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SYNTHESIS AND EVALUATION OF DIAMINOPYRIMIDINE DERIVATIVES AS DUAL INHIBITORS OF EGFR AND SRC FOR ANTITUMOR TREATMENT

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Abstract – In this paper, a series of novel diaminopyrimidine derivatives was designed and synthesized using palladium-catalyzed Buchwald–Hartwig-type heteroarylamination procedure. And then, they were evaluated for antitumor activity *in vitro* on wild type epidermal growth factor receptor tyrosine kinase ($EGFR^{wt}$-TK), c-Src and four human cancer cell lines including A549, PC-3, SMMC-7721 and K562. The results displayed that some of the compounds had good activities. Especially 2-(2-cycloheptylamino-5-trifluoromethylpyrimidin-4-ylamino)-*N*-methylbenzamide (**1h**) showed high antitumor activities against four cancer cell lines with 2.33, 7.46, 1.13 and 1.28 μM. Furthermore, the IC_{50} values of compound **1h** for EGFR and Src reached in 0.86 μM and 0.22 μM.

INTRODUCTION

Epidermal growth factor receptor (EGFR) tyrosine kinase (TK) plays an indispensable role in cancer cell proliferation, survival, adhesion, migration and differentiation. Overexpression and mutation of EGFR have been associated with a variety of cancers.[1,2] At present, several drugs such as Gefitinib, Afatinib, Osimertinib and Avitinib have been approved by the Food and Drug Administration (FDA) for clinical use as EGFR inhibitors (Figure 1).[3] However, the emergence of acquired point mutations has weakened

their therapeutic efficacy, leading to drug resistance and toxicity burden. c-Src is a type of non-receptor tyrosine kinase, which functions in diverse cellular processes.[4] It is an important anticancer target, because of its key roles in signaling pathways including apoptosis, migration and invasion. The EGFR/Src/STAT3 signaling pathway not only affects the proliferation, differentiation, invasion and metastasis of tumor cells, but also plays a key role in the treatment of tumors.[5] Dual EGFR and Src inhibition may not be sufficient for sustained inhibition of STAT3 activation. In clinic, the combination of Lapatinib (EGFR inhibitor) and Saracatinib (Src inhibitor) could induce the Lapatinib-resistant cells apoptosis.[6] Therefore, targeting EGFR/Src pathway is one of the effective ways to overcome the resistance to EGFR inhibitors in certain cancers. It has been proved that development of drugs targeted both EGFR and Src may offer a better therapeutic advantage.[7,8]

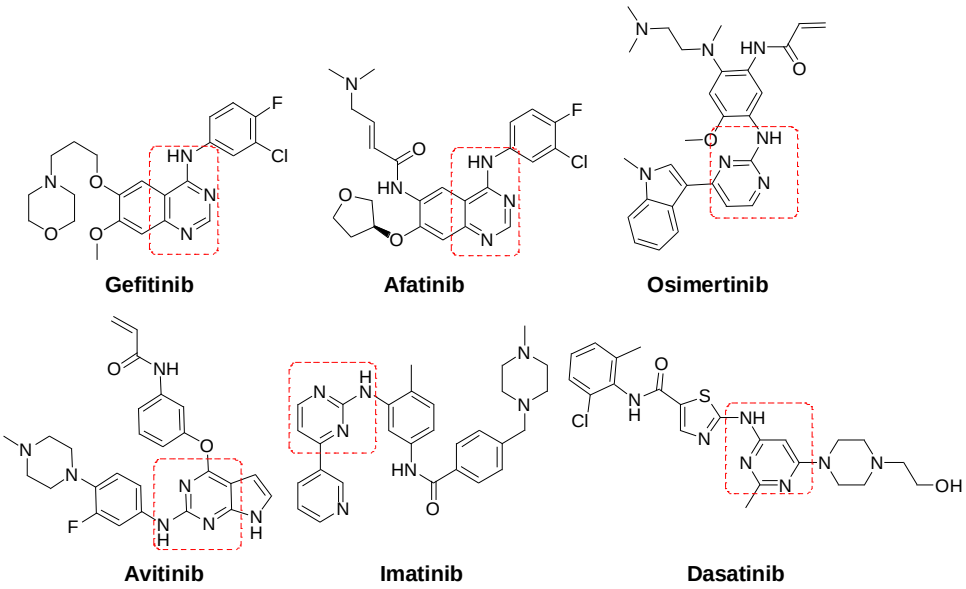


Figure 1. Tyrosine kinase inhibitors in drug

Pyrimidine, the privileged heterocycyclic scaffold, has been widely studied because of their interesting pharmacological activities.[9-11] Polysubstituted pyrimidines, especially 2- and 4-aminopyrimidines, constitute important biological and pharmaceutical compounds that are also being increasingly studied in materials research.[12] For example, Imatinib the first marketed tyrosine kinase inhibitor and Dasatinib a c-Src inhibitor approved in 2006 (Figure 1) were all contained the 2-amino or 4-aminopyrimidine structures.[13] Our group has been involved for several years in the design and synthesis of antitumor agents, including several different kinase inhibitors.[14-16] Based on our group previous research and initial screening, we found that compound **I** (Figure 2) showed 64% inhibition at 10 μM for Src. Rational drug design which is according to the known three-dimensional structure of drug target protein or DNA to find

and design drug candidates has been the most effective strategy in modern medicinal chemistry. Recently, a large number of tyrosine kinase inhibitors were reported through the method of rational drug design.[17] In this paper, we designed a new series of diaminopyrimidine derivatives **1** using this method and the previous result of compound **I**, which could efficiently bind with the key residues of EGFR and Src. As shown in Figure 2, we firstly changed the chlorine atom to trifluoromethyl group to increase hydrophilic activity. In literature, we found that *N*-methylbenzamide was important for EGFR activity and thus we studied the different substituent groups of 4-morpholinophenyl group in the left side of compound **I**.[18] The total of 8 novel diaminopyrimidine compounds **1a-h** were designed and synthesized. Preliminary in vitro antiproliferative ability of synthetic compounds against A549 (Human non-small cell lung cancer) cell, PC-3 (Human prostate cancer) cell, SMMC-7721 (Human liver cancer) cell and K562 (Human erythroleukemic cancer) cell and the structure-activity relationship (SAR) was disclosed.

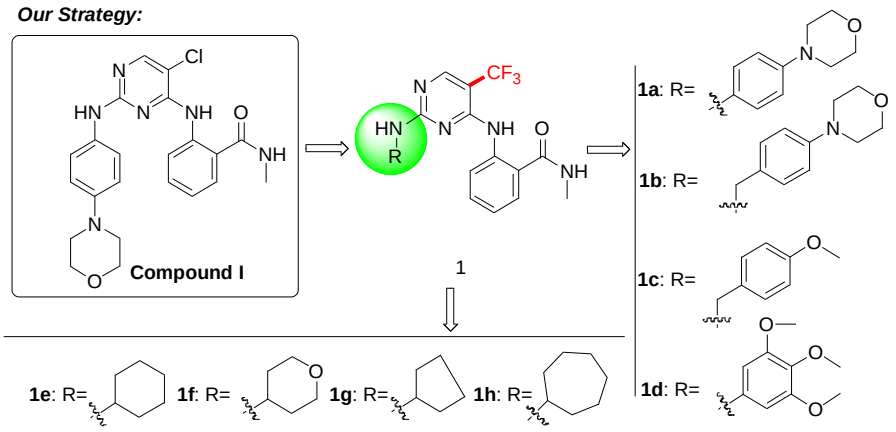
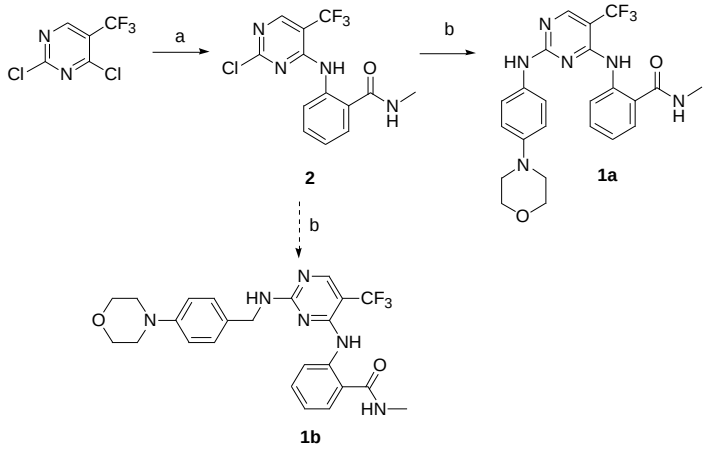


Figure 2. Our strategy to optimize compound **I**

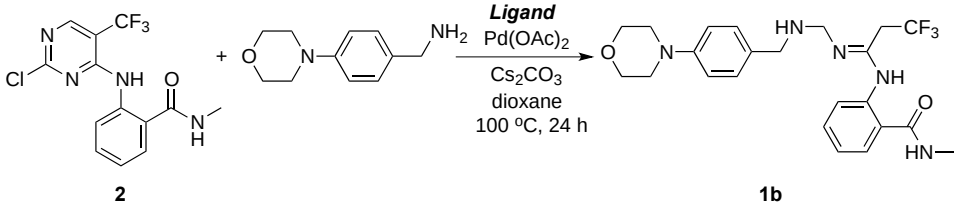
RESULTS AND DISCUSSION

The synthetic route of the target compound **1a** was shown in Scheme 1. Starting from 2,4-dichloro-5-trifluoromethylpyrimidine, the first chlorine atom in position 4 is displaced by substituted 2-amino-*N*-methylbenzamide in the presence of $NaHCO_3$ in EtOH at room temperature to afford monosubstituted compound **2** in 54% yield. The chlorine atom in position 2 of compound **2** was further substituted to the compound **1a** in the presence of TFA in TFE through the agency of the corresponding arylamine in 68% yield. However, it was no product **1b** when compound **2** reacted with 4-(morpholin-4-yl)benzylamine at the same condition.



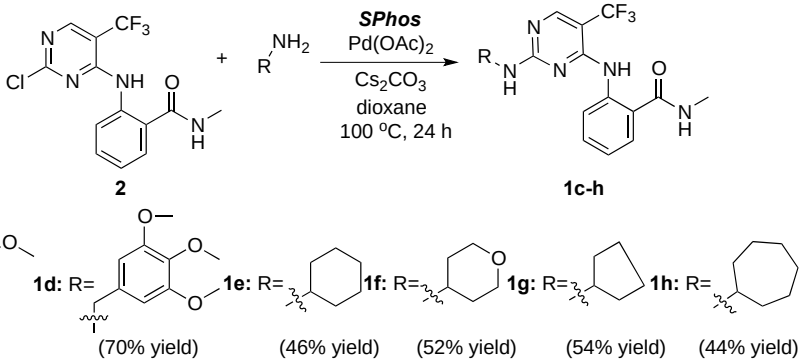
Scheme 1. Synthetic route of target compounds **1a**. Reagents and conditions: a) 2-amino-*N*-methylbenzamide, $NaHCO_3$, EtOH, rt, overnight; b) corresponding amine, TFA, TFE, reflux, overnight.

In order to access **1b-h**, we began our investigation with Pd-catalyzed Buchwald–Hartwig-type heteroarylamination procedure.[19] Under the classical condition of 10 mol% $Pd(OAc)_2$, 20 mol% BINAP and 1.5 equiv of Cs_2CO_3 in dioxane, the reaction proceeded and we were delighted to obtain the product **1b** with 21% yield. We subsequently investigated the effect of various ligands, and SPhos ligand gave better result 62% yield. Lower yield (39-45%) was obtained when SPhos ligand was replaced by XPhos,



Ligand: a) BINAP, 21% yield; b) XPhos, 39% yield; c) XantPhos, 44% yield; d) SPhos, 62% yield; e) DavePhos, 45% yield

(b)

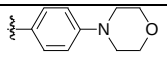
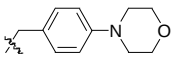
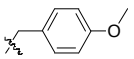
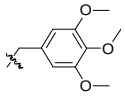


Scheme 2. Synthetic route of target compounds **1b-h**

XantPhos and DavePhos. Having determined the optimal reaction conditions, the target compounds **1c-h** were explored with different substituted aliphatic amine derivatives. They could work well and afforded corresponding products in good yields with 4-methoxybenzyl (**1c**), 3,4,5-trimethoxybenzyl (**1d**) and cyclic substituents such as cyclohexyl (**1e**), tetrahydropyranyl (**1f**), cyclopentyl (**1g**) and cycloheptyl (**1h**) groups. All the products **1a-h** were fully characterized by NMR, mass spectroscopy and elemental analysis. The details of operation and analytical data were given in experimental part.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate the antiproliferative activities of these novel compounds against four human tumor cell lines including A549, PC-3, SMMC-7721 and K562. Gefitinib was used as reference compound. The IC_{50} values of synthesized compounds were listed in Table 1. The results indicated that all compounds exhibited moderate activities. Against A549 cells, compounds **1a**, **1d**, **1f** and **1h** (with IC_{50} values of 5.68, 4.54, 3.05 and 2.33 μM, respectively) were more potent than Getitinib (IC_{50} = 7.22 μM), while compounds **1b**, **1c**, **1e** and **1g** were less than Gefitinib. Against PC-3 cells, compounds **1a-d**, **1f** and **1h** were more potent than Gefitinib (IC_{50} = 9.29 μM), while **1e** and **1g** were less than Gefitinib. The inhibitory efficacy of compounds **1a**, **1f** and **1h** against SMMC-7721 cells were higher than Gefitinib (IC_{50} = 6.04 μM), and the IC_{50} values were 5.87, 4.11 and 1.13 μM. Also, the compounds **1a**, **1f** and **1h** showed the same inhibitory effect against K562 compared to Gefitinib. Overall, the anti-proliferative activities of compounds **1a**, **1d**, **1f** and **1h** against all tested tumor cells were higher than that of Gefitinib, which suggested that cyclic structure of R was well for anti-tumor activity.

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

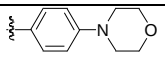
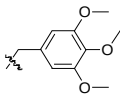
Entry	Comp.	R	IC_{50} (μM)			
			A549	PC-3	SMMC-7721	K562
1	**1a**		5.68 ± 0.18	6.51 ± 0.24	5.87 ± 0.16	4.31 ± 0.11
2	**1b**		>10	5.32 ± 0.44	8.56 ± 0.77	>10
3	**1c**		>10	7.24 ± 0.58	9.49 ± 0.66	>10
4	**1d**		4.54 ± 0.11	8.96 ± 0.28	7.03 ± 0.22	6.84 ± 0.35
5	**1e**		>10	>10	9.89 ± 0.73	>10
6	**1f**		3.05 ± 0.27	8.27 ± 0.46	4.11 ± 0.38	2.34 ± 0.15
7	**1g**		>10	>10	8.71 ± 0.64	>10
8	**1h**		2.33 ± 0.15	7.46 ± 0.43	1.13 ± 0.14	1.28 ± 0.17

9	Gefitinib	7.22 ± 3.51	9.29 ± 1.12	6.04 ± 0.55	6.83 ± 0.75

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

As the designed compounds **1a**, **1d**, **1f** and **1h** had good anti-cancer activity, *in vitro* EGFR and Src kinase inhibition capability was firstly evaluated to see whether their cytotoxicity was due to kinase inhibition. As shown in Table 2, all the compounds were higher than Gefitinib for $EGFR^{wt}$-TK (IC_{50} = 0.0061 μM) and Dasatinib for Src (IC_{50} = 0.001 μM). However, the best desired compound **1h** showed IC_{50} value of 0.86 μM for EGFR and 0.22 μM for c-Src. These data suggested that the seven-member ring was more effective than the others. This result is consistent with the previous structure-activity relationship of tumor cell assay.

Table 2. IC_{50} Values for $EGFR^{wt}$-TK and c-Src[a]

Entry	Comp.	R	IC_{50} (μM)	
			$EGFR^{wt}$-TK	c-Src
1	**1a**		8.22 ± 0.25	0.24 ± 0.061
2	**1d**		5.29 ± 0.31	8.58 ± 0.34
3	**1f**		3.36 ± 0.28	0.25 ± 0.042
4	**1h**		0.86 ± 0.07	0.22 ± 0.058
5		Gefitinib	0.0061 ± 0.0003	-
6		Dasatinib	-	0.001 ± 0.0001

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

CONCLUSION

In summary, a series of novel diaminopyrimidine derivatives were designed and synthesized under Pd-catalyzed Buchwald-Hartwig-type heteroarylamination method. *In vitro* biological activities of novel compounds on cell level and enzyme level were evaluated. Four human cancer cell lines including A549, PC-3, SMMC-7721 and K562 showed that **1a**, **1d**, **1f** and **1h** have good anti-proliferative activity. The result for EGFR and Src kinase assay showed that compound **1h** was the strongest activities with IC_{50} values of 0.86 μM and 0.22 μM, respectively. Other further studies are going on in our lab.

EXPERIMENTAL SECTION

NMR spectroscopic data were recorded with Bruker 400 MHz NMR spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) in DMSO-d_6 or $CDCl_3$ solution, with TMS serving as the internal standard. Mass spectrometry was recorded with SHIMADZU DUIS-2020. Melting points were determined with X-4X digital display micro melting point analyzer (uncorrected, Shanghai Microelectronics Technology Co., Ltd.). Elemental analysis was recorded with Vario ELcube.

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

2,4-Dichloro-5-trifluoromethylpyrimidine (4 mmol) was added to a stirred solution of 2-amino-*N*-methylbenzamide (4.4 mmol) and $NaHCO_3$ (4.4 mmol) in anhydrous EtOH (5 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 light yellow solid (713 mg; 54% yield); mp 208-209 °C; ^{1}H NMR (400 MHz, DMSO) 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); ESI-MS *m/z* 331.1 $[M + H]^+$. Spectral properties were in accordance with the literature.[20]

N-Methyl-2-[2-(4-morpholin-4-ylphenylamino)-5-trifluoromethylpyrimidin-4-ylamino]benzamide (1a)

To a solution of compound **2** (1 mmol) in TFE (2,2,2-trifluoroethanol, 4 mL) was added 4-(morpholin-4-yl)phenylamine (1.2 mmol) and TFA (trifluoroacetic acid, 3 mmol). The resulted mixture was heated to reflux and stirred overnight before cooled to room temperature. The mixture was added EtOAc (50 mL) and washed with saturated $NaHCO_3$ (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 title compound as white solid (208 mg; 44% yield); mp 223-224 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.33 (s, 1H), 9.62 (s, 1H), 8.73 (s, 1H), 8.39 (s, 1H), 7.71 (d, *J* = 7.2 Hz, 1H), 7.47 (br, 3H), 7.18 – 7.14 (m, 1H), 6.88 (d, *J* = 8.8 Hz, 2H), 3.80 – 3.65 (m, 4H), 3.10 – 2.97 (m, 4H), 2.79 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 169.3, 161.4, 156.5, 156.3, 147.6, 139.3, 131.9, 131.7, 128.3, 126.5, 123.8, 122.9, 122.6, 122.5, 115.8, 110.0, 66.6 (2C), 49.5 (2C), 26.7; ESI-MS *m/z* 473.1 $[M + H]^+$. Anal. Calcd for $C_{23}H_{23}F_3N_6O_2$ C 58.47, H 4.91, N, 17.79. Found C 58.50, H 4.93, N 17.68.

A 10-mL Schlenk tube equipped with a stir-bar was charged with compound **2** (0.5 mmol), corresponding amine (0.75 mmol), $Pd(OAc)_2$ (0.025 mmol), SPhos (0.05 mmol), Cs_2CO_3 (0.75 mmol), anhydrous dioxane (2.5 mL). The reaction tube was purged with argon. The Schlenk tube was placed in an oil-bath at 100 °C for 24 h and then cooled to room temperature. The reaction mixture was extracted with EtOAc

(3 × 20 mL). The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure and the crude residue purified by flash chromatography on silica gel. The purified material was dried in vacuo to afford the corresponding products **1b-h**.

***N*-Methyl-2-[2-((4-morpholin-4-yl)benzylamino)-5-(trifluoromethylpyrimidin-4-yl)amino]benzamide (1b)** White solid 75 mg; 31% yield; mp 212-213 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.08 (s, 1H), 8.65 (d, *J* = 4.4 Hz, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 8.22 (s, 1H), 7.82 (t, *J* = 6.0 Hz, 1H), 7.65 (d, *J* = 8.8 Hz, 1H), 7.37 (t, *J* = 8.4 Hz, 1H), 7.20 (d, *J* = 8.8 Hz, 2H), 7.02 (t, *J* = 7.6 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 2H), 4.56 (d, *J* = 6.0 Hz, 2H), 3.75 – 3.65 (m, 4H), 3.07 – 3.00 (m, 4H), 2.78 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 169.4, 160.7, 158.4, 155.1 (q, *J* = 5.2 Hz), 150.4, 140.2, 131.9, 130.3, 128.4, 128.0, 126.6, 124.0, 121.4, 120.5, 120.4, 115.5, 66.5 (2C), 49.1(2C), 43.7, 26.7; ESI-MS *m/z* 487.1 $[M + H]^+$. Anal. Calcd for $C_{24}H_{25}F_3N_6O_2$ C 59.25, H 5.18, N, 17.27. Found C 59.21, H 5.12, N 17.33.

2-[2-(4-Methoxybenzylamino)-5-(trifluoromethylpyrimidin-4-yl)amino]-*N*-methylbenzamide (1c) White solid 71 mg; 33% yield; mp 205-206 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.08 (s, 1H), 8.64 (s, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 8.23 (s, 1H), 7.84 (s, 1H), 7.66 (d, *J* = 12.0 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.05 – 7.00 (m, 1H), 6.89 (d, *J* = 8.8 Hz, 2H), 4.58 (d, *J* = 6.0 Hz, 2H), 3.70 (s, 3H), 2.79 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 169.4, 160.7, 158.5, 158.4, 155.2 (q, *J* = 5.2 Hz), 140.1, 131.9, 131.6, 128.4, 128.3, 126.6, 124.0, 121.4, 120.6, 120.4, 114.1, 55.4, 43.6, 26.7; ESI-MS *m/z* 432.1 $[M + H]^+$. Anal. Calcd for $C_{21}H_{20}F_3N_5O_2$ C 58.47, H 4.67, N, 16.23. Found C 58.41, H 4.65, N 16.29.

***N*-Methyl-2-[5-trifluoromethyl-2-((3,4,5-trimethoxybenzylamino)pyrimidin-4-yl)amino]benzamide (1d)** White solid 93 mg; 38% yield; mp 208-209 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.13 (s, 1H), 8.64 (d, *J* = 4.4 Hz, 1H), 8.47 (d, *J* = 8.4 Hz, 1H), 8.24 (s, 1H), 7.85 (t, *J* = 6.0 Hz, 1H), 7.66 (d, *J* = 9.2 Hz, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 7.03 (t, *J* = 7.6 Hz, 1H), 6.68 (s, 2H), 4.57 (d, *J* = 6.0 Hz, 2H), 3.67 (s, 6H), 3.59 (s, 3H), 2.78 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 169.4, 160.7, 158.4, 155.2 (q, *J* = 5.2 Hz), 153.2, 140.2, 136.7, 135.6, 131.8, 128.4, 126.6, 123.9, 121.4, 120.6, 120.4, 105.0, 60.4, 56.1 (2C), 44.4, 26.6; ESI-MS *m/z* 492.1 $[M + H]^+$. Anal. Calcd for $C_{23}H_{24}F_3N_5O_4$ C 56.21, H 4.92, N, 14.25. Found C 56.22, H 4.95, N 14.18.

2-[2-(4-Cyclohexylphenylamino)-5-(trifluoromethylpyrimidin-4-yl)amino]-*N*-methylbenzamide (1e) White solid 80 mg; 41% yield; mp 201-202 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.11 (s, 1H), 8.69 (d, *J* = 4.4 Hz, 1H), 8.63 (d, *J* = 8.0 Hz, 1H), 8.21 (s, 1H), 7.70 (d, *J* = 6.8 Hz, 1H), 7.47 (t, *J* = 8.4 Hz, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.56 (d, *J* = 7.6 Hz, 1H), 4.10 – 4.02 (m, 1H), 2.82 (d, *J* = 4.4 Hz, 3H), 1.89 (d, *J* = 10.2 Hz, 2H), 1.77 (d, *J* = 12.8 Hz, 2H), 1.65 (d, *J* = 12.8 Hz, 1H), 1.52 – 1.40 (m, 2H), 1.34 (d, *J* = 12.8 Hz, 2H), 1.21 – 1.11 (m, 1H); ^{13}C NMR (100 MHz, DMSO) δ 169.4, 160.7, 157.7, 155.4 (q, *J* = 5.2 Hz),

140.4, 131.7, 128.5, 126.6, 123.9, 121.5, 120.8, 120.2, 50.7, 32.1, 26.7, 25.7, 25.5; ESI-MS *m/z* 394.1 [M + H]$^+$. Anal. Calcd for $C_{19}H_{22}F_3N_5O$ C 58.01, H 5.64, N, 17.80. Found C 57.99, H 5.66, N 17.87.

***N*-Methyl-2-[2-(tetrahydropyran-4-ylamino)-5-(trifluoromethylpyrimidin-4-yl)amino]benzamide (1f)** White solid 77 mg; 39% yield; mp 203-204 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.09 (s, 1H), 8.67 (d, *J* = 4.0 Hz, 1H), 8.58 (d, *J* = 8.4 Hz, 1H), 8.23 (s, 1H), 7.69 (d, *J* = 7.6 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.07 (t, *J* = 7.6 Hz, 1H), 6.79 (d, *J* = 7.6 Hz, 1H), 4.34 – 4.25 (m, 1H), 3.93 (d, *J* = 10.8 Hz, 2H), 3.40 (t, *J* = 10.8 Hz, 2H), 2.80 (d, *J* = 4.4 Hz, 3H), 1.82 – 1.70 (m, 4H); ^{13}C NMR (100 MHz, DMSO) δ 169.4, 160.7, 157.8, 155.5 (q, *J* = 5.2 Hz), 140.3, 131.8, 128.5, 126.5, 123.8, 121.6, 120.9, 120.4, 67.0 (2C), 48.2, 32.3 (2C), 26.7; ESI-MS *m/z* 396.1 [M + H]$^+$. Anal. Calcd for $C_{18}H_{20}F_3N_5O_2$ C 54.68, H 5.10, N, 17.71. Found C 54.70, H 5.14, N 17.68.

2-(2-Cyclopentylamino-5-(trifluoromethylpyrimidin-4-yl)amino)-*N*-methylbenzamide (1g) White solid 61 mg; 32% yield; mp 199-200 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.13 (s, 1H), 8.70 – 8.65 (m, 2H), 8.22 (s, 1H), 7.70 (d, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.05 (t, *J* = 7.2 Hz, 1H), 6.64 (d, *J* = 7.2 Hz, 1H), 4.54 – 4.44 (m, 1H), 2.82 (d, *J* = 4.4 Hz, 3H), 2.00 – 1.97 (m, 2H), 1.71 – 1.56 (m, 6H); ^{13}C NMR (100 MHz, DMSO) δ 169.4, 160.7, 158.2, 155.2 (q, *J* = 5.2 Hz), 140.4, 131.8, 128.4, 126.6, 123.9, 121.4, 120.7, 120.4, 53.0, 32.1, 26.7, 24.0; ESI-MS *m/z* 380.1 [M + H]$^+$. Anal. Calcd for $C_{18}H_{20}F_3N_5O$ C 56.99, H 5.31, N, 18.46. Found C 57.02, H 5.30, N 18.41.

2-(2-Cycloheptylamino-5-(trifluoromethylpyrimidin-4-yl)amino)-*N*-methylbenzamide (1h) White solid 59 mg; 29% yield; mp 211-212 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.11 (s, 1H), 8.69 (d, *J* = 4.4 Hz, 1H), 8.64 (d, *J* = 8.4 Hz, 1H), 8.20 (s, 1H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 1H), 7.06 (t, *J* = 7.2 Hz, 1H), 6.57 (d, *J* = 7.6 Hz, 1H), 4.28 – 4.20 (m, 1H), 2.81 (d, *J* = 4.4 Hz, 3H), 1.92 – 1.84 (m, 2H), 1.72 – 1.60 (m, 6H), 1.55 – 1.46 (m, 4H); ^{13}C NMR (100 MHz, DMSO) δ 169.4, 160.8, 157.4, 155.3 (q, *J* = 5.2 Hz), 140.3, 131.7, 128.5, 126.6, 123.9, 121.5, 120.7, 120.3, 52.6, 34.2, 27.8, 26.7, 24.7; ESI-MS *m/z* 408.1 [M + H]$^+$. Anal. Calcd for $C_{20}H_{24}F_3N_5O$ C 58.96, H 5.94, N, 17.19. Found C 58.97, H 5.99, N 17.23.

***In vitro* EGFR^{wt}-TK and Src assay** Recombinant EGFR and Src were purchased from Sino Biology Inc. Antiphosphotyrosine mouse mAb was purchased from PTM Bio. The effects of compounds on the activity of wild type EGFR tyrosine kinase and Src were determined by enzyme-linked immunosorbent assays (ELISAs) with recombinant EGFR and Src according to reported methods.[21]

Cytotoxicity Evaluation (MTT Assay) A549 (Human non-small cell lung cancer cell line) cell was purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences; PC-3 (Human prostate cancer cell line) cell was donated by the Key Laboratory of Natural Product Chemistry of the Chinese

Academy of Sciences of Guizhou Province; SMMC-7721 (Human liver cancer cell line) cell was donated by the Department of Immunology, the Third Military Medical University; K562 (Human erythroleukemic cancer cell line) cell was purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences; All cell lines were kept by the laboratory. Cells were maintained in RPMI 1640 or DMEM complete medium. In vitro cytotoxicity of synthesized compounds against three kinds of human tumor cell lines (A549, PC-3, SMMC-7721 and K562) was determined by MTT assay described as previous article.[16] Gefitinib were used as positive controls.

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