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新型 2,4,6,-取代嘧啶衍生物的设计、合成和抗肿瘤活性研究

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摘要 为了寻找高效的新型抗肿瘤药物，设计合成了一系列 2,4,6-取代嘧啶衍生物，并使用噻唑蓝（MTT）法对四种人的肿瘤细胞 SW-620（人结肠癌细胞）, PC-3（人前列腺癌细胞）, A549（人非小细胞肺癌细胞）和 MGC-803（人胃癌细胞）进行了体外抗肿瘤活性研究。其中化合物 5i，5o 和 5r 对 4 种测试的癌细胞系显示出高的抗肿瘤增殖活性，特别是化合物 5r 具有最高的抑制活性，与阳性对照药 5-氟尿嘧啶（5-Fu）相比，对 SW-620 的 IC_{50} 值最低，为 1.46μM。进一步机制研究表明，化合物 5r 诱导 SW-620 凋亡，使细胞周期阻滞在 S 期。分子对接揭示了化合物 5r 可以很好地结合 EGFR 的活性位点，化合物 5r 可能被认为是一种有前途的化合物，可用于进一步研究开发新的抗癌药物。

关键词 嘧啶衍生物；合成；抗肿瘤活性；细胞周期；凋亡

Design, Synthesis and Antitumor Activity Evaluation of 2,4,6-substitute Pyrimidine Derivatives

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Abstract With the expectation to find out novel and effective anti-tumor agents, a series of novel 2,4,6-substituted pyrimidine derivatives were synthesized and evaluated for their anti-tumor activity against four human cancer cells (SW-620, PC-3, A549 and MGC-803) using MTT assay. Among compounds, 5i, 5o and 5r displayed strong antiproliferative activity on 4 tested cancer cell lines, in particular, compound 5r did the highest inhibitive activity, and possessed the lowest IC_{50} value of 1.46 μM towards SW-620 cells compared to that of the positive reference drug 5-Fluorouracil(5-Fu). Further mechanism research showd compound 5r induced SW-620 apoptosis, arrested cell cycle at S phase. Molecular docking revealed that 5r can bind well to the active site of EGFR, and compound 5r may be considered as a promising compound amenable for further investigation for the development of new anticancer agents.

Keywords Pyrimidine derivatives; Synthesis; Antitumor activity; Cell cycle; Apoptosis

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1 Introduction

According to the latest Annual World Cancer Report, there were approximately 18 million new cases and 9.6 million cancer deaths worldwide in 2018 [1]. Current studies showed that chemotherapy was still one of the most effective methods for cancer treatment. The adverse side effects and the development of resistance to traditional anticancer drugs call for an urgent exploration of new anticancer agents [2-3]. The epidermal growth factor receptor (EGFR) plays an important role in cellular functions, and it is often over-expressed and uncontrolled in a variety of human tumor cells [4-8]. In recent years, many quinazoline-based compounds have been approved for clinical use by US Food and Drug Administration (FDA) including Gefitinib, Erlotinib and so on (**Figure 1**) [9-12]. Based on the strategy of the scaffold hopping, the 4-aminopyrimidine was selected as the scaffold to study.

Urea has the capacity to exert various non-covalent interactions such as hydrogen bonding and dipole interaction which can improve the physicochemical property and the ability of binding to biomolecular targets [13-15]. Compound **1** exhibited the strongest activity (IC_{50}=5.21±0.47μM against A549), effectively regulated the expression of apoptosis- and cell cycle-related proteins, and influenced the Raf/MEK/ERK pathway [16]. Sorafenib **5** with the aryl urea group, as a key pharmacophore, is a systemic agent approved for the treatment of Hepatocellular Carcinoma (HCC) with good tolerance and safety [17]. Considering that the active site of EGFR exhibited great adaptability to the aryl urea group, it was expected that the 4-ethyl aryl urea could make hydrophobic interactions with EGFR compared with other alkyl by molecular docking. Besides, it has been well established that fluorinated in particular CF_3 substituted heterocycles have got significant place in modern medicinal chemistry [18]. Their biological studies clearly indicate that the presence of trifluoromethyl group gives useful biological activity and are the subject of considerable growing interest, such as Rociletinib [19-20].

Therefore, we have used scaffold hopping approach through changing the core structure , and synthesized a series of pyrimidine derivatives containing 4-ethyl aryl urea and trifluoromethyl by using the combination principles and evaluated the antiproliferative activity of target compounds in vitro by MTT assay[21-23].

2 Results and discussion

2.1 Chemistry

The general synthetic procedures for the target compounds **5a-5r** were outlined in **Scheme 1**. Taking commercially available 4,4,4-trifluoromethylacetoacetate as starting material, compound **3** was synthesized by mechanical stirring with thiourea at 80 ℃ for 3 h. Chloroacetamide and oxalyl chloride were reacted in 1,2-dichloroethane at 90℃ for 4 h to obtain 2-chloroacetyl isocyanate, then different anilines were added at 0℃ for 0.5 h to get compound **2**. Compound **2** and **3** were dissolve in 1,4-dioxane at 70℃ for

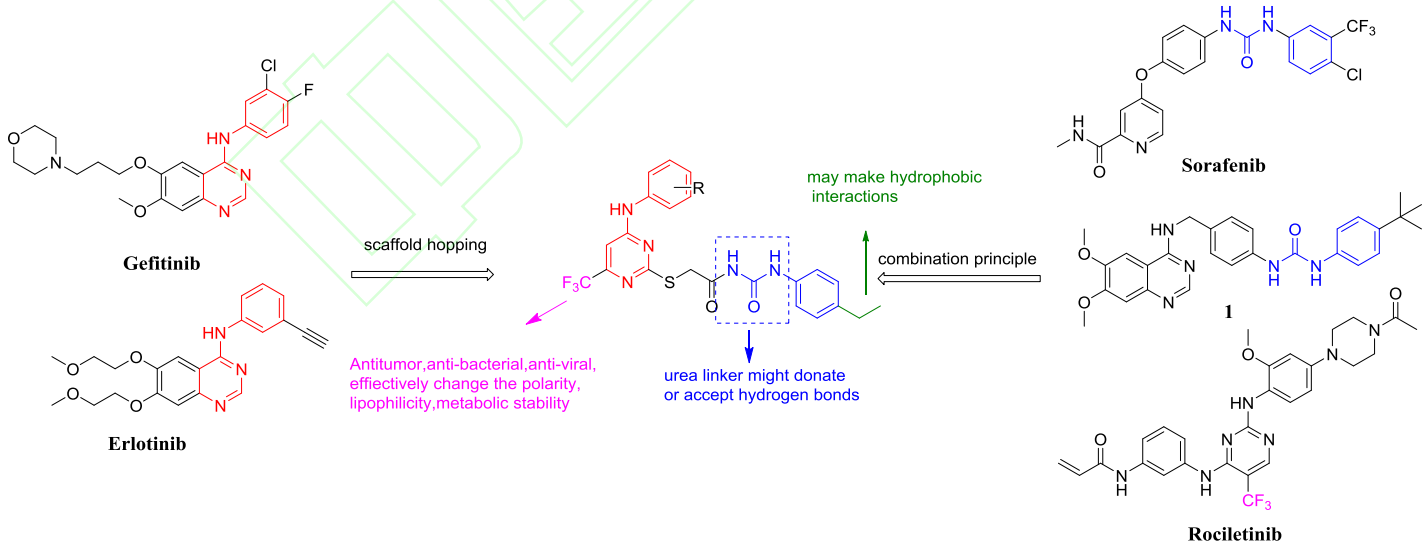

Figure1 Design of pyrimidine derivatives

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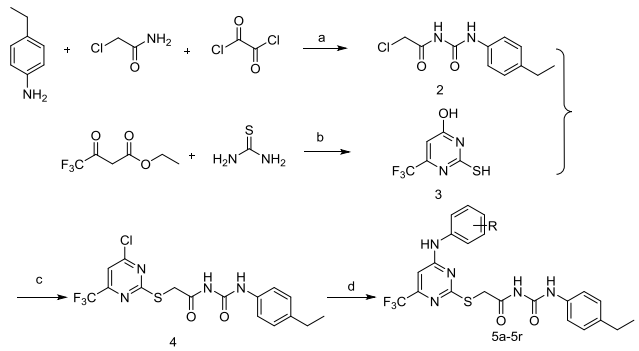

Scheme 1 Synthesis of 2,4,6-substituted pyrimidine derivatives Reagents and conditions;(a) 1,2-Dichloroethane, 90℃, 4 h;(b) CH3CH2OH, KOH, 80℃, 3 h;(c) 1,4-Dioxane, KOH, POCl3, 90℃, 2 h;(d) DMF, K2CO3, 80℃, 6 h.

1 h, and then phosphorus oxychloride was added to the mixture and slowly heated to 90 ℃ for 2 h to give compound **4**. Compound **4** and different anilines were added to DMF, and potassium carbonate was used as catalyst to obtain compounds **5a-5r** by nucleophilic substitution reaction. All the structures were fully characterized by ^{1}H-NMR, ^{13}C-NMR and HRMS.

2.2 Biological evaluation

2.2.1 In vitro antiproliferative activity

The in vitro antiproliferative activities of all the prepared compounds (**5a-5r**) were evaluated against PC-3, SW-620, MGC-803, and A549 cells, which was reported that EGFR protein was overexpressed in these cell lines. 5-Fluorouracil was selected as positive reference drugs. The IC_{50} values (concentration required to achieve 50% inhibition of the tumor cell proliferation) of the tested compounds for each cell line are presented in **Table 1**.

From IC_{50} value of compounds **5a-5c**, it could be found that the activity of *meta*-substitution was better than *ortho*-substitution and *para*-substitution except for SW-620. As the electron withdrawing ability of substituents increased such as compounds **5d-5f**, the antiproliferative activity of most compounds slightly decreased. When the substituent was an electron withdrawing double substituent in compounds **5k** and **5l**, the antiproliferative activity was not greatly improved. Notably, from the biological data of compounds **5g-5i** and **5m-5r**, we could know that the electron donating substituents contribute to significantly increase activity. In addition, the activity of para-substitution was better than ortho-substitution and meta-substitution. Compared with compounds **5i**, **5o** and **5r**, the contribution to enhance antiproliferative activity was 4-OCH_3 **>** 4-OCH_2CH_3 **>** 4-CH_3.

2.2.2 Effect of compound 5r against normal human cell line

Table 2 In vitro antiproliferative activity of compound **5r** against human normal cell [IC_{50}/μM]

compound	Ges-1	HEEC
5r	50.38±1.02	>100
5-Fu	22.61±0.56	>100

To further investigate the antitumor activity of these compounds, we picked out compound **5r** which had best antitumor activity against tumor cell line in **Table 1**, and further been determined their effects on GES-1 (normal human gastric epithelial cell) and HEEC (human normal esophageal cell). As shown in **Table 2**, we found it showed weak or no cytotoxicity against GES-1 and HEEC compared with 5-Fluorouracil.

2.2.3 Induced apoptosis and cell cycle arrest by compound 5r

Compound **5r** was chosen to further evaluate its possible anticancer mechanism of action against SW-620 cell based on the above results. After 72 h treatment with compound **5r**, SW-620 cells were arrested in S phase (**Figure 2**). Then, the apoptosis induction of compound **5r** against SW-620 cells was next investigated. After 72 h treatment with different concentrations of compound **5r**, the apoptosis rate was elevated to about 58.8% with high dose treatment group of compound **5r** (**Figure 2**).

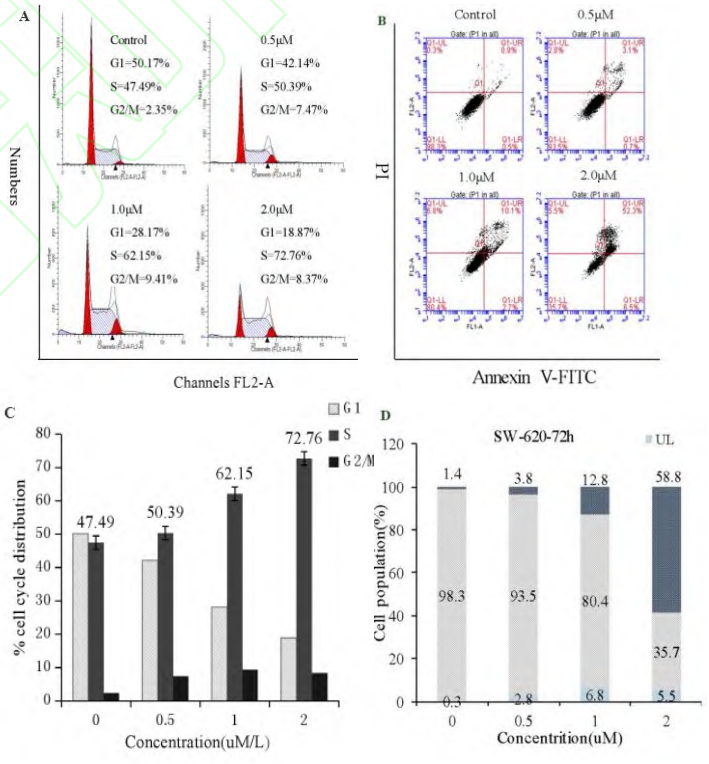

Figure 2 Cell cycle arrestment and apoptosis induction of compound **5r** after 72 h treatment with different concentrations(A) Cells were treated with different concentrations (0, 0.5, 1.0, 2μM/L) for 72 h. Then analyze DNA content by flow cytometry. (B) SW-620 cells apoptosis was detected by Annexin V/PI on flow cytometry. Cells treated with 0, 0.5, 1, 2μM/L for 72h were processed for analysis. (C) Quantitative analysis of the cell cycle distribution of SW-620 cells for 72h. (D) Quantitative analysis of

apoptosis cells of SW-620 cells for 72 h.

2.2.4 Molecular modeling

In order to predict the possible binding mode of this series of compounds with EGFR, molecular docking was performed using MOE 2014. EGFR (PDB code:5GNK) was retrieved from the Protein Data Bank (http://www.rcsb.org/pdb) for the docking calculations. Based on the antiproliferative activity results, compound **5r** (the most potent compound) was selected as ligand (**Figure 3**). As shown in **Figure 3**, compound **5r** was able to be tightly embedded in the active pocket of EGFR in the given posture. Compound **5r** can bind to Lys745 (3.21Å) residue and Asp855 (2.57Å) residue by hydrogen bonds and formed π-H interaction with Cys797 (3.66Å). The result suggested that compound **5r** may be a valuable lead compound.

3 Conclusions

In summary, a series of novel ~~novel~~ pyrimidine derivatives were designed, synthesized, and evaluated for their antitumor activity against MGC-803, PC-3, SW-620 and A549. The antiproliferative evaluation revealed that compound **5r** displayed the most potent and broad-spectrum proliferative inhibition against the tested cell lines, especially was sensitive to SW-620(IC_{50}=1.46μM), and was higher potent than 5-Fluorouracil. Further mechanism research showd compound **5r** induced SW-620 apoptosis, arrested cell cycle at S phase. Molecular docking revealed that compound **5r** could bind well to the active site of EGFR. Taken together, these results suggested that compound **5r** might be a valuable lead compound for antitumor agents.

Table 1 antiproliferative activities [IC_{50}/(μmol•L-1)] of target compounds against four cancer cell lines.

Compd.	R	MGC-803	PC-3	SW-620	A549
5a	2-Cl	13.81±1.14	25.66±1.41	6.73±1.22	23.13±1.37
5b	3-Cl	4.77±0.67	17.67±1.67	24.05±1.38	19.82±1.29
5c	4-Cl	5.05±0.70	18.39±1.70	7.30±0.86	20.19±1.31
5d	2-F	9.37±0.97	19.99±1.89	19.31±1.28	10.13±0.91
5e	3-F	28.52±1.45	32.57±1.55	>50	>50
5f	4-F	6.83±0.83	15.30±1.11	22.77±1.35	24.40±1.38
5g	2-CH_3	16.88±1.83	26.01±1.41	9.25±0.86	>50
5h	3-CH_3	13.69±1.13	20.20±1.12	7.78±0.89	29.12±1.46
5i	4-CH_3	4.96±0.69	7.33±1.00	3.97±0.59	10.38±1.03
5j	3,4,5-3OCH_3	4.45±0.64	3.02±0.83	16.42±1.33	22.52±1.35
5k	3-Cl-4-F	9.49±0.97	12.33±1.13	12.71±1.24	10.18±0.89
5l	3,4-di-Cl	7.37±0.88	8.73±0.13	7.57±0.87	13.48±1.13
5m	2-OCH_2CH_3	16.91±1.22	21.03±1.31	>50	>50
5n	3-CH_2CH_3	9.51±0.97	17.23±1.28	13.18±1.12	29.30±1.46
5o	4-OCH_2CH_3	4.04±0.60	7.23±0.63	9.87±1.47	5.84±0.76
5p	2-OCH_3	>50	24.38±1.23	>50	>50
5q	3-OCH_3	9.54±0.98	17.34±1.33	10.97±1.32	23.01±1.36
5r	4-OCH_3	1.53±0.13	5.62±0.11	1.46±0.11	3.68±0.56
5-Fu	-	12.24±1.23	9.65±0.57	10.32±0.79	13.38±1.25

[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}). Dates are presented as the mean ±SDs of three independent experiments. [b] Positive control.

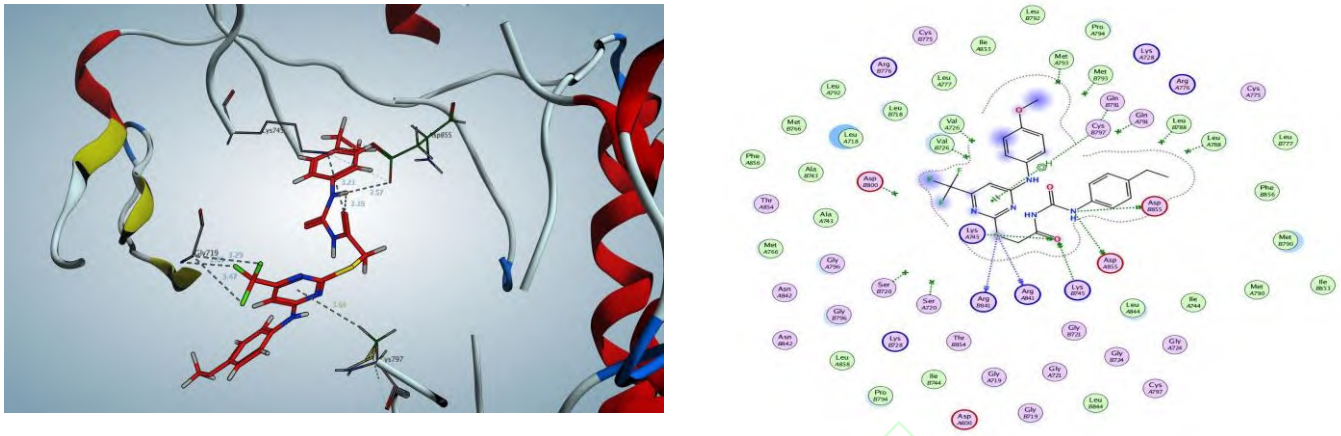

Figure 3 The 3D and 2D representation of EGFR crystal structure in complex with 5r (PDB code: 5GNK).

4 Experimental section

4.1 Materials

Silica gel: Qingdao Kangyexin Medicinal Silicone Desiccant Company Limited. Column chromatography silica gel: Yantai Jiangyou Silicone Development Company Limited. Potassium hydroxide: Tianjin Yongda Chemical Reagent Company Limited; Anhydrous Ethanol: Tianjin Yongda Chemical Reagent Company Limited. N, N-Dimethylformamide: Tianjin Yongda Chemical Reagent Company Limited. All reagents and solvents were purchased from commercial sources and were used without further purification. Melting points were determined on an X-5 micro melting apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured using a DPX-DPX -400 superconducting nuclear magnetic resonance instrument, Chemical shifts (δ) are given relative to TMS. High-resolution mass spectrometry was measured using a Waters-Micromass Q-TofMicro High Resolution Determination of tetragonal-flight time tandem mass spectrometer.

4.2 Antitumor activity evaluation

4.2.1 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, different concentrations of compounds were treated for 72h, respectively. MTT(3-[4,5-dimethylthia-zol-2-yl]-2,5-diphenyltetrazolium bromide, Solarbio) was added to each well at a final concentration of 0.5 mg/ml. After 4 h in a 37℃ incubator, the medium was aspirated. 150 ml DMSO was then added to each well to dissolve the formazan, and the plate was shaken on a shaker for 10 min. The absorbance was measured by an enzyme-linked immunosorbent assay reader (BioTek, USA) at a wavelength of 490 nm, and the cell survival rate was measured. The concentration-response curve generated by SPSS 16.0 software was used to determine the concentration of compound (IC_{50}) required to inhibit cell growth by 50%. Results were Mean ± SD of three independent experiments.

4.2.2 Cell cycle distribution assay

SW-620 cells were seeded in 6-well culture plate and treated with compound for 72 h. Then cells were harvested and fixed with 70% ethanol for 8 h at 4 ℃. The fixed cells were washed and resuspended using PBS containing 50 mg/mL and PI 10 mg/mL RNaseA. Then cell suspension was incubated for 40 min in a dark place at room temperature. After that, samples were analyzed for DNA content with flow cytometry (Becton, Dickinson and Company, NJ).

4.2.3 Cell apoptosis assay

SW-620 cells were seeded in 6-well culture plate and treated with compound for 72 h. Then cells were harvested and resuspended in binding buffer containing 0.5 mg/mL Annexin V-FITC and 0.5 mg/mL PI, then incubated for 40 min in a dark place. After that, samples were analyzed with flow cytometry (Becton, Dickinson and Company, NJ).

4.3 Chemistry

2-chloro-N-((4-ethylphenyl)carbamoyl)acetamide(**2**) [24],2-Mercapto-6-(trifluoromethyl)pyrimidin-4-ol(**3**)[25] were synthesized according to the literatures.

4.3.1Synthesis of 2-((4-chloro-6-(trifluoromethyl)pyrimidin-2-yl)thio)-N-((4-ethylphenyl)carbamoyl)acetamide (4)

In a dry flask, Compound **2**(2.45，10.20mmol) and **3**(2.00g，10.20mmol) were reacted in the 1,4-dioxane solution of KOH(1.14g，20.40mmol) at 70℃ for 1 h. The mixture was not further purified and phosphorus oxychloride (1.83 mL, 20mmol) were added slowly, and then the mixture was stirred at 90 ℃ for 2 h. The reaction mixture was cooled to room temperature. Ice water (50 mL) was added to the mixture with constant stirring. After 10 min, the precipitate was collected through filtration and washed with water to yield product **4**.

2-((4-chloro-6-(trifluoromethyl)pyrimidin-2-yl)thio)-N-((4-ethylphenyl)carbamoyl)acetamide**(4)**:white solid, yield 71.2%; m.p.159.1-160.3℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.03 (s, 1H), 10.23 (s, 1H), 8.01 (s, 1H), 7.34 (s, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.20 (t, *J* = 7.8 Hz, 1H), 6.90 (d, *J* = 7.5 Hz, 1H), 4.25 (s, 2H), 3.70 (s, 2H),

2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.37, 169.89, 162.74, 155.60, 155.24, 150.32, 138.27, 137.39, 128.76, 128.63, 124.38, 121.00, 120.03, 118.26, 116.67, 114.29, 35.62, 21.01. HRMS (ESI) Calcd for $C_{16}H_{14}ClF_3N_4O_2Na$ $[M+Na]^+$：441.0376 found: 441.0378.

4.3.2 General procedure for synthesis of 5a-5r

A solution of compound **4** (200 mg, 0.48 mmol) and different substituted-anilines (0.96 mmol) dissolved in DMF (2 mL) was slowly added to a solution of potassium carbonate (132mg, 0.96 mmol) dissolved in DMF（10 mL）. Then, the mixture was stirred at 80 ℃ for 6 h. After the reaction was completed, 20 mL of water was added, and the products were extracted with ethyl acetate (3 × 30 mL) and then purified by chromatography on silica gel ($V_{\text{petroleum ether}}$: $V_{\text{ethyl acetate}}$ = 5:1) to get the target compounds **5a-5r**.

2-((4-((2-chlorophenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)-N-((4-ethylphenyl)carbamoyl)acetamide **(5a)**:white solid, yield 75.3%; m.p.165.6-166.4℃； ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 10.39 (s, 1H), 10.18 (s, 1H), 7.79 (t，J = 2.1 Hz，1H), 7.74 - 7.71 (m，1H), 7.57 (dd，J = 8.2，2.0 Hz, 1H), 7.44 (dd，J = 8.2、2.1 Hz，1H), 7.37(d，J = 6.6 Hz，2H)，7.14(d，J = 6.6 Hz，2H), 6.86 (s, 1H), 4.13 (s, 2H), 2.53 (q, *J* = 7.6 Hz , 2H), 1.13 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.26, 170.98, 170.37, 160.06, 159.98, 150.45, 139.75, 139.20, 135.04, 133.17, 130.56, 130.52, 128.10, 123.37, 119.74, 118.70, 117.52, 46.13, 27.48, 15.57. HRMS (ESI) Calcd for $C_{22}H_{20}ClF_3N_5O_2S$ $[M+H]^+$：510.0978, found: 510.0977.

2-((4-((3-chlorophenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)-N-((4-ethylphenyl)carbamoyl)acetamide (**5b**):white solid, yield 77.1%; m.p.180.9-181.3℃； ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.97 (s, 1H), 10.40 (s, 1H), 10.22 (s, 1H), 7.75 (d, *J* = 12.5 Hz, 1H), 7.59 (d, *J* = 8.2 Hz, 1H), 7.44 – 7.33 (m, 3H), 7.19-7.11 (m, 3H), 6.88 (s, 1H), 4.15 (s, 2H), 2.55 (q, *J* = 7.7 Hz, 2H), 1.17 (t, *J* = 7.7 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.22, 170.35, 160.09, 150.45, 139.81, 139.12, 139.04, 135.13, 133.16, 130.56, 128.10, 123.36, 121.78, 119.72, 119.15, 118.76, 118.20, 35.03, 27.49, 15.61. HRMS (ESI) Calcd for $C_{22}H_{20}ClF_3N_5O_2S$ $[M+H]^+$：510.0978, found: 510.0974.

2-((4-((4-chlorophenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)-N-((4-ethylphenyl)carbamoyl)acetamide **(5c)**:white solid, yield 70.9%; m.p.179.3-180.2℃； ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H), 10.33 (s, 1H), 10.19 (s, 1H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.45 – 7.37 (m, 4H), 7.15 (d, *J* = 8.3 Hz, 2H), 6.85 (s, 1H), 4.15 (s, 2H), 2.55 (q, *J* = 7.6 Hz, 2H), 1.15 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.05, 170.18, 160.06, 159.68, 150.46, 139.14, 137.22, 135.11, 128.80, 128.73, 128.09, 121.83, 120.67, 119.74, 100.27, 32.70, 19.95, 15.27. HRMS (ESI) Calcd for $C_{22}H_{20}ClF_3N_5O_2S$ $[M+H]^+$：510.0978, found:510.0977.

N-((4-ethylphenyl)carbamoyl)-2-((4-((2-fluorophenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)acetamide **(5d)**:white solid, yield 79.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H), 10.20 (s, 1H), 10.01(s, 1H)，7.83 (s, 1H) ,7.41(d，*J* = 8.4 Hz，2H)，7.32 -7.25(m，1H)，7.25 -7.19(m，2H)，7.16(d，*J* = 8.3 Hz，2H)，6.99（s，1H)，4.07（s，2H)，2.54(q, *J* = 7.6 Hz , 2H)，1.16（t，*J* = 7.6 Hz，3H）. ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.91, 170.16, 160.92, 159.96, 150.43, 139.10, 135.17, 128.08, 126.29, 125.19, 124.42, 122.72, 121.85, 119.71, 119.12, 115.87, 115.68, 35.09, 27.49, 15.56. HRMS (ESI) Calcd for $C_{22}H_{20}F_4N_5O_2S$ $[M+H]^+$： 494.1274, found: 494.1275.

N-((4-ethylphenyl)carbamoyl)-2-((4-((3-fluorophenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)acetamide **(5e)**:white solid, yield 64.9%; m.p.163.6-164.1℃； ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H), 10.42 (s, 1H), 10.25 (s, 1H), 8.31 (d, *J* = 1.6 Hz, 1H), 7.63-7.52 (m, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 6.2 Hz, 2H), 4.15 (s, 2H), 2.55 (q, *J* = 7.5 Hz, 2H), 1.15 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.00, 170.33, 162.90, 162.24, 159.57, 150.69, 140.19, 138.72, 134.89, 130.16, 127.96, 119.79, 114.84, 110.26, 109.94，106.18, 105.92， 35.03, 27.10, 15.84. HRMS (ESI) Calcd for $C_{22}H_{20}F_4N_5O_2S$ $[M+H]^+$： 494.1274, found: 494.1277.

N-((4-ethylphenyl)carbamoyl)-2-((4-((4-fluorophenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)acetamide **(5f)**: white solid, yield 65.3%；m.p.159.9-160.6℃； ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (s, 1H), 10.24 (s, 1H), 10.20 (s, 1H), 7.61 (d, *J* = 7.9 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.24 – 7.12 (m, 4H), 6.82 (s, 1H), 4.12 (s, 2H), 2.55 (q, *J* = 7.6 Hz, 2H), 1.15 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.98, 170.23, 150.46, 139.13, 137.98, 135.11, 134.72, 134.51, 128.10, 124.12, 122.51, 121.94, 119.73, 115.66, 115.43, 34.77, 27.10, 15.60. HRMS (ESI) Calcd for $C_{22}H_{20}F_4N_5O_2S$ $[M+H]^+$： 494.1274, found: 494.1276.

N-((4-ethylphenyl)carbamoyl)-2-((4-(o-tolylamino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)acetamide**(5g)**:white solid, yield 66.7%; m.p.189.6-190.3℃； ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 10.22 (s, 1H), 10.15 (s, 1H), 7.49 (d, *J* = 7.2 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.33 (s, 1H), 7.26 (t, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 2H), 6.93 (d, *J* = 7.5 Hz, 1H), 6.84 (s, 1H), 4.13 (s, 2H), 2.55 (q, *J* =7.6 Hz, 2H), 2.30 (s, 3H), 1.15 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.92, 170.80, 167.13, 164.87, 159.69, 145.94, 138.93, 138.25, 138.03, 136.79, 131.84, 128.28, 126.95, 124.50, 124.47, 120.77, 117.80, 35.02, 27.49, 21.04, 15.61. HRMS (ESI) Calcd for $C_{23}H_{23}F_3N_5O_2S$ $[M+H]^+$： 490.1525, found: 490.1528.

N-((4-ethylphenyl)carbamoyl)-2-((4-(m-tolylamino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)acetamide**(5h)**:white solid, yield 57.4%；m.p.193.2-193.3℃； ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H), 10.24 (s, 1H), 9.68 (s, 1H), 7.41 (t, *J* = 9.3 Hz, 3H), 7.26 (d, *J* = 7.2 Hz, 1H), 7.21 (d, *J* = 6.7 Hz, 1H), 7.16 (d, *J* = 8.4 Hz, 4H), 3.99 (s, 2H), 2.56 (q, *J* = 7.6 Hz, 2H), 2.20 (s, 3H), 1.16 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.82, 170.69, 170.33, 161.38, 150.57, 150.42, 139.06, 135.50, 135.21, 130.65, 128.11, 126.26, 125.35, 123.76, 121.92, 119.66,

119.21, 34.97, 27.50, 17.72, 15.59. HRMS (ESI) Calcd for $C_{23}H_{23}F_3N_5O_2S$ $[M+H]^+$: 490.1521, found: 490.1528.

N-((4-ethylphenyl)carbamoyl)-2-((4-(p-tolylamino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)acetamide**(5i)**:white solid, yield 61.2%; m.p.189.9-190.7℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 10.21 (s, 1H), 10.12 (s, 1H), 7.46 (d, *J* = 5.2 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.16 (t, *J* = 7.8 Hz, 4H), 6.81 (s, 1H), 4.13 (s, 2H), 2.55 (q, *J* = 7.6 Hz, 2H),2.28(s, 3H) 1.15 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.95, 170.26, 159.98, 150.47, 139.09, 135.55, 135.18, 132.77, 129.32, 128.09, 121.88, 120.66, 120.54, 119.67, 119.16, 35.20, 27.49, 20.44, 15.60. HRMS (ESI) Calcd for $C_{23}H_{23}F_3N_5O_2S$ $[M+H]^+$: 490.1521, found: 490.1528.

N-((4-ethylphenyl)carbamoyl)-2-((4-(trifluoromethyl)-6-((3,4,5-trimethoxyphenyl)amino)pyrimidin-2-yl)thio)acetamide(**5j**):white solid, yield 65.3%; m.p.201.1-202.3℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 10.24 (s, 1H), 10.18 (s, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 2H), 7.00 (s, 2H), 6.82 (s, 1H), 4.13 (s, 2H), 3.80(s, 6H)3.66(s, 3H)2.56 (q, *J* = 7.6 Hz, 2H), 1.16 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.78, 170.63, 160.03, 152.86, 150.42, 142.68, 139.10, 135.14, 134.14, 128.08, 127.01, 121.85, 119.69, 119.12, 96.56, 60.09, 55.76, 34.95, 27.49, 15.59. HRMS (ESI) Calcd for $C_{25}H_{27}F_3N_5O_5S$ $[M+H]^+$: 566.1685, found:566.1687.

2-((4-((3-chloro-4-fluorophenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)-N-((4-ethylphenyl)carbamoyl)acetamide**(5k)**:white solid, yield 55.4%; m.p.199.3-200.4℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 10.37 (s, 1H), 10.21 (s, 1H), 7.82 (dd, *J* = 6.7, 2.2 Hz, 1H), 7.62 – 7.56 (m, 1H), 7.39 (d, *J* = 8.6 Hz, 3H), 7.15 (d, *J* = 8.3 Hz, 2H), 6.83 (s, 1H), 4.13 (s, 2H), 2.55 (q, *J* = 7.7 Hz, 2H), 1.14 (d, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.98, 170.31, 160.06, 159.83, 154.70, 152.25, 150.67, 150.43, 139.12, 135.44, 135.11, 128.09, 120.58, 119.72, 119.44, 117.15, 116.93, 35.04, 27.49, 15.60. HRMS (ESI) Calcd for $C_{22}H_{19}ClF_4N_5O_2S$ $[M+H]^+$: 528.0884; found: 528.0885.

2-((4-((3,4-dichlorophenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)-N-((4-ethylphenyl)carbamoyl) acetamide **(5l)**:white solid, yield 56.3%; m.p.201.4-201.9℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 1H), 10.45 (s, 1H), 10.19 (s, 1H), 7.89 (d, *J* = 1.9 Hz, 1H), 7.62 (dd, *J* = 6.3, 3.3 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 2H), 6.86 (s, 1H), 4.15 (s, 2H), 2.55 (q, *J* = 7.6 Hz, 2H), 1.15 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.05, 170.27, 160.00, 150.44, 145.06, 139.13, 138.46, 135.10, 131.09, 130.76, 128.10, 125.17, 121.51, 120.34, 119.94, 119.73, 100.57, 35.09, 27.49, 15.61. HRMS (ESI) Calcd for $C_{22}H_{19}Cl_2F_3N_5O_2S$ $[M+H]^+$: 544.0589, found: 544.0588.

2-((4-((2-ethoxyphenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)-N-((4-ethylphenyl)carbamoyl)acetamide **(5m):**white solid, yield 75.3%; m.p.158.2-160.2℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 10.23 (s, 1H), 9.50 (s, 1H), 7.73 (s, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 3H), 7.07 (d, *J* = 8.0 Hz, 1H), 7.02 (t, *J* = 3.4 Hz, 1H), 6.95 (t, *J* = 8.1 Hz, 1H), 4.11 (d, *J* = 7.0 Hz, 2H), 2.56 (q, *J* = 7.6 Hz, 2H), 1.38 (q, *J* = 7.0 Hz, 2H), 1.30 (t, *J* = 6.7 Hz, 3H), 1.14 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.56, 170.38, 160.10, 150.44, 147.59, 139.09, 135.17, 128.10, 127.11, 123.99, 120.25, 121.97, 119.69, 119.24, 116.18, 113.91, 112.69, 112.00, 63.81, 27.50, 15.61, 14.58, 14.47. HRMS (ESI) Calcd for $C_{24}H_{25}F_3N_5O_3S$ $[M+H]^+$: 520.1630, found: 520.1630.

2-((4-((3-ethylphenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)-N-((4-ethylphenyl)carbamoyl)acetamide **(5n):**white solid, yield 70.3%; m.p.159.9-160.7℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (s, 1H), 10.22 (s, 1H), 10.17 (s, 1H), 7.49 (s, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.35 (s, 1H), 7.28 (t, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 2H), 6.96 (d, *J* = 7.5 Hz, 1H), 6.83 (s, 1H), 4.12 (s, 2H), 2.62-2.56 (m, 4H), 1.18 – 1.12 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.82, 170.42, 154.52, 150.44, 144.49, 144.62, 139.11, 138.23, 137.57, 135.14, 128.82, 128.11, 125.84, 124.50, 123.23, 119.70, 118.68, 112.40, 34.98, 28.10, 27.49, 15.62, 15.39. HRMS (ESI) Calcd for $C_{24}H_{25}F_3N_5O_2S$ $[M+H]^+$: 504.1681, found: 504.1679.

2-((4-((4-ethoxyphenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)-N-((4-ethylphenyl)carbamoyl)acetamide **(5o)**:white solid, yield 69.7%; m.p.157.4-158.3℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1H NMR (400 MHz, DMSO) δ 10.89 (s, 1H), 10.23 (s, 1H), 10.07 (s, 1H), 7.47 (s, 2H), 7.41 (d, J = 8.4 Hz, 3H), 7.15 (d, J = 8.4 Hz, 2H), 6.91 (d, J = 8.8 Hz, 3H), 6.78 (s, 1H), 4.12 (s, 2H), 3.98 – 3.92 (m, 2H), 2.55 (q, J = 7.6 Hz, 2H), 1.33 – 1.29 (m, 3H), 1.16 (t, J = 7.6 Hz, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 170.94, 170.26, 150.50, 149.87, 142.11, 139.12, 135.17, 128.09, 121.91, 119.68, 115.25, 114.99, 114.54, 63.26, 27.50, 15.61, 14.84, 14.61. HRMS (ESI) Calcd for $C_{24}H_{25}F_3N_5O_3S$ $[M+H]^+$: 520.1630, found: 520.1632.

N-((4-ethylphenyl)carbamoyl)-2-((4-((2-methoxyphenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)acetamide**(5p)**:white solid, yield 68.3%; m.p.167.1-167.3℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 10.22 (s, 1H), 9.60 (s, 1H), 7.80 (s, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 3H), 7.08 (d, *J* = 7.9 Hz, 2H), 6.96 (t, *J* = 7.6 Hz, 1H), 4.06 (s, 2H), 3.83 (s, 3H), 2.56 (q, *J* = 7.6 Hz, 2H), 1.15 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.58, 170.35, 150.44, 139.09, 135.17, 128.11, 125.84, 124.37, 121.53, 120.24, 119.70, 117.45, 113.14, 111.65, 70.51, 69.75, 69.63, 55.63, 35.04, 27.49, 15.62. HRMS (ESI) Calcd for $C_{23}H_{23}F_3N_5O_3S$ $[M+H]^+$: 506.1474, found: 506.1475.

N-((4-ethylphenyl)carbamoyl)-2-((4-((3-methoxyphenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)acetamide**(5q)**:white solid, yield 65.3%; m.p.159.1-160.3℃; m.p.169.1-170.3℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 10.22 (s, 1H), 9.60 (s, 1H), 7.79 (s, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 4H), 7.09 (s, 1H), 6.96 (t, *J* = 7.6 Hz, 1H), 4.07 (s, 2H), 3.83 (s, 3H), 2.56 (q, *J* = 7.6 Hz, 2H), 1.14 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR

(101 MHz, DMSO-d_6) δ 170.62, 170.35, 160.97, 150.44, 139.09, 135.17, 128.10, 126.08, 124.50, 121.95, 120.24, 119.70, 119.22, 111.65, 70.51, 69.75, 69.64, 55.62, 35.04, 27.49, 15.61. HRMS (ESI) Calcd for $C_{23}H_{23}F_3N_5O_3S$ $[M+H]^+$：506.1474, found: 506.1475.

N-((4-ethylphenyl)carbamoyl)-2-((4-((4-methoxyphenyl)amino)-6-(trifluoromethyl)pyrimidin-2-yl)thio)acetamide**(5r)**:white solid, yield 55.3%；m.p.161.4-162.2℃；^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 10.23 (s, 1H), 10.06 (s, 1H), 7.48 (s, 2H), 7.40 (d, *J* = 8.5 Hz, 2H), 7.15 (d, *J* = 8.5 Hz, 2H), 6.93 (d, *J* = 8.9 Hz, 2H), 6.77 (s, 1H), 4.10 (s, 2H), 3.72 (s, 3H), 2.55 (q, *J* = 7.6 Hz, 2H), 1.15 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.92, 170.26, 150.49, 142.25, 139.12, 135.15, 134.05, 128.11, 121.90, 119.70, 119.18, 116.73, 114.91, 114.45, 114.06, 55.24, 35.15, 27.49, 15.62. HRMS (ESI) Calcd for $C_{23}H_{23}F_3N_5O_3S$ $[M+H]^+$：506.1474, found: 506.1475.

Supporting Information ^{1}H NMR, ^{13}C NMR and HRMS of compounds 4 and 5a-5r are available for free download from our website(http://sioc-journal.cn/.)

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图文摘要模板

Design, Synthesis and Antitumor Activity Evaluation of 2,4,6-substitute Pyrimidine Derivatives

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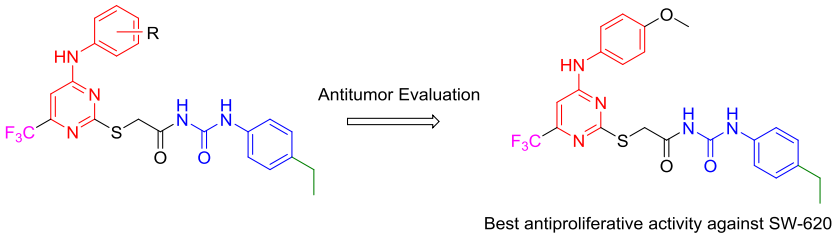

Best antiproliferative activity against SW-620

A series of novel pyrimidine derivatives were designed, synthesized, and evaluated for antitumor activity, among which compound **5r** possessed antiproliferative activity against SW-620 (IC_{50}=1.46μmol•L^{-1}), and the antiproliferative activity was better than the positive control drug 5-fluorouracil.

