d, $J = 7.8$ Hz, 1H), 7.50 (t, $J = 7.7$ Hz, 1H), 7.39 (s, 1H), 7.35–7.26 (m, 2H), 6.88 (s, 1H), 6.45 (dd, $J = 17.0$, 10.1 Hz, 1H), 6.26 (dd, $J = 17.0$, 1.9 Hz, 1H), 5.76 (dd, $J = 10.1$, 2.0 Hz, 1H), 4.49 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.18, 163.18, 160.26, 152.20, 139.72, 139.49, 138.54, 133.04, 131.76, 129.27, 129.12, 128.77, 126.91, 125.58, 123.68, 122.69, 121.95, 119.23, 115.94, 114.95, 111.74, 33.51. Calcd for $C_{22}H_{17}F_6N_4OS$ $[M + H]^+$: 499.1027, found: 499.1048.

*N-(3-((6-(trifluoromethyl)-2-((2-(trifluoromethyl)benzyl)thio) pyrimidin-4-yl)amino)phenyl)acrylamide* ***(16f)*** white solid, yield 54%, mp174.6–175.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.31 (s, 1H), 10.19 (s, 1H), 8.12 (s, 1H), 7.73 (t, $J = 8.3$ Hz, 2H), 7.61 (t, $J = 7.5$ Hz, 1H), 7.49 (t, $J = 7.6$ Hz, 1H), 7.43 (s, 1H), 7.30–7.24 (m, 2H), 6.92 (s, 1H), 6.43 (dd, $J = 17.0$, 10.1 Hz, 1H), 6.22 (dd, $J = 17.0$, 1.9 Hz, 1H), 5.73 (dd, $J = 10.1$, 1.9 Hz, 1H), 4.61 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.04, 163.16, 160.35, 152.21, 139.51, 138.56, 135.57, 132.76, 131.73, 129.08, 127.96, 127.32, 126.87, 126.09, 125.68, 122.95, 121.93, 119.20, 115.90, 115.00, 111.79, 31.02. Calcd for $C_{22}H_{17}F_6N_4OS$ $[M + H]^+$: 499.1027, found: 499.1098.

*N-(3-((2-((4-methoxybenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16 g)*** light yellow solid, yield 70%, mp 132.6–133.4℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.24 (s, 1H), 10.21 (s, 1H), 8.13 (s, 1H), 7.44 (d, $J = 6.3$ Hz, 1H), 7.34–7.27 (m, 4H), 6.87 (s, 1H), 6.83 (d, $J = 8.7$ Hz, 2H), 6.45 (dd, $J = 17.0$, 10.1 Hz, 1H), 6.27 (dd,

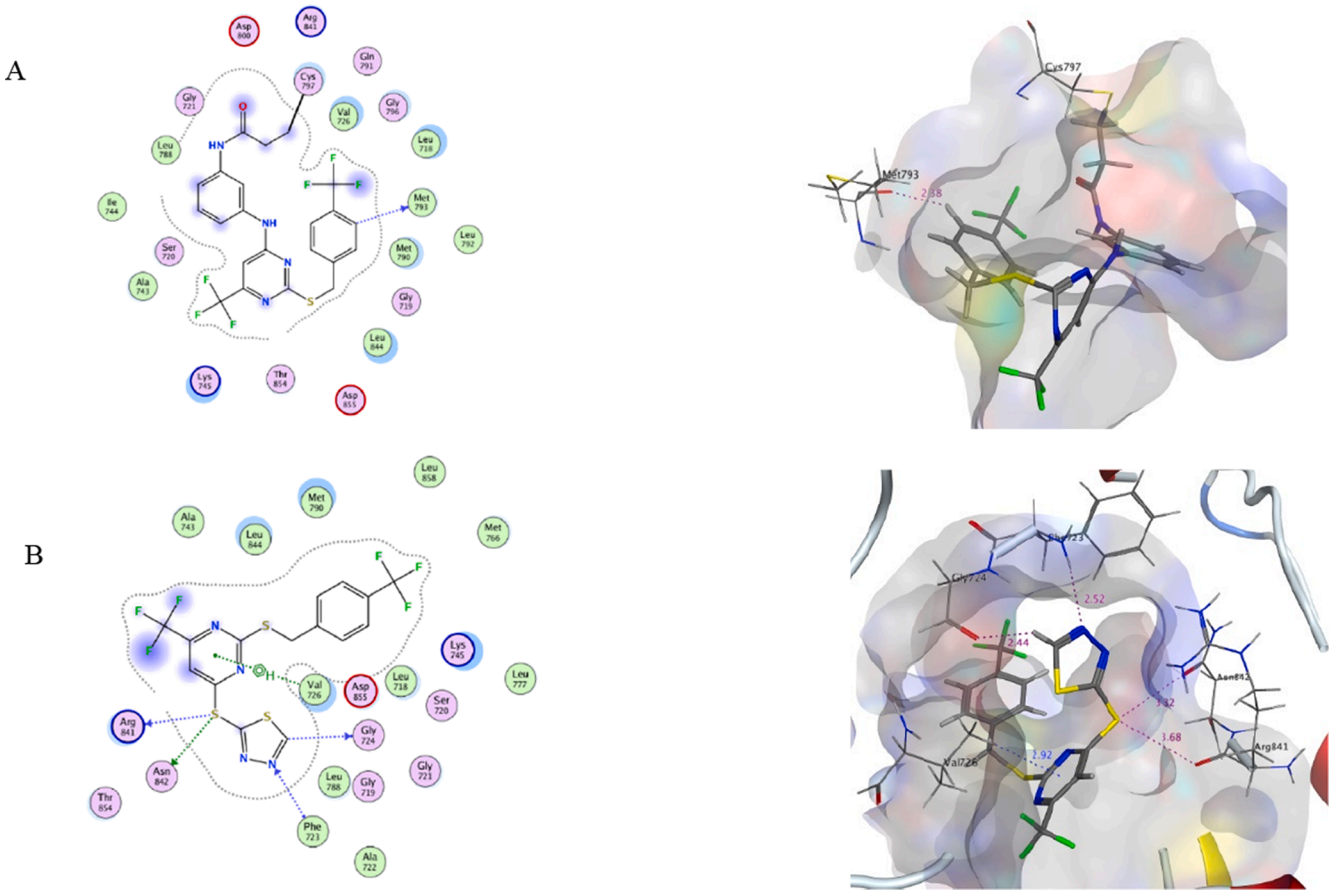



**Figure 3.** Compound **17v** induced apoptosis in H1975 cells and possible mechanisms. (A) Analysis of apoptosis of H1975 Cells with DAPI staining. Yellow arrow indicates nuclear lysis. (B) Apoptotic H1975 cells were analyzed with Annexin V-FITC/PI double staining. Quantitative analysis of apoptosis rates by flow cyomerty. (C) Expression of apoptosis-related proteins including Bax, Bcl-2 and P53. The GAPDH was used as a control. The dates were represented as the Mean ± SD. All experiments were performed at least three times. *p < 0.5, **p < 0.01, ***p < 0.001 compared with the control.

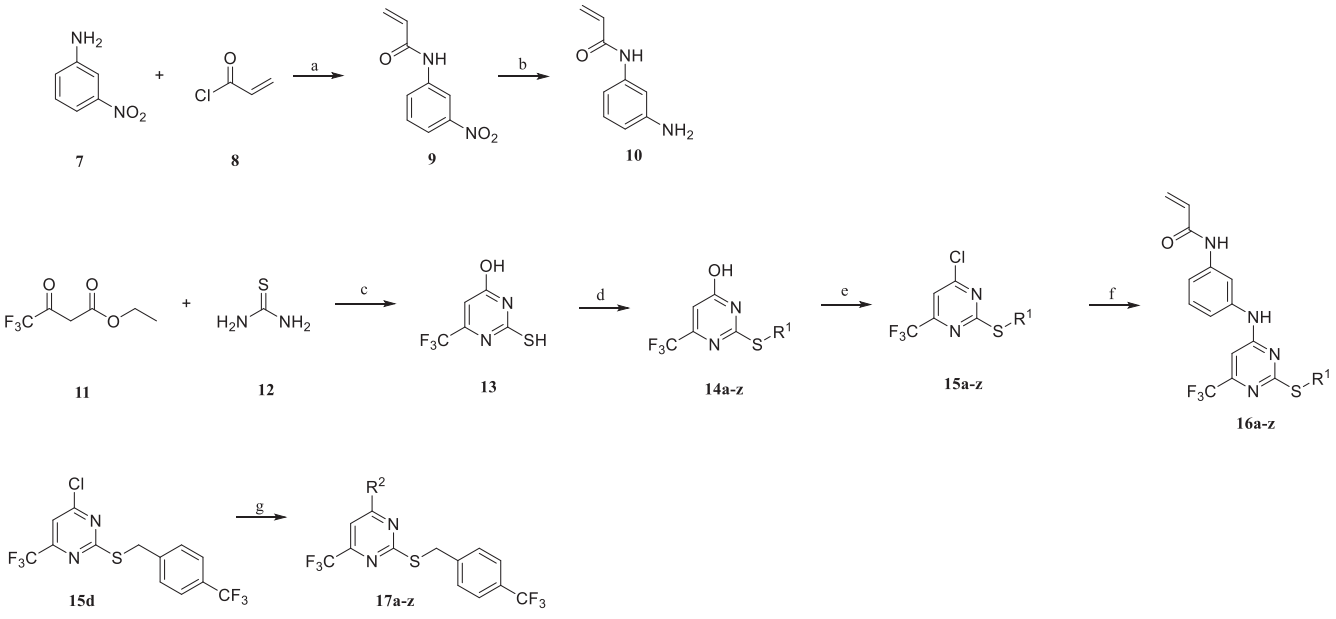



**Figure 4.** The 2D and 3D representation of the predicted binding model of compounds **16d (A)** and **17v (B)** with EGFR (PDB code: 5GNK).

$J$ = 17.0, 2.0 Hz, 1H), 5.76 (dd, $J$ = 10.1, 2.0 Hz, 1H), 4.35 (s, 2H), 3.71 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.79, 163.18, 160.26, 158.32, 152.27, 139.48, 138.64, 131.76, 130.10, 129.14, 126.98, 124.53, 121.99, 119.26, 116.02, 114.94, 113.71, 111.82, 54.98, 33.77. Calcd for $C_{22}H_{20}F_3N_4O_2S$ $[M + H]^+$: 461.1259, found: 461.1258.

*N-(3-((2-((3-methoxybenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl)*

*amino)phenyl)acrylamide* ***(16 h)*** white solid, yield 72%, mp 151.7–152.4℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.26 (s, 1H), 10.21 (s, 1H), 8.16 (s, 1H), 7.44 (d, $J$ = 6.3 Hz, 1H), 7.34–7.26 (m, 2H), 7.19 (t, $J$ = 7.9 Hz, 1H), 6.95 (d, $J$ = 7.1 Hz, 2H), 6.88 (s, 1H), 6.81–6.76 (m, 1H), 6.45 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.27 (dd, $J$ = 17.0, 2.0 Hz, 1H), 5.76 (dd, $J$ = 10.1, 2.0 Hz, 1H), 4.39 (s, 2H), 3.66 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.62, 163.18, 160.22, 159.14, 152.25, 139.50, 139.20, 138.63, 131.76, 129.36, 129.14, 126.97, 121.97, 121.12, 119.25, 115.88, 114.88, 114.31, 112.73, 111.67, 54.80, 34.17. Calcd for $C_{22}H_{20}F_3N_4O_2S$ [M + H]$^+$: 461.1259, found:461.1260.

*N-(3-((2-((2-methoxybenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16i)*** yellow solid, yield 57%, mp 151.2–152.6℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.23 (s, 1H), 10.21 (s, 1H), 8.07 (s, 1H), 7.46 (s, 1H), 7.33–7.28 (m, 3H), 7.27–7.22 (m, 1H), 7.00 (d, $J$ = 8.1 Hz, 1H), 6.87 (s, 1H), 6.82 (t, $J$ = 7.4 Hz, 1H), 6.44 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.25 (dd, $J$ = 17.0, 2.0 Hz, 1H), 5.75 (dd, $J$ = 10.1, 1.9 Hz, 1H), 4.34 (s, 2H), 3.82 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 172.06, 163.18, 160.24, 157.20, 151.88, 139.49, 138.66, 131.76, 130.16, 129.14, 128.79, 126.97, 124.93, 122.01, 120.07, 119.28, 115.99, 114.91, 111.77, 110.85, 55.43, 29.44. Calcd for $C_{22}H_{20}F_3N_4O_2S$ [M + H]$^+$: 461.1259, found: 461.1258.

*N-(3-((2-((4-fluorobenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16j)*** light yellow solid, yield 58%, mp 178.5–179.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.26 (s, 1H), 10.21 (s, 1H), 8.13 (s, 1H), 7.44–7.38 (m, 3H), 7.34–7.26 (m, 2H), 7.11–7.06 (m, 2H), 6.88 (s, 1H), 6.45 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.26 (dd, $J$ = 17.0, 2.0 Hz, 1H), 5.76 (dd, $J$ = 10.1, 2.0 Hz, 1H), 4.39 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.48, 163.18, 162.40, 160.33, 159.99, 152.24, 139.48, 138.59, 133.87, 131.75, 130.88, 129.15, 126.98, 121.97, 119.24, 115.12, 111.87, 64.98, 33.37. Calcd for $C_{21}H_{17}F_4N_4OS$[M + H]$^+$: 449.1059, found: 449.1058.

*N-(3-((2-((3-fluorobenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16 k)*** white solid, yield 48%, mp 145.8–146.3℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.26 (s, 1H), 10.22 (s, 1H), 8.15 (s, 1H), 7.41 (d, $J$ = 6.0 Hz, 1H), 7.34–7.27 (m, 3H), 7.21 (t, $J$ = 8.4 Hz, 2H), 7.08–7.01 (m, 1H), 6.89 (s, 1H), 6.46 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.30–6.24 (m, 1H), 5.78–5.74 (m, 1H), 4.42 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.31, 163.18, 163.10, 160.68, 160.29, 152.24, 140.76, 139.50, 138.56, 131.76, 130.08, 129.13, 126.95, 124.97, 121.96, 119.23, 115.57, 114.98, 113.68, 111.83, 33.60. Calcd for $C_{21}H_{17}F_4N_4OS$[M + H]$^+$: 449.1059, found: 449.1060.

*N-(3-((2-((2-fluorobenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16 l)*** white solid, yield 69%, mp 165.1–166.0℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.27 (s, 1H), 10.21 (s, 1H), 8.11 (s, 1H), 7.49–7.39 (m, 2H), 7.34–7.27 (m, 3H), 7.22–7.16 (m, 1H), 7.09 (td, $J$ = 7.5, 1.0 Hz, 1H), 6.89 (s, 1H), 6.44 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.25 (dd, $J$ = 17.0, 2.0 Hz, 1H), 5.75 (dd, $J$ = 10.1, 2.0 Hz, 1H), 4.43 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.19, 163.18, 161.72, 160.30, 159.27, 152.26, 139.50, 138.58, 131.74, 131.17, 129.42, 129.15, 126.95, 124.48, 124.25, 121.95, 119.22, 115.98, 114.98, 111.82, 27.87. Calcd for $C_{21}H_{17}F_4N_4OS$[M + H]$^+$: 449.1059, found: 449.1054.

*N-(3-((2-((4-chlorobenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16 m)*** white solid, yield 68%, mp 164.5–165.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.25 (s, 1H), 10.22 (s, 1H), 8.13 (s, 1H), 7.39 (d, $J$ = 8.4 Hz, 3H), 7.33 (t, $J$ = 3.5 Hz, 2H), 7.31–7.27 (m, 2H), 6.88 (s, 1H), 6.45 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.27 (dd, $J$ = 17.0, 1.9 Hz, 1H), 5.78–5.72 (m, 1H), 4.39 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.35, 163.19, 160.30, 152.29, 139.49, 138.56, 136.85, 131.75, 131.59, 130.73, 129.15, 128.17, 126.99, 121.95, 119.23, 116.09, 115.03, 111.91, 33.40. Calcd for $C_{21}H_{17}ClF_3N_4OS$[M + H]$^+$: 465.0764, found: 465.0763.

*N-(3-((2-((3-chlorobenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16n)*** white solid, yield 73%, mp 168.1–169.2℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.26 (s, 1H), 10.22 (s, 1H), 8.15 (s, 1H), 7.44 (s, 1H), 7.39 (d, $J$ = 6.1 Hz, 1H), 7.36–7.26 (m, 5H), 6.88 (s, 1H), 6.45 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.27 (dd, $J$ = 17.0, 2.0 Hz, 1H), 5.76 (dd, $J$ = 10.1, 2.0 Hz, 1H), 4.40 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.25, 163.18, 160.28, 152.22, 140.54, 139.50, 138.53, 132.74, 131.76, 130.06, 129.14, 128.83, 127.58, 126.97, 126.92, 121.96, 119.23, 116.05, 114.99, 111.84, 33.48. Calcd for $C_{21}H_{17}ClF_3N_4OS$[M + H]$^+$: 465.0764, found: 465.0765.

*N-(3-((2-((2-chlorobenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16o)*** white solid, yield 73%, mp 173.2–173.9℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.28 (s, 1H), 10.21 (s, 1H), 8.10 (s, 1H), 7.54–7.46 (m, 2H), 7.41 (s, 1H), 7.34–7.27 (m, 3H), 7.25–7.20 (m, 1H), 6.89 (s, 1H), 6.44 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.25 (dd, $J$ = 17.0, 1.9 Hz, 1H), 5.75 (dd, $J$ = 10.1, 2.0 Hz, 1H), 4.49 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.21, 163.18, 160.32, 152.23, 139.51, 138.56, 134.86, 133.35, 131.75, 131.19, 129.38, 129.17, 128.62, 127.13, 126.97, 121.96, 119.23, 116.01, 115.02, 111.84, 32.35. Calcd for $C_{21}H_{17}ClF_3N_4OS$[M + H]$^+$: 465.0764, found: 465.0765.

*N-(3-((2-((4-bromobenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16p)*** white solid, yield 64%, mp 163.4–164.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.25 (s, 1H), 10.21 (s, 1H), 8.12 (s, 1H), 7.45 (d, $J$ = 8.4 Hz, 2H), 7.40 (d, $J$ = 6.5 Hz, 1H), 7.31 (dd, $J$ = 15.1, 8.1 Hz, 4H), 6.88 (s, 1H), 6.45 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.27 (dd, $J$ = 17.0, 2.0 Hz, 1H), 5.76 (dd, $J$ = 10.1, 2.0 Hz, 1H), 4.37 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.33, 170.27, 163.19, 160.31, 152.28, 139.49, 138.56, 137.29, 131.76, 131.09, 129.15, 126.97, 121.95, 120.07, 119.23, 116.12, 115.04, 111.95, 33.46. Calcd for $C_{21}H_{17}BrF_3N_4OS$[M + H]$^+$: 509.0259, found: 509.0231.

*N-(3-((2-((3-bromobenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16q)*** white solid, yield 54%, mp 168.1–169.3℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.25 (s, 1H), 10.21 (s, 1H), 8.14 (s, 1H), 7.59 (s, 1H), 7.40 (dd, $J$ = 10.6, 8.6 Hz, 3H), 7.31 (q, $J$ = 8.2 Hz, 2H), 7.23 (t, $J$ = 7.8 Hz, 1H), 6.88 (s, 1H), 6.45 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.27 (dd, $J$ = 17.0, 2.0 Hz, 1H), 5.76 (dd, $J$ = 10.1, 2.0 Hz, 1H), 4.39 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.25, 163.19, 160.27, 152.21, 140.80, 139.50, 138.53, 131.76, 131.71, 130.35, 129.82, 129.14, 127.97, 126.97, 121.96, 121.33, 119.23, 116.03, 114.99, 111.84, 33.43. Calcd for $C_{21}H_{17}BrF_3N_4OS$[M + H]$^+$: 509.0259, found: 509.0247.

*N-(3-((2-((2-bromobenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16r)*** white solid, yield 51%, mp 180.8–181.7℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.27 (s, 1H), 10.21 (s, 1H), 8.09 (s, 1H), 7.64 (d, $J$ = 7.8 Hz, 1H), 7.52 (d, $J$ = 7.3 Hz, 1H), 7.42 (s, 1H), 7.34–7.24 (m, 3H), 7.21 (t, $J$ = 7.6 Hz, 1H), 6.89 (s, 1H), 6.44 (dd, $J$ = 16.9, 10.1 Hz, 1H), 6.25 (d, $J$ = 16.9 Hz, 1H), 5.75 (d, $J$ = 10.2 Hz, 1H), 4.48 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.19, 163.19, 160.34, 152.24, 139.51, 138.56, 136.48, 132.66, 131.75, 131.26, 129.39, 129.19, 127.70, 126.96, 124.11, 121.97, 119.24, 116.03, 115.04, 111.88, 34.98. Calcd for $C_{21}H_{17}BrF_3N_4OS$[M + H]$^+$: 509.0259, found: 509.0266.

*N-(3-((2-((2,4-dichlorobenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16 s)*** white solid, yield 75%, mp 166.2–167.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.28 (s, 1H), 10.22 (s, 1H), 8.10 (s, 1H), 7.63 (d, $J$ = 2.1 Hz, 1H), 7.51 (d, $J$ = 8.3 Hz, 1H), 7.40 (d, $J$ = 6.9 Hz, 1H), 7.34–7.27 (m, 3H), 6.89 (s, 1H), 6.45 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.25 (dd, $J$ = 17.0, 2.0 Hz, 1H), 5.76 (dd, $J$ = 10.1, 2.0 Hz, 1H), 4.46 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.97, 163.18, 160.35, 152.20, 139.52, 138.52, 134.30, 134.17, 132.72, 132.32, 131.74, 129.17, 128.83, 127.19, 126.96, 121.94, 119.21, 116.04, 115.09, 111.92, 31.80. Calcd for $C_{21}H_{16}Cl_2F_3N_4OS$[M + H]$^+$: 499.0374, found: 499.0514.

*N-(3-((2-((2,6-dichlorobenzyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16 t)*** white solid, yield 76%, mp 179.1–179.9℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.33 (s, 1H), 10.19 (s, 1H), 8.22 (s, 1H), 7.52 (d, $J$ = 8.1 Hz, 2H), 7.47 (d, $J$ = 7.2 Hz, 1H), 7.40–7.31 (m, 2H), 7.25 (d, $J$ = 8.0 Hz, 1H), 6.94 (s, 1H), 6.42 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.18 (dd, $J$ = 17.0, 1.9 Hz, 1H), 5.71 (dd, $J$ = 10.1, 1.9 Hz, 1H), 4.76 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.18,

163.09, 160.23, 152.27, 139.46, 138.67, 135.08, 132.12, 131.71, 130.24, 129.14, 128.68, 126.82, 124.72, 121.93, 119.20, 115.78, 114.97, 111.69, 30.95. Calcd for $C_{21}H_{16}Cl_2F_3N_4OS[M + H]^+$: 499.0374, found: 499.0373.

*N-(3-((2-(benzylthio)-6-(trifluoromethyl)pyrimidin-4-yl)ami-no) phenyl)acrylamide* ***(16u)*** white solid, yield 59%, mp 170.6–171.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.25 (s, 1H), 10.21 (s, 1H), 8.14 (s, 1H), 7.45 (d, $J$ = 19.1 Hz, 1H), 7.38 (d, $J$ = 7.0 Hz, 2H), 7.34–7.21 (m, 5H), 6.88 (s, 1H), 6.45 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.27 (dd, $J$ = 17.0, 2.0 Hz, 1H), 5.77–5.73 (m, 1H), 4.41 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.62, 163.18, 160.27, 152.22, 139.48, 138.62, 137.51, 131.76, 129.13, 128.92, 128.29, 127.01, 126.95, 121.98, 119.25, 115.99, 114.94, 111.98, 34.23. Calcd for $C_{21}H_{18}F_3N_4OS[M + H]^+$: 431.1153, found: 431.1154.

*N-(3-((2-(((2-chlorothiazol-5-yl)methyl)thio)-6-(trifluoromethyl) pyrimidin-4-yl)amino)phenyl)acrylamide* ***(16v)*** white solid, yield 76%, mp 164.5–165.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.34 (s, 1H), 10.22 (s, 1H), 8.17 (s, 1H), 7.51 (s, 1H), 7.41–7.27 (m, 3H), 6.92 (s, 1H), 6.45 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.27 (dd, $J$ = 17.0, 2.0 Hz, 1H), 5.77 (dd, $J$ = 10.1, 2.0 Hz, 1H), 4.60 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.63, 163.21, 160.36, 152.26, 149.62, 140.11, 139.52, 139.25, 138.40, 131.75, 129.22, 127.02, 121.94, 119.22, 116.20, 115.12, 112.00, 26.24. Calcd for $C_{18}H_{14}ClF_3N_5OS_2[M + H]^+$: 472.0280, found: 472.0279.

*N-(3-((2-((cyclohexylmethyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16w)*** white solid, yield 66%, mp 186.1–187.5℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.19 (s, 2H), 8.05 (s, 1H), 7.49 (s, 1H), 7.36–7.25 (m, 2H), 6.84 (s, 1H), 6.46 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.28 (dd, $J$ = 17.0, 1.7 Hz, 1H), 5.82–5.72 (m, 1H), 3.03 (d, $J$ = 6.8 Hz, 2H), 1.78 (d, $J$ = 12.4 Hz, 2H), 1.65 (d, $J$ = 11.3 Hz, 2H), 1.60–1.50 (m, 2H), 1.21–1.08 (m, 3H), 0.96 (dd, $J$ = 22.4, 10.9 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 172.38, 163.16, 160.22, 152.25, 139.45, 138.70, 131.81, 128.97, 126.90, 121.96, 119.23, 115.83, 114.91, 111.82, 37.06, 36.86, 31.91, 25.78, 25.43. Calcd for $C_{21}H_{24}F_3N_4OS[M + H]^+$: 437.1623, found: 437.1622.

*N-(3-((2-(((tetrahydro-2H-pyran-4-yl)methyl)thio)-6-(trifluoro-methyl)pyrimidin-4-yl)amino)phenyl)acrylamide* ***(16x)*** white solid, yield 51%, mp 103.5–104.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.21 (s, 2H), 8.06 (s, 1H), 7.48 (d, $J$ = 6.6 Hz, 1H), 7.35–7.26 (m, 2H), 6.85 (s, 1H), 6.46 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.28 (dd, $J$ = 16.9, 1.9 Hz, 1H), 5.77 (dd, $J$ = 10.1, 1.9 Hz, 1H), 3.80 (dd, $J$ = 11.3, 2.8 Hz, 2H), 3.20 (t, $J$ = 10.9 Hz, 2H), 3.07 (t, $J$ = 5.8 Hz, 2H), 1.78 (ddt, $J$ = 14.9, 7.7, 3.7 Hz, 1H), 1.65 (d, $J$ = 12.8 Hz, 2H), 1.26–1.20 (m, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 172.14, 163.21, 160.26, 152.22, 139.45, 138.66, 131.78, 129.03, 126.98, 121.97, 119.22, 115.93, 114.96, 111.87, 66.71, 36.23, 34.50, 31.84. Calcd for $C_{20}H_{22}F_3N_4O_2S[M + H]^+$:439.1416, found: 439.1416.

*N-(3-((2-(((tetrahydrofuran-2-yl)methyl)thio)-6-(trifluorome-thyl)pyr-imidin-4-yl)amino)phenyl)acrylamide* ***(16y)*** yellow solid, yield 53%, mp 94.6–95.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.28 (s, 1H), 10.22 (s, 1H), 8.08 (s, 1H), 7.45 (d, $J$ = 6.6 Hz, 1H), 7.33 (dt, $J$ = 13.0, 7.7 Hz, 2H), 6.89–6.84 (m, 1H), 6.51–6.42 (m, 1H), 6.28 (dd, $J$ = 17.0, 1.8 Hz, 1H), 5.77 (dd, $J$ = 10.1, 1.9 Hz, 1H), 4.30 (d, $J$ = 4.3 Hz, 1H), 3.62–3.56 (m, 2H), 2.06–1.74 (m, 4H), 1.26 (dd, $J$ = 16.7, 12.9 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.00, 163.22, 160.29, 151.89, 139.52, 138.49, 131.80, 129.19, 126.99, 121.90, 119.17, 115.95, 114.97, 111.76, 61.56, 44.65, 37.65, 33.70, 28.95. Calcd for $C_{19}H_{19}F_3N_4O_2S$ $[M + Na]^+$: 447.1079, found: 447.1087.

*N-(3-((2-((cyclopropylmethyl)thio)-6-(trifluoromethyl)pyrimidin-4-yl) amino)phenyl)acrylamide* ***(16z)*** white solid, yield 70%, mp 90.5–91.7℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.20 (s, 1H), 10.19 (s, 1H), 8.10 (s, 1H), 7.47 (d, $J$ = 6.9 Hz, 1H), 7.35–7.25 (m, 2H), 6.85 (s, 1H), 6.47 (dd, $J$ = 17.0, 10.1 Hz, 1H), 6.28 (dd, $J$ = 17.0, 2.0 Hz, 1H), 5.77 (dd, $J$ = 10.1, 2.0 Hz, 1H), 3.08 (d, $J$ = 7.2 Hz, 2H), 1.12 (ddd, $J$ = 10.2, 6.0, 2.7 Hz, 1H), 0.54–0.46 (m, 2H), 0.30–0.23 (m, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 172.41, 160.16, 152.28, 139.41, 138.72, 131.80, 129.05, 126.92, 124.69, 121.97, 119.24, 115.79, 114.89, 111.72, 99.36, 35.66, 10.44, 5.40. Calcd for $C_{18}H_{18}F_3N_4OS[M + H]^+$: 395.1153, found: 395.1151.

*General procedure for the synthesis of compounds* ***17a-z***

Compound **15d** (536.61 μmol, 1.00 eq) and absolute $K_2CO_3$ (804.92 μmol, 1.50 eq) were dissolved in 5 mL DMF and then diverse heterocyclics and anilines (1.20 eq) were added, next, the temperature were heated to 80 °C for 6 h. When the reaction was completed, extracted with water and ethyl acetate, and then purified by column chromatography to get the target compounds **17a-z**. All of the target compounds have never been reported in published articles.

*4-(piperazin-1-yl)-6-(trifluoromethyl)-2-((4-(trifluorome-thyl)benzyl) thio)pyrimidine* ***(17a)*** light green solid, yield 67%, mp 147.3–148.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.69–7.61 (m, 4H), 6.95 (s, 1H), 4.43 (s, 2H), 3.61 (s, 4H), 2.71 (s, 4H), 1.23 (s, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.14, 160.85, 152.96, 143.45, 129.41, 127.66, 125.06, 122.98, 122.21, 119.53, 95.94, 45.18, 33.52. Calcd for $C_{17}H_{17}F_6N_4S[M + H]^+$: 423.1078, found: 423.1078.

*4-(6-(trifluoromethyl)-2-((4-(trifluoromethyl)benzyl)thio)py-rimidin-4-yl)morpholine* ***(17b)*** white solid, yield 56%, mp 154.3–155.2℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.69–7.62 (m, 4H), 7.00 (s, 1H), 4.44 (s, 2H), 3.68 (s, 4H), 3.64 (s, 4H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.23, 161.22, 153.07, 143.38, 129.53, 127.70, 125.12, 122.87, 122.22, 119.49, 96.20, 65.60, 33.52. Calcd for $C_{17}H_{16}F_6N_3OS$ $[M + H]^+$: 424.0918, found: 424.0917.

*4-(6-(trifluoromethyl)-2-((4-(trifluoromethyl)benzyl)thio)py-rimidin-4-yl)thiomorpholine* ***(17c)*** white solid, yield 67%, mp 168.1–169.2℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.68 (d, $J$ = 8.8 Hz, 2H), 7.65 (d, $J$ = 9.0 Hz, 2H), 7.03 (s, 1H), 4.43 (s, 2H), 3.99 (s, 4H), 2.60 (s, 4H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.31, 160.76, 153.23, 143.40, 129.36, 127.66, 125.16, 122.88, 122.23, 119.50, 96.44, 33.56, 26.02. Calcd for $C_{17}H_{16}F_6N_3S_2[M + H]^+$: 440.0690, found: 440.0689.

*4-(4-methylpiperazin-1-yl)-6-(trifluoromethyl)-2-((4-(triflu-oromethyl) benzyl)thio)pyrimidine* ***(17d)*** light green solid, yield 67%, mp 94.5–95.1℃; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.65 (s, 2H), 7.64 (d, $J$ = 9.8 Hz, 2H), 6.99 (s, 1H), 4.43 (s, 2H), 3.67 (s, 4H), 2.32 (s, 4H), 2.19 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.21, 160.99, 153.08, 143.40, 129.40, 127.35, 125.12, 122.87, 122.23, 119.50, 96.15, 53.94, 45.44, 33.55. Calcd for $C_{18}H_{19}F_6N_4S[M + H]^+$: 437.1235, found: 437.1233.

*4-(4-ethylpiperazin-1-yl)-6-(trifluoromethyl)-2-((4-(trifle-oromethyl) benzyl)thio)pyrimidine* ***(17e)*** white solid, yield 59%, mp 84.5–85.3℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.68–7.62 (m, 4H), 6.99 (s, 1H), 4.43 (s, 2H), 3.67 (s, 4H), 2.35 (d, $J$ = 5.7 Hz, 4H), 2.32 (d, $J$ = 7.1 Hz, 2H), 1.01 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.29, 160.79, 153.06, 143.40, 129.38, 127.65, 125.12, 122.87, 122.23, 96.10, 51.72, 51.33, 43.76, 33.54, 11.78. Calcd for $C_{19}H_{21}F_6N_4S[M + H]^+$: 451.1391, found: 451.1390.

*4-(4-phenylpiperazin-1-yl)-6-(trifluoromethyl)-2-((4-(triflu-oromethyl) benzyl)thio)pyrimidine* ***(17f)*** yellow solid, yield 82%, mp 92.3–93.2℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.66 (d, $J$ = 7.0 Hz, 4H), 7.24 (t, $J$ = 7.6 Hz, 2H), 7.06 (s, 1H), 6.97 (d, $J$ = 8.1 Hz, 2H), 6.82 (t, $J$ = 7.2 Hz, 1H), 4.46 (s, 2H), 3.85 (s, 4H), 3.20 (s, 4H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.28, 161.04, 153.13, 150.52, 143.40, 129.43, 128.95, 127.74, 125.10, 122.88, 122.25, 119.52, 119.27, 115.67, 96.28, 47.79, 33.60. Calcd for $C_{23}H_{21}F_6N_4S[M + H]^+$: 499.1391, found: 499.1390.

*tert-butyl 4-(6-(trifluoromethyl)-2-((4-(trifluoromethyl)benzyl) thio) pyrimidin-4-yl)piperazine-1-carboxylate* ***(17 g)*** white solid, yield 81%, mp 117.2–118.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.66 (s, 4H), 6.99 (s, 1H), 4.44 (s, 2H), 3.69 (s, 4H), 3.39 (s, 4H), 1.42 (s, 9H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.26, 161.09, 153.75, 153.13, 143.37, 129.42, 127.70, 125.07, 122.87, 122.21, 119.48, 96.24, 79.19, 33.58, 27.96. Calcd for $C_{22}H_{25}F_6N_4O_2S[M + H]^+$: 523.1602, found:523.1603.

*4-(4-(pyridin-2-yl)piperazin-1-yl)-6-(trifluoromethyl)-2-((4-(tri-fluoromethyl)benzyl)thio)pyrimidine* ***(17 h)*** light yellow solid, yield 82%,

mp 116.2–117.0℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.14 (d, $J$ = 3.8 Hz, 1H), 7.67 (s, 4H), 7.57 (t, $J$ = 7.8 Hz, 1H), 7.04 (s, 1H), 6.85 (d, $J$ = 8.6 Hz, 1H), 6.67 (t, $J$ = 5.8 Hz, 1H), 4.46 (s, 2H), 3.82 (s, 4H), 3.60 (s, 4H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.18, 161.09, 158.49, 153.06, 147.54, 143.42, 137.57, 129.45, 127.72, 125.10, 122.87, 122.26, 119.53, 113.22, 107.08, 96.28, 43.82, 33.58. Calcd for $C_{22}H_{20}F_6N_5S$[M + H]$^+$: 500.1344, found: 500.1345.

*4-(4-(pyrimidin-2-yl)piperazin-1-yl)-6-(trifluoromethyl)-2-((4-(trifluoromethyl)benzyl)thio)pyrimidine* ***(17i)*** white solid, yield 83%, mp 138.3–138.9℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.41 (d, $J$ = 4.2 Hz, 2H), 7.67 (s, 4H), 7.04 (s, 1H), 6.68 (t, $J$ = 4.2 Hz, 1H), 4.46 (s, 2H), 3.82 (s, 8H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.26, 161.12, 161.00, 157.93, 153.08, 143.40, 129.45, 127.72, 125.11, 122.87, 122.25, 119.52, 110.45, 96.29, 42.60, 33.59. Calcd for $C_{21}H_{19}F_6N_6S$[M + H]$^+$: 501.1296, found: 501.1295.

*N-(4-fluorophenyl)-6-(trifluoromethyl)-2-((4-(trifluorometh-yl)benzyl)thio)pyrimidin-4-amine* ***(17j)*** yellow solid, yield 77%, mp 123.5–124.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.22 (s, 1H), 7.68–7.55 (m, 6H), 7.20 (t, $J$ = 8.6 Hz, 2H), 6.81 (s, 1H), 4.45 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.06, 160.29, 152.22, 142.92, 134.45, 129.52, 127.76, 127.44, 125.53, 123.00, 121.93, 119.20, 115.64, 115.41, 33.53. Calcd for $C_{19}H_{13}F_7N_3S$ [M + H]$^+$: 448.0718, found: 448.0719

*N-(p-tolyl)-6-(trifluoromethyl)-2-((4-(trifluoromethyl)benz-yl)thio)pyrimidin-4-amine* ***(17 k)*** yellow solid, yield 72%, mp 112.3–113.2℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.12 (s, 1H), 7.64 (d, $J$ = 8.1 Hz, 2H), 7.59 (d, $J$ = 8.0 Hz, 2H), 7.44 (d, $J$ = 5.8 Hz, 2H), 7.16 (d, $J$ = 7.9 Hz, 2H), 6.80 (s, 1H), 4.44 (s, 2H), 2.28 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.04, 143.00, 135.56, 129.55, 129.31, 127.74, 127.42, 125.55, 125.04, 122.85, 121.97, 121.10, 120.10, 119.22, 33.50, 20.40. Calcd for $C_{20}H_{16}F_6N_3S$ [M + H]$^+$: 444.0969, found: 444.0970

*N-(4-methoxyphenyl)-6-(trifluoromethyl)-2-((4-(trifluorometh-yl)benzyl)thio)pyrimidin-4-amine* ***(17 l)*** light yellow solid, yield 69%, mp 104.5–105.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.05 (s, 1H), 7.63 (d, $J$ = 7.6 Hz, 2H), 7.58 (s, 2H), 7.46 (s, 2H), 6.94 (d, $J$ = 8.2 Hz, 2H), 6.76 (s, 1H), 4.42 (s, 2H), 3.75 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.99, 160.24, 156.09, 143.09, 129.56, 127.71, 127.40, 127.08, 125.55, 125.04, 122.89, 121.99, 119.21, 114.09, 55.18, 33.59. Calcd for $C_{20}H_{16}F_6N_3OS$[M + H]$^+$: 460.0918, found: 460.0919.

*N-(3-chloro-4-fluorophenyl)-6-(trifluoromethyl)-2-((4-(triflu-oromethyl)benzyl)thio)pyrimidin-4-amine* ***(17 m)*** yellow solid, yield 67%, mp 132.4–133.2℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.36 (s, 1H), 7.96 (d, $J$ = 5.8 Hz, 1H), 7.63 (dd, $J$ = 15.2, 8.5 Hz, 4H), 7.48 (d, $J$ = 8.7 Hz, 1H), 7.40 (t, $J$ = 9.0 Hz, 1H), 6.84 (s, 1H), 4.47 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.14, 160.14, 152.33, 142.72, 135.46, 129.54, 127.83, 125.52, 125.11, 122.82, 122.44, 121.86, 121.23, 119.36, 117.13, 116.92, 33.58. Calcd for $C_{19}H_{12}ClF_7N_3S$[M + H]$^+$: 482.0329, found: 482.0328.

*N-(2,4-dichlorophenyl)-6-(trifluoromethyl)-2-((4-(trifluorome-thyl)benzyl)thio)pyrimidin-4-amine* ***(17n)*** yellow solid, yield 68%, mp 135.8–136.5℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.01 (s, 1H), 7.77 (s, 1H), 7.70 (d, $J$ = 8.6 Hz, 1H), 7.58 (d, $J$ = 7.9 Hz, 2H), 7.46 (d, $J$ = 8.7 Hz, 1H), 7.40 (d, $J$ = 7.7 Hz, 2H), 6.94 (s, 1H), 4.31 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.96, 161.20, 152.56, 143.01, 133.65, 130.74, 129.62, 129.43, 129.29, 128.78, 127.76, 124.89, 122.82, 121.88, 119.15, 99.13, 33.44. Calcd for $C_{19}H_{12}Cl_2F_6N_3S$[M + H]$^+$: 498.0033, found: 498.0034.

*N1-(6-(trifluoromethyl)-2-((4-(trifluoromethyl)benzyl)thio) pyrimidin-4-yl)benzene-1,3-diamine* ***(17o)*** brown liquid, yield 68%. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.95 (s, 1H), 7.70–7.58 (m, 4H), 7.00 (t, $J$ = 7.9 Hz, 1H), 6.81 (s, 1H), 6.77 (s, 1H), 6.37 (d, $J$ = 7.7 Hz, 1H), 5.38 (s, 2H), 4.45 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 170.98, 160.42, 149.32, 143.15, 138.62, 129.69, 129.22, 127.70, 127.38, 125.04, 122.85, 121.99, 119.26, 110.35, 108.99, 106.68, 33.51. Calcd for $C_{19}H_{14}F_6N_4S$ [M + H]$^+$: 445.0922, found: 445.0923.

*6-(trifluoromethyl)-2-((4-(trifluoromethyl)benzyl)thio)-N-(3,4,5-trimethoxyphenyl)pyrimidin-4-amine* ***(17p)*** brown solid, yield 74%, mp 146.4–147.2℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.17 (s, 1H), 7.63 (d, $J$ = 7.9 Hz, 2H), 7.59 (d, $J$ = 7.8 Hz, 2H), 6.99 (s, 2H), 6.82 (s, 1H), 4.48 (s, 2H), 3.75 (s, 6H), 3.64 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.11, 160.33, 152.87, 142.86, 134.18, 129.74, 128.23, 127.83, 127.51, 125.52, 125.13, 124.68, 122.82, 121.95, 119.22, 100.19, 60.07, 55.73, 33.52. Calcd for $C_{22}H_{20}F_6N_3O_3S$[M + H]$^+$: 520.1130, found: 520.1130.

*4-methyl-2-((6-(trifluoromethyl)-2-((4-(trifluoromethyl)ben-zyl)thio) pyrimidin-4-yl)thio)thiazole* ***(17q)*** white solid, yield 88%, mp 86.4–87.2℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.76 (s, 1H), 7.66 (d, $J$ = 8.5 Hz, 3H), 7.54 (d, $J$ = 7.9 Hz, 2H), 4.38 (s, 2H), 2.40 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.49, 153.99, 153.33, 150.17, 142.30, 142.23, 129.54, 127.98, 125.11, 122.78, 121.70, 118.62, 110.16, 33.54, 16.58. Calcd for $C_{17}H_{12}F_6N_3S_3$[M + H]$^+$: 468.0098, found: 468.0097.

*4-(pyrimidin-2-ylthio)-6-(trifluoromethyl)-2-((4-(trifluoro-methyl)benzyl)thio)pyrimidine* ***(17r)*** white solid, yield 85%, mp 102.5–103.4℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.85 (d, $J$ = 4.8 Hz, 2H), 8.32 (s, 1H), 7.67 (d, $J$ = 8.1 Hz, 2H), 7.64 (d, $J$ = 8.3 Hz, 2H), 7.52 (t, $J$ = 4.8 Hz, 1H), 4.47 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.26, 170.55, 165.61, 158.85, 153.66, 142.31, 129.74, 127.96, 125.14, 121.50, 120.36, 118.76, 113.08, 33.81. Calcd for $C_{17}H_{11}F_6N_4S_2$[M + H]$^+$: 449.0329, found: 449.0330.

*4-(pyridin-2-ylthio)-6-(trifluoromethyl)-2-((4-(trifluoromethyl) benzyl)thio)pyrimidine* ***(17 s)*** white solid, yield 80%, mp 77.3–78.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.67 (d, $J$ = 3.9 Hz, 1H), 7.92 (t, $J$ = 7.6 Hz, 1H), 7.85 (d, $J$ = 7.7 Hz, 1H), 7.64 (d, $J$ = 11.8 Hz, 3H), 7.51 (d, $J$ = 6.0 Hz, 1H), 7.47 (d, $J$ = 8.2 Hz, 2H), 4.30 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 172.97, 171.23, 153.16, 150.87, 149.92, 142.31, 138.27, 130.21, 129.55, 127.91, 125.12, 124.60, 121.43, 118.70, 110.55, 33.51. Calcd for $C_{18}H_{12}F_6N_3S_2$[M + H]$^+$: 448.0377, found: 448.0377.

*N-(1H-tetrazol-5-yl)-6-(trifluoromethyl)-2-((4-(trifluorome-thyl)benzyl)thio)pyrimidin-4-amine* ***(17 t)*** white solid, yield 62%, mp 175.1–175.9℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 16.14 (s, 1H), 11.95 (s, 1H), 7.66 (s, 4H), 7.38 (s, 1H), 4.52 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.59, 159.42, 153.81, 142.98, 129.74, 127.82, 125.09, 122.83, 121.82, 119.01, 99.77, 33.60. Calcd for $C_{14}H_{10}F_6N_7S$ [M + H]$^+$: 422.0623, found: 422.0623.

*4-((1-phenyl-1H-tetrazol-5-yl)thio)-6-(trifluoromethyl)-2-((4-(trifluoromethyl)benzyl)thio)pyrimidine* ***(17u)*** white solid, yield 57%, mp 106.5–107.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.01 (s, 1H), 7.62 (d, $J$ = 7.7 Hz, 4H), 7.58 (d, $J$ = 6.8 Hz, 3H), 7.51 (d, $J$ = 7.9 Hz, 2H), 4.27 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.59, 168.72, 153.60, 146.58, 141.77, 133.05, 130.83, 129.60, 129.43, 127.91, 124.92, 122.76, 121.21, 118.44, 111.13, 33.56. Calcd for $C_{20}H_{13}F_6N_6S_2$[M + H]$^+$: 515.0547, found: 515.0551.

*2-((6-(trifluoromethyl)-2-((4-(trifluoromethyl)benzyl)thio) pyrimidin-4-yl)thio)-1,3,4-thiadiazole* ***(17v)*** white solid, yield 65%, mp 123.1–124.0℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.89 (s, 1H), 8.05 (s, 1H), 7.66 (d, $J$ = 7.8 Hz, 2H), 7.56 (d, $J$ = 7.9 Hz, 2H), 4.41 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.58, 168.71, 158.78, 155.81, 153.34, 141.96, 129.65, 128.03, 125.19, 122.78, 121.37, 111.24, 33.70. Calcd for $C_{15}H_9F_6N_4S_3$[M + H]$^+$: 454.9894, found: 454.9922.

*2-methyl-5-((6-(trifluoromethyl)-2-((4-(trifluoromethyl)benzyl) thio) pyrimidin-4-yl)thio)-1,3,4-thiadiazole* ***(17w)*** white solid, yield 82%, mp 143.4–144.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.99 (s, 1H), 7.66 (d, $J$ = 8.0 Hz, 2H), 7.55 (d, $J$ = 7.9 Hz, 2H), 4.41 (s, 2H), 2.75 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.60, 170.68, 169.22, 157.00, 154.88, 153.37, 142.07, 129.57, 128.00, 125.18, 121.37, 111.05, 33.66, 15.36. Calcd for $C_{16}H_{11}F_6N_4S_3$[M + H]$^+$: 469.0050, found: 469.0048.

*2-((6-(trifluoromethyl)-2-((4-(trifluoromethyl)benzyl)thio) pyrimidin-4-yl)thio)-4,5-dihydrothiazole* ***(17x)*** yellow solid, yield 83%, mp 107.2–108.1℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.85 (s, 1H), 7.70 (s, 4H), 4.78 (t, $J$ = 7.5 Hz, 2H), 4.57 (s, 2H), 3.51 (t, $J$ = 7.5 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 201.67, 171.55, 159.60, 154.12, 142.45, 129.66, 127.97, 125.25, 121.61, 118.87, 104.68, 57.86, 33.90, 28.35.

Calcd for $C_{16}H_{12}F_6N_3S_3[M + H]^+$: 456.0098, found: 456.0099.

*N-(6-methoxypyridin-3-yl)-6-(trifluoromethyl)-2-((4-(triflu-oromethyl) benzyl)thio)pyrimidin-4-amine* ***(17y)*** white solid, yield 72%, mp 129.6–130.3℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.78 (s, 1H), 8.65 (d, $J$ = 5.0 Hz, 2H), 7.96 (s, 1H), 7.90 (d, $J$ = 5.1 Hz, 2H), 7.56 (d, $J$ = 8.0 Hz, 2H), 7.47 (d, $J$ = 8.0 Hz, 2H), 4.36 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.51, 170.49, 153.34, 153.31, 152.98, 152.74, 150.38, 142.08, 142.07, 139.74, 129.42, 127.99, 125.05, 124.60, 120.10, 110.72, 33.54. Calcd for $C_{21}H_{13}F_6N_4S_3[M + H]^+$: 531.0207, found: 531.0208.

*5-(pyridin-4-yl)-2-((6-(trifluoromethyl)-2-((4-(trifluorometh-yl)benzyl) thio)pyrimidin-4-yl)thio)thiazole* ***(17z)*** light yellow solid, yield 77%, mp 92.9–93.7℃. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.14 (s, 1H), 8.34 (s, 1H), 7.87 (d, $J$ = 8.3 Hz, 1H), 7.62 (d, $J$ = 7.8 Hz, 2H), 7.57 (d, $J$ = 7.4 Hz, 2H), 6.84 (d, $J$ = 8.8 Hz, 1H), 6.79 (s, 1H), 5.76 (s, 1H), 4.42 (s, 2H), 3.86 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 171.09, 160.62, 152.21, 142.93, 139.88, 129.50, 128.91, 127.71, 127.45, 125.52, 125.00, 122.82, 121.93, 119.20, 110.25, 53.18, 33.52. Calcd for $C_{19}H_{16}F_6N_4OS$ $[M + H]^+$:461.0871, found:461.0870.

### *Cell culture and reagents*

RPMI-1640 and MTT were purchased from Solarbio. Fetal Bovine Serum (FBS) was purchased from Tianhang Biotechnology Co. Annexin V-FITC/PI Apoptosis Detection Kit and Cell Cycle Detection Kit were bought from Keygen Biotech. PC-3 (human prostate cancer cell line), MGC-803 (human gastric cancer cell line), MCF-7 (human breast cancer cell line) and H1975 (non-small cell lung cancer cell line) were obtained from our laboratories (originally purchased from the CCTCC). The antibodies used in western blotting (Bcl-2, Bax and p53) were all purchased from CST. All cancer cells were cultured in RPMI 1640 medium with 10% FBS and all of them were cultured at 37℃ in a humidified incubator of 95% air and 5% $CO_2$. Compounds were dissolved in DMSO to prepare a stock solution with an initial concentration of 10 mM.

### *MTT assay*

Cells in the logarithmic growth phase were seeded in 96-well plates at 3000–5000 cells per well. After the cells were cultured for 24 h at 37℃, removed the medium and added 200 μL of medicated medium at different concentrations to each well. After 72 h incubation, 20 μL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Solarbio) was added to each well at a final concentration of 5 mg/mL, and cells were cultured for 4 h in a 37℃ incubator. After that, the medium was aspirated, 150 μL DMSO was then added to each well, and the plate was shaken on the shaker for 10 min until the formazan dissolved completely. The absorbance was measures at a wavelength of 490 nm with a microplate reader, and the cell survival rate was measured. The date of $IC_{50}$ was calculated using SPASS 16.0 software, and the results were Mean ± SD of three independent experiments.

### *Colony formation assay*

Cells in the logarithmic growth phase were seeded in a 6-well plate at 1000 cells per well. After approximately 24 h incubation, different concentrations (0, 1, 2, 4 μM) of compound **17v** were treated for 9 days. After washing 2–3 times with PBS, cells were fixed with ice methanol for 30 min and stained with crystal violet for 30 min. Images were photographed after drying the plate. After that, morphological analysis was performed through microscopy (Leica EZ4W, China).

### *Cell cycle analysis*

H1975 cells in the logarithmic growth phase were seeded in 6-well plates at a concentration of $2 \times 10^5$ per well. After 24 h incubation, treated with different concentrations (0, 1, 2, 4 μM) of compound **17v** for 24 h. After that, collected cells, fixed with pre-cooled 70% ethanol at 4℃ overnight and stained with 20 mg/mL PI and 20 mg/mL RNase A. Then cell suspension was incubated for 40 min in the dark. Finally, samples were analyzed for DNA content with flow cytometry (Becton, Dickinson and Company, N*J*).

### *Scratch assay*

H1975 cells in the logarithmic growth phase were seeded in 6-well plates at a density of $3.5 \times 10^5$ cells per well. After 24 h incubation, the cell surface was scratched by a 200 μL pipet tip. Cells were washed three or four times with PBS and cultured in RPMI-1640 medium containing 2% FBS. Taken photos with a microscope as control groups. After cells were treated with different concentrations (0, 2, 4 μM) of compound **17v** for 24 or 48 h, take photos again as experimental groups. Photoshop was used to process the images.

### *DAPI staining assay*

H1975 cells in the logarithmic growth phase were seeded in 6-well plates at $2 \times 10^5$ cells per well. After 24 h incubation, cells were treated with different concentrations (0, 2, 4 μM) of compound **17v** for 48 h. After that, the cells were washed two or three times with PBS and fixed with pre-cooled methanol at 4℃ for 10 min, then incubated in the DAPI dye solution in the dark. Photos were taken with a fluorescence microscope (Nikon Eclipse TieS, Nikon Ltd, Japan), and the images were processed with Photoshop.

### *Analysis of apoptosis by flow cytometry*

H1975 cells in the logarithmic growth phase were seeded in 6-well plates at a density of $1.5 \times 10^5$ cells per well. After 24 h incubation, cells were treated with different concentrations (0, 1, 2, 4 μM) of compound **17v** for 48 or 72 h. The cells were collected, washed with PBS and measured with the Annexin V-FITC/PI Apoptosis Detection Kit (Keygen Biotech, China), according to the protocol. Then, the cells were detected using the FAC-SCalibur flow cytometer (BD Biosciences, San Jose, CA, USA), and data was analyzed by the Flow J*o* software.

### *Western blot analysis*

H1975 cells were seeded in cell culture dishes (100 mm). After 24 h incubation, the cells were treated with different concentrations (0, 1, 2, 4 μM) of compound **17v** for 48 h. Then, the cells were harvested with trypsin and lysed with cell/tissue RIPA lysis buffer (Beyotime Biotechnology) for 30 min. The supernatant was determined by a BCA Protein Assay kit (Beyotime Biotechnology), denatured at 100 °C for 10 min, and incubated at − 20 °C for Western Blot. After being separated by SDS-PAGE, proteins were transferred to nitrocellulose (NC) membranes and blocked by 5% skim milk at room temperature for 2 h. The membranes were then incubated overnight at 4 °C with primary antibodies. After washing the membrane with the secondary antibody (1: 10000) at room temperature for 2 h, the membranes were washed with PBST. Blots exposure was performed with an ECL chemiluminescence solution. The data were analyzed using Image*J* software.

### *Molecular docking*

Molecular docking study was performed with Molecular Operating Environment software (MOE 2020). The co-crystal structure of the small molecule with the EGFR (PDB ID: 5GNK) was obtained from the RCSB protein database (www.rcsb.org). The “QuickPrep” function was used for structural correction and protonation of receptor proteins. After that, all solvent molecules were deleted and the protonation states of the ionizable residues with $pK_a$ = 7 were set up. We used the co-crystal ligand site as the docking site. The highest score conformation of the

docking result was used to explain the binding model.

## Declaration of Competing Interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128268.

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