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SYTHESIS AND BIOLOGICAL EVALUATION OF NEW PYRIMIDINE DERIVATIVES AS FAK INHIBITORS FOR DEVELOPMENT OF ANTITUMOR AGENTS

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Abstract – In this paper, a set of new pyrimidine derivatives was designed and synthesized. Subsequently, all the final targets were evaluated for antitumor activities *in vitro* on four human cancer cell lines including U-87 MG, MDA-MB-231, PC-3, and MCF-7, which were high expressed with focal adhesion kinase (FAK). The results were shown that these compounds performed well antitumor activities. Especially 2-((2-((4-((2-((2-acrylamidoethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (**7b**) exhibited the highest antitumor activities with 2.16 μM, 2.03 μM, 6.19 μM, and 21.31 μM, respectively. In addition, all the compounds were tested activities against FAK and compound **7b** was also the best candidate with IC_{50} value of 5.9 nM.

INTRODUCTION

Focal adhesion kinase (FAK) is a kind of non-receptor tyrosine kinase with 125 kDa. It plays an important role in the cell signal transition, and integrin.[1-3] Many studies have shown that FAK is overexpressed in a variety of tumors, such as ovarian cancer, head cancer, cervical squamous cell carcinoma, and liver cancer.[4] In these malignant tumors, FAK performed a crucial effect on cell growth, proliferation, migration, and adhesion.[5] Therefore, it is necessary to seek high active and selective inhibitors for the development of anti-tumor reagents.[6-8]

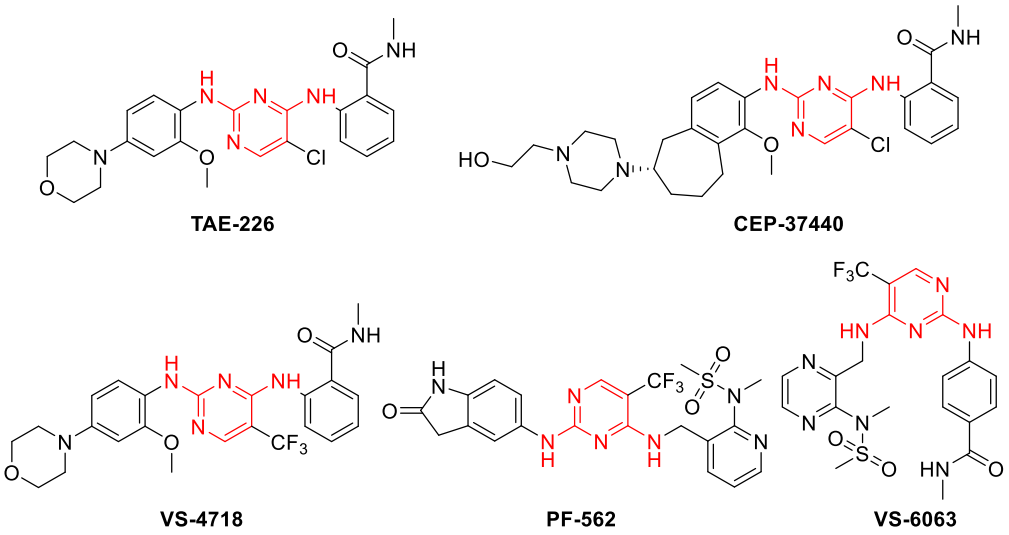


Figure 1. FAK inhibitors in market

Recently, there are lots of FAK inhibitors have been developed in clinical research.[9] As shown in **Figure 1**, TAE-226 and CEP-37440 were ATP competitive inhibitors, which worked mainly by blocking the entry of ATP into the catalytic sites of FAK.[10] Besides, TAE-226 and CEP-37440 have been proved good anti-tumor activity in preclinical studies *in vivo* and *in vitro*. They also could treat many cancers including glioma, ovarian cancer, neuroblastoma, and esophageal cancer in clinic.[11] VS-4718 (**Figure 1**), as the bioisostere of TAE-226 was behaved in antitumor activities by inhibiting the binding of ATP with FAK.[12] The other 5-trifluoromethylpyrimidine derivatives such as PA-562 and VS-6063 (**Figure 1**) could effectively inhibit the phosphorylation of FAK on Y397 amino acids, the growth and metastasis of breast cancer in mice, the growth of prostate cancer, the growth of lung cancer cells, and alter the tumor microenvironment.[13,14]

In 2018, Yen-Pon and coauthors reported the first irreversible inhibitor of FAK kinase.[15] As shown in **Figure 2**, the IC_{50} value of highly potent compound A reached in 0.6 nM. Our group has been focused on the synthesis of antitumor reagents for several years.[16-19] Inspired by the rapid growth of FAK inhibitor, we are now interested in development of new FAK inhibitors. In this paper, compound A was considered as lead compound. Subsequently, the chloride atom was replaced by CF_3 group according the bioisosterism in the new drug research. The cocrystal structure of compound A with FAK kinase revealed that the methylene ($-CH_2-$) linker was not necessary.[15] Therefore, we reduced the methylene group and designed the new pyrimidine derivatives as FAK inhibitors. At last, all the targets were synthesized and evaluated the antitumor activities against tumor cells and FAK kinase.

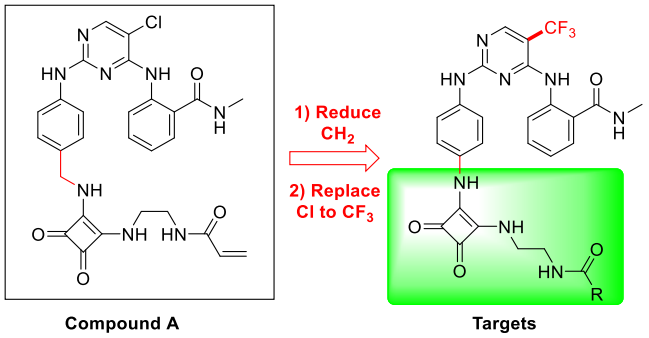
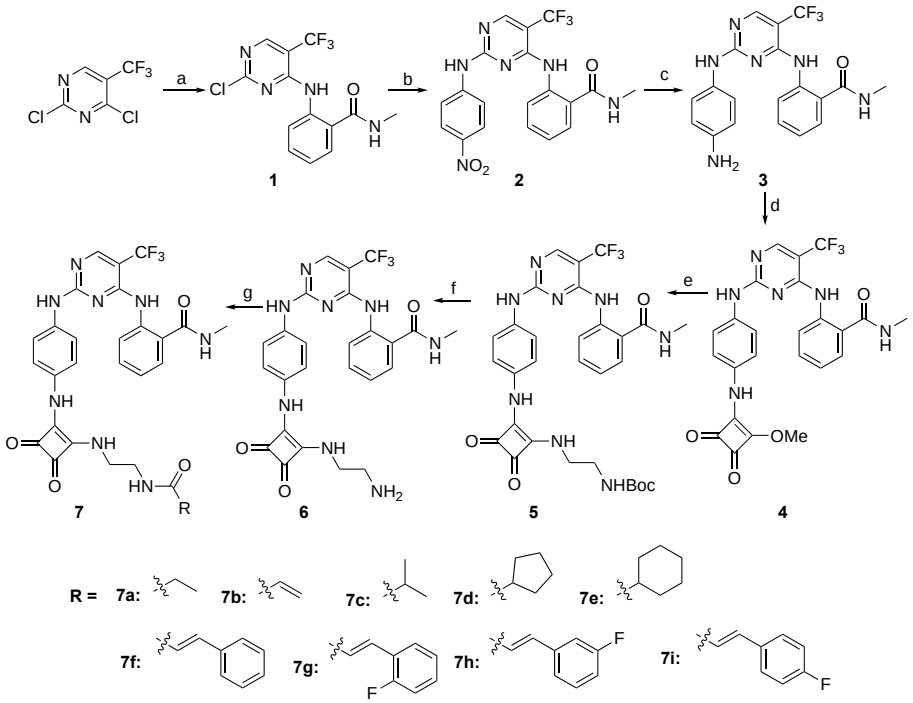


Figure 2. Our strategy to optimize compound **A**

RESULTS AND DISCUSSION

Starting from 2,4-dichloro-5-trifluoromethylpyrimidine, the target compounds **7a-7i** were synthesized by seven steps. The details of synthetic route were shown in Scheme 1. Firstly, the nucleophilic substitution reaction of 2,4-dichloro-5-trifluoromethylpyrimidine with 2-amino-*N*-methylbenzamide gave compound **1** in 55% yield. In the presence of TFA and TFE, compound **1** reacted with 4-nitroaniline to obtain the compound **2** with 49% yield. And then, the intermediate **3** was prepared in good yield by reduction of the nitro group with Pd/C under hydrogen atmosphere. Subsequently, compound **3** was converted to compound **5** with 62% and 43% yields respectively, which was experienced two substituted reactions by dimethyl squarate and *tert*-butyl (2-aminoethyl)carbamate.

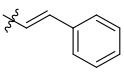
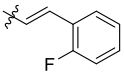
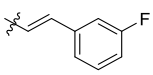
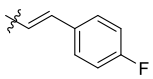


Scheme 1. Synthetic route of target compounds **7a-7i**. Reagents and conditions: a) 2-amino-*N*-methyl-benzamide, $NaHCO_3$, EtOH, rt, overnight; b) 4-nitroaniline, TFA, TFE, reflux, overnight; c) Pd/C, MeOH, rt, 24 h; d) dimethyl squarate, DIEA, DMF, rt, 12 h; e) *tert*-butyl (2-aminoethyl)carbamate, DIEA, DMF, 80 °C, 12 h; f) hydrogen chloride-EtoAc solution; g) corresponding acyl chloride, TEA, DCM or corresponding acid, HATU, DIEA, DMF, rt, 12 h.

After deprotected Boc group under HCl, we got the key intermediate **6** with high yield. At last, the final compounds **7a-7e** were synthesized in corresponding acyl chloride and compounds **7f-7i** were synthesized in corresponding acid with moderate yields. All the new products including **2-6**, **7a-7i** were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. The spectrum was shown in experimental part.

With the targets **7a-7i** in hand, we evaluated the anti-tumor activities of the compounds against four tumor cell lines including human glioblastoma (U-87MG), human metastatic breast cancer (MDA-MB-231), and human prostate cancer (PC-3), MCF-7 (Human breast adenocarcinoma cell line) cell, which were overexpressed with FAK kinase. The antiproliferative activities were tested by methyl thiazolyltetrazolium colorimetric assay (MTT)[20] as previous report and TAE-226 was used as the positive control. The IC_{50} values were summarized in Table 1. Against U-87 MG and MDA-MB-231 cells compound **7b** (IC_{50} values of 2.16 μM and 2.03 μM) was more potent than the other compounds. Against PC-3 cells compound **7g** (IC_{50} value of 4.03 μM) was more active than the compounds **7a-7f** and **7h-7i**. Against MCF-7 cells compound **7g** (IC_{50} value of 8.29 μM) was the more potential compound than TAE-226 (IC_{50} value of 8.33 μM) and the other compounds.

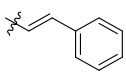
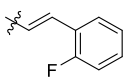
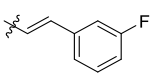
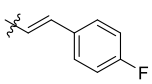
Table 1. IC_{50} values for cancer cell lines[a]

Entry	Comp.	R	IC_{50} (μM) [a]			
			U-87 MG	MDA-MB-231	PC-3	MCF-7
1	**7a**		5.47 ± 0.39	8.42 ± 0.47	7.63 ± 0.52	15.02 ± 3.92
2	**7b**		2.16 ± 0.14	2.03 ± 0.15	6.19 ± 0.75	21.31 ± 4.83
3	**7c**		8.33 ± 0.81	7.94 ± 1.03	9.52 ± 0.85	18.47 ± 0.36
4	**7d**		9.09 ± 0.81	8.15 ± 0.87	7.43 ± 0.62	19.84 ± 3.77
5	**7e**		9.36 ± 0.88	8.41 ± 1.05	9.89 ± 0.74	20.68 ± 4.11
6	**7f**		4.15 ± 0.47	4.08 ± 0.36	4.11 ± 0.38	9.44 ± 2.45
7	**7g**		3.89 ± 0.53	3.92 ± 0.64	4.03 ± 0.55	8.29 ± 1.67
8	**7h**		4.33 ± 0.48	4.16 ± 0.52	4.43 ± 0.43	10.07 ± 2.19
9	**7i**		4.45 ± 0.61	4.24 ± 0.55	4.49 ± 0.53	11.18 ± 2.56
10	**TAE-226**		1.42 ± 0.15	1.58 ± 0.22	2.67 ± 0.29	8.33 ± 2.35

[a] The values are mean ± SD of three replicates

To investigate the possible mechanism of antitumor activity, we used ADP-Glo kinase kit to test the ability of targets to inhibit FAK kinase.[21] As shown in Table 2, compound **7b** gave the best IC_{50} value of 5.9 nM, which was higher than TAE-226 (IC_{50} value of 3.5 nM). The preliminary result is consistent with the tumor cell assay. Compared **7b** with compound A (**Figure 2**),[15] the IC_{50} value against FAK decreased from 5.9 nM to 0.6 nM. This phenomenon suggested that the strategy of removement a methylene group and replacement of Cl with CF_3 was not successful for developing new FAK inhibitor. However, when the substituents were styrene **7f-7i**, the IC_{50} values surprisedly could reach 11-16 nM. And the most active compound **7g** was 11 nM, which was very closed to the positive control TAE-226. These results indicated that the compounds **7f-7i** bearing enone fragment might exist covalent interaction with Cys427 like compound A (**Figure 2**).[15] Whether the compounds **7f-7i** were covalent inhibitors required more kinetic experiments. But, the enone moiety indeed gave a choice to design new FAK inhibitor. In addition, the calculated values of clog P for **7a-7i** were the range of 3.35-5.71, which were well to discovery new anti-tumor reagents.

Table 2. IC_{50} values for FAK

Entry	Comp.	R	clog P	FAK IC_{50} (μM) [a]
1	**7a**		3.51	0.042 ± 0.011
2	**7b**		3.35	0.0059 ± 0.0014
3	**7c**		3.81	0.088 ± 0.023
4	**7d**		4.45	0.17 ± 0.064
5	**7e**		5.01	0.18 ± 0.063
6	**7f**		5.57	0.013 ± 0.0065
7	**7g**		5.71	0.011 ± 0.0069
8	**7h**		5.71	0.016 ± 0.0082
9	**7i**		5.71	0.015 ± 0.0086
10	**TAE-226**		4.30	0.0035 ± 0.0007

[a] The values are mean ± SD of three replicates

CONCLUSION

In summary, a new series of pyrimidine derivatives as FAK inhibitors were designed and synthesized. All the structures of new compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS. The final compounds were tested antitumor activities against U-87 MG, MDA-MB-231, PC-3, and MCF-7 cancer cells. The results indicate that compound **7b** was the strongest antiproliferative activities with IC_{50} values of 2.16 μM, 2.03 μM, 6.19 μM, and 21.3 μM, respectively. Moreover, the result for FAK kinase assay showed that compound **7b** was the best enzyme inhibitory activity with IC_{50} value of 5.9 nM. Further researches are still underway in our lab.

EXPERIMENTAL

All commercial materials were used without further purification. Melting points were determined with X-4X digital display micro melting point analyzer (uncorrected, Shanghai Microelectronics Technology Co., Ltd.). Analytical thin-layer chromatography was performed on precoated 250 μm layer thickness silica gel 60 F254 plates and visualized with UV light. Column chromatography was performed silica gel 300-400 mesh. ^{1}H NMR and ^{13}C NMR spectroscopic data were recorded with Bruker 400 MHz NMR spectrometer and JEOL-ECX 500 NMR spectrometer in DMSO-d_6 solution, with TMS serving as the internal standard. The high-resolution mass spectrometer (HRMS) was tested in TSQ 8000 high-resolution mass spectrometer and AB SCIEX X500R QTOF.

2-(2-Chloro-5-trifluoromethylpyrimidin-4-ylamino)-*N*-methylbenzamide (1)

2,4-Dichloro-5-trifluoromethylpyrimidine (8 mmol) was added to a stirred solution of 2-amino-*N*-methylbenzamide (8.8 mmol) and $NaHCO_3$ (8.8 mmol) in anhydrous EtOH (10 mL) at room temperature. The resulted mixture was heated to reflux and stirred overnight before cooled to room temperature. The precipitate was filtered out, washed with water give the title compound as yellow solid (1.452 g; 55% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 8.86 (q, J = 4.4 Hz, 1H), 8.68 (s, 1H), 8.38 (d, J = 8.4 Hz, 1H), 7.78 (dd, J = 8.0, 1.2 Hz, 1H), 7.59 (td, J = 8.0, 1.2 Hz, 1H), 7.26 (td, J = 8.0, 1.2 Hz, 1H), 2.34 (d, J = 4.4 Hz, 3H). Spectral properties were in accordance with the literature.[22]

***N*-Methyl-2-((2-((4-nitrophenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)benzamide (2)**

To a solution of compound **1** (5 mmol) in TFE (2,2,2-trifluoroethanol, 20 mL) was added 4-nitroaniline (6 mmol) and TFA (trifluoroacetic acid, 15 mmol). The resulted mixture was heated to reflux under nitrogen atmosphere and stirred overnight before cooled to room temperature. The mixture was added EtOAc (100 mL) and washed with saturated aqueous $NaHCO_3$ (50 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo to afford the crude compound. The residue was purified by silica-gel column using DCM/MeOH = 30/1 to give the product. 1.058 g yellow solid; 49% yield; mp > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 8.86 (q, J = 4.4 Hz, 1H), 8.68 (s, 1H), 8.38 (d, J

= 8.4 Hz, 1H), 7.78 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.59 (m, 1H), 7.26 (m, 1H), 2.34 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (100MHz, DMSO-d_6) δ 169.1, 160.6, 156.8, 156.4, 146.7, 141.5, 138.7, 131.7, 128.5, 126.0, 125.1, 123.9, 123.8, 123.4, 123.3, 119.3, 26.7; ESI-HRMS $C_{19}H_{15}F_3N_6O_3$ ([M+Na]$^+$): calcd 455.1056, found 455.1037.

2-((2-((4-Aminophenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (3)

To a solution of compound **2** (864 mg, 2 mmol) in MeOH (10 mL) was added Pd/C (86 mg). The mixture was stirred at room temperature under hydrogen atmosphere for 24 h. The solution was filtered with celite and the filtration was evaporated under vacuum. The crude solid was recrystallized with MeOH to afford compound **3**. 0.611 g yellow solid; 76% yield; mp 225.4-226.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.30 (s, 1H), 9.42 (s, 1H), 8.72 (d, *J* = 4.4 Hz, 1H), 8.36 (d, *J* = 23.4 Hz, 2H), 7.70 (d, *J* = 7.6 Hz, 1H), 7.47 – 7.07 (m, 4H), 6.51 (d, *J* = 8.8 Hz, 2H), 4.89 (s, 2H), 2.78 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (100MHz, DMSO-d_6) δ 169.3, 161.9, 160.1, 156.3, 145.5, 139.3, 132.0, 128.2, 126.6, 125.7, 123.7, 122.6, 118.8, 116.1, 114.7, 114.2, 26.7; ESI-HRMS $C_{19}H_{17}F_3N_6O$ ([M+H]$^+$): calcd 403.1488, found 403.1478.

2-((2-((4-((2-Methoxy-3,4-dioxocyclobut-1-en-1-yl)amino)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (4)

To a solution of compound **3** (804 mg, 2 mmol) in DMF (10 mL) was added dimethyl squarate (284 mg, 2 mmol) and DIEA (258 mg, 2 mmol). The mixture was stirred at room temperature for 12 h. The mixture was extracted with EtOAc (100 mL x 3) and the combined organic phase was washed with saturated brine (50 mL x 3). The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo to afford the crude compound. The residue was purified by silica-gel column using DCM/MeOH = 30/1 to give the product. White solid 635 mg; 62% yield; mp 234.8-235.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 10.72 (s, 1H), 9.87 (s, 1H), 8.75 (s, 1H), 8.44 (s, 1H), 7.68 (dd, *J* = 43.2, 7.8 Hz, 3H), 7.50 (t, *J* = 7.6 Hz, 1H), 7.31 – 7.16 (m, 4H), 4.39 (s, 3H), 2.79 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 186.6, 170.2, 166.8, 165.8, 155.4, 150.3, 148.9, 145.4, 145.2, 140.5, 131.2, 129.0, 125.3, 122.7, 121.0, 119.2, 118.5, 117.2, 116.4, 68.8, 41.4. ESI-HRMS $C_{24}H_{19}F_3N_6O_4$ ([M+H]$^+$): calcd 513.1492, found 513.1492.

***tert*-Butyl (2-((2-((4-((4-((2-(methylcarbamoyl)phenyl)amino)-5-(trifluoromethyl)pyrimidin-2-yl)amino)phenyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)ethyl)carbamate (5)**

To a solution of compound **4** (528 mg, 1 mmol) in DMF (10 mL) was added *tert*-butyl (2-aminoethyl)carbamate (160 mg, 1 mmol) and DIEA (129 mg, 1 mmol). The mixture was stirred at 80 °C for 12 h. The mixture was extracted with EtOAc (50 mL x 3) and the combined organic phase was washed with saturated brine (20 mL x 3). The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo to afford the crude compound. The residue was purified by silica-gel column using DCM/MeOH = 30/1 to give the product. White solid 397 mg; 43% yield; mp > 250 °C; ^{1}H NMR (400 MHz,

DMSO-d_6) δ 11.33 (s, 1H), 9.83 (s, 1H), 9.66 (s, 1H), 8.74 (d, *J* = 4.4 Hz, 1H), 8.43 (s, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.65 – 7.48 (m, 5H), 7.34 (d, *J* = 8.6 Hz, 2H), 7.17 (t, *J* = 7.8 Hz, 1H), 6.98 (s, 1H), 3.62 (d, *J* = 0.8 Hz, 2H), 3.16 (dd, *J* = 11.2, 5.6 Hz, 2H), 2.79 (d, *J* = 4.4 Hz, 3H), 1.36 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 187.0, 169.7, 164.8, 155.9, 155.4, 151.2, 150.3, 149.0, 145.4, 145.1, 128.0, 127.7, 125.3, 124.9, 122.7, 121.0, 119.3, 118.5, 117.7, 114.9, 82.7, 49.0, 45.0, 43.0, 41.4. ESI-HRMS $C_{30}H_{31}F_3N_8O_5$ ([M+H]$^+$): calcd 641.2442, found 641.2442.

2-((2-((4-((2-((2-Aminoethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (6)

A mixture of compound **5** (640 mg, 1 mmol) and hydrogen chloride-EtoAc solution (5 mL, 1 mol/L) was stirred at room temperature for 12 h. The mixture was evaporated under vacuum and recrystallized with MeOH to afford compound **6**. White solid 454 mg; 84% yield; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.35 (s, 1H), 10.07 – 9.81 (m, 2H), 8.75 (d, *J* = 4.4 Hz, 1H), 8.43 (s, 2H), 7.90 (d, *J* = 2.8 Hz, 4H), 7.73 (d, *J* = 9.2 Hz, 1H), 7.62 (d, *J* = 7.2 Hz, 2H), 7.51 (t, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 7.6 Hz, 2H), 7.17 (t, *J* = 7.2 Hz, 1H), 3.81 (dd, *J* = 11.6, 6.0 Hz, 4H), 3.15 – 3.05 (m, 4H), 2.79 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 184.0, 169.7, 169.2, 164.6, 161.1, 159.0, 158.7, 157.4, 142.1, 135.1, 134.5, 128.3, 126.4, 123.7, 123.1, 122.0, 118.9, 118.1, 115.2, 41.7, 27.3, 26.7; ESI-HRMS $C_{25}H_{23}F_3N_8O_3$ ([M+H]$^+$): calcd 541.1917, found 541.1923.

A mixture of compound **6** (270 mg, 0.5 mmol), DCM (4 mL) and TEA (101 mg, 1 mmol) was stirred at room temperature. And then the corresponding acyl chloride (0.5 mmol) was added into the solution. The mixture was stirred at room temperature for 12 h and purified by silica-gel column using DCM/MeOH = 30/1 to give the product **7a-7e**.

2-((2-((4-((3,4-Dioxo-2-((2-propionamidoethyl)amino)cyclobut-1-en-1-yl)amino)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (7a)

Yellow solid 125 mg; 42% yield; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 9.83 (d, *J* = 1.6 Hz, 1H), 9.71 (s, 1H), 8.74 (d, *J* = 4.4 Hz, 1H), 8.43 (s, 1H), 7.96 (d, *J* = 1.6 Hz, 1H), 7.62 (m, 6H), 7.35 (d, *J* = 8.8 Hz, 2H), 7.17 (t, *J* = 8.0 Hz, 1H), 3.63 (d, *J* = 4.4 Hz, 2H), 3.31 – 3.25 (m, 2H), 2.79 (d, *J* = 4.4 Hz, 3H), 2.08 (q, *J* = 7.6 Hz, 2H), 0.99 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.2, 181.0, 176.7, 169.3, 164.0, 161.2, 156.7, 156.3, 142.3, 139.1, 137.5, 135.0, 134.5, 131.7, 128.3, 127.6, 126.5, 124.0, 123.1, 122.0, 118.7, 116.6, 28.9, 26.7, 10.3; ESI-HRMS $C_{28}H_{27}F_3N_8O_4$ ([M+H]$^+$): calcd 597.2179, found 597.2180.

2-((2-((4-((2-((2-Acrylamidoethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (7b)

Yellow solid 92 mg; 31% yield; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 9.84 (s, 1H), 9.68 (s, 1H), 8.74 (d, *J* = 4.4 Hz, 1H), 8.37 (d, *J* = 43.2 Hz, 2H), 7.76 – 7.48 (m, 6H), 7.34 (d, *J* = 8.8 Hz,

2H), 7.17 (t, *J* = 7.6 Hz, 1H), 6.25 – 6.17 (m, 1H), 6.10 (d, *J* = 17.2 Hz, 1H), 5.60 (d, *J* = 10.0 Hz, 1H), 3.69 (d, *J* = 4.4 Hz, 2H), 3.41 (d, *J* = 22 Hz, 2H), 2.79 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.1, 178.1, 176.0, 169.3, 165.4, 161.2, 159.3, 157.6, 148.0, 147.9, 146.0, 145.9, 144.3, 140.7, 138.0, 136.0, 134.8, 133.7, 132.1, 128.3, 125.7, 123.6, 122.1, 118.6, 26.7; ESI-HRMS $C_{28}H_{25}F_3N_8O_4$ ([M+H]$^+$): calcd 595.2023, found 595.2023.

2-((2-((4-((2-((2-Isobutyramidoethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (7c)

Yellow solid 134 mg; 44% yield; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 9.77 (d, *J* = 44.4 Hz, 2H), 8.74 (d, *J* = 4.4 Hz, 1H), 8.42 (s, 1H), 7.93 (s, 1H), 7.72 (d, *J* = 6.8 Hz, 1H), 7.67 – 7.47 (m, 4H), 7.34 (d, *J* = 8.8 Hz, 2H), 7.17 (t, *J* = 7.6 Hz, 1H), 3.64 (d, *J* = 3.6 Hz, 2H), 3.27 (d, *J* = 5.6 Hz, 2H), 2.79 (d, *J* = 4.4 Hz, 3H), 2.34 (m , 1H), 0.99 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 187.4, 167.2, 164.8, 163.9, 163.6, 161.6, 155.9, 155.4, 151.2, 149.0, 145.1, 139.6, 131.3, 128.0, 127.7, 125.4, 122.7, 121.0, 119.3, 118.5, 117.7, 115.0, 47.7, 41.4, 36.0; ESI-HRMS $C_{29}H_{29}F_3N_8O_4$ ([M+H]$^+$): calcd 611.2336, found 611.2336.

2-((2-((4-((2-((2-(Cyclopentanecarboxamido)ethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (7d)

Yellow solid 153 mg; 48% yield; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 9.86 (d, *J* = 11.2 Hz, 2H), 8.76 (s, 1H), 8.43 (s, 1H), 7.96 (d, *J* = 4.8 Hz, 1H), 7.66 (m , 6H), 7.35 (d, *J* = 7.6 Hz, 2H), 7.17 (d, *J* = 7.2 Hz, 1H), 3.63 (d, *J* = 1.6 Hz, 2H), 3.27 (d, *J* = 4.0 Hz, 2H), 2.78 (d, *J* = 3.6 Hz, 3H), 1.62 (m , 8H), 1.22 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 187.1, 169.4, 167.2, 164.8, 161.0, 155.9, 155.4, 151.2, 149.0, 145.3, 145.1, 131.3, 127.9, 127.8, 125.4, 122.7, 119.3, 119.0, 118.5, 117.7, 115.9, 114.9, 55.9, 44.3, 41.4, 40.9; ESI-HRMS $C_{31}H_{31}F_3N_8O_4$ ([M+H]$^+$): calcd 637.2492, found 637.2493.

2-((2-((4-((2-((2-(Cyclohexanecarboxamido)ethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (7e)

Yellow solid 169 mg; 52% yield; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.33 (s, 1H), 9.85 (s, 1H), 9.71 (s, 1H), 8.76 (q, *J* = 3.6 Hz, 1H), 8.43 (s, 1H), 7.97 – 7.87 (m, 1H), 7.76 – 7.46 (m, 6H), 7.34 (d, *J* = 8.8 Hz, 2H), 7.22 – 7.12 (m, 1H), 3.63 (d, *J* = 2.8 Hz, 2H), 3.26 (d, *J* = 5.2 Hz, 2H), 2.79 (d, *J* = 4.4 Hz, 3H), 2.07 (t, *J* = 11.2 Hz, 1H), 1.67 (d, *J* = 9.6 Hz, 4H), 1.38 – 1.06 (m, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 187.4, 167.2, 164.8, 160.9, 155.9, 155.4, 151.2, 150.3, 149.0, 147.6, 145.3, 145.1, 129.9, 128.0, 127.7, 125.4, 122.7, 121.0, 119.3, 118.5, 117.7, 115.0, 55.6, 43.7, 41.4, 40.8, 40.6; ESI-HRMS $C_{32}H_{33}F_3N_8O_4$ ([M+H]$^+$): calcd 651.2649, found 651.2649.

A mixture of compound **6** (270 mg, 0.5 mmol) and DMF (4 mL) and DIEA (129 mg, 1 mmol) was stirred at room temperature. And then the corresponding acid (0.5 mmol) and HATU (380 mg, 2 mmol) were added into the solution. The mixture was stirred at room temperature for 12 h. The solution was extracted

with EtOAc (50 mL x 3) and the combined organic phase was washed with saturated brine (20 mL x 3). The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo to afford the crude compound. The residue was purified by silica-gel column using DCM/MeOH = 30/1 to give the product **7f-7i**.

2-((2-((4-((2-((2-Cinnamamidoethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (7f)

Yellow solid 124 mg; 37% yield; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 10.11 (d, J = 0.4 Hz, 1H), 9.81 (d, J = 4.4 Hz, 1H), 8.81 – 8.70 (m, 2H), 8.46 – 8.32 (m, 3H), 8.09 (s, 1H), 7.72 (d, J = 6.8 Hz, 1H), 7.65 – 7.34 (m, 10H), 7.16 (t, J = 7.2 Hz, 1H), 6.64 (d, J = 15.6 Hz, 1H), 3.71 (d, J = 5.2 Hz, 2H), 3.43 (d, J = 5.4 Hz, 2H), 2.78 (d, J = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.6, 169.8, 169.3, 165.8, 164.2, 161.2, 156.9, 156.6, 151.6, 147.4, 146.0, 139.3, 136.9, 135.3, 134.7, 133.4, 131.7, 130.0, 129.4, 129.3, 128.3, 128.0, 123.8, 123.1, 122.5, 122.0, 121.2, 118.6, 26.7; ESI-HRMS $C_{34}H_{29}F_3N_8O_4$ ([M+H]$^+$): calcd 671.2336, found 671.2336.

(*E*)-2-((2-((4-((2-((2-(3-(2-Fluorophenyl)acrylamido)ethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (7g)

Yellow solid 110 mg; 32% yield; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 9.78 (d, J = 35.6 Hz, 2H), 8.74 (d, J = 4.4 Hz, 1H), 8.45 (d, J = 22.0 Hz, 3H), 7.76 – 7.24 (m, 12H), 7.16 (t, J = 7.6 Hz, 1H), 6.74 (d, J = 16.0 Hz, 1H), 3.72 (s, 2H), 3.44 (d, J = 5.4 Hz, 2H), 2.79 (d, J = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.3, 169.7, 169.3, 167.7, 165.6, 164.1, 162.2, 161.0, 159.7, 156.6, 156.1, 148.6, 136.2, 134.6, 132.7, 131.8, 131.8, 129.7, 128.3, 126.6, 125.5, 123.2, 122.9, 122.8, 122.1, 121.3, 120.4, 119.4, 118.7, 116.7, 116.4, 26.7; ESI-HRMS $C_{34}H_{28}F_4N_8O_4$ ([M+H]$^+$): calcd 689.2242, found 689.2242.

(*E*)-2-((2-((4-((2-((2-(3-(3-Fluorophenyl)acrylamido)ethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (7h)

Yellow solid 96 mg; 28% yield; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 10.71 (s, 1H), 9.82 (s, 1H), 8.84 – 8.74 (m, 2H), 8.68 (s, 1H), 8.48 – 8.40 (m, 2H), 7.64 – 7.41 (m, 10H), 7.29 – 7.13 (m, 2H), 6.66 (m , 1H), 3.72 – 3.68 (m, 2H), 3.59 (m , 2H), 2.78 (d, J = 4.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 187.3, 170.6, 167.0, 164.1, 159.8, 155.9, 155.5, 152.5, 151.4, 149.0, 145.1, 142.7, 141.3, 135.4, 130.4, 128.8, 127.0, 126.4, 125.3, 125.1, 123.5, 122.7, 119.3, 118.5, 117.7, 114.8, 113.3, 111.6, 111.5, 41.4; ESI-HRMS $C_{34}H_{28}F_4N_8O_4$ ([M+H]$^+$): calcd 689.2242, found 689.2242.

(*E*)-2-((2-((4-((2-((2-(3-(4-Fluorophenyl)acrylamido)ethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-*N*-methylbenzamide (7i)

Yellow solid 103 mg; 30% yield; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (s, 1H), 10.69 (s, 1H), 9.81 (s, 1H), 8.82 – 8.62 (m, 2H), 8.42 (m, 4H), 7.72 (d, J = 7.8 Hz, 1H), 7.65 – 7.38 (m, 10H), 6.58

(d, J = 15.6 Hz, 1H), 3.72 (d, J = 10.8 Hz, 2H), 3.45 (d, J = 5.6 Hz, 2H), 2.78 (d, J = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 188.5, 169.9, 169.3, 165.7, 164.3, 161.9, 161.2, 157.4, 156.0, 147.9, 145.7, 142.6, 139.7, 138.0, 135.0, 133.1, 132.5, 131.7, 130.2, 130.1, 128.3, 127.9, 122.4, 121.1, 119.5, 118.6, 116.5, 116.2, 26.7; ESI-HRMS $C_{34}H_{28}F_4N_8O_4$ ($[M+H]^+$): calcd 689.2242, found 689.2242.

Cytotoxicity Evaluation (MTT Assay) U-87 MG (human glioblastoma cancer cell line), MDA-MB-231 (human metastatic breast cancer cell line), and PC-3 (human prostate cancer cell line), MCF-7 (Human breast adenocarcinoma cell line) were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. All the cells were maintained in RPMI 1640 or DMEM complete medium. *In vitro* cytotoxicity of synthesized compounds against tumor cell lines (U-87 MG, MDA-MB-231, PC-3, and MCF-7) was determined by MTT assay described as previous article. TAE-226 was used as positive control.

***In vitro* FAK assay** The final compounds were prepared into different concentrations of 5% DMSO solution. The different concentration solution (1 μL) was added into 384 well plate. And then, each hole was added FAK (2 ng/μL, 2 μL), adenosine triphosphate (ATP) (250 μmol/L, 1 μL), the substrate (1 μg/μL, 1 μL). The solution was incubated in 30 ℃ shaker for 60 min, and added 5 μL ADP-Glo reagent, incubated at 25 ℃ for 40 min, and added 10 μL enzyme detection reagent. The data was collected and IC_{50} value was calculated by Graphpad Prism 5 Software.

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