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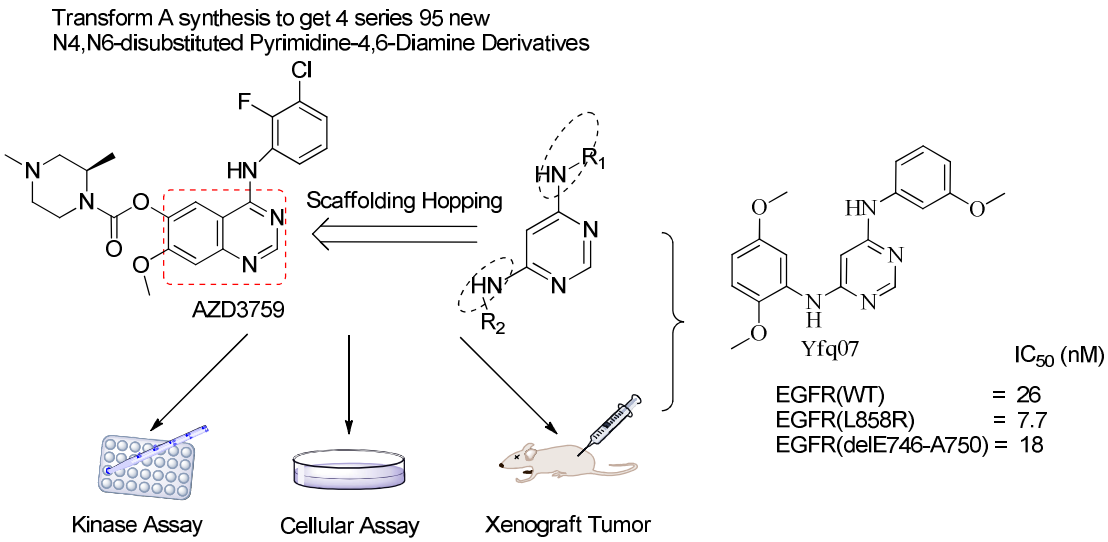


Design, Synthesis and pharmacological evaluation of N4,N6-disubstituted Pyrimidine-4,6-Diamine Derivatives as potent EGFR Inhibitors in Non-Small Cell Lung Cancer

Yuan Zhang [a,#], Handeng Lv [a,#], Lu Luo [a], Yong Xu [a], Yaqian Pan [a], Yuewu Wang [a], Han Lin [c], Jianhua Xiong [b], Ping Guo [a], Jinsan Zhang [a,*], Xiaokun Li [a,*], Faqing Ye [a,*]

[a] School of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou, Zhejiang, 325035, China.

[b] Wenzhou Peoples Hospital, Wenzhou, Zhejiang, 325000, China

c Department of pharmacy. The first affiliated Hospital of Wenzhou Medical University. Wenzhou, China

[#] These authors contributed equally to this work.

* Corresponding author:

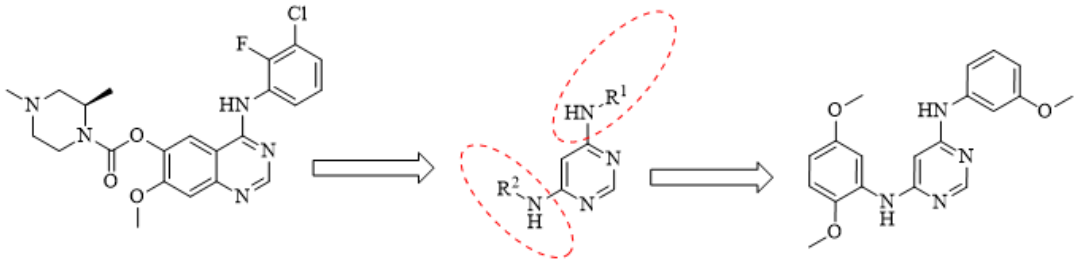
Faqing Ye, Professor

School of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou, Zhejiang, 325035, China

Tel: (+86)-577-86689793; Fax: (+86)-577-86689793

E-Mail: yfq664340@163.com

ABSTRACT:



A novel series of 4, 6-disubstituted pyrimidines derivatives were designed, synthesized, and evaluated as epidermal growth factor receptor (EGFR) inhibitors for non-small cell lung cancer(NSCLC). 4, 6-disubstituted pyrimidines as core structure was utilized to substitute the lead structure AZD3759 of the quinazoline basic skeleton via an approach involving scaffold hopping. It was found that compound **Yfq07** exhibited the best inhibitory effect compared with AZD3759 *in vitro* and *in vivo*: **Yfq07** exhibited a competitive ATP inhibitory effect, multiple target effects, and further featured a stronger activity against H3255, A431, HCC827, PC-9 and H1975 compared to AZD3759. Moreover, a stronger pro-apoptotic effect, inhibition of cell G2 / M phase on A431, H3255, HCC827 and H1975 could also be observed. In this study, the ultimate goal was changing the core structure to improve other epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) properties while retaining the overall potency. **Yfq07** was further explored as an effective 4, 6-pyrimidine anticancer agent for the treatment of human NSCLC.

Keywords: EGFR-TKIs; Scaffold hopping; NSCLC

1. INTRODUCTION

Lung cancer represents a cancer type exhibiting the highest morbidity rate and non–small cell lung cancer (NSCLC) types, accounting for approximately 90% of all lung cancer cases [1]. NSCLC targeted therapy has now become critically important, and the epidermal growth factor receptor (EGFR) tyrosine kinase is a particular popular target [2-5].

Presently, small molecule inhibitors, used for EGFR triggered NSCLC, are mainly based on two types of core structures (Figure 1). One type is based on 2,4-pyrimidines, such as Rociletinib [6] and Osimertinib [7]. Another type stems from quinazolines compounds, including Gefitinib [8], Erlotinib [9] and Afatinib [10]. In recent years, a new potent quinazoline EGFR inhibitor, AZD3759 was discovered and evaluated by Xiaolin Zhang et al. [11]. AZD3759 has demonstrated an effective inhibitory activity on NSCLC. Moreover, this investigational compound is currently evaluated in a phase 1 clinical trial in patients suffering from EGFR triggered NSCLC [12-14].

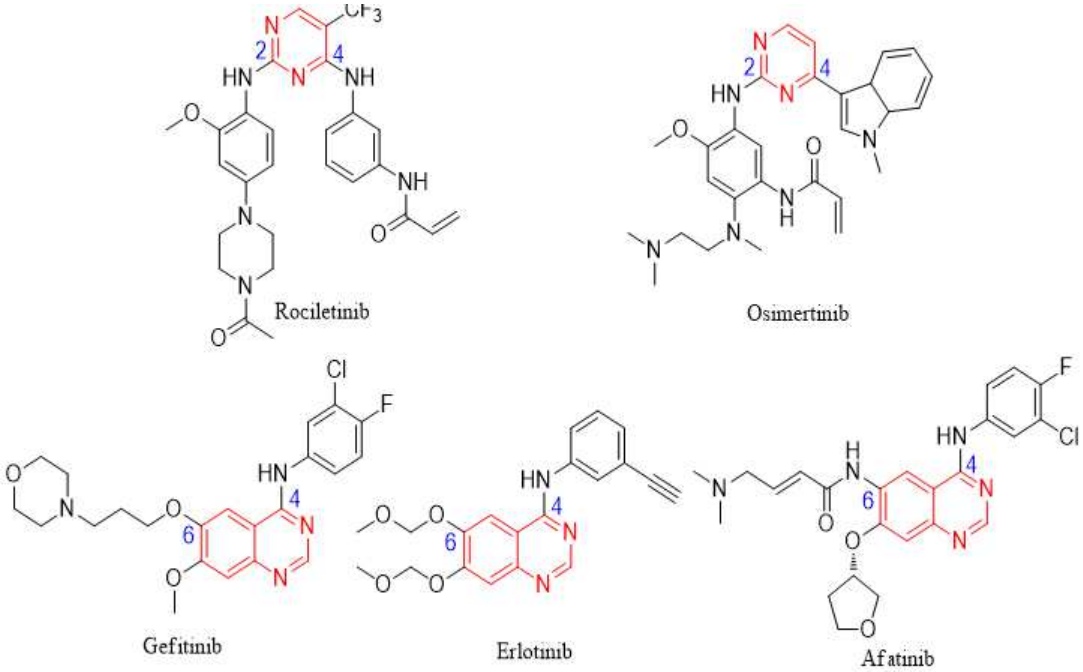


Figure 1. Chemical structures of representative examples of the two EGFR inhibitor types

Using molecular docking simulation to mimic the binding mode of inhibitors AZD3759 and EGFR L858R protein, we found that N-1 of the quinazoline forms an H-bond with back bond NH of Met-798. H-2 of the quinazoline group was shown to be acidic and could form an oxygen bridge with water molecules to form H-bonding interactions with Thr-766. However, N6-methylpiperazine unit was found to be completely exposed to solvent molecules and did not form interactions with the protein. Thus, besides pyrimidines [15], triazines, purines, and pyrazines may be selected to replace the core structure of the quinazoline. Interestingly, one report published by Qiong Zhang et al. [16] showed that 4,6-disubstituted pyrimidines [17] derivatives exhibited a more potent and selective inhibitory effect in both enzymatic and cellular EGFR studies compared to other kinase-directed heterocycles including 2,4-pyrimidines [18], triazines, purines, quinazolines [19], pyrazines [20], etc. In this study, we have used scaffold hopping approach through changing the core structure and in an effort to improve other structure properties while retaining the overall potency [21-25]. The ultimate goal was to develop novel compounds with potentially higher efficacy.

In summary, we selected 4, 6-disubstituted pyrimidines as core structure to synthesize four series of new 4, 6-diamine pyrimidines derivatives (Figure 2). All compounds were evaluated for their efficacy as EGFR-TKIs for NSCLC therapy. Our study showed that 4, 6-disubstituted pyrimidines may indeed be used as new small molecule inhibitors in EGFR triggered NSCLC.

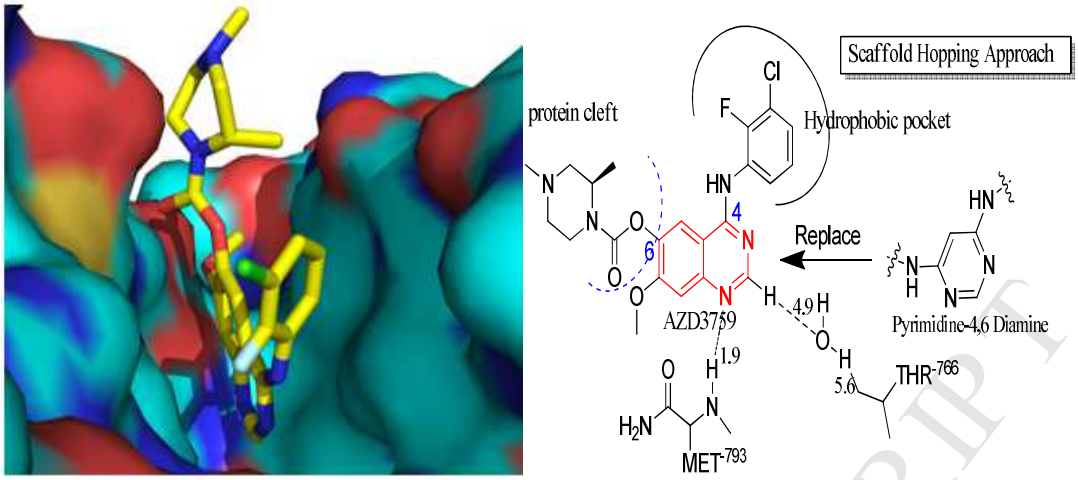
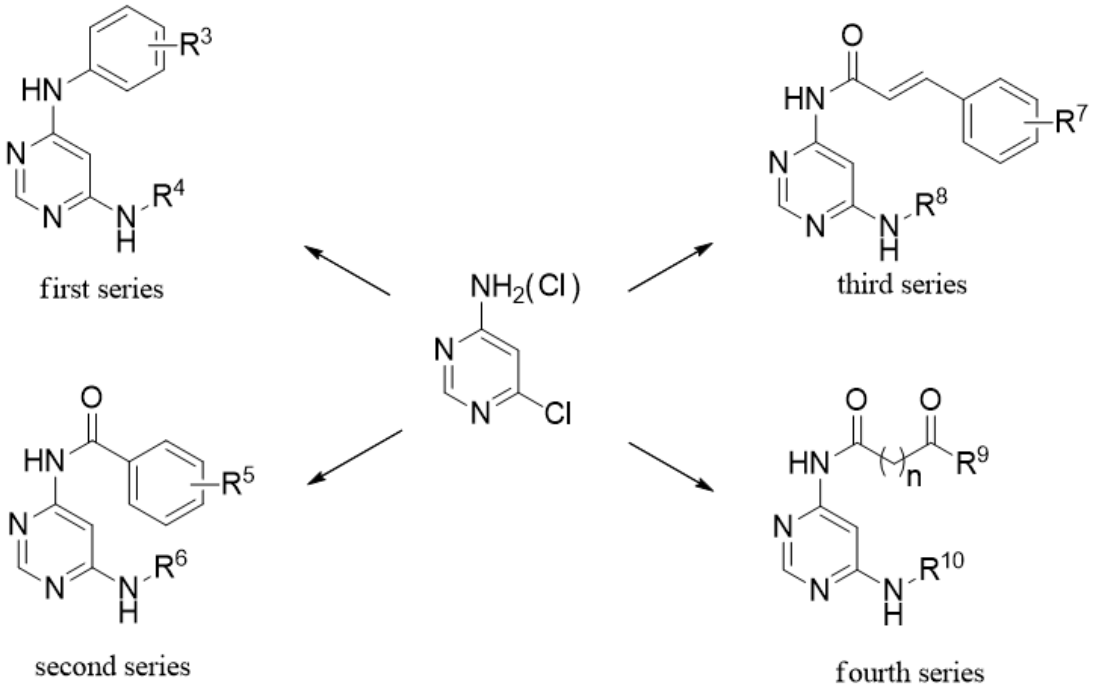


Figure 2. Schematic representation of the rational design of novel compounds

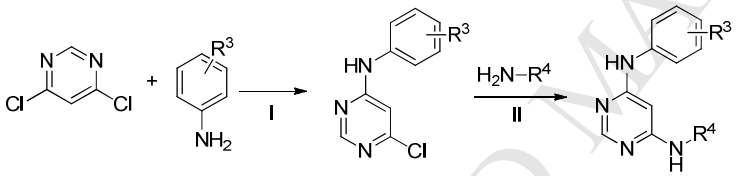
2. RESULTS AND DISCUSSION

2.1 CHEMISTRY

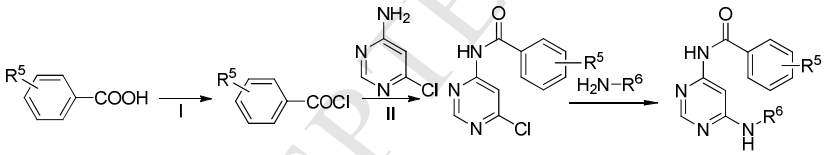
The synthetic routes for the production of the four series of target compounds used in this study are shown in Scheme 3. The corresponding structures are shown in Tables 1-4. 4-pyrimidinamine derivatives, as the key intermediates of the first series of compounds (**Yfq01-Yfq24**), were prepared from commercially available 4,6-dichloropyrimidine with different anilines in the presence of a sufficient amount of potassium iodide. Nucleophilic substitution of these intermediates with appropriately substituted anilines in the presence of p-toluenesulfonic acid afforded the target compounds **Yfq01-Yfq24**. Acyl chlorination of commercially available or previously synthesized benzoic acids or cinnamic acids with thionyl chloride provided the corresponding acyl chlorides, which were coupled with 6-chloropyrimidin-4-amine, followed by nucleophilic substitution with substituted anilines to yield the second (**Yfq25-Yfq40**) and third (**Yfq41-Yfq61**) series of compounds. Furthermore, after esterification of fatty acid chains with various alcohols, the forth series of compounds were synthesized using a similar procedure the first series of compounds.



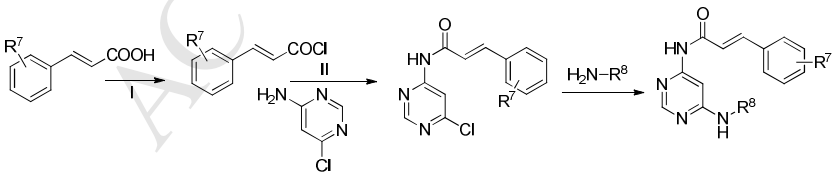
Scheme 2. Synthesis of target compounds.



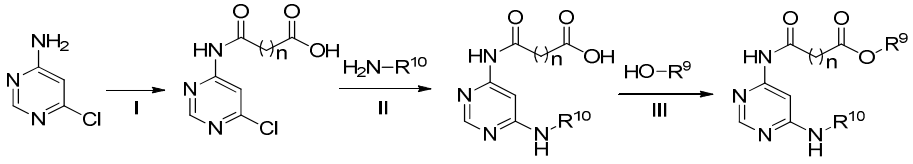
Reagents and conditions: (I) KI, EtOH, reflux; (II) EtOH, TsOH, reflux.



Reagents and conditions: (I) $SOCl_2$, reflux; (II) 4- chloro- 6- amine pyrimidine, acetone, Et_3N, reflux; (III) substitued amines, dioxane, TsOH, reflux.



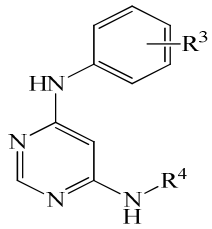
Reagents and conditions: (I) $SOCl_2$, reflux; (II) 4- chloro-6-aminepyrimidine, acetone, Et_3N, reflux; (III) substitued amines, dioxane, TsOH, reflux.

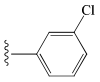
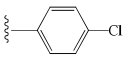
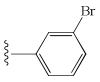
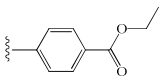
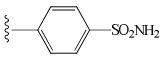
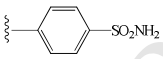
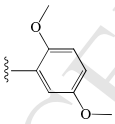
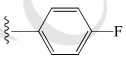
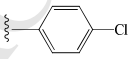
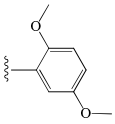
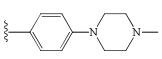
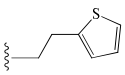


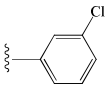
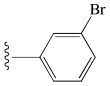
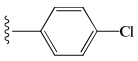
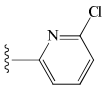
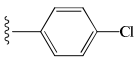
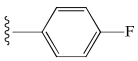
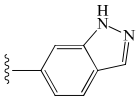
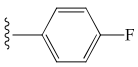
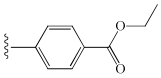
Reagents and conditions: (I) Glutaric anhydride or succinic anhydride, dioxane, reflux; (II) substituted amines, sulfuric acid, KI, acetone, reflux; (III) EtOH; sulfuric acid; reflux.

Scheme 3. Synthetic route for the target compounds of the four series.

Table 1. Chemical structures of target compounds **Yfq01-Yfq24**.

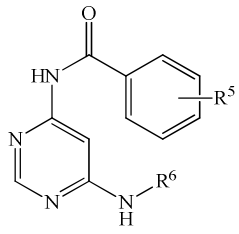


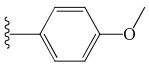
Compd.	R^3	R^4	EGFR		$EGFR^{delE746-A750}$		$EGFR^{L858R}$	
			%inh	IC_{50} (nM)	%inh	IC_{50} (nM)	%inh	IC_{50} (nM)
Yfq01	3’-OMe		46.8	>10000	84.7	974	71.6	1573
Yfq02	3’- OMe		16.7	--	48.3	--	32.8	--
Yfq03	3’- OMe		30.7	--	46.2	--	48.4	--
Yfq04	3’- OMe		30.5	>10000	72.5	1188	76.6	1745
Yfq05	3’- OMe		48.7	>10000	90.2	1229	89.8	1847
Yfq06	2’,5’- OMe		50.2	779	99.3	467	96.7	619
Yfq07	3’- OMe		96.9	26	97.9	7.7	97.0	18
Yfq08	2’,5’- OMe		72.5	336	55.9	208	51.6	230
Yfq09	2’,5’- OMe		6.2	--	26.6	--	32.1	--
Yfq10	2’,5’- OMe		30.4	--	43.4	--	46.9	--
Yfq11	2’,5’- OMe		79.0	288	43.3	>10000	72.1	167
Yfq12	2’,5’- OMe		54.7	125	57.9	102	58.2	167

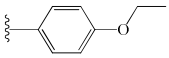
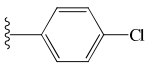
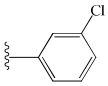
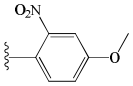
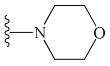
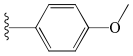
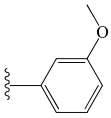
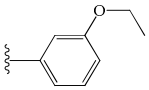
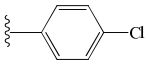
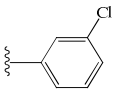
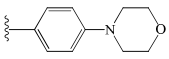
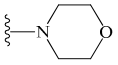
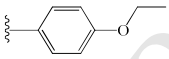
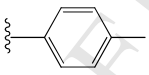
Yfq13	3',4',5'-O Me		79.6	901	10.9	>10000	16.2	>10000
Yfq14	2',5'- OMe		19.6	--	44.3	--	43.9	--
Yfq15	3'- Cl		65.5	170	44.2	>10000	43.8	>10000
Yfq16	3'- Cl		-2.9	--	-12.1	--	-4.5	--
Yfq17	3'- Cl		6.8	--	-12.0	--	-8.3	--
Yfq18	3'- Cl		9.5	--	0.7	--	12.8	--
Yfq19	4'- Cl		89.8	324	97.5	229	97.5	236
Yfq20	4'- Cl		12.3	--	14.2	--	35.4	--
Yfq21	4'- Cl		-4.9	--	12.6	--	8.5	--
Yfq22	4'- Cl		34.6	--	40.9	--	45.5	--
Yfq23	4'- F		25.3	--	33.0	--	41.6	--
Yfq24	4'-ethyl formate		-0.4	--	-14.4	--	-1.5	--

Note: "--" indicates IC_{50} greater than 30000 nM

Table 2. Chemical structures of target compounds **Yfq25-Yfq40.**

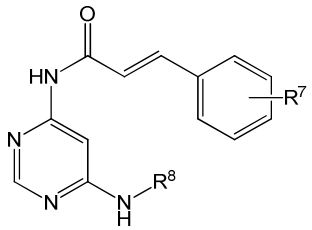


Compd.	R^5	R^6	EGFR		$EGFR^{delE746-A750}$		$EGFR^{L858R}$	
			%inh	IC_{50} (nM)	%inh	IC_{50} (nM)	%inh	IC_{50} (nM)
Yfq25	H		18.6	--	47.8	--	39.6	--
Yfq26	H		96.0	81	98.0	26	97.9	60

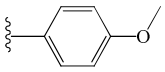
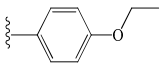
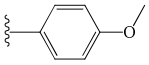
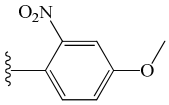
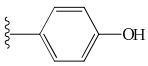
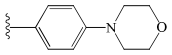
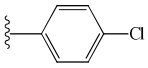
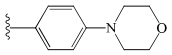
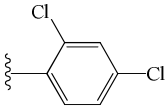
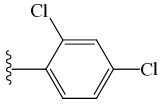
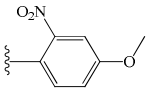
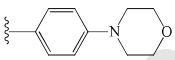
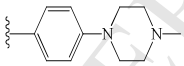
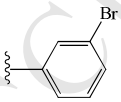
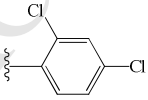
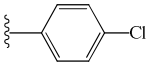
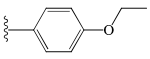
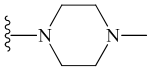
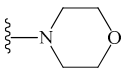
Yfq27	H		12.8	>10000	61.2	754	73.2	1209
Yfq28	H		-1.2	--	-7.2	--	-0.2	--
Yfq29	H		-2.9	--	-17.6	--	-4.2	--
Yfq30	H		11.2	--	-3.3	--	2.4	--
Yfq31	H		10.1	--	3.8		2.1	--
Yfq32	4'- F		2.7	--	-8.8		-0.8	--
Yfq33	4'- F		14.0	--	32.5		37.0	--
Yfq34	4'- F		-4.2	--	-15.7		-3.7	--
Yfq35	4'- F		34.4	>10000	79.7	1409	82.7	1608
Yfq36	4'- F		-8.1	--	-5.2	--	-0.5	--
Yfq37	4'- F		-3.3	--	-6.4	--	-1.0	--
Yfq38	4'-F		83.3	131	99.0	63	97.7	96
Yfq39	4'- F		-1.2	--	-7.3	--	-5.5	--
Yfq40	4'- F		1.7	--	-11.3	--	-10.3	--

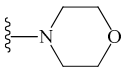
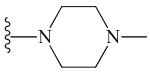
Note: "--" indicates IC_{50} greater than 30000 nM

Table 3. Chemical structures of target compounds **Yfq41-Yfq61**.



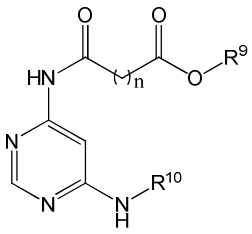
Compd.	R^7	R^8	EGFR		$EGFR^{delE746-A750}$		$EGFR^{L858R}$	
			%inh	IC_{50} (nM)	%inh	IC_{50} (nM)	%inh	IC_{50} (nM)

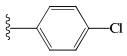
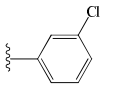
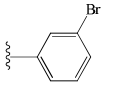
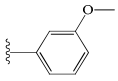
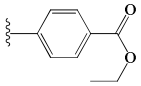
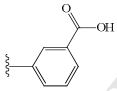
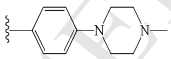
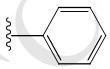
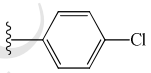
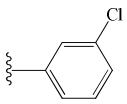
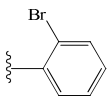
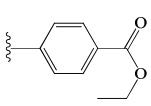
Yfq41	H		-0.3	--	13.0	--	-2.1	--
Yfq42	H		-0.4	--	28.2	--	-4.0	--
Yfq43	H		1.9	--	24.3	--	-6.5	--
Yfq44	H		-4.0	--	-5.2	--	-3.5	--
Yfq45	H		-5.7		24.3	--	-8.7	--
Yfq46	H		-11.1	--	40.5	--	5.8	--
Yfq47	4’-F		7.3	--	42.7	--	7.7	--
Yfq48	4’-F		-5.1	--	47.7	--	2.2	--
Yfq49	4’-F		-7.6	--	-12.5	--	-9.0	--
Yfq50	2’-Br-4’-F		2.7	--	-12.7	--	-11.8	--
Yfq51	2’-Br-4’-F		2.1	--	-12.2	--	-9.2	--
Yfq52	2’-Br-4’-F		-7.7	--	-12.9	--	-12.2	--
Yfq53	4’- F		2.9	--	-9.0	--	-10.3	--
Yfq54	4’-OMe		22.7	--	36.6	--	0.1	--
Yfq55	4’- OMe		-6.9	--	0.8	--	-9.0	--
Yfq56	4’- OMe		-2.9	--	-13.3	--	-8.4	--
Yfq57	4’- OMe		4.1	--	17.0	--	-2.8	--
Yfq58	4’- F		7.1	--	47.3	--	11.3	--
Yfq59	2’-Br-4’-F		-1.6	--	-4.5	--	-9.8	--

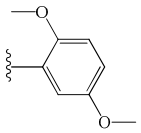
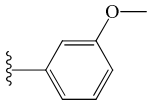
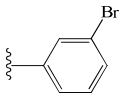
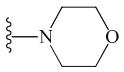
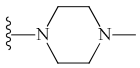
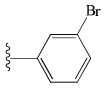
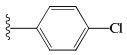
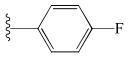
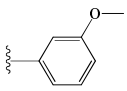
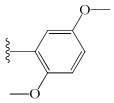
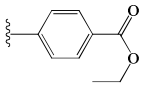
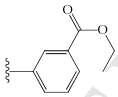
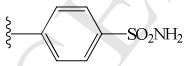
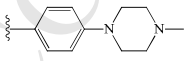
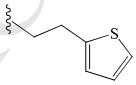
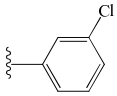
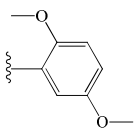
Yfq60	4'-OMe		7.6	--	-7.9	--	-7.2	--
Yfq61	4'-OMe		-1.8	--	8.9	--	26.1	--

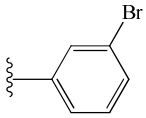
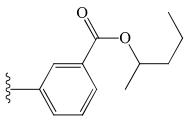
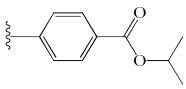
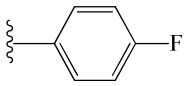
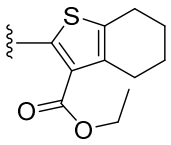
Note: "--" indicates IC_{50} greater than 30000 nM

Table 4. Chemical structures of target compounds **Yfq62-Yfq95**.



Compd.	n	R^9	R^{10}	EGFR		$EGFR^{delE746-A750}$		$EGFR^{L858R}$	
				%inh	IC_{50} (nM)	%inh	IC_{50} (nM)	%inh	IC_{50} (nM)
Yfq62	2	H		34.7	>10000	90.8	792	84.0	825
Yfq63	2	H		-17.8	--	-6.3	--	-4.5	--
Yfq64	2	H		11.7	--	-11.9	--	-4.4	--
Yfq65	2	H		35.2	--	28.6	--	39.3	--
Yfq66	2	H		36.5	--	35.6	--	47.9	--
Yfq67	2	H		26.3	--	1.7	--	34.6	--
Yfq68	2	H		10.2	--	25.4	--	38.9	--
Yfq69	3	H		88.3	>10000	96.5	105	96.0	183
Yfq70	3	H		56.0	>10000	69.7	944	79.1	1322
Yfq71	3	H		73.1	27	98.9	7	98.4	21
Yfq72	3	H		39.4	--	39.6	--	43.6	--
Yfq73	3	H		91.3	48	100.4	21	100.6	69

Yfq74	3	H		68.4	68	60.6	>10000	56.7	>10000
Yfq75	3	H		96.4	37	79.5	12	83.9	31
Yfq76	3	H		90.5	70	-5.2	>10000	-1.5	>10000
Yfq77	3	H		68.3	53	95.8	11	99.5	27
Yfq78	3	H		41.4	--	-10.2	--	-4.5	--
Yfq79	2	ethyl		65.9	>10000	82.3	933	82.1	1345
Yfq80	2	ethyl		46.8	--	8.8	--	-8.7	--
Yfq81	1	ethyl		43.6	--	5.9	--	-4.4	--
Yfq82	2	ethyl		39.4	--	-7.2	--	-9.5	--
Yfq83	2	ethyl		41.0	--	-1.1	--	-6.2	--
Yfq84	2	ethyl		24.2	--	5.9	--	-5.6	--
Yfq85	2	ethyl		45.0	--	-6.5	--	-8.9	--
Yfq86	2	ethyl		6.0	--	-4.9	--	-4.9	--
Yfq87	2	ethyl		3.6	--	3.7	--	-4.0	--
Yfq88	2	ethyl		85.7	27	45.6	>10000	10.6	>10000
Yfq89	3	ethyl		-1.4	--	-19.0	--	-10.9	--
Yfq90	3	ethyl		7.2	--	-4.9	--	-7.3	--

Yfq91	2			3.6	--	24.7	--	0.1	--
Yfq92	2			-2.4	--	1.6	--	-10.4	--
Yfq93	2			-7.3	--	-0.2	--	-13.0	--
Yfq94	2			8.8	--	-3.0	--	-4.9	--
Yfq95	2			-2.5	--	-9.8	--	-5.0	--

Note: "--" indicates IC_{50} greater than 30000 nM

2.2. Biological evaluation

2.2.1 Kinase Inhibitory Activity and Kinase Selectivity Profile Compounds Yfq**01-95** were screened using a mobility shift assay against EGFR (wt), EGFR (L858R), EGFR (delE746-A750), FGFR1 and KDR, AZD3759 as positive control and an ATP concentration at Km. As shown in Supplementary Table S1 and Table 1-4, all compounds suppressed EGFR (wt), EGFR (L858R), and EGFR (delE746-A750) more selectively than FGFR1 and KDR. As shown in Table 1-4, **Yfq07** and **Yfq71** suppressed EGFR (wt) (IC_{50} = 26 nM), (IC_{50} = 27 nM) respectively and EGFR (delE746-A750) (**Yfq07** IC_{50} = 18 nM), (**Yfq71** IC_{50} = 21 nM) more effectively than AZD3759 (IC_{50} = 30 nM, IC_{50} = 41 nM). As for EGFR (L858R), the inhibitory activity of **Yfq07** (IC_{50} = 7.7 nM) and **Yfq71** (IC_{50} = 7 nM) were close to **AZD3759** (IC_{50} = 5.5 nM).

In order to further verify the effects of the drug targets, AURA, EGFR, FGFR1, FGFR2, FGFR4, FLT3, HER2, HER4, IKKβ, JAK1, JAK2, MET, MAP4K2, MAP4K4, PAK2, PDGFRb, RET, SRC, TYK2, TRK-A, BRAF, FLT1 (VEGFR1), FLT4 (VEGFR3), IGF1R, PDGFRa, AKT1 and cKIT 27 as different statuses of RTKs were selected. As expected, all compounds exhibited a more potent inhibitory activity to EGFR than other kinase species (Table 5.).

Table 5. *In vitro* percent inhibitory activity of partial compounds against 27 kinase species.

RTK.	%inhibiton					
	Yfq07	**Yfq26**	**Yfq71**	**Yfq73**	**Yfq75**	**Yfq77**
PDGFRa	26.9	21.7	43.0	4.7	8.0	9.2
PDGFRb	30.4	29.4	74.1	24.2	10.3	10.0
EGFR	86.2	99.0	96.5	93.2	86.5	84

HER2	-4.2	55.8	57.3	4.9	-1.9	-1.5
HER4	11.3	71.4	88.6	27.1	21.7	8.3
FGFR1	4.8	-1.6	13.5	5.6	24.0	5.6
FGFR2	9.8	8.3	16.5	7.2	21.4	5.9
FGFR4	4.8	1.8	8.8	-0.6	21.6	4.0
RET	16.3	2.9	16.8	10.4	7.7	8.5
MET	7.2	3.3	4.0	4.4	12.0	9.2
SRC	33.2	27.7	83.7	11.4	16.6	11.7
JAK2	0.7	-4.2	12.7	4.2	17.7	0.2
TRK-A	6.8	5.6	9.6	8.4	18.8	6.0
IGF1R	6.3	5.3	13.2	9.2	10.1	4.0
AKT1	-2.1	-3.4	0.0	4.7	6.8	-3.0
FLT3	59.1	6.9	35.5	11.5	10.5	16.4
JAK1	-1.4	-0.8	-1.0	-3.1	-0.7	-2.4
TYK2	0.0	-7.9	-0.6	-1.4	-4.4	2.5
CKIT	0.4	26.0	13.3	5.9	13.3	-4.4
MAP4K2	5.4	-3.2	9.2	5.5	10.5	3.3
MAP4K4	53.5	44.8	78.2	56.3	13.5	21.6
BRAF	47.5	16.6	79.3	12.7	11.3	16.7
IKKB	-2.1	3.5	1.1	1.2	13.3	-3.2
PAK2	4.7	13.4	16.9	15.7	18.9	9.4
FLT1	18.1	2.3	12.3	2.9	11.8	-9.5
FLT4	47.3	21.6	29.4	28.2	29.4	9.5
AURa	52.3	2.6	8.7	17.3	19.8	49.0

Note: Compound concentrations were 1μM

2.2.2 Cellular Activities Compounds **Yfq01** to **Yfq95** were screened against H3255, A431, HCC827, H1975, PC-9 and BEAS-2B, AZD3759 as positive control using an MTT assay. As shown in Table 6, compound **Yfq07** inhibits A431 (IC_{50} = 0.74±0.43 μM) and H1975 (IC_{50} = 0.89±0.03 μM) more effectively than AZD3759 (IC_{50} = 3.50±0.30 μM, IC_{50} = 24.16±0.35 μM). Although compound **Yfq07** exhibited a reduced inhibitory activity against H3255 and HCC827 cells compared to **AZD3759**, an overall suitable inhibitory effect could be observed. Compound **Yfq07** featured an IC_{50} = 21.43±0.15 μM towards Beas-2B meaning that **Yfq07** exhibited a low toxicity towards normal lung bronchial cells.

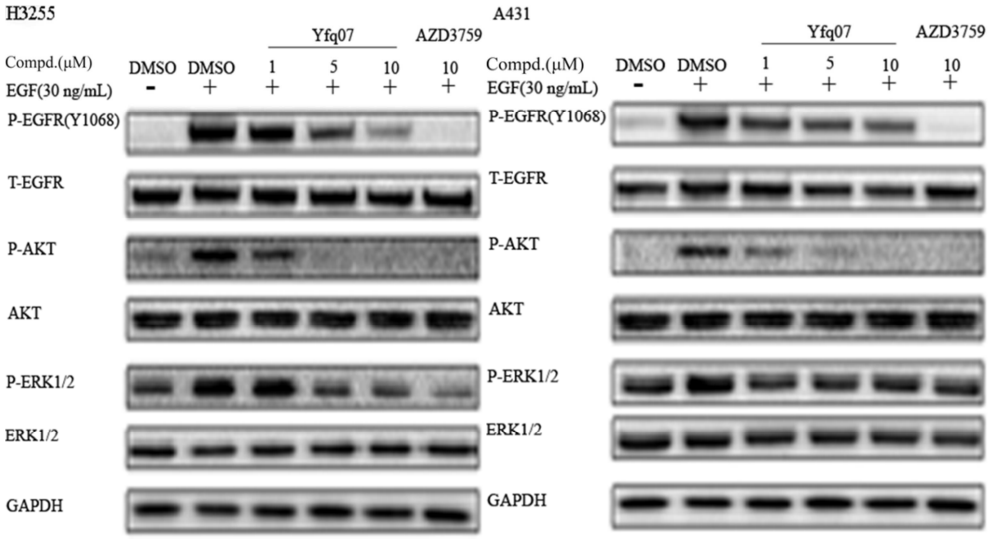
Table 6. Anti-proliferative activities of partial **Yfq01-95** against cells using a different status of EGFR.

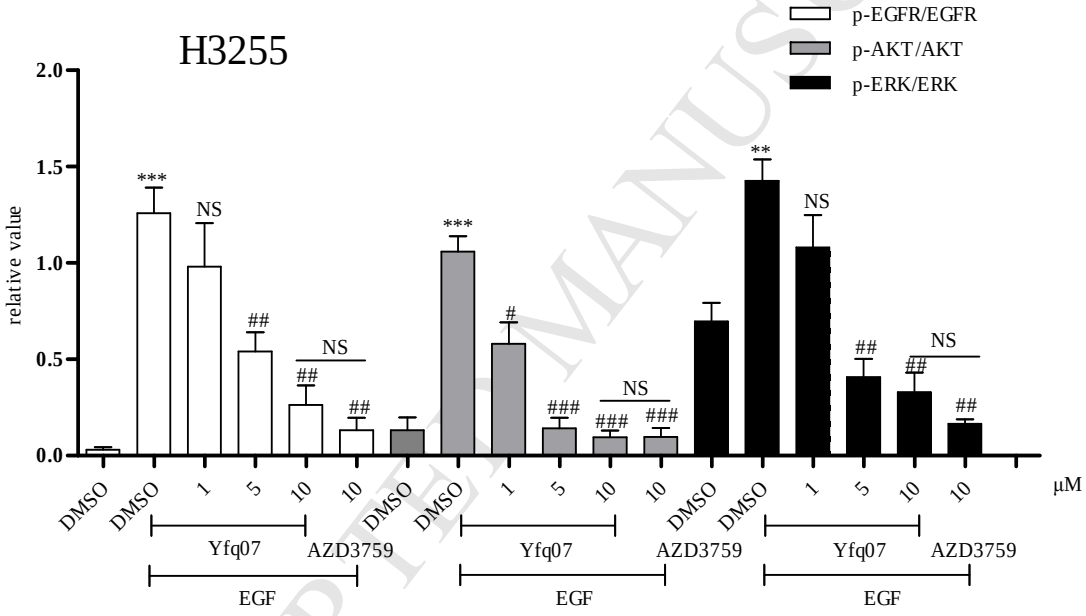
Compound	IC_{50}±SD(nM)a					
	H3255[b]	A431[c]	HCC827[d]	H1975[e]	PC-9[f]	BEAS-2B[g]
Yfq01	--	15.20±0.32	4.04±0.18	--	1.66±0.21	17.60±0.18
Yfq07	0.86±0.09	0.74±0.43	1.14±0.17	0.89±0.03	0.45±0.07	21.43±0.
Yfq08	--	--	1.72±0.16	--	0.64±0.09	17.96±0.10
Yfq11	--	--	24.29±0.53	--	--	18.10±0.30
Yfq19	--	--	--	--	1.18±0.10	18.05±0.22
Yfq26	8.88±0.27	9.46±0.25	5.35±0.16	11.40±0.26	0.66±0.08	18.52±0.20
Yfq27	9.17±0.45	6.54±0.15	7.00±0.13	7.07±0.21	2.09±0.12	17.43±0.31
Yfq35	--	--	--	17.57±0.32	0.82±0.08	18.56±0.16
Yfq38	1.87±0.36	1.13±0.10	1.96±0.09	1.97±0.06	0.87±0.04	20.95±0.29
Yfq62	4.37±0.12	1.90±0.13	3.13±0.14	2.73±0.11	0.79±0.06	18.98±0.23
Yfq69	2.94±0.22	1.19±0.12	2.22±0.13	1.85±0.09	0.94±0.09	17.91±0.30
Yfq71	--	--	4.02±0.16	--	2.00±0.10	18.02±0.21
Yfq77	5.69±0.13	0.78±0.07	1.56±0.09	1.16±0.08	0.56±0.04	17.90±0.20
Yfq88	--	0.49±0.02	--	--	--	21.07±0.12
AZD3759	0.09±0.02	3.50±0.30	0.03±0.01	24.16±0.35	0.05±0.02	18.66±0.28

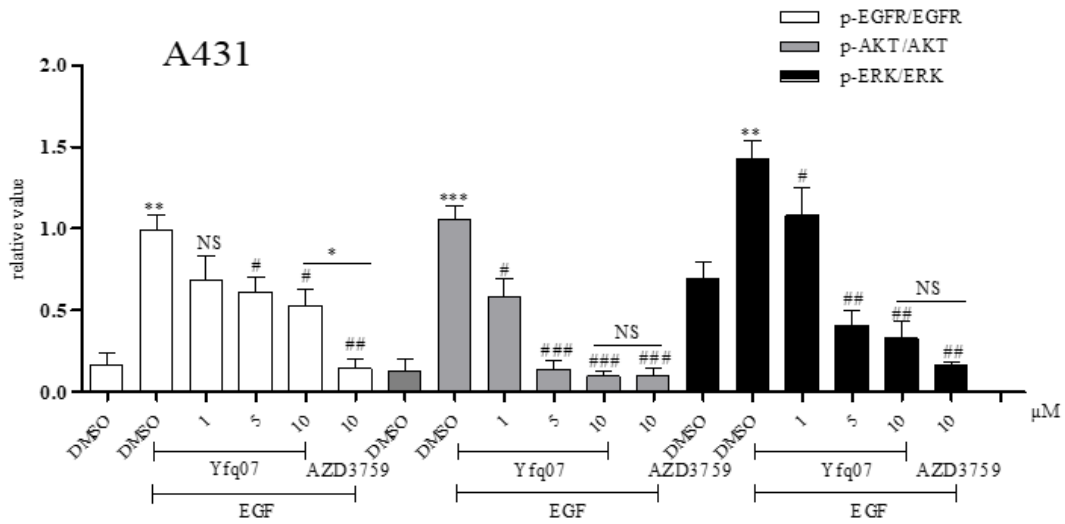
Note: "--" indicates IC_{50} greater than 30000 nM. [a]The inhibitory effects of individual compounds on the proliferation of cancer cell lines were determined by an MTT assay. The data are represented as means ± SD from at least three independent experiments. H3255[b] is a human lung cancer cell line (EGFR L858R). A431[c] is a human epithelial carcinoma cell line (overexpressed WT EGFR). HCC827[d] is a human lung cancer cell line (EGFR del E746_A750). H1975[e] is a human lung cancer cell line (EGFR L858R/T790M). PC-9[f] is a human lung cancer cell line (EGFR del E746_A750). BEAS-2B[g] is a human bronchial epithelial cell line

2.2.3 Structure–activity relationship analysis Four series of 95 novel N4, N6-disubstituted pyrimidine-4, 6-diamine derivatives were evaluated using kinases and EGFR triggered NSCLC. Most of these species exhibited EGFR inhibitory activity, indicating that N4, N6-disubstituted pyrimidine-4, 6-diamine as the core structure could effectively inhibit EGFR and EGFR triggered NSCLC. The first series of these compounds, namely compound **Yfq07,** was found to be the most prominent active structure, with the methoxy groups on the benzene ring substituted by 4,6-diamine, respectively. Moreover, the substitution of the methoxy group on the benzene ring was more prominent than chlorine substitution, and the methoxy groups in 3-position on the benzene ring exhibited an improved EGFR inhibitory activity. Different from the first series of compounds, the second series bearing a phenylamide group attached on the N4-position of the N4,N6-disubstituted pyrimidine-4,6-diamine, featured reduced overall activities compared to the first series. Among them, compound **Yfq26** also featured a methoxyphenyl group, and surprisingly, it still exhibited an excellent inhibitory activity. As for the third series, upon increasing the length of the branch in N4-position by introducing a Michael acceptor molecule, we found that the overall inhibitory activities of the third series were not enhanced. Presumably, the Michael acceptor molecule was not positioned in its right place. Moreover, the fourth series of compounds without aniline substitution and selected straight chain, further increased the length of the side chain and retained a Michael acceptor molecule. However, compounds of the fourth series of compounds exhibited a reasonable inhibitory activity on EGFR kinase but did not exhibit the same inhibitory activities in cellular assays of **Yfq73**, **Yfq75** etc. Furthermore, compounds featuring a branched 3-carbon chain proved to be exhibit a more potent inhibitory activity than others. Taken in concert, via structure–activity relationship analysis of the four series, compounds of the first series with a relatively simple structure could be further modified to obtain compounds with improved characteristics.

2.2.4 Inhibitory Effects of Yfq07 on EGFR Mediated Signaling Pathways in Cancer Cells In order to investigate the inhibitory effect on the phosphorylation of EGFR and the downstream signaling transduction, Western blot analysis of the representative compound **Yfq07** in H3255, HCC827, A431 and H1975 cells was used (Figure 6.). A dosage of 30 ng/ml of EGF was used for the following set of experiments. The results indicated that **Yfq07** significantly inhibited EGFR auto-phosphorylation at Y1068, including the downstream AKT and ERK1/2 phosphorylation, in a dose-dependent manner.






A431
p-EGFR/EGFR
p-AKT/AKT
p-ERK/ERK
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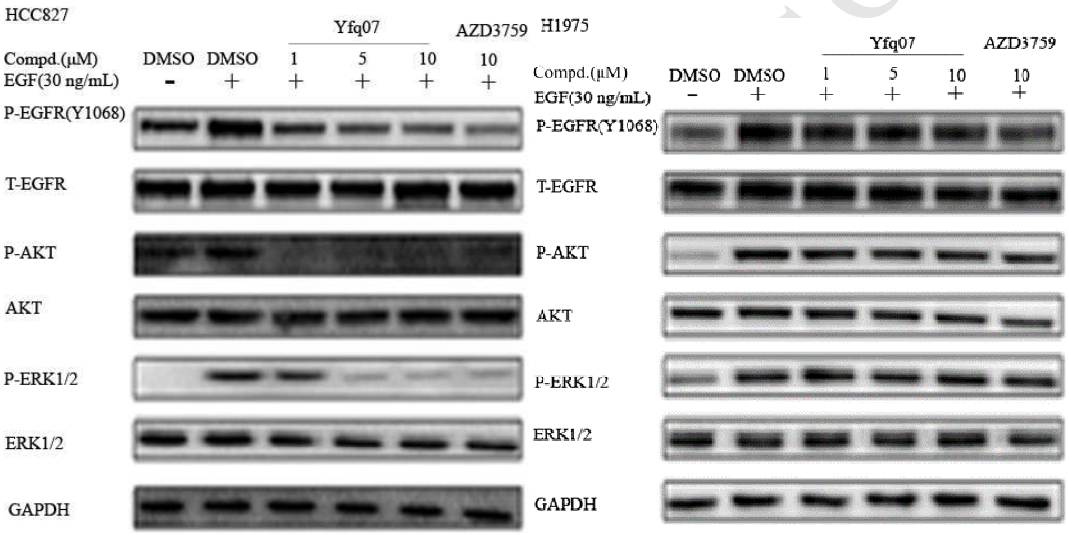
1.5
1.0
0.5
0.0
DMSO
Yfq07
AZD3759
EGF
μM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H1975
Compd.(μM)
EGF(30 ng/mL)
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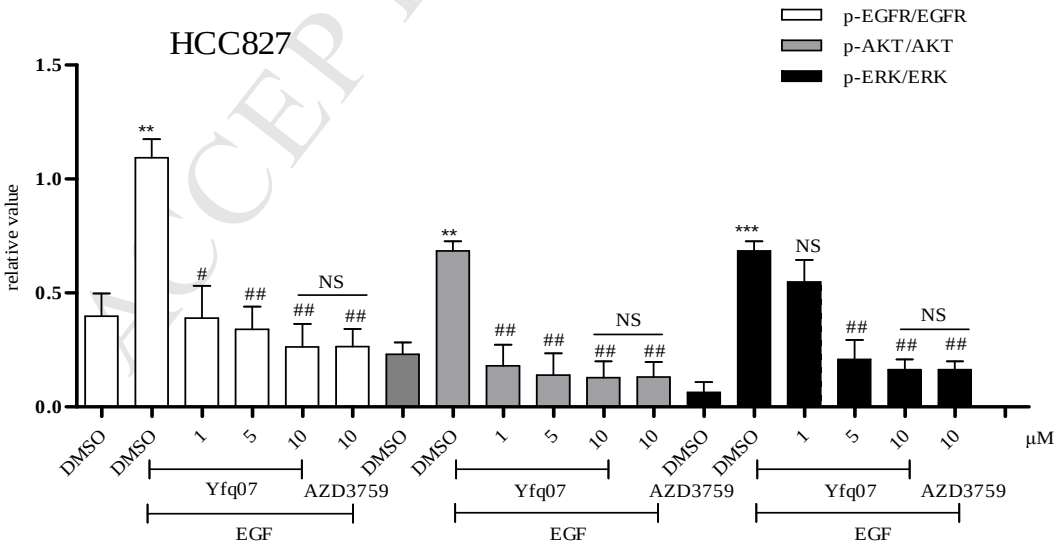
AZD3759
P-EGFR(Y1068)
T-EGFR
P-AKT
AKT
P-ERK1/2
ERK1/2
GAPDH


HCC827
p-EGFR/EGFR
p-AKT/AKT
p-ERK/ERK
relative value
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1.0
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DMSO
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EGF
μM

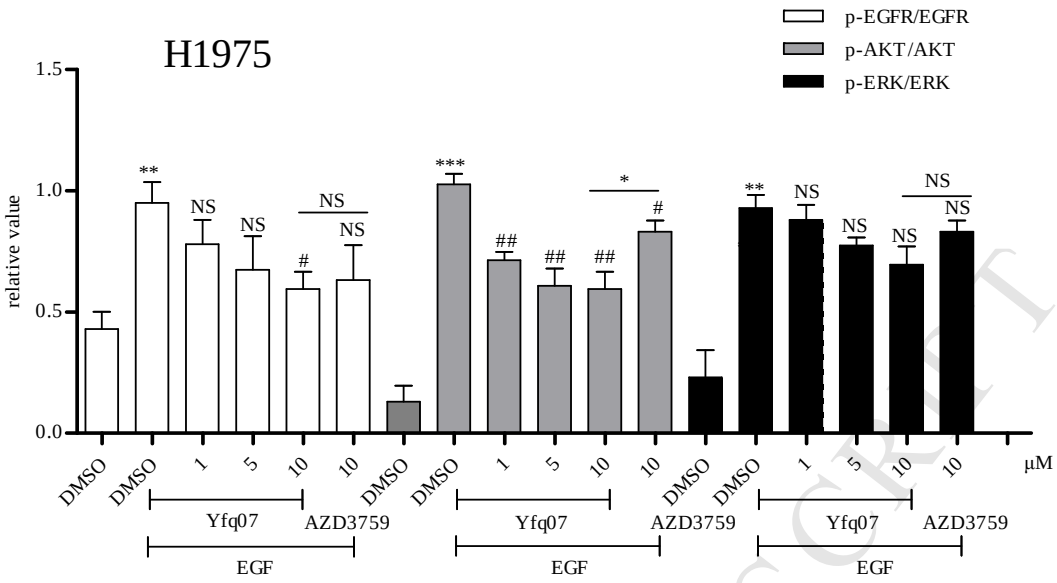
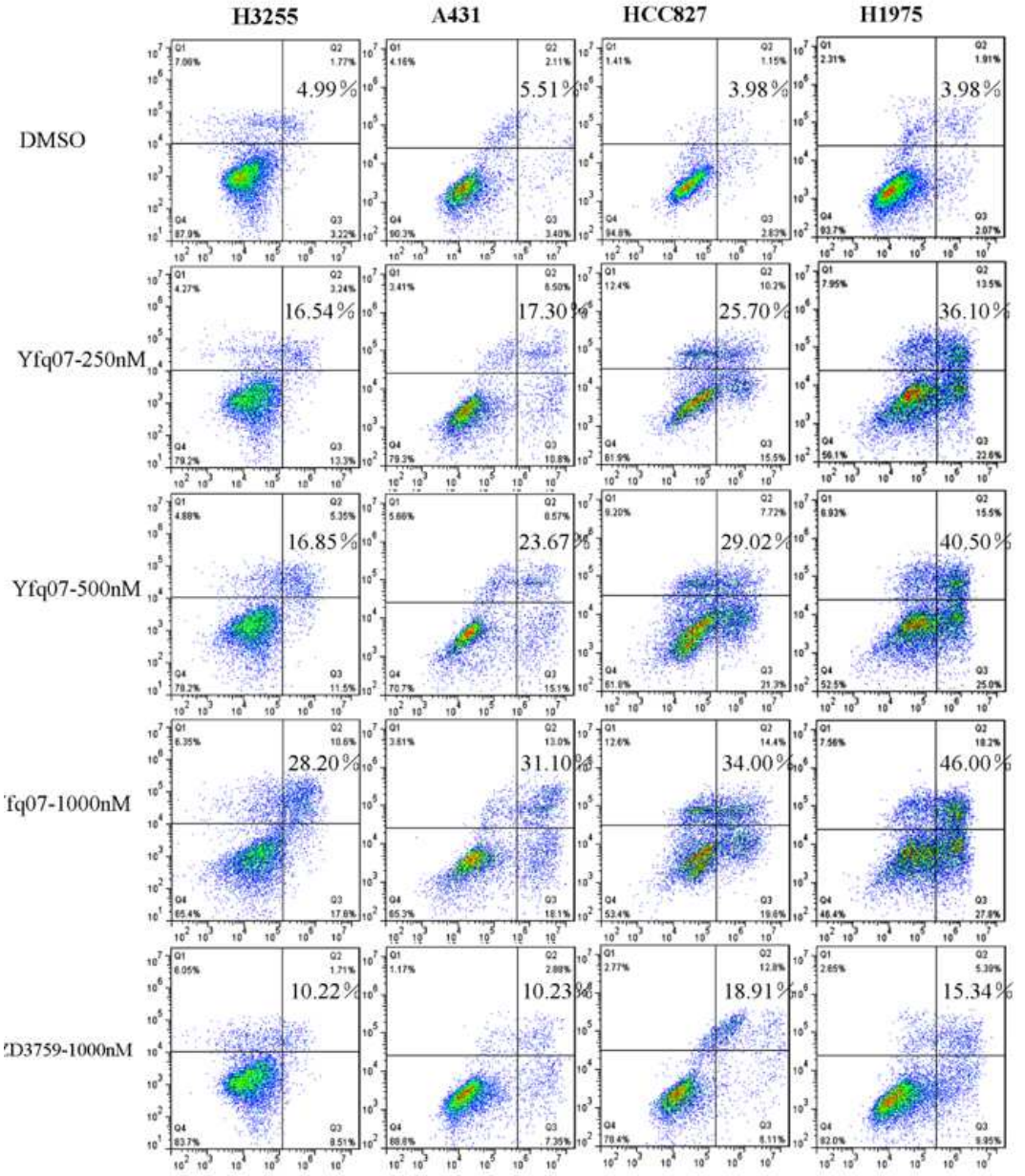


Figure 3. Inhibitory effect of **Yfq07** on EGFR and its downstream ERK1/2 and AKT phosphorylation levels in H3255, A431, HCC827 and H1975 cell lines ;* P < 0.05, ** p < 0.01, *** p <0.001 compared with the blank control group; # P < 0.05, ## p < 0.01, ### p <0.001 compared with the positive drug **AZD3759**.

2.2.5 Compound Yfq07 Induces Cellular Apoptosis and Cell Cycle Arrest in G2/M Phase in NSCLC We examined the pro-apoptotic effects of compound **Yfq07** using an Annexin V/propidium iodide (PI) assay. Non-treated cells were used as a negative control and AZD3759 was used as a positive control. As shown in Figure 4, the cell lines H3255, A431, HCC827 and H1975 were treated for 48 h. We found that a concentration of 500 nM exhibited a significant effect on the overall apoptosis rate. Compound **Yfq07** was found to promote apoptosis in a dose-dependent manner. These results revealed that **Yfq07** was the most effective apoptotic inducer, exhibiting an apoptotic potential higher than **AZD3759**.

We used flow cytometry to determine the anti-mitogenic effects of **Yfq07** in H3255, A431, HCC827 and H1975 cell lines. As shown in Figure 5, compound **Yfq07** was found to block the cell cycle in a dose-dependent manner. Furthermore, it was found that compound **Yfq07** blocked the cells in the G2/M phase, while **AZD3759** demonstrated no significant effect. Compound **Yfq07** in particular blocked cells in the G2/M phase in H1975, which would be the main reason for inhibiting the proliferation of H1975 cells. Therefore, these results revealed that **Yfq07** may represent a potential cell cycle inhibitor blocking G2/M phase.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HCC827
H1975
DMSO
Yfq07-250nM
Yfq07-500nM
fq07-1000nM
ZD3759-1000nM
4.99%
5.51%
3.98%
3.98%
16.54%
17.30%
25.70%
36.10%
16.85%
23.67%
29.02%
40.50%
28.20%
31.10%
34.00%
46.00%
10.22%
10.23%
18.91%
15.34%

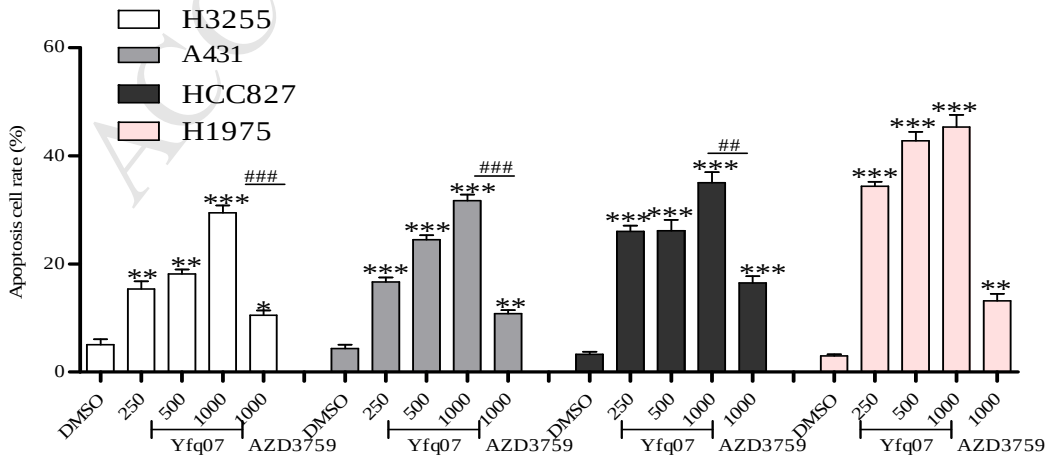
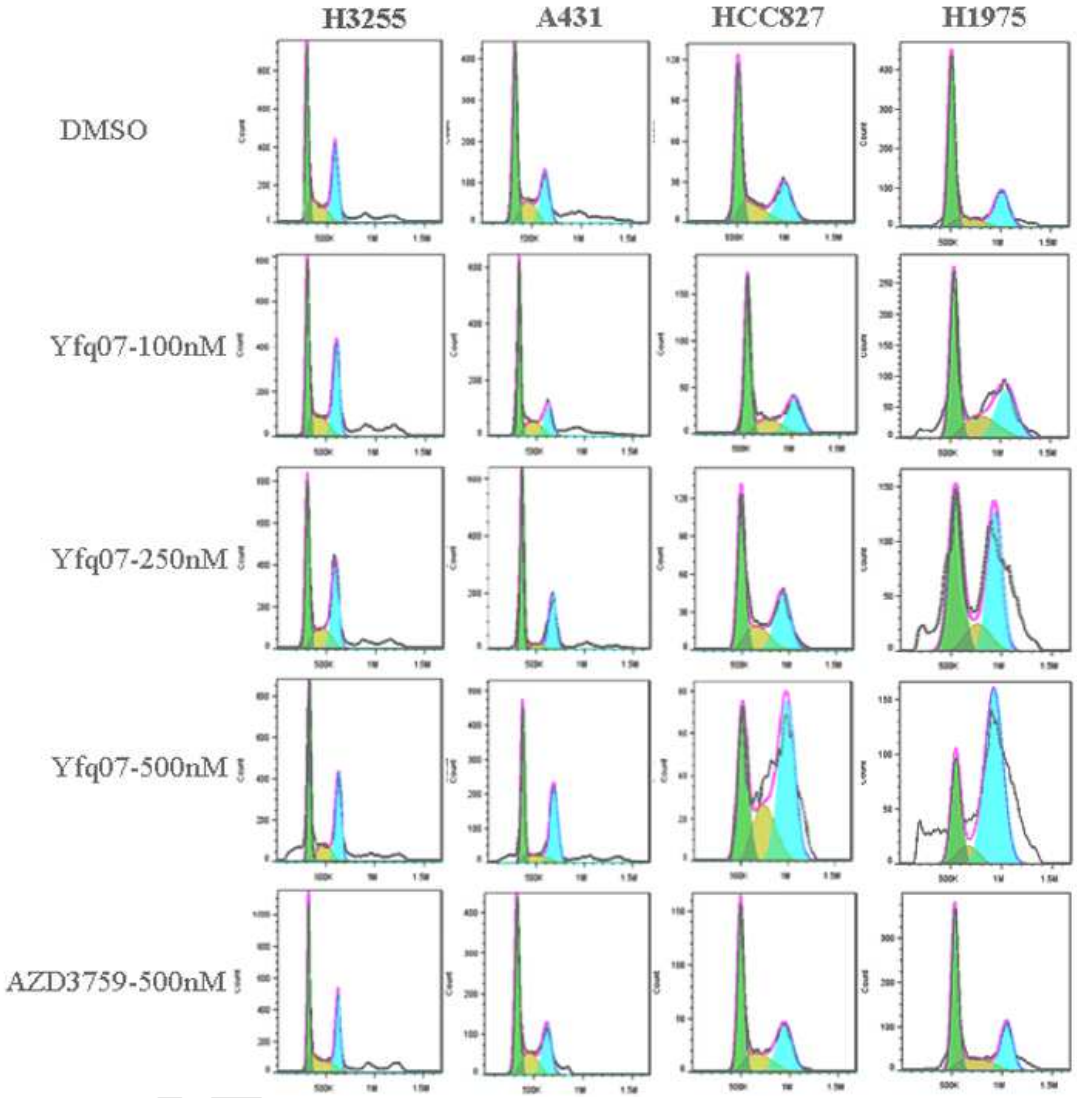

H3255
A431
HCC827
H1975
Apoptosis cell rate (%)
60
40
20
0
DMSO
250
500
1000
1000
Yfq07
AZD3759

Figure 4. Effects of **Yfq07** on apoptosis in H3255, A431, HCC827 and H1975 cell lines; * P < 0.05, ** p < 0.01, *** p < 0.001 compared with the blank control group; # P < 0.05, ## p < 0.01; ### p < 0.001 compared with the positive drug **AZD3759**.



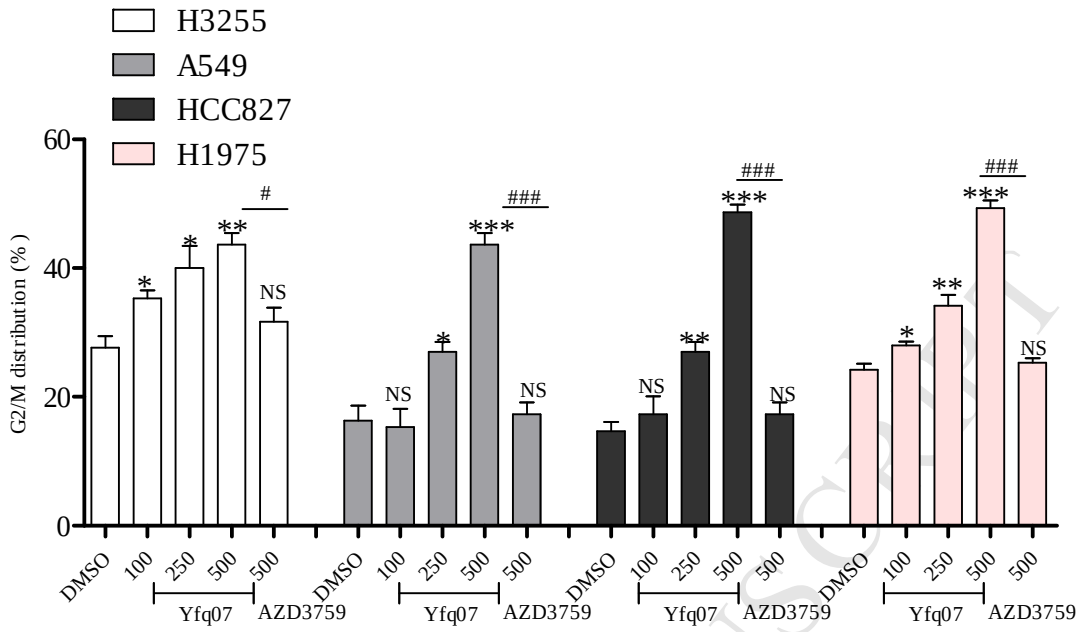


Figure 5. Effects of compound **Yfq07** on cell cycle arrest in A431, H3255, HCC827 and H1975 cell lines; * P < 0.05, ** p < 0.01, *** p < 0.001 compared with the blank control group.

2.2.6 Molecular Docking and Molecular Dynamics Simulation To kinetically validate that drug binding could block ATP from binding to this site, we carried out ATP competition experiments by measuring the inhibitory capacity of **Yfq07** and **Yfq071** at increasing ATP concentrations. As shown at Figures 6A and 6B, we found that compounds **Yfq07** and **Yfq071** were dependent on the ATP concentration. Hence, both compounds were confirmed to be ATP-competitive inhibitors.

Through docking results of the active compound **Yfq07** and EGFR L858R (Figure 6C), it could be concluded that the 2,5-dimethoxyphenyl group introduced to the nitrogen atom in 4-position of the modified **Yfq07** pyrimidine nucleus penetrated into the ATP binding cleft of the receptor protein and one of the methoxy groups formed a hydrogen bond with Asp-855. Moreover, 3-methoxyaniline in 6-position of the pyrimidine moiety formed one H-bond with the Met-793 residue. In addition, the binding pattern of **Yfq07** with the receptor differed from the lead compound AZD3759, suggesting that the modified 4,6-pyrimidine diamines may exhibit an improved molecular flexibility.

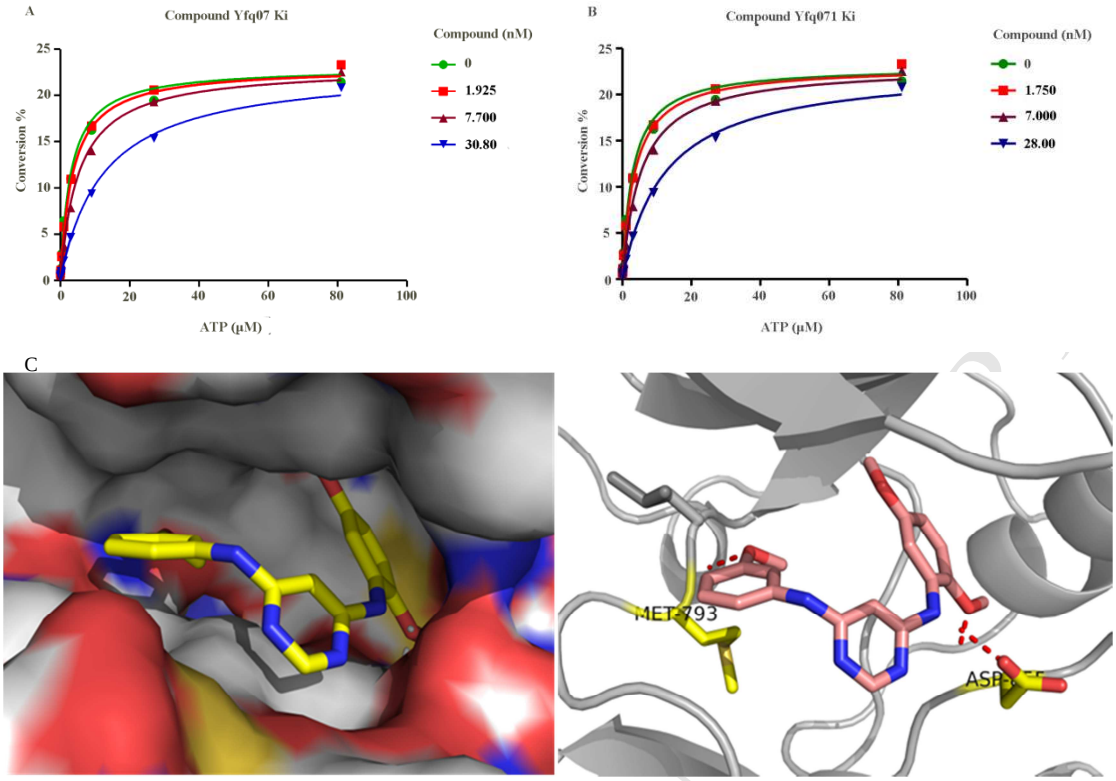


Figure 6. Yfq07 and **Yfq071** inhibit EGFR L858R through a mechanism that proved to be ATP-concentration-dependent. Selective ATP-competitive kinase assay of compounds (A) **Yfq07**, (B) **Yfq071**. (C) Molecular docking diagram and two-dimensional diagram highlighting the binding modes of **Yfq07** and EGFRL858R.

2.2.7 Pharmacokinetic Evaluation and *In Vivo* Antitumor Efficacy Study We evaluated the PK properties of compound **Yfq07** in rats following oral administration (Table 8 and Figure 7). Upon oral administration of compound **Yfq07**, a C_{max} value of 1056.37 ± 394.23 ng/mL was obtained. After oral administration, a half-life of 4.17 ± 2.06 hours was obtained.

Furthermore, compound **Yfq07** was evaluated for antitumor efficacy evaluation *in vivo* using a PC-9 cell xenograft mouse model. Over a 23-day period, compound **Yfq07** (15 mg/kg and 30 mg/kg) was administered intraperitoneally. Moreover, the positive control compound **AZD3759** (30 mg/kg) was administered intraperitoneally [14]. As shown in Figure 8, we found that compound **Yfq07** exhibited a significant antitumor efficacy *in vivo*. Furthermore, the mice exhibited no body weight loss throughout the experiment. The immunoblotting assays performed on the tumor tissue confirmed that the treatment with compound **Yfq07** effectively inhibited phosphorylation of EGFR, AKT and ERK1/2 (Figure 9). Immunohistochemical analysis confirmed these results (Figure 10).

Table 8. The pharmacokinetic parameters of compound **Yfq07** in rat plasma after oral administration at a dosage of 30 mg/kg, n=6.

Parameters	Mean ± SD
$t_{1/2}$ (h)	4.17 ± 2.06
C_{max} (ng/mL)	1056.37 ± 394.23
$MRT_{0\rightarrow t}$ (h)	6.66 ± 2.74
$AUC_{0\rightarrow t}$ (ng/mL•h)	5700.32
$AUC_{0\rightarrow \infty}$ (ng/mL•h)	5822.04
V_d (L/kg)	0.03

$t_{1/2}$, elimination half-life; C_{max}, peak concentration; MRT(0–t), mean residence time (from time point 0 to time t); AUC (0–t), area under the curve from time zero to the last sampling time point, AUC (0–∞), area under the curve from time zero to the infinity; V_d, volume of distribution.

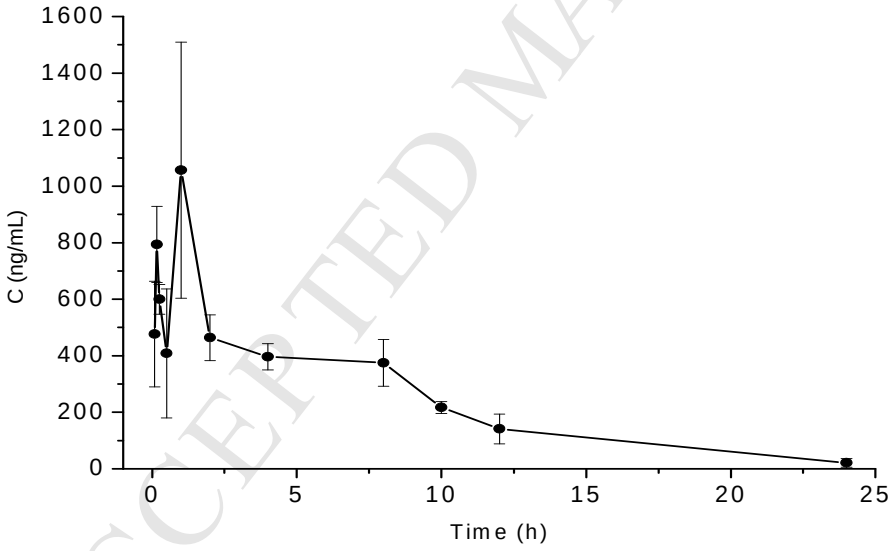


Figure 7. Concentration versus time curves of compound **Yfq07** in rats after a single dose (30 mg/kg) via oral administration.

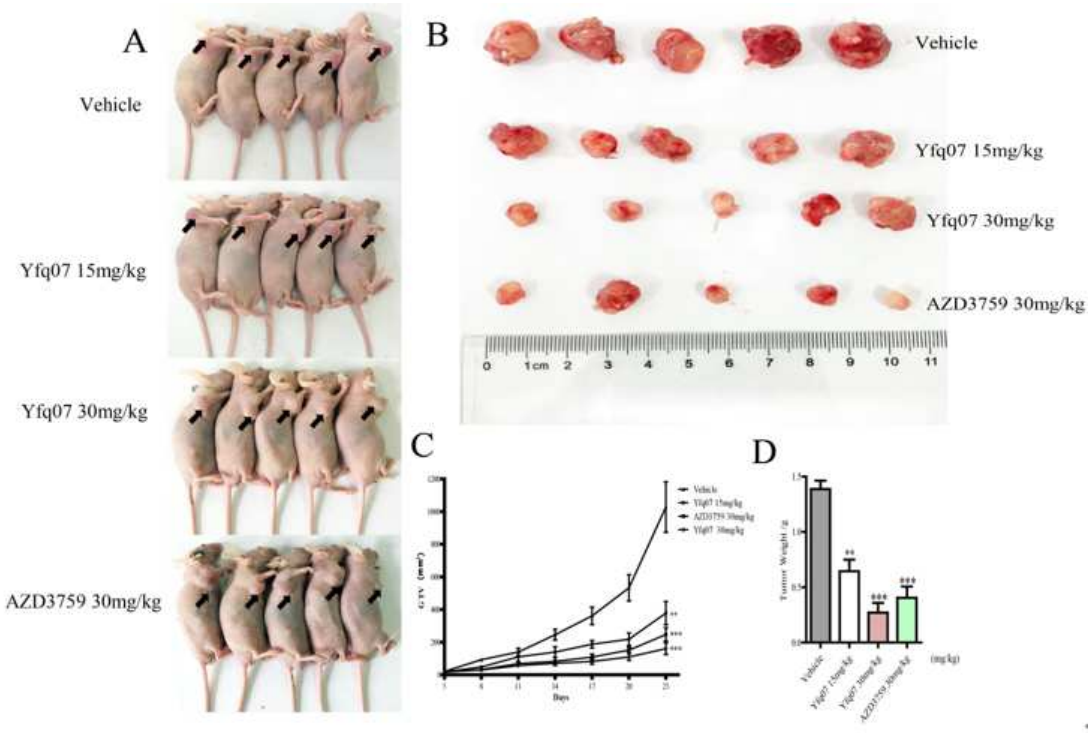
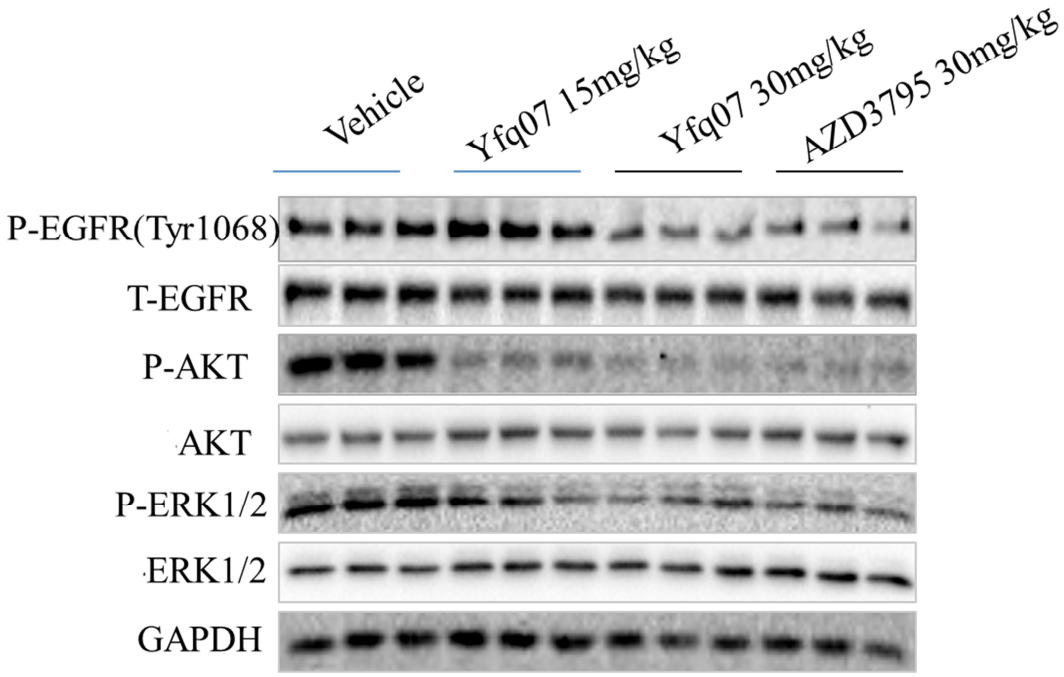


Figure 8. Effects of **Yfq07** and **AZD3759** on the growth of nude mice; (A) Representative tumor-bearing mice after various treatments. (B) Tumor weights. (C) Tumor volumes. (D) Tumor weight statistics. * P < 0.05, ** p < 0.01, *** p < 0.001 compared with the blank control group.



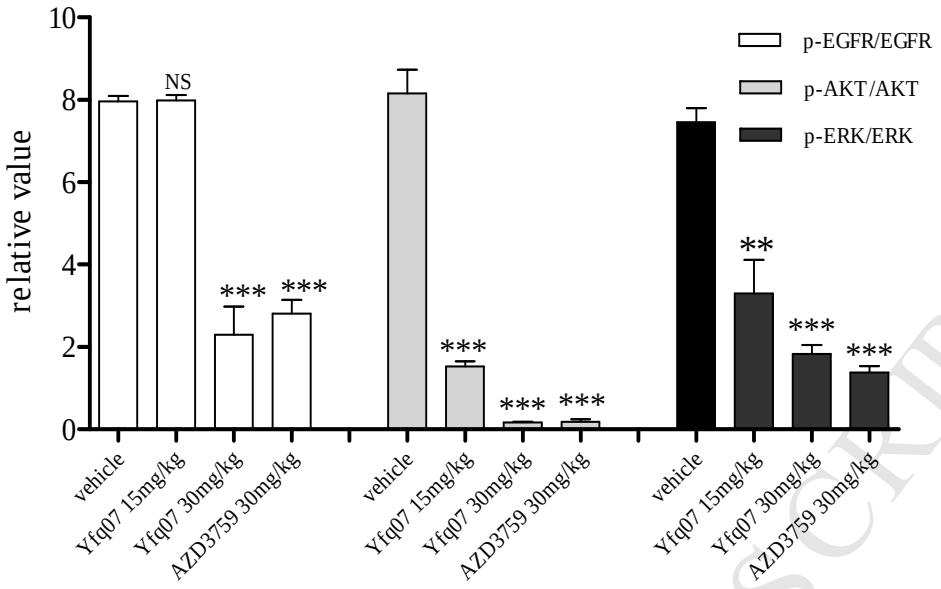


Figure 9. Effects of **Yfq07** on phosphorylation of EGFR and downstream AKT as well as ERK1/2 in tumor tissue; * $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the blank control group.

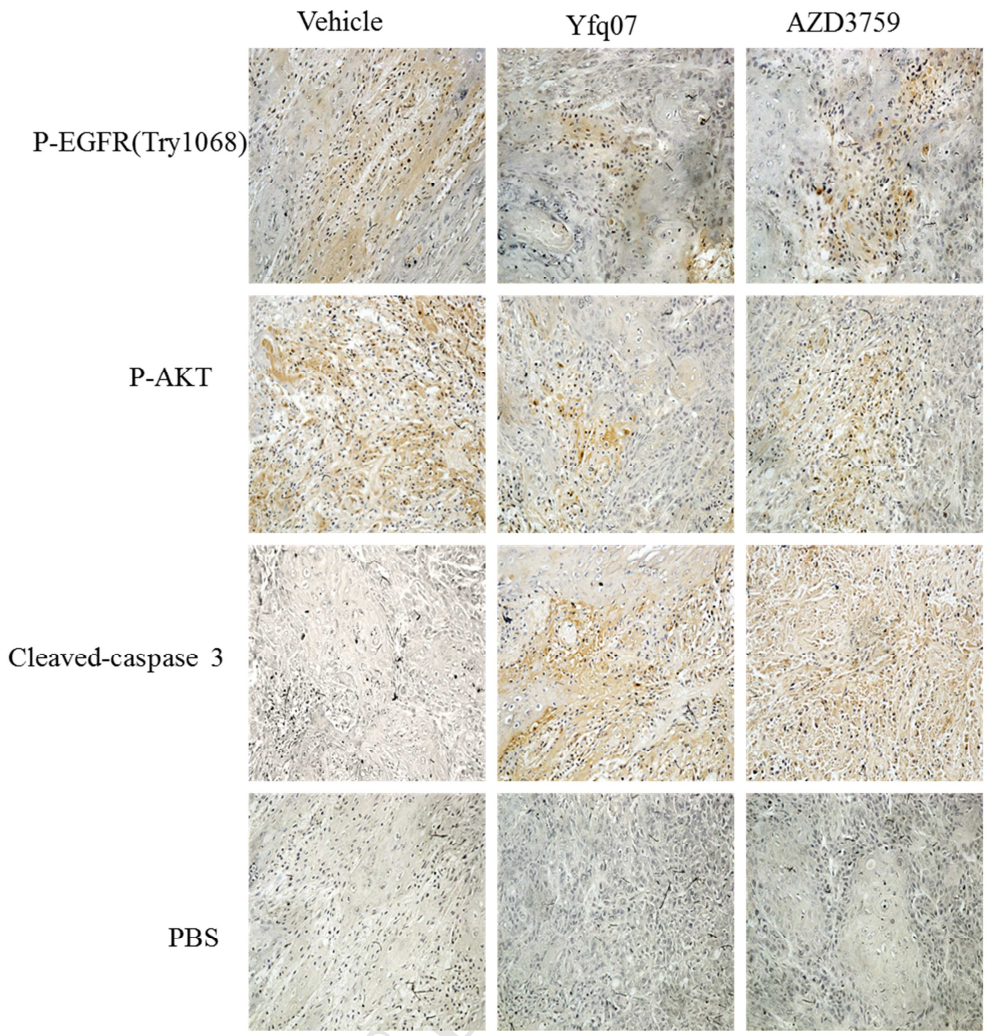


Figure 10. Subcutaneous PC-9 xenografts, treated with compound **Yfq07** and the positive-control **AZD3759**. The statuses of p-EGFR, p-AKT and cleaved-caspase 3 were evaluated by immunohistochemistry.

3. CONCLUSIONS

In this study, we reported the synthesis of a series of N4, N6-disubstituted pyrimindine-4, 6-diamines and evaluation of their biological activities. Most of the compounds exhibited inhibitory activities towards non-small cell lung cancer cells, similar to AZD3759. Using a mobility shift assay and Latha screen assay to screen the compounds demonstrated that an inhibitory effect was only observed on EGFR kinase *in vitro*. The anti-proliferative activity of the test compounds in non-small cell lung cancer cells including different types of EGFR mutation cells (H3255 (EGFR ^{L858R}), A431 (overexpressed WT EGFR), HCC827 (EGFR $^{del\ E746\_A750}$), H1975 (EGFR $^{L858R/T790M}$) and PC-9 (EGFR $^{del\ E746\_A750}$)) was evaluated using an MTT assay. In doing so, similar effects as using AZD3759 could be observed. While compound Yfq**07** significantly increased apoptosis and blockage in the G2/M phase in above mentioned NSCLC cell lines. Furthermore, compound **Yfq07** demonstrated the ability to down-regulate expression of

EGFR, AKT and ERK1/2 phosphorylation. Moreover, compound **Yfq07** significantly inhibited a PC-9-driven xenograft mouse model. Overall, these results showed that compound Yfq**07** exhibits advantages over similar compounds, particularly with respects to its anti-EGFR kinase activity, anti-proliferative effects against non-small cell lung cancer cell lines, induction of apoptosis and blocking of cell cycles. The preliminary biological activity screenings of our novel series of N4, N6-disubstituted pyrimindine-4,6-diamine derivatives demonstrate that these compounds may potentially be useful for treatment of non-small cell lung cancer.

4. EXPERIMENTAL SECTION

4.1 Chemistry

4.1.1 General Melting points were determined using a SGWX-4 microscopic melting point meter and are reported in uncorrected form. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 600 MHz NMR spectrometer, using $CDCl_3$ or DMSO-d_6 as solvents. Chemical shifts are expressed in ppm with TMS as internal reference. J values are provided in hertz. Mass spectra were recorded on a Waters Xevo TQ-S Micro mass spectrometer. High resolution mass spectrometric data were obtained on a Thermo Fisher Scientific LTQ FTICR-MS instrument. IR spectra were recorded on a Varian 660 IR spectrometer. Reactions were monitored by thin layer chromatography (TLC) on glass plates coated with silica gel GF-254. The purity of the all biologically tested compounds was ≥ 95%. Column chromatography was performed with 200-300 mesh silica gel.

4.1.2 General Procedure for the Synthesis of All Compounds in the First Series To a round bottom flask, 4, 6-dichloropyrimidine 1.00 g was added along with ethanol 30 ml and KI 5 mg. The substituted aniline was then added dropwise and the resulting mixture was stirred at 80 °C for 2 h. The resulting crude product was filtered off, recrystallized and used in the next reaction step without further purification. A mixture of the above crude product, another substituted amine, p-methylbenzene sulfonicacid in ethanol was stirred at 80 °C for 6 h. The mixture was cooled to room temperature and the organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purifed by silica gel chromatography to provide the desired product.

4.1.3 General Procedure for the Synthesis of All Compounds in the Second and the Third Series To a round-bottomed flask was added 4-chloro-6-aminopyrimidine (500 mg), benzoyl chloride, acetone 20 ml, and triethylamine 100 μL. The resulting solution was stirred at 0 °C for 8 h. Then, the solution was concentrated using a rotary evaporator and upon addition of saturated aqueous Na_2CO_3, precipitation occurred. The mixture was stirred for 3 h and then washed with 10ml dichloromethane and ethyl acetate and filtered. The resulting crude product was recrystallized from a mixture of dichloromethane and ethyl acetate three times to obtain the

desired intermediate. Then, to a solution of the above intermediate (1.00 mmol), the substituted amines (2.5 mmol) and benzoic acid hydrate (2.00 g) were added. The mixture was stirred at 80 °C for 10 h. After reaction completion, the solution was concentrated on a rotary evaporator, washed with the saturated aqueous Na_2CO_3, and concentrated *in vacuo*. The residue was purified by silica gel chromatography to provide the desired product.

4.1.4 General Procedure for the Synthesis of All Compounds in the Fourth Series A mixture of 4-chloro-6-aminopyrimidine (2.00 g), glutaric anhydride (3.50 g) or succinic anhydride (3.10 g) in dioxane (80 ml) was refluxed for 20 h. The solution was concentrated on a rotary evaporator, washed with the saturated aqueous Na_2CO_3, and concentrated *in vacuo*. To the filtrate, 5% hydrochloric acid was added and the pH was adjusted to 2-3. The forming precipitate was filtered off and dried to obtain the desired intermediate. To a round-bottomed flask was added intermediate I, the substituted amines, sulfuric acid 60 μL, and KI 200 mg in acetone (35 ml). The mixture was refluxed for 20 h. The resulting crude product was filtered off and recrystallized to obtain intermediate II. To a solution of intermediate II in ethanol was added the substitute and a small amount of concentrated sulfuric acid. The reaction was refluxed for 5 h. Upon reaction completion, the solution was concentrated using a rotary evaporator, washed with the saturated aqueous Na_2CO_3, and concentrated *in vacuo*. The residue was purified by silica gel chromatography to provide the desired product.

4.1.5 *N4-(3-Chlorophenyl)-N6-(3-methoxyphenyl)pyrimidine-4,6-diamine (**Yfq01**)* Yield: 87.5%; mp: 183-185 °C; ^{1}H NMR (600 MHz, DMSO-d_6), δ 9.71 (s, 1H, NH), 9.21(s, 1H, H-2), 8.33 (s, 1H, H-5), 7.88-7.89 (m, 1H, N4-5'-PhH), 7.43-7.55 (m, 1H, N6-5'-PhH), 7.29-7.30 (m, 1H, N6-4'-PhH), 7.21-7.22 (m, 2H, N4-5',6'-PhH), 7.12 (s, 1H, N4-2'-PhH), 6.97-6.99 (m, 1H, N4-4'-PhH), 6.57 (s, 1H, N6-2'-PhH), 6.21 (s, 1H, -NH), 3.74 (s, 3H, OCH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.5, 160.2, 159.7, 157.6, 142.2, 141.5, 133.0, 130.2, 129.5, 120.7, 118.5, 117.5, 112.3, 107.2, 105.7, 87.3, 55.0. HRMS (ESI) *m/z*: [M-H]$^-$ calc = 325.0856; found 325.0850 for $C_{17}H_{15}N_4ClO$.

4.1.6 *N4-(4-Chlorophenyl)-N6-(3-methoxyphenyl)pyrimidine-4,6-diamine (**Yfq02**)* Yield: 86.8%; mp: 227-228 °C; ESI-MS [M+H]+: 326; ^{1}H NMR (600 MHz, DMSO-d_6), δ 9.30 (s, 1H, NH), 9.17 (s, 1H, H-2), 8.29 (s, 1H, H-5), 7.63(d, 2H, *J* = 9.0 Hz, N4-2', 6'-PhH), 7.33 (d, 2H, *J* = 9.0 Hz, N4-3',5'-PhH), 7.20-7.21 (m, 2H, N6-5'-PhH, N6-2'-PhH), 7.02 (d, 1H, *J* = 9.0 Hz, N6-4'-PhH), 6.54 (d, 1H, *J* = 9.0 Hz, N6-6'-PhH), 6.18 (s, 1H, -NH), 3.74 (s, 3H, -OCH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.5, 160.3, 159.7, 157.6, 141.5, 139.6, 129.5, 126.5, 125.0, 120.9, 112.2, 107.2, 105.8, 89.0, 54.9.

4.1.7 *N4-(3-Bromophenyl)-N6-(3-methoxyphenyl)pyrimidine-4,6-diamine (**Yfq03**)* Yield: 82.8 %;

mp: 181-183 °C; ESI-MS [M+H]+: 370; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.34 (s, 1H, NH), 9.22 (s, 1H, H-2), 8.33 (s, 1H, H-5), 8.02 (m, 1H, N6-6'- PhH), 7.51 (m, 1H, N4-6'-PhH), 7.19-7.23 (m, 3H, N4-5'-PhH+N6-4',5'-PhH)), 7.10-7.12 (m, 2H, N4-4'-PhH+N6-2'-PhH), 6.20 (s, 2H, N4-2'-PhH), 3.74 (s, 3H, -OCH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.6, 160.2, 159.7, 157.7, 142.4, 141.5, 130.6, 121.7, 121.4, 118.0, 107.3, 105.9, 87.4.

4.1.8 *Ethyl-4-((6-((3-methoxyphenyl)amino)pyrimidin-4-yl)amino)benzoate* ***(Yfq04)*** Yield:78.6 %; mp: 242-243 °C; ESI-MS [M+H]+:364; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.62 (s, 1H, NH), 9.25 (s, 1H, H-2), 8.36 (s, 1H, H-5), 7.87-7.89 (d, 2H, *J* = 9.0 Hz, N6-2',6'-PhH), 7.76-7.78 (d, 2H, *J* = 9.0 Hz, N6-3',5'-PhH), 7.21-7.22 (m, 3H, N4-4',5',6'-PhH), 6.56-6.58 (m, 1H, N4-2'-PhH), 6.29 (s, 1H, NH), 4.27-4.28 (q, 2H, *J* = 7.2 Hz, CH2), 3.74 (s, 3H, OCH3), 1.31 (t, 3H, *J* = 7.2 Hz, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.5, 160.2, 159.7, 157.6, 142.2, 141.5, 133.0, 130.2, 129.5, 120.7, 118.5, 117.5, 112.3, 107.2, 105.7, 87.3, 62.7, 55.0, 14.1.

4.1.9 *4-((6-((3-Methoxyphenyl)amino)pyrimidin-4-yl)amino)benzenesulfonamide* ***(Yfq05)*** Yield: 87.6 %; mp: 167-168 °C; ESI-MS [M+H]+:371; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.25 (s, 1H, H-2), 8.35 (s, 1H, H-5), 7.76-7.78 (d, 2H, *J* = 9.0 Hz, N6-2',6'-PhH), 7.71-7.72 (d, 2H, *J* = 9.0 Hz, N6-3',5'-PhH), 7.10-7.12 (m, 1H, N4-4'-PhH), 7.09-7.10 (m, 2H, N4-5',6'-PhH), 6.57-6.59 (m, 1H, N4-2'-PhH), 6.27 (s, 2H, -NH), 3.75 (s, 3H, -OCH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.6, 160.0, 159.6, 157.6, 143.8, 141.3, 136.1, 129.5, 126.7, 118.3, 112.3, 105.9, 87.8, 55.6.

4.1.10 *4-((6-((2,5-Dimethoxyphenyl)amino)pyrimidin-4-yl)amino)benzenesulfonamide* ***(Yfq06)*** Yield: 82.4 %; mp: 169-170 °C; ESI-MS [M+H]+: 401; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.49-9.52 (s, 2H, SO2NH2), 9.25 (s, 1H, H-2), 8.36 (s, 1H, H-5), 7.76-7.79 (d, 2H, *J* = 9.0 Hz, N6-2',6'-PhH), 7.70-7.72 (d, 2H, *J* = 9.0 Hz, N6-3',5'-PhH), 7.10-7.13 (m, 1H, N4-4'-PhH), 7.01-7.11 (m, 2H, N4-5',6'-PhH), 6.57-6.61 (m, 1H, N4-2'-PhH), 6.25 (s, 1H, -NH), 3.78 (s, 3H, N4-2'-OCH3), 3.16 (s, 3H, N4-5'-OCH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.6, 160.0, 159.6, 157.6, 143.8, 141.3, 136.1, 129.5, 126.7, 118.3, 112.3, 105.9, 87.8, 55.6.

4.1.11 *N4-(2,5-Dimethoxyphenyl)-N6-(3-methoxyphenyl)pyrimidine-4,6-diamine* ***(Yfq07)*** Yield: 82.7 %; mp: 166-168 °C; ^{1}H NMR (600 MHz, DMSO-d_6), δ 9.08 (s, 1H, H-2), 8.23 (s, 1H, H-5), 7.45-7.48 (m, 1H, N6-3'-PhH), 7.17-7.20 (m, 1H, N4-5',6'-PhH), 7.10-7.17 (m, 1H, N4-4'-PhH), 6.95-6.96 (m, 1H, N6-4'-PhH), 6.65 (m, 1H, N4-2'-PhH), 6.55 (m, 1H, N6-6'-PhH), 6.21 (s, 1H, NH), 3.70-3.76 (m, 9H, OCH3×3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.9, 160.4, 157.4, 153.1, 145.0, 141.7, 112.1, 109.7, 107.4, 105.5, 86.6, 59.1, 55.3, 54.9. HRMS (ESI) *m/z*: $[M+H]^+$ calc = 353.1613; found 353.1607 for $C_{19}H_{20}N_4O_3$.

4.1.12 *N4-(2,5-Dimethoxyphenyl)-N6-(4-fluorophenyl)pyrimidine-4,6-diamine* ***(Yfq08)*** Yield:

80.4 %; mp: 168-169 °C; ESI-MS [M+H]+: 340; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.10 (s, 1H, H-2), 8.30 (s, 1H, H-5), 8.21 (s, 1H, NH),7.53-7.55 (d, 2H, *J* = 9.0 Hz, N6-2',6'-PhH),7.48 (s, 1H, N4-3'-PhH), 7.10-7.13 (d, 2H, *J* = 9.0 Hz, N6-3',5'-PhH), 6.95-6.96 (m, 1H, N4-4'-PhH), 6.60-6.62 (m, 1H, N4-6'-PhH), 6.13 (s, 1H, NH), 3.76 (s, 3H, N4-2'-OCH3), 3.70 (s, 3H, N4-5'-OCH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.8, 160.6, 157.4, 145.0, 136.9, 129.3, 121.6, 121.5, 115.3, 115.1, 112.1, 109.6, 107.4, 86.2, 56.2, 56.0.

4.1.13 *N4-(4-Chlorophenyl)-N6-(2,5-dimethoxyphenyl)pyrimidine-4,6-diamine* ***(Yfq09)*** Yield: 88.1 %; mp: 175-177 °C; ESI-MS [M+H]+:356; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.24 (s, 1H, H-2), 8.35 (s, 1H, H-5), 8.25 (s, 1H, NH), 7.60-7.62 (d, 2H, *J* = 9.0 Hz, N6-2',6'-PhH), 7.46-7.47 (m, 1H, N4-3'-PhH), 7.31-7.32 (d, 2H, *J* = 9.0 Hz, N6-3',5'-PhH), 6.96-6.97 (m, 1H, N4-4'-PhH), 6.62-6.63 (m, 1H, N4-6'-PhH), 6.16 (s, 1H, -NH), 3.71 (s, 6H, -OCH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.9, 160.2, 157.4, 153.1, 145.2, 139.6, 129.1, 128.5, 120.9, 112.2, 109.8, 107.6, 86.9, 56.1, 55.3.

4.1.14 *N4, N6-Bis (2,5-dimethoxyphenyl)pyrimidine-4,6-diamine* ***(Yfq10)*** Yield: 88.9 %; mp: 171-173 °C; ESI-MS [M+H]+: 382; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.20 (m, 3H, H+-NH), 7.47-7.48 (m, 2H, 6'-PhH), 6.93-6.95 (m, 2H, 3'-PhH), 6.55-6.60 (m, 2H, 5'-PhH), 6.26 (s, 1H, NH), 3.75 (s, 6H, 2'-Ph-OCH3×2), 3.67 (s, 6H, 5'-Ph-OCH3×2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.7, 157.2, 153.0, 144.8, 129.9, 112.0, 109.3, 107.2, 86.6, 56.1, 55.3.

4.1.15 *N4-(2,5-Dimethoxyphenyl)-N6-(4-(4-methylpiperazin-1-yl)phenyl)pyrimidine-4,6-diamine* ***(Yfq11)*** Yield: 79.2 %; mp: 181-183 °C; ESI-MS [M+H]+: 420; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.78 (s, 1H, H-2), 8.18 (s, 1H, H-5), 8.16 (s, 1H, -NH), 7.35-7.36 (m, 1H, N4-3'-PhH), 7.28-7.30 (d, 2H, *J* = 9.0 Hz, N6-2',6'-PhH), 6.92-6.93 (d, 2H, *J* = 9.0 Hz, N6-3',5'-PhH), 6.88-6.89 (m, 1H, N4-4'-PhH), 6.56-6.58 (m, 1H, N4-6'-PhH), 6.10 (s, 1H, -NH), 3.75 (s, 3H, N4-2'-OCH3), 3.07 (s, 3H, N4-5'-OCH3), 3.06-3.07 (m, 4H, H), 2.44-2.50 (m, 4H, H), 2.22 (s, 3H, N-CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 161.0, 160.6, 157.4, 153.04,146.8, 144.6, 131.9, 129.6, 128.0, 122.1, 116.0,112.0, 109.1, 106.9, 85.3, 55.1, 54.7, 48.7, 45.8.

4.1.16 *N4-(2,5-Dimethoxyphenyl)-N6-(2-(thiophen-2-yl)ethyl)pyrimidine-4,6-diamine* ***(Yfq12)*** Yield: 76.4 %; mp: 186-188 °C; ESI-MS [M+H]+: 356; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.55 (s, 1H, H-2), 8.08 (s, 1H, H-5), 7.32-7.33 (m, 1H, N4-3'-PhH), 6.91-6.96 (m, 4H, H+N4-4'-PhH), 6.55 (s, 1H, N4-6'-PhH), 3.75 (s, 6H, OCH3), 3.10-3.03 (t, 2H, *J* = 7.2 Hz, 1'-CH2), 2.50-2.50 (t, 2H, *J* = 7.2 Hz, 2'-CH2). ^{13}C NMR (150 MHz, DMSO-d6) δ 163.7, 159.7, 158.0, 130.6, 130.3, 124.5, 112.3, 110.0, 108.0, 92.8, 56.1, 55.3, 45.9, 33.7.

4.1.17 *N4-(4-Chlorophenyl)-N6-(3,4,5-trimethoxyphenyl)pyrimidine-4,6-diamine* ***(Yfq13)*** Yield:

68.8 %; mp: 197-198 °C; ESI-MS [M+H]+: 386; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.27 (s, 1H, -NH), 9.05 (s, 1H, H-2), 8.27 (s, 1H, H-5), 7.62-7.64 (d, 2H, *J* = 9.0 Hz, N4-2',6'-PhH), 7.31-7.33 (d, 2H, *J* = 9.0 Hz, N4-3',5'-PhH), 6.87 (s, 2H, N6-2',6'-PhH), 6.15 (s, 1H, NH), 3.76 (s, 6H, N6-3',5'-OCH3), 3.63 (s, 3H, N6-4'-OCH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.6, 160.2, 157.7, 52.86, 139.6, 136.2, 132.9, 128.5, 125.0, 120.9, 98.3, 86.5, 60.1, 55.8.

4.1.18 *N4-(2,5-Dimethoxyphenyl)-N6-ethylpyrimidine-4,6-diamine (**Yfq14**)* Yield: 86.7 %; mp: 165-167 °C; ESI-MS [M+H]+: 274; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.20 (s, 1H, H-2), 8.43 (s, 1H, H-5), 7.55 (m, 1H, N4-2'PhH), 6.99-7.01 (m, 1H, N4-4'PhH), 6.69-6.71 (m, 1H, N4-6'PhH), 6.12 (s, 1H, -NH), 4.39 (q, 2H, CH2), 3.78 (s, 3H, N4-2'-OCH3), 3.70 (s, 3H, N4-5'-OCH3), 1.32 (t, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.6, 161.6, 157.8, 141.7, 133.1, 130.3, 121.4, 118.7, 117.7, 88.8, 61.7, 55.6, 55.1, 14.1.

4.1.19 *N4-(3-Chlorophenyl)-N6-ethylpyrimidine-4,6-diamine (Yfq15)* Yield: 84.6 %; mp: 169-171 °C; ESI-MS [M+H]+: 248; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.59 (s, 1H, -NH), 8.41 (s, 1H, H-2), 7.90 (m, 1H, N4-6'PhH), 7.43 (m, 1H, N4-5'PhH), 7.23-7.33 (m, 1H, N4-4'PhH), 6.99-7.00 (m, 1H, N4-2'PhH), 6.07 (s, 1H, H-5), 4.32-4.35 (q, 2H, CH2), 1.31 (t, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.6, 161.6, 157.8, 141.7, 133.1, 130.3, 121.4, 118.7, 117.7, 88.8, 61.7, 14.3.

4.1.20 *N4,N6-bis(3-Chlorophenyl)pyrimidine-4,6-diamine (**Yfq16**)* Yield: 77.3 %; mp: 182-184 °C; ESI-MS [M+H]+: 330; ^{1}H NMR (600 MHz, DMSO-d_6) 9.44-9.46 (d, 2H, *J* = 7.8 Hz, -NH×2), 8.38 (s, 1H, H-2), 8.02-8.03 (t, 1H, *J* = 3.6 Hz, PhH), 7.88-7.88 (t, 1H, *J* = 3.6 Hz, PhH), 7.49-7.50 (dd, 1H, J_1 = 1.2 Hz, J_2 = 7.8 Hz, PhH), 7.43-7.45 (dd, 1H, J_1 = 3.6 Hz, J_2 = 7.8 Hz, PhH), 7.29-7.32 (t, 1H, *J* = 8.4 Hz, PhH), 7.24-7.26 (t, 1H, *J* = 7.8 Hz, PhH), 7.12-7.14 (dd, 1H, J_1 = 1.2 Hz, J_2 = 7.8 Hz, PhH), 6.99-7.01 (dd, 1H, J_1 = 1.8 Hz, J_2 = 7.8 Hz, PhH), 6.18 (s, 1H, H-5). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.1, 157.6, 142.2, 142.2, 130.5, 130.2, 124.0, 121.5, 121.5, 121.1, 118.6, 118.1, 117.6, 88.8.

4.1.21 *N4-(3-Bromophenyl)-N6-(3-chlorophenyl) pyrimidine-4,6-diamine (**Yfq17**)* Yield: 79.1 %; mp: 189~191 °C; ESI-MS [M+H]+: 374; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.44~9.46 (d, 2H, *J* = 7.8 Hz, -NH×2), 8.38 (s, 1H, H-2), 8.00~8.01 (t, 1H, *J* = 3.6 Hz, PhH), 7.88~7.89 (t, 1H, *J* = 3.6 Hz, PhH), 7.50~7.51 (dd, 1H, J_1 = 1.2 Hz, J_2 = 7.8 Hz, PhH), 7.44~7.45 (dd, 1H, J_1 = 3.6 Hz, J_2 = 7.8 Hz, PhH), 7.29~7.32 (t, 1H, *J* = 8.4 Hz, PhH), 7.23~7.26 (t, 1H, *J* = 7.8 Hz, PhH), 7.12~7.14 (dd, 1H, J_1 = 1.2 Hz, J_2 = 7.8 Hz, PhH), 6.99~7.00 (dd, 1H, J_1 = 1.8 Hz, J_2 = 7.8 Hz, PhH), 6.19 (s, 1H, H-5). ^{13}C NMR (600 MHz, DMSO-d_6) δ 160.2, 157.6, 142.4, 142.2, 130.6, 130.3, 124.0, 121.6, 121.5, 121.1, 118.7, 118.1, 117.7, 89.0.

4.1.22 *N4-(3-Bromophenyl)chlorophenyl)-N6-(4-chlorophenyl)pyrimidine-4,6-diamine* ***(Yfq18)*** Yield: 75.1 %; mp: 240-241 °C; ESI-MS [M+H]+: 330; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.34 (s, 1H, NH), 9.334 (s, 1H, 2-NH), 8.35 (s, 1H, H-2), 7.89 (s, 1H, N4-2'-PhH), 7.62-7.64 (d, 2H, J = 9.0 Hz, N6-2'-6'-PhH), 7.43-7.44 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.2 Hz, N4-PhH), 7.33-7.35 (d, 2H, J = 9.0 Hz, N6-3',5'-PhH), 7.29-7.33 (m, 1H, N4-4'-PhH), 7.00-7.01 (m, 1H, N4-2'-PhH), 6.17 (s, 1H, NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.3, 160.1, 157.6, 142.1, 139.4, 133.1, 130.3, 128.5, 125.2, 121.1, 121.0, 118.6, 117.6, 87.6.

4.1.23 *N4-(4-Chlorophenyl)-N6-(6-chloropyridin-2-yl)pyrimidine-4,6-diamine* ***(Yfq19)*** Yield: 78.4 %; mp: 252-253 °C; ESI-MS [M+H]+: 331; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.34 (s, 1H, H-2), 8.31 (s, 1H, H-5), 7.62-7.63 (m, 4H, N4-2',6'- PhH+ H-4',5'), 7.34 (s, 1H, H-3'), 7.33-7.34 (d, 2H, J = 9.0 Hz, N4-3',5'-PhH), 6.149 (s, 1H, -NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.9, 160.2, 157.4, 153.1, 142.4, 137.7, 133.0, 130.2, 128.1, 125.5, 118.4, 117.5, 109.9, 107.7, 87.3.

4.1.24 *N4, N6-Bis(4-chlorophenyl)pyrimidine-4,6-diamine* ***(Yfq20)*** Yield: 83.4 %; mp: 165-166 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.25 (s, 2H, NH), 8.43 (s, 1H, H-2), 7.55-7.43 (m, 4H, ArH), 7.43-7.44 (m, 4H, ArH), 6.24 (s, 1H, H-5). ^{13}C NMR (150 MHz, DMSO-d_6) δ 157.7, 153.1, 137.2, 129.1, 123.4, 85.4. HRMS (ESI) *m/z*: [M+H] $^{+}$ calc = 331.0517; found 331.0510 for $C_{16}H_{12}Cl_2N_4$.

4.1.25 *N4-(4-Chlorophenyl)-N6-(4-fluorophenyl) pyrimidine-4,6-diamine* ***(Yfq21)*** Yield: 72.1 %; mp: 255-256 °C; ESI-MS [M+H]+: 314; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.29 (s, 1H, NH), 9.18 (s, 1H, NH), 8.27 (s, 1H, H-2), 7.62-7.63 (d, 2H, J = 8.4 Hz, N6-2',6'-PhH), 7.55-7.57 (q, 2H, J_1 = 5.4 Hz, J_2 = 9 Hz, N4-2',6'-PhH), 7.32-7.34 (t, 2H, J = 6.0 Hz, N6-3',5'-PhH), 7.13-7.16 (t, 2H, J = 9.0 Hz, N4-3',5'-PhH), 6.09 (s, 1H, H-5). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.6, 160.2, 157.6, 152.6, 139.6, 136.7, 128.5, 121.9, 121.8, 120.9, 115.3, 115.2, 88.5.

4.1.26 *N4-(4-Chlorophenyl)-N6-(1H-indazol-5-yl) pyrimidine-4,6-diamine* ***(Yfq22)*** Yield: 75.4 %; mp: 200-201 °C; ESI-MS [M+H]+: 336; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.73 (s, 1H, N6-1'-IndazoleH), 9.34 (s, 2H, NH×2), 7.09-8.35 (m, 9H, H-2+ N4-PhH+ N6- IndazoleH), 6.22 (s, 1H, H-5). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.6, 160.0, 159.6, 157.6, 143.8, 141.3, 136.1, 129.5, 126.7, 118.3, 112.3, 105.9, 87.8.

4.1.27 *N4,N6-Bis(4-fluorophenyl)pyrimidine-4,6-diamine* ***(Yfq23)*** Yield: 85.3 %; mp: 263-264 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.12 (s, 2H, 2-NH), 8.14-8.22 (m, 4H, H-2), 7.53-7.54 (d, 2H, J = 9.0 Hz, N4- 2',6'- PhH), 7.11-7.14 (t, 2H, J = 9.0 Hz, N6-3',5'-PhH), 6.03 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.5, 157.6, 156.6, 136.8, 121.7, 121.7, 115.3, 115.1, 85.7. HRMS (ESI) *m/z*: [M+H] $^{+}$ calc = 299.1108; found 299.1101 for $C_{16}H_{12}F_2N_4$.

4.1.28 *Diethyl-4,4'-(pyrimidine-4,6-diylbis(azanediyl)) dibenzoate (**Yfq24**)* Yield: 72.4 %; mp: 241-242 °C; ESI-MS [M+H]+: 406; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.25 (s, 1H, NH), 9.76 (s, 1H, -NH), 8.58 (s, 1H, H-2), 8.12-8.13 (d, 2H, *J* = 9.0 Hz, PhH), 8.00.-8.01 (d, 2H, *J* = 9.0 Hz, 2',6'-PhH), 7.89-7.95 (d, 2H, *J* = 9.0 Hz, N6-3', 5'-PhH), 7.79-7.83 (d, 2H, *J* = 9.0 Hz, 2',6'-PhH), 6.37 (s, 1H, H-5), 4.27-4.29 (q, 4H, *J* = 7.2 Hz, -CH2CH3×2), 1.30-1.32 (t, 6H, *J* = 7.2 Hz, -CH2CH3×2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.1, 167.3, 166.4, 163.2, 145.4, 132.2, 122.9, 121.1, 113.9, 84.2, 61.3, 14.2.

4.1.29 *N-(6-((3-Methoxyphenyl) amino) pyrimidin-4-yl) benzamide (**Yfq25**)* Yield: 78.3 %; mp: 245-249 °C; ESI-MS [M+H]+: 320; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.81 (s, 1H, N4-NH), 9.66 (s, 1H, N6-NH), 8.48 (s, 1H, H-2), 8.00-8.01 (d, 2H, *J* = 7.2 Hz, N4-2',6'-PhH), 7.76 (s, 1H, N4-4'-PhH), 7.60-7.62 (t, 1H, *J* = 7.2 Hz, N6-5'-PhH), 7.50-7.53 (t, 2H, *J* = 7.2 Hz, N4-3',5'-PhH), 7.40 (s, 1H, N6-2'-PhH), 7.22-7.23 (m, 2H, N6-4',6'-PhH), 6.60 (s, 1H, H-5), 3.75 (s, 3H, N6-OCH3); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.6, 163.5, 161.8, 157.5, 156.8, 147.0, 130.9, 130.8, 130.2, 121.6, 115.6, 115.3, 115.2, 66.0.

4.1.30 *N-(6-((4-Methoxyphenyl) amino)pyrimidin-4-yl)benzamide (**Yfq26**)* Yield: 77.5 %; mp: 243-245 °C; ESI-MS [M+H]+: 320; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.77 (s, 1H, CONH), 9.85 (s, 1H, H-2), 8.02 (d, *J* = 8.2 Hz, 2H,ArH), 7.72 (t, *J* = 8.2 Hz, 1H, ArH), 7.65 (t, J = 8.2 Hz, 2H, ArH), 7.49 (d, *J* = 7.6 Hz, 2H, ArH), 6.72 (d, *J* = 7.6 Hz, 2H, ArH), 5.56 (s, 1H, H-5), 4.05 (s, 1H, NH), 3.92 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.7, 163.6, 161.8, 157.6, 156.9, 147.0, 131.0, 130.9, 130.3, 121.8, 115.6, 115.4, 115.2, 66.1.

4.1.31 *N-(6-((4-Ethoxyphenyl) amino)pyrimidin-4-yl)benzamide (**Yfq27**)* Yield: 76.3 %; mp: 240-242 °C; ESI-MS [M+H]+: 334; ^{1}H NMR (600 MHz, $CDCl_3$-d_6) δ 8.90 (s, 1H, CONH), 8.25 (s, 1H, H-2), 7.88 (d, *J* = 7.2 Hz, 2H, ArH), 7.67 (s, 1H, H-5), 7.59 (t, *J* = 7.2 Hz, 1H, ArH), 7.57 (t, *J* = 7.2 Hz, 1H, ArH), 7.56 (t, *J* = 7.2 Hz, 1H, ArH), 6.94 (d, *J* = 10.8 Hz, 2H, ArH), 6.72 (d, *J* = 10.8 Hz, 2H, ArH), 4.05 (s, 1H, NH), 3.92 (q, 2H, OCH2), 1.26 (t, 3H, *J* = 7.2 Hz, CH3), ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.0, 164.7, 161.7, 160.4, 152.0, 134.2, 132.5, 128.8, 127.5, 121.3, 115.2, 82.6, 64.6, 14.8.

4.1.32 *N-(6-((4-Chlorophenyl) amino) pyrimidin-4-yl)benzamide(**Yfq28**)* Yield: 81.5 %; mp: 255-257 °C; ESI-MS [M+H]+: 324; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.88 (s, 1H, NH), 9.88 (s, 1H, H-2), 8.54 (s, 1H, H-5), 8.02 (d, *J* = 7.8 Hz, 1H, ArH), 8.00 (d, *J* = 7.8 Hz, 1H, ArH), 7.95 (d, *J* = 7.2 Hz, 2H, ArH), 7.35 (t, *J* = 7.8 Hz, 1H, ArH), 7.33 (t, *J* = 7.8 Hz, 1H, ArH), 7.32 (t, *J* = 7.8 Hz, 1H, ArH), 7.53 (d, *J* = 7.8 Hz, 1H, ArH), 7.51 (t, *J* = 7.8 Hz, 1H, ArH), 7.04 (dd, *J* = 7.8 Hz, 1H, NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.4, 164.8, 162.1, 157.6, 140.5, 130.1, 129.0,

128.2, 127.5, 121.3, 116.0, 92.8, 59.6, 20.7, 14.0.

4.1.33 *N-(6-((3-Chlorophenyl) amino) pyrimidin-4-yl)benzamide* ***(Yfq29)*** Yield: 71.5 %; mp: 235-237 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.88 (s, 1H, NH), 9.88 (s, 1H, H-2), 8.54 (s, 1H, ArH), 8.02 (dd, *J* = 7.8 Hz, 2H, ArH), 7.94 (d, *J* = 7.2 Hz, 1H, ArH), 7.77 (s, 1H, NH), 7.61 (t, *J* = 7.2 Hz, 1H, ArH), 7.53 (m, *J* = 3.6 Hz, 2H, ArH), 7.49 (s, 1H, H-5), 7.33 (t, *J* = 7.8 Hz, 1H, ArH), 7.04 (d, *J* = 6.6 Hz, 1H, ArH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.4, 164.8, 162.0, 157.6, 140.5, 130.1, 129.2, 128.2, 121.3, 116.2, 92.8, 59.8, 20.8, 14.1.

4.1.34 *N-(6-((4-Methoxy-2-nitrophenyl) amino) pyrimidin-4-yl) benzamide* ***(Yfq30)*** Yield: 71.8 %; mp: 236-238 °C; ESI-MS [M+H]+: 365; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.85 (s, 1H, NH), 9.68 (s, 1H, H-2), 8.28 (s, 1H, ArH), 8.00 (d, *J* = 7.8 Hz, 2H, ArH), 7.99 (s, 1H, H-5), 7.62 (t, *J* = 7.2 Hz, 1H, ArH), 7.61 (t, *J* = 7.2 Hz, 1H, ArH), 7.60 (t, *J* = 7.2 Hz, 1H, ArH), 7.57 (d, *J* = 9.0 Hz, 1H, ArH), 7.56 (d, *J* = 9.0 Hz, 1H, ArH), 7.32 (dd, *J* = 6 Hz, 1H, NH), 3.86 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.4, 165.0, 160.6, 156.3, 152.4, 137.2, 135.1, 134.9, 133.3, 129.2, 128.1, 127.3, 125.2, 118.6, 110.7, 86.8, 56.2.

4.1.35 *N-(6-Morpholinopyrimidin-4-yl) benzamide* ***(Yfq31)*** Yield: 67.8 %; mp: 210-212 °C; ESI-MS [M+H]+: 284; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.93 (s, 1H, NH), 8.56 (s, 1H, H-2), 7.97 (d, *J* = 7.6 Hz, 2H, ArH), 7.85 (d, *J* = 7.6 Hz, 2H, ArH), 7.49 (s, 1H, H-5), 3.70 (m, 4H, CH2), 3.57 (m, 4H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.4, 162.0, 157.6, 154.3, 130.2, 130.1, 129.2, 129.1, 127.6, 127.3, 88.8, 68.7, 68.6, 48.8, 48.7.

4.1.36 *4-Fluoro-N-(6-((4-methoxyphenyl) amino) pyrimidin-4-yl)benzamide* ***(Yfq32)*** Yield: 76.1 %; mp: 240-241 °C; ESI-MS [M+H]+: 338; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.77 (s, 1H, NH), 9.44 (s, 1H, H-2), 8.03 (d, *J* = 7.6 Hz, 2H, ArH), 7.63 (d, *J* = 7.6 Hz, 2H, ArH), 7.36 (t, *J* = 7.6 Hz, 2H, ArH), 7.01 (d, *J* = 7.6 Hz, 1H, ArH), 6.98 (d, *J* = 7.6 Hz, 2H, ArH), 5.52 (d, 1H, H-5), 4.01 (s, 1H, NH), 3.83 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.8, 166.3, 164.8, 161.5, 155.9, 144.8, 129.2, 120.8, 115.2, 108.1, 104.5, 99.8, 83.1, 56.5.

4.1.37 *4-Fluoro-N-(6-((3-methoxyphenyl) amino) pyrimidin-4-yl)benzamide* ***(Yfq33)***` Yield: 65.6 %; mp: 228-230 °C; ESI-MS [M+H]+: 338; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.77 (s, 1H, NH), 9.44 (s, 1H, H-2), 8.03 (d, *J* = 7.6 Hz, 2H, ArH), 7.63 (d, *J* = 7.6 Hz, 2H, ArH), 7.35 (t, *J* = 7.6 Hz, 2H, ArH), 6.99 (d, *J* = 7.6 Hz, 2H, ArH), 5.52 (d, 1H, H-5), 4.01 (s, 1H, NH), 3.84 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.9, 166.2, 164.9, 161.5, 156.0, 144.9, 129.2, 120.8, 115.3, 108.2, 104.67, 99.8, 83.1, 56.7.

4.1.38 *N-(6-((3-Ethoxyphenyl) amino)pyrimidin-4-yl)-4-fluorobenzamide* ***(Yfq34)*** Yield: 69.5 %;

mp: 231-233 °C; ESI-MS [M+H]+: 352; ^{1}H NMR (600 MHz, DMSO-d_6) 10.77 (s, 1H, NH), 9.44 (s, 1H, H-2) 8.02 (d, *J* = 7.6 Hz, 2H, ArH), 7.63 (d, *J* = 7.6 Hz, 2H, ArH), 7.35 (t, *J* = 7.6 Hz, 2H, ArH), 7.06 (d, *J* = 7.6 Hz, 1H, ArH), 6.99 (d, *J* = 7.6 Hz, 2H, ArH), 5.53 (d, 1H, H-5), 4.03 (s, 1H, NH), 3.83 (s, 3H, *J* = 6.6 Hz, CH3), 1.26 (t, 3H, *J* = 6.6 Hz, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.7, 167.0, 164.6, 161.0, 158.5, 156.8, 144.6, 130.7, 120.9, 116.3, 108.0, 105.3, 98.8, 88.1, 61.0, 15.2.

4.1.39 *N-(6-((4-Chlorophenyl)amino pyrimidin-4-yl)-4-fluorobenzamide* ***(Yfq35)*** Yield: 73.5 %; mp: 240-242 °C; ESI-MS [M+H]+: 342; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.94 (s, 1H, NH), 9.88 (s, 1H, H-2), 8.54 (s, 1H, H-5), 8.09 (dd, *J* = 7.6 Hz, 2H, ArH), 7.75 (s, 1H, NH), 7.55 (dd, *J* = 6.6 Hz, 2H, ArH), 7.36 (d, *J* = 9.0 Hz, 1H, ArH), 7.33 (d, *J* = 8.4 Hz, 1H, ArH), 7.04 (dd, *J* = 6.6 Hz, 2H, ArH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.2, 166.2, 164.2, 160.1, 156.5, 146.7, 130.0, 129.6, 129.6, 129.1, 129.1, 125.4, 117.7, 117.6, 115.6, 115.6, 83.2.

4.1.40 *N-(6-((3-Chlorophenyl) amino) pyrimidin-4-yl)-4-fluorobenzamide* ***(Yfq36)*** Yield: 78.1 %; mp: 244-246 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.94 (s, 1H, NH), 9.88 (s, 1H, H-2), 7.41 (d, *J* = 7.6 Hz, 1H, ArH), 6.62 (s, 1H, ArH), 6.49 (d, *J* = 7.6 Hz, 2H, ArH), 6.04 (d, *J* = 7.6 Hz, 1H, ArH), 5.52 (s, 1H, H-2), 5.28 (d, *J* = 7.6 Hz, 1H, ArH), 5.11 (t, *J* = 7.6 Hz, 1H, ArH), 4.68 (d, *J* = 7.6 Hz, 1H, ArH), 4.03 (s, 1H, NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.2, 166.2, 164.2, 160.1, 155.8, 146.9, 137.1, 129.8, 119.0, 125.4, 116.8, 114.9, 86.4. HRMS (ESI) *m/z*: $[M+H]^+$ calc = 343.0762; found 343.0755 for $C_{17}H_{12}ClFN_4O$.

4.1.41 *4-Fluoro-N-(6-((4-morpholinophenyl) amino) pyrimidin-4-yl)benzamide* ***(Yfq37)*** Yield: 77.2 %; mp: 222-224 °C; ESI-MS [M+H]+: 393; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.77 (s, 1H, NH), 9.40 (s, 1H, H-2), 8.38 (s, 1H, H-5), 8.07 (dd, *J* = 3.0 Hz, 2H, ArH), 7.46 (d, *J* = 7.8 Hz, 2H, ArH), 7.33 (d, *J* = 8.4 Hz, 2H, ArH), 7.61 (s, 1H, NH), 6.93 (d, *J* = 9.0 Hz, 2H, ArH), 3.74 (t, *J* = 4.2 Hz, 4H, CH2), 3.18 (t, *J* = 4.8 Hz, 4H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.2, 166.1, 164.1, 160.0, 155.7, 139.9, 131.7, 129.3, 129.1, 129.1, 117.2, 117.2, 115.5, 115.5, 115.4, 115.4, 83.4, 66.7, 66.7, 47.8.

4.1.42 *4-Fluoro-N-(6-morpholinopyrimidin-4-yl) benzamide* ***(Yfq38)*** Yield: 77.2 %; mp: 222-224 °C; ESI-MS [M+H]+: 393; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.77 (s, 1H, NH), 9.40 (s, 1H, H-2), 8.38 (s, 1H, H-5), 8.07 (dd, *J* = 3.0 Hz, 2H, ArH), 7.46 (d, *J* = 7.8 Hz, 2H, ArH), 7.33 (d, *J* = 8.4Hz, 2H, ArH), 7.61 (s, 1H, NH), 6.93 (d, *J* = 9.0 Hz, 2H, ArH), 3.74 (t, *J* = 4.2 Hz, 4H, CH2), 3.18 (t, *J* = 4.8 Hz, 4H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.2, 166.1, 164.1, 160.0, 155.7, 139.9, 131.7, 129.3, 129.1, 129.1, 117.2, 117.2, 115.5, 115.5, 115.4, 115.4, 83.4, 66.7, 66.7, 47.8.

4.1.43 *N-(6-((4-Ethoxyphenyl) amino) pyrimidin-4-yl)-4-fluorobenzamide (**Yfq39**)* Yield: 87.8 %; mp: 225-227 °C; ESI-MS [M+H]+: 352; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.77 (s, 1H, NH), 9.44 (s, 1H, H-2), 8.38 (s, 1H, H-5), 8.07 (dd, *J* = 5.4 Hz, 2H, ArH), 7.60 (s, 1H, NH), 7.49 (d, *J* = 8.4 Hz, 2H, ArH), 7.33 (t, *J* = 9.0 Hz, 2H, ArH), 6.91 (d, *J* = 9.0 Hz, 1H, ArH), 6.89 (d, *J* = 9.0 Hz, 1H, ArH), 4.00 (q, 2H, *J* = 6.6 Hz, CH2), 1.32 (t, 3H, *J* = 6.6 Hz, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.9, 166.9, 164.9, 161.5, 155.9, 149.7, 135.7, 130.9, 118.3, 116.2, 86.0, 64.2, 15.6.

4.1.44 *4-Fluoro-N-(6-(p-tolylamino) pyrimidin-4-yl) benzamide (**Yfq40**)* Yield: 77.3 %; mp: 214-216 °C; ESI-MS [M+H]+: 322; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.69 (s, 1H, NH), 9.64 (s, 1H, H-2), 8.43 (s, 1H, H-5), 7.62 (d, *J* = 7.2 Hz, 2H, ArH), 7.47 (t, *J* = 6.6 Hz, 2H, ArH), 7.44 (d, *J* = 5.4 Hz, 2H, ArH), 7.21 (d, *J* = 6.0 Hz, 2H, ArH), 7.71 (s, 1H, NH), 1.75 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.8, 160.6, 158.7, 156.8, 141.5, 141.2, 134.2, 130.0, 127.9, 121.5, 112.0, 107.4, 105.7, 93.1, 20.4.

4.1.45 *N-(6-((4-Methoxyphenyl) amino) pyrimidin-4-yl) cinnamamide (**Yfq41**)* Yield: 72.8 %; mp: 248-250 °C; ESI-MS [M+H]+: 346; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.70 (s, 1H, NH), 9.65 (s, 1H, H-2), 8.43 (s, 1H, H-2), 7.71 (s, 1H, NH), 7.63 (d, *J* = 7.2 Hz, 2H, ArH), 7.47 (t, *J* = 6.6 Hz, 3H, ArH), 7.44 (d, *J* = 5.4 Hz, 2H, ArH), 7.22 (d, *J* = 6.0 Hz, 2H, ArH), 7.20 (d, 1H, *J* = 3.0 Hz, CH=CH), 6.59 (d, 1H, *J* = 3.0 Hz, CH=CH), 3.75 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.9, 161.5, 157.7, 141.7, 134.5, 130.1, 127.9, 121.5, 112.2, 107.4, 105.8, 93.3, 55.0.

4.1.46 *N-(6-((4-Ethoxyphenyl)amino)pyrimidin-4-yl)cinnamamide (**Yfq42**)* Yield: 70.6 %; mp: 206-208 °C; ESI-MS [M+H]+: 360; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.75 (s, 1H, NH), 9.65 (s, 1H, H-2), 8.44 (s, 1H, H-5), 7.97 (s, 1H, NH), 7.77 (d, *J* = 7.2 Hz, 2H, ArH), 7.56 (t, *J* = 6.6 Hz, 3H, ArH), 7.52 (d, *J* = 5.4 Hz, 2H, ArH), 7.35 (d, *J* = 6.0 Hz, 2H, ArH), 7.27 (d, 1H, *J* = 3.0 Hz, CH=CH), 6.63 (d, 1H, *J* = 3.0 Hz, CH=CH), 3.88 (q, 2H, *J* = 6.6 Hz, CH2), 1.31 (t, 3H, *J* = 6.6 Hz, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.7, 163.3, 160.9, 157.6, 143.1, 131.5, 130.3, 130.0, 119.6, 116.4, 116.0, 103.9, 66.9, 51.0, 48.8.

4.1.47 *N-(6-((3-Methoxyphenyl)amino)pyrimidin-4-yl)cinnamamide (**Yfq43**)* Yield: 69.4 %; mp: 238-240 °C; ESI-MS [M+H]+: 346; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.68 (s, 1H, NH), 9.612 (s, 1H, H-2), 7.60 (d, *J* = 7.6 Hz, 2H, ArH), 7.55 (d, *J* = 7.6 Hz, 2H, ArH), 7.40 (t, *J* = 7.6 Hz, 1H, ArH), 7.356 (d, 1H, = 7.6 Hz, CH=CH), 7.28 (t, *J* = 7.6 Hz, 1H, ArH), 6.89 (d, 1H, *J* = 7.6 Hz, CH=CH), 6.82 (d, *J* = 7.6 Hz, 1H, ArH), 6.60 (s, 1H, ArH), 6.65 (s, 1H, ArH), 5.52 (s, 1H, H-5), 4.02 (s, 1H, NH), 3.85 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.9, 161.9, 160.2, 158.4, 157.9, 144.6, 143.9, 135.5, 130.6, 128.1, 126.4, 118.3, 108.2, 104.2, 99.5, 83.7, 55.8.

4.1.48 *N-(6-((4-Methoxy-2-nitrophenyl)amino)pyrimidin-4-yl)cinnamamide(**Yfq44**)* Yield: 86.3 %;

mp: 229-231 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.74 (s, 1H, NH), 9.67 (s, 1H, H-2), 8.23 (s, 1H, H-2), 7.66 (s, 1H, ArH), 7.63 (t, *J* = 5.4 Hz, 3H, ArH), 7.55 (d, *J* = 9.0 Hz, 2H, ArH), 7.46 (dd, *J* = 4.2 Hz, 1H, CH=CH), 7.44 (dd, *J* = 4.2 Hz, 1H, CH=CH), 7.32 (dd, *J* = 6.0 Hz, 2H, ArH), 7.03 (d, 1H, NH), 3.85 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.9, 166.4, 160.2, 156.9, 150.0, 145.1, 136.5, 135.2, 134.4, 128.3, 126.8, 121.8, 118.9, 111.0, 85.7, 56.0. HRMS (ESI) *m/z*: [M+H] $^{+}$ calc = 392.1359; found 392.1352 for $C_{20}H_{17}N_5O_4$.

4.1.49 *N-(6-((4-Hydroxyphenyl)amino)pyrimidin-4-yl)cinnamamide* ***(Yfq45)*** Yield: 81.6 %; mp: 252-253 °C; ESI-MS [M+H]+: 332; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.71 (s, 1H, NH), 9.75 (s, 1H, H-2), 7.60 (d, *J* = 7.6 Hz, 2H, ArH), 7.40 (d, *J* = 7.6 Hz, 2H, ArH), 7.37 (d, 1H, CH=CH), 7.33 (t, *J* = 7.6 Hz, 1H, ArH), 7.22 (d, *J* = 7.6 Hz, 2H, ArH), 6.89 (d, 1H, CH=CH), 6.70 (d, *J* = 7.6 Hz, 2H, ArH), 5.52 (s, 1H, H-5), 5.35 (s, 1H, OH), 4.00 (s, 1H, NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.4, 165.5, 160.2, 156.8, 148.4, 145.1, 136.4, 135.6, 128.3, 128.2, 126.8, 118.6, 117.1, 116.8, 85.7.

4.1.50 *N-(6-((4-Morpholinophenyl) amino) pyrimidin-4-yl) cinnamamide* ***(Yfq46)*** Yield: 75.2 %; mp: 235-236 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.61 (s, 1H, NH), 9.38 (s, 1H, H-2), 8.33 (s, 1H, H-5), 7.64 (d, *J* = 12.0 Hz, 1H, CH=CH), 7.61 (d, *J* = 3.6 Hz, 2H, ArH), 7.59 (d, *J* = 8.4 Hz, 1H, CH=CH), 7.46 (m, 5H, ArH), 7.04 (s, 1H, NH), 6.93 (d, *J* = 9.0 Hz, 2H, ArH), 3.74 (t, 4H, CH2), 3.06 (t, 4H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.5, 165.4, 161.9, 158.9, 157.6, 142.9, 142.1, 131.8, 131.0, 129.8, 127.0, 121.8, 118.4, 114.6, 102.7, 55.4. HRMS (ESI) *m/z*: [M+H] $^{+}$ calc = 402.1930; found 402.1923 for $C_{23}H_{23}N_5O_2$.

4.1.51 *(E)-N-(6-((4-Chlorophenyl)amino)pyrimidin-4-yl)-3-(4-fluorophenyl)acrylamide* ***(Yfq47)*** Yield: 64.2 %; mp: 257-259 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.78 (s, 1H, NH), 8.42 (s, 1H, H-2), 7.69 (d, *J* = 7.6 Hz, 2H, ArH), 7.43 (d, *J* = 7.6 Hz, 2H, ArH), 7.35 (d, 1H, CH=CH), 7.23 (d, *J* = 7.6 Hz, 2H, ArH), 6.89 (d, *J* = 7.6 Hz, 2H, ArH), 5.78 (d, 1H, CH=CH), 5.84 (s, 1H, H-5), 5.71 (s, 1H, NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.3, 165.9, 165.2, 163.6, 161.5, 157.6, 141.8, 133.1, 131.1, 131.0, 130.2, 121.5, 118.7, 117.8, 115.4, 94.5. HRMS (ESI) *m/z*: [M+H] $^{+}$ calc = 369.0918; found 369.0911 for $C_{19}H_{14}ClFN_4O$.

4.1.52 *(E)-3-(4-Fluorophenyl)-N-(6-((4-morpholinophenyl)amino)pyrimidin-4-yl)acrylamide* ***(Yfq48)*** Yield: 87.1 %; mp: 234-236 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.74 (s, 1H, NH), 9.40 (s, 1H, H-2), 7.72 (d, *J* = 7.6 Hz, 2H, ArH), 7.37 (d, 1H, CH=CH), 7.19 (d, *J* = 7.6 Hz, 2H, ArH), 6.89 (d, 1H, CH=CH), 6.66 (d, *J* = 7.6 Hz, 2H, ArH), 6.45 (d, *J* = 7.6 Hz, 2H, ArH), 5.52 (s, 1H, H-5), 4.00 (s, 1H, NH), 3.65 (t, 4H, CH2), 3.18 (t, 4H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.2, 164.9, 161.4, 157.7, 156.8, 146.3, 141.7, 137.8, 134.5, 133.9, 130.9, 129.1, 128.6, 121.9, 121.5, 117.3, 93.1. HRMS (ESI) *m/z*: [M+H] $^{+}$ calc = 420.1836; found 420.1828 for $C_{23}H_{22}FN_5O_2$.

4.1.53 *(E)-N-(6-((2,4-Dichlorophenyl)amino)pyrimidin-4-yl)-3-(4-fluorophenyl)acrylamide **(Yfq49)*** Yield: 76.4 %; mp: 245-246 °C; ESI-MS [M+H]+: 402; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.80 (s, 1H, NH), 8.44 (s, 1H, H-2), 7.73 (m, 3H, ArH), 7.69 (s, 1H, ArH), 7.65 (d, *J* = 15.6 Hz, 1H, ArH), 7.39 (m, 2H, ArH), 7.24 (m, 2H, ArH), 69 (d, *J* = 15.6 Hz, 1H, ArH), 5.84 (s, 1H, NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.3, 165.7, 163.6, 161.5, 157.5, 141.7, 133.0, 131.0, 131.0, 130.1, 126.3, 121.4, 118.5, 117.7, 115.4, 94.5.

4.1.54 *(E)-3-(2-Bromo-4-fluorophenyl)-N-(6-((2,4-dichlorophenyl)amino)pyrimidin-4-yl) acrylamide **(Yfq50)*** Yield: 76.4 %; mp: 263-265 °C; ESI-MS [M+H]+: 479. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.81 (s, 1H, NH), 9.34 (s, 1H, H-2), 8.34 (s, 1H, ArH), 7.83 (s, 1H, ArH), 7.81 (s, 1H, H-5), 7.74 (m, *J* = 6.4Hz, 2H, ArH), 7.72 (d, *J* = 11.4 Hz, 1H, CH=CH), 7.69 (d, *J* = 1.8 Hz, 1H, ArH), 7.65 (s, 1H, NH), 7.44 (dd, *J* = 6.6 Hz, 1H, ArH), 6.98 (d, *J* = 15.6 Hz, 1H, CH=CH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 167.6, 165.3, 162.8, 161.6, 145.9, 136.3, 134.0, 132.7, 131.9, 129.1, 127.6, 126.4, 122.6, 122.6, 120.5, 119.8, 116.4, 83.5.

4.1.55 *(E)-3-(2-Bromo-4-fluorophenyl)-N-(6-((4-methoxy-2-nitrophenyl)amino)pyrimidin-4-yl) acrylamide **(Yfq51)*** Yield: 76.4 %; mp: 251-252 °C; ESI-MS [M+H]+: 487. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.72 (s, 1H, NH), 9.33 (s, 1H, H-2), 8.35 (s, 1H, ArH), 7.83 (s, 1H, ArH), 7.80 (s, 1H, H-5), 73 (m, *J* = 6.4 Hz, 2H, ArH), 7.73 (d, *J* = 11.4 Hz, 1H, CH=CH), 7.69 (d, *J* = 1.8 Hz, 1H, ArH), 7.64 (s, 1H, NH), 7.46 (dd, *J* = 6.6 Hz, 1H, ArH), 6.98 (d, *J* = 15.6 Hz, 1H, CH=CH), 3.79 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.6, 165.6, 162.9, 161.7, 156.3, 145.9, 141.2, 138.0, 133.0, 132.0, 123.1, 122.4, 119.8, 118.7, 115.5, 110.2, 84.5, 56.9.

4.1.56 *(E)-3-(2-Bromo-4-fluorophenyl)-N-(6-((4-morpholinophenyl)amino)pyrimidin-4-yl) acrylamide **(Yfq52)*** Yield: 84.2 %; mp: 265-268 °C; ESI-MS [M+H]+: 497; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.77 (s, 1H, NH), 9.15 (s, 1H, ArH), 8.45 (s, 1H, H-2), 7.80 (s, 1H, H-5), 7.72 (d, *J* = 7.6 Hz, 2H, ArH), 7.37 (d, 1H, CH=CH), 7.19 (d, *J* = 7.6 Hz, 2H, ArH), 6.89 (d, 1H, CH=CH), 6.66 (d, *J* = 7.6 Hz, 2H, ArH), 4.00 (s, 1H, NH), 3.65 (t, 4H, CH2), 3.18 (t, 4H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.2, 164.9, 161.4, 157.7, 156.8, 146.3, 141.7, 137.77, 134.5, 133.9, 130.9, 128.6, 121.9, 121.5, 117.3, 93.1.

4.1.57 *(E)-3-(4-Fluorophenyl)-N-(6-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl) acrylamide **(Yfq53)*** Yield: 76.3 %; mp: 254-254 °C; ESI-MS [M+H]+: 432; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.59 (s, 1H, NH), 9.35 (s, 1H, H-2), 8.32 (s, 1H, H-5), 7.67 (d, *J* = 3.0 Hz, 2H, ArH), 7.63 (d, *J* = 13.8 Hz, 2H, CH=CH), 7.42 (d, *J* = 7.2 Hz, 2H, ArH), 7.30 (t, *J* = 9.0 Hz, 2H, ArH), 6.98 (s, 1H, NH), 6.91 (d, 2H, ArH), 3.08 (t, 4H, CH2), 2.50 (t, 4H, CH2), 2.44 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.1, 168.8, 163.1, 160.4, 143.4, 140.5, 132.7, 132.3, 132.3, 120.1,

120.0, 119.9, 117.7, 117.7, 115.1, 115.1, 82.8, 58.3, 58.3, 52.0, 52.0, 47.1.

4.1.58 *(E)-N-(6-((3-Bromophenyl)amino)pyrimidin-4-yl)-3-(4-methoxyphenyl)acrylamide(**Yfq54**)* Yield: 79.6 %; mp: 251-253 °C; ESI-MS [M+H]+: 424. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.63 (s, 1H, NH), 9.39 (s, 1H, H-2), 8.39 (s, 1H, H-5), 7.68 (d, *J* = 10.2 Hz, 1H, CH=CH), 7.43-7.64 (d, *J* = 4.2 Hz, 4H, ArH), 7.42 (d, *J* = 7.8 Hz, 1H, ArH), 7.02 (t, *J* = 7.8 Hz, 1H, ArH), 6.97 (d, *J* = 7.8 Hz, 1H, ArH), 6.86 (s, 1H, ArH), 6.79 (d, *J* = 10.8 Hz, 1H, CH=CH), 4.25 (s, 1H, NH), 3.83 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.2, 167.3, 162.9, 161.1, 159.3, 142.3, 134.8, 132.4, 129.2, 124.3, 119.7, 117.8, 83.4, 58.2.

4.1.59 *(E)-N-(6-((2,4-Dichlorophenyl)amino)pyrimidin-4-yl)-3-(4-methoxyphenyl)acrylamide (**Yfq55**)* Yield: 81.5 %; mp: 253-253 °C; ESI-MS [M+H]+: 414; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.73 (s, 1H, NH), 9.43 (s, 1H, H-2), 8.33 (s, 1H, H-5), 7.86 (s, 1H, ArH), 7.79 (d, *J* = 10.2 Hz, 1H, CH=CH), 7.55 (d, *J* = 4.2 Hz, 3H, ArH), 7.52 (d, *J* = 8.4 Hz, 1H, ArH), 7.02 (d, *J* = 9.0 Hz, 2H, ArH), 6.76 (d, *J* = 10.8 Hz, 1H, CH=CH), 4.43 (s, 1H, NH), 3.91 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.5, 167.5, 163.7, 161.9, 160.3, 143.8, 134.4, 132.8, 129.8, 124.5, 120.1, 117.6, 83.7, 57.4.

4.1.60 *(E)-N-(6-((4-Chlorophenyl)amino)pyrimidin-4-yl)-3-(4-methoxyphenyl)acrylamide (**Yfq56**)* Yield: 83.4 %; mp: 249-249 °C; ESI-MS [M+H]+: 380; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.71 (s, 1H, NH), 9.39 (s, 1H, H-2), 8.42 (s, 1H, H-5), 7.69 (d, *J* = 10.2 Hz, 1H, CH=CH), 7.43 (d, *J* = 4.2 Hz, 3H, ArH), 7.41 (d, *J* = 8.4 Hz, 1H, ArH), 7.02 (d, *J* = 9.0 Hz, 2H, ArH), 6.80 (d, *J* = 3.0 Hz, 2H, ArH), 6.78 (d, *J* = 10.8 Hz, 1H, CH=CH), 4.41 (s, 1H, NH), 3.80 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.0, 167.9, 163.8, 161.9, 160.7, 144.0, 134.3, 132.9, 129.4, 124.6, 120.2, 117.6, 84.0, 57.3.

4.1.61 *(E)-N-(6-((4-Ethoxyphenyl)amino)pyrimidin-4-yl)-3-(4-methoxyphenyl)acrylamide(**Yfq57**)* Yield: 85.1 %; mp: 261-263 °C; ESI-MS [M+H]+: 390; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.53 (s, 1H, NH), 9.41 (s, 1H, H-2), 8.33 (s, 1H, H-5), 7.58 (d, *J* = 10.2 Hz, 1H, CH=CH), 7.56 (d, *J* = 4.2 Hz, 3H, ArH), 7.49 (d, *J* = 8.4 Hz, 1H, ArH), 7.02 (d, *J* = 9.0 Hz, 2H, ArH), 6.90 (d, *J* = 3.0 Hz, 2H, ArH), 6.88 (d, *J* = 10.8 Hz, 1H, CH=CH), 4.01 (s, 1H, NH), 4.0 (q, *J* = 6.6 Hz, 2H, CH2), 3.80 (s, 3H, CH3), 1.32 (t, *J* = 6.6 Hz, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.3, 168.7, 163.7, 162.2, 161.5, 154.3, 143.8, 134.5, 132.6, 128.7, 123.8, 120.5, 117.1, 84.0, 66.2, 57.3, 16.2.

4.1.62 *(E)-3-(4-Fluorophenyl)-N-(6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)acrylamide (**Yfq58**)* Yield: 76.4 %; mp: 245-246 °C; ESI-MS [M+H]+: 341; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.61 (s, 1H, NH), 8.30 (s, 1H, H-2), 7.66 (dd, 2H, ArH), 7.61 (d, *J* = 15.6 Hz, 1H, CH=CH), 7.56 (s, 1H, H-5), 7.30 (d, *J* = 8.4 Hz, 2H, ArH), 6.96 (d, *J* = 15.6 Hz, 1H, CH=CH), 3.57 (t, *J* = 4.8 Hz, 4H,

CH2), 2.37 (t, *J* = 4.8 Hz, 4H, CH2), 2.21 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.4, 164.9, 161.7, 157.8, 154.3, 141.6, 134.5, 132.8, 130.1, 127.9, 122.2, 114.5, 63.2, 59.8, 20.8, 14.1.

4.1.63 *(E)-3-(2-Bromo-4-fluorophenyl)-N-(6-morpholinopyrimidin-4-yl)acrylamide (**Yfq59**)* Yield: 81.2 %; mp: 251-251 °C; ESI-MS [M+H]+: 406; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.81 (s, 1H, NH), 9.34 (s, 1H, H-2), 8.34 (s, 1H, ArH), 7.81 (s, 1H, H-5), 7.74 (m, *J* = 6.0 Hz, 1H, ArH), 7.72 (d, *J* = 11.4 Hz, 1H, CH=CH), 7.69 (d, *J* = 6.0 Hz, 1H, ArH), 6.98 (d, *J* = 15.6 Hz, 1H, CH=CH), 3.82 (t, *J* = 4.8 Hz, 4H, CH2), 3.70 (t, *J* = 4.8 Hz, 4H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 167.6, 165.3, 162.8, 145.9, 136.3, 134.0, 132.7, 129.1, 127.6, 126.4, 122.6, 120.5, 116.4, 83.5.

4.1.64 *(E)-3-(4-Methoxyphenyl)-N-(6-morpholinopyrimidin-4-yl) acrylamide (**Yfq60**)* Yield: 78.6; mp %: 248-249 °C; ESI-MS [M+H]+: 340; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.73 (s, 1H, NH), 9.39 (s, 1H, H-2), 8.44 (s, 1H, H-5), 7.58 (d, *J* = 10.2 Hz, 1H, CH=CH), 7.47 (d, *J* = 4.2 Hz, 2H, ArH), 7.40 (d, *J* = 4.2 Hz, 2H, ArH), 6.78 (d, *J* = 10.8 Hz, 1H, CH=CH), 3.82 (s, 3H, CH3), 3.82 (t, *J* = 4.8 Hz, 4H, CH2), 3.69 (t, *J* = 4.8 Hz, 4H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.5, 167.9, 163.8, 162.0, 160.4, 143.8, 134.4, 132.8, 129.7, 124.8, 120.2, 117.6, 83.6, 57.8.

4.1.65 *(E)-3-(4-Methoxyphenyl)-N-(6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)acrylamide (**Yfq61**)* Yield: 77.1 %; mp: 251-253 °C; ESI-MS [M+H]+: 353; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.67 (s, 1H, NH), 8.45 (s, 1H, H-2), 7.68 (dd, 2H, ArH), 7.61 (d, *J* = 15.6 Hz, 1H, CH=CH), 7,55 (s, 1H, H-5), 7.28 (d, *J* = 8.4 Hz, 2H, ArH), 6.88 (d, *J* = 15.6 Hz, 1H, CH=CH), 3.80 (s, 3H, CH3), 3.68 (t, *J* = 4.8 Hz, 4H, CH2), 2.48 (t, *J* = 4.8 Hz, 4H, CH2), 2.47 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.4, 164.9, 161.7, 157.8, 154.3, 141.6, 134.5, 130.1, 127.9, 122.2, 114.5, 63.2, 59.8, 20.8, 14.1.

4.1.66 *4-((6-((4-Chlorophenyl)amino)pyrimidin-4-yl)amino)-4-oxobutanoic acid (**Yfq62**)* Yield: 73.9 %; mp: 224-227 °C; ESI-MS [M+H]+: 320; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.64 (s, 1H, COOH), 9.80 (s, 1H, H-2), 8.41 (s, 1H, H-5), 7.69-7.71 (d, *J* = 9.0 Hz, 2H, Ph-H), 7.52 (s, 1H, CONH), 7.34-7.35 (d, *J* = 9.0 Hz, 2H, Ph-H), 3.42-3.45 (q, *J* = 7.2 Hz, 1H, NH), 2.62-2.65 (t, *J* = 12.6 Hz, 2H, CH2), 2.50-2.52 (m, 2H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.6, 175.6, 172.2, 163.0, 153.0, 142.2, 132.7, 129.2, 124.0, 90.0, 33.9, 29.8.

4.1.67 *4-((6-((3-Chlorophenyl)amino)pyrimidin-4-yl) amino)-4-oxobutanoic acid (**Yfq63**)* Yield: 77.5 %; mp: 201-202 °C; ESI-MS [M+H]+: 320; ^{1}H NMR (600 MHz, DMSO-d6) δ 10.61 (s, 1H, COOH), 9.77 (s, 1H, H-2), 8.45-8.45 (d, *J* = 0.6 Hz, 1H, Ph-H), 7.97 (s, 1H, Ph-H), 7.50 (m, 1H, Ph-H), 7.30-7.33 (t, *J* = 8.0 Hz, 1H, Ph-H), 6.61 (s, 1H, H-5), 6.53 (s, 1H, NH), 6.52 (s, 1H, ArH), 2.65-2.66 (t, *J* = 3.0 Hz, 2H, CH2), 2.50 (t, *J* = 3.0 Hz, 2H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6)

δ 176.2, 175.0, 72.0, 162.7, 152.8, 142.9, 133.7, 129.8, 125.1, 88.4, 33.6, 28.8.

4.1.68 *4-((6-((3-Bromophenyl)amino)pyrimidin-4-yl)amino)-4-oxobutanoic acid (**Yfq64**)* Yield: 80.4 %; mp: 175-177 °C; ESI-MS [M+H]+:364; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.69 (s, 1H, COOH), 9.88 (s, 1H, -NH), 8.46 (s, 1H, H-2), 8.09 (s, 1H, H-5), 7.53-7.54 (m, 1H, PhH), 7.52 (s, 1H, 2'-PhH), 7.26-7.27 (m, 1H, PhH), 7.15-7.163 (m, 1H, PhH), 6.73 (s, 1H, NH), 2.64-2.66 (t, 2H, J = 6.0 Hz, CH2CH2COOH), 2.52-2.53 (m, 2H, CH2CH2COOH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.8, 177, 170.4, 160.3, 151.8, 144.6, 130.6, 123.9, 121.6, 116.6, 115.5, 88.5, 28.7.

4.1.69 *4-((6-((3-Methoxyphenyl)amino)pyrimidin-4-yl)amino)-4-oxobutanoic acid (**Yfq65**)* Yield: 60.1 %; mp: 203-204 °C; ESI-MS [M+H]+: 316; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.67 (s, 1H, OH), 9.71 (s, 1H, H-2), 8.40 (s, 1H, H-5), 7.50 (s, 1H, ArH), 7,32 (s, 1H, NH), 7.21 (t, J = 7.8 Hz, 1H, ArH), 7.17 (d, J = 8.4 Hz, 1H, ArH), 6.76 (s, 1H, NH), 6.60 (dd, *J* = 6.0 Hz, 1H, ArH), 3.58 (s, 3H, CH3), 2.64 (t, *J* = 6.6 Hz, 2H, CH2), 2.05 (dd, *J* = 1.8 Hz, 2H, CH2). 13C NMR (150 MHz, DMSO-d_6) δ 174.6, 173.7, 172.5, 163.3, 152.1, 143.9, 130.1, 125.9, 121.5, 117.1, 115.4, 89.3, 55.9, 28.0.

4.1.70 *4-((6-((4-(Ethoxycarbonyl)phenyl)amino)pyrimidin-4-yl)amino)-4-oxobutanoic acid (**Yfq66**)* Yield: 57.3 %; mp: 213-216 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.64 (s, 1H, OH), 9.99 (s, 1H, NH), 8.48 (s, 1H, H-2), 7.83-7.90 (q, 4H, ArH), 7.64 (s, 1H, H-5), 6.81 (s, 1H, NH), 4.26-4.29 (q, 2H, *J* = 7.2 Hz, OCH2CH3), 2.64-2.66 (t, 2H, *J* = 6.6 Hz, NHCOCH2CH2COOH), 2.50-2.53 (m, 2H, NHCOCH2CH2COOH), 1.29-1.32 (t, 3H, *J* = 7.2 Hz, COOCH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.9, 173.8, 170.4, 165.9, 160.3, 151.8, 145.2, 130.7, 120.1, 111.7, 60.4, 28.9, 14.1. HRMS (ESI) *m/z*: $[M+H]^+$ calc = 359.1355; found 359.1348 for $C_{17}H_{18}N_4O_5$.

4.1.71 *3-((6-(3-Carboxypropanamido)pyrimidin-4-yl)amino)benzoic acid (**Yfq67**)*Yield: 60.2 %; mp: 195-197 °C; ESI-MS [M+H]+: 330; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.72 (s, 1H, COOH), 10.67 (s, 1H, OH), 9.92 (s, 1H, NH), 8.44 (s, 1H, H-2), 8.28 (s, 1H, H-5), 7.90-7.91 (m, 1H, ArH), 7.57-7.58 (m, 1H, ArH), 7.51 (s, 1H, ArH), 7.42-7.44 (m, 1H, ArH), 6.81 (s, 1H, NH), 2.64-2.66 (t, 2H, *J* = 7.2 Hz, CH2CH2COOH), 2.52-2.53 (m, 2H, CH2CH2COOH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.9, 173.8, 170.4, 166.4, 160.3, 151.2, 142.4, 133.8, 128.7, 123.1, 120.5, 114.6, 88.3, 28.9.

4.1.72 *4-((6-((4-(4-Methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)amino)-4-oxobutanoic acid (**Yfq68**)* Yield: 56.8 %; mp: 186-187 °C; ESI-MS [M+H]+: 384. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.77 (s, 1H, COOH), 9.85 (s, 1H, NH), 8.42 (s, 1H, H-2), 8.28 (s, 1H, H-5), 7.90-7.91 (m, 1H, ArH), 7.57-7.59 (m, 1H, ArH), 7.51 (s, 1H, ArH), 7.42-7.44 (m, 1H, ArH), 6.81 (s, 1H, NH), 2.64-2.66 (t, 2H, *J* = 72 Hz, CH2CH2COOH), 3.68 (t, *J* = 4.8 Hz, 4H, CH2), 2.51-2.52 (m, 2H,

CH2CH2COOH), 2.48 (t, *J* = 4.8 Hz, 4H, CH2), 2.47 (s, 3H, CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.9, 173.8, 170.4, 160.3, 151.8, 139.7, 130.4, 118.5, 113.7, 88.5, 61.4, 57.3, 52.1, 46.7, 28.9.

4.1.73 *5-Oxo-5-((6-(phenylamino)pyrimidin-4-yl)amino)pentanoic acid (**Yfq69**)* Yield: 67.5 %; mp: 175-177 °C; ESI-MS [M+H]+: 300; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.53 (s, 1H, COOH), 8.42 (s, 1H, H-2), 8.39 (s, 1H, NH), 8.28 (s, 1H, H-5), 7.70-7.71 (d, *J* = 9.0 Hz, 2H, PhH), 6.55-6.57 (t, *J* = 7.8 Hz, 3H, PhH), 2.50 (s, 1H, NH), 2.41-2.44 (t, *J* = 7.2 Hz, 2H, CH2), 2.27-2.42 (t, *J* = 3.0 Hz, 2H, CH2), 1.75-1.80 (m, 2H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.3, 173.7, 170.2, 160.4, 151.7, 139.3, 130.3, 118.8, 113.5, 88.7, 61.2, 57.9, 52.3, 46.7, 28.6.

4.1.74 *5-((6-((4-Chlorophenyl)amino)pyrimidin-4-yl)amino)-5-oxopentanoic acid (**Yfq70**)* Yield: 79.4 %; mp: 181-184 °C; ESI-MS [M+H]+: 334; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.53 (s, 1H, COOH), 9.74 (s, 1H, H-2), 8.39 (s, 1H, NH), 7.70-7.71 (d, *J* = 9.0 Hz, 2H, PhH), 7.55 (s, 1H, H-5), 7.33-7.34 (d, *J* = 9.0 Hz, 2H, PhH), 2.50 (s, 1H, NH), 2.41-2.44 (t, *J* = 7.2 Hz, 2H, CH2), 2.27-2.42 (t, *J* = 3.0 Hz, 2H, CH2), 1.75-1.80 (m, 2H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.3, 170.3, 166.1, 160.7, 151.1, 142.4, 133.5, 128.78, 123.0, 20.4, 114.7, 88.2, 28.9.

4.1.75 *5-((6-((3-Chlorophenyl)amino)pyrimidin-4-yl)amino)-5-oxopentanoic acid (**Yfq71**)* Yield: 82.2 %; mp: 184-185 °C; ESI-MS [M+H]+: 334; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.54 (s, 1H, COOH), 9.78 (s, 1H, H-2), 8.44 (s, 1H, NH), 7.99 (s, 1H, PhH), 7.55 (s, 1H, ArH), 7.33-7.35 (dd, *J* = 9.0 Hz, 1H, PhH), 6.57-6.58 (m, 1H, ArH), 6.55-6.57 (t, *J* = 9.5 Hz, 1H, PhH), 6.52 (s, 1H, NH), 2.49-2.51 (m, 2H, CH2), 2.44-2.45 (t, *J* = 3.0 Hz, 2H, CH2), 2.27-2.42 (t, *J* = 7.8 Hz, 2H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.8, 173.7, 170.4, 166.1, 160.3, 151.8, 144.6, 130.6, 123.9, 121.6, 116.6, 115.5, 88.5, 28.7.

4.1.76 *5-((6-((2-Bromophenyl)amino)pyrimidin-4-yl)amino)-5-oxopentanoic acid (**Yfq72**)* Yield: 80.3 %; mp: 189-191 °C; ESI-MS [M+H]+: 378; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.73 (s, 1H, COOH), 9.42 (s, 1H, H-2), 8.47 (s, 1H, NH), 7.98 (s, 1H, H-5), 7.35-7.36 (dd, J = 9.0 Hz, 2H, PhH), 6.57 -6.58 (m, 1H, PhH), 6.55-6.57 (t, *J* = 9.5 Hz, 1H, PhH), 6.52 (s, 1H, NH), 2.49-2.51 (m, 2H, CH2), 2.45 (t, *J* = 3.0 Hz, 2H, CH2), 2.27-2.42 (t, *J* = 7.8 Hz, 2H,CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.8, 173.9, 170.6, 166.3, 160.3, 151.5, 144.5, 130.2, 123.2, 121.5, 116.9, 115.2, 88.9, 28.9.

4.1.77 *5-((6-((4-(Ethoxycarbonyl)phenyl)amino)pyrimidin-4-yl)amino)-5-oxopentanoic acid (**Yfq73**)* Yield: 58.6 %; mp: 248-249 °C; ESI-MS [M+H]+: 372; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.53 (s, 1H, COOH), 9.74 (s, 1H, 2-pyrimindineH), 8.39 (s, 1H, NH), 7.70-7.71 (d, *J* = 9.0 Hz, 2H, PhH), 7.55 (s, 1H, 5-pyrimindineH), 7.33-7.34 (d, *J* = 9.0 Hz, 2H, PhH), 4.26-4.29 (q, 2H, *J* =

7.2 Hz, OCH2CH3), 2.50 (s, 1H, NH), 2.41-2.44 (t, *J* = 7.2 Hz, 2H, CH2), 2.27-2.42 (t, *J* = 3.0 Hz, 2H, CH2), 1.75-1.80 (m, 2H, CH2), 1.29-1.32 (t, 3H, *J* = 7.2 Hz, COOCH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.8, 173.3, 170.5, 166.3, 160.4, 151.5, 144.5, 130.3, 123.3, 121.5, 116.8, 115.3, 88.5, 28.9.

4.1.78 *5-((6-((2,5-Dimethoxyphenyl)amino)pyrimidin-4-yl)amino)-5-oxopentanoic acid (**Yfq74**)* Yield: 57.9 %; mp: 240-242 °C; ESI-MS [M+H]+: 360; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.97 (s, 1H, COOH), 9.56 (s, 1H, H-2), 8.40 (s, 1H, H-5), 7.30 (s, 2H, PhH), 7.02 (d, *J* = 10.2 Hz, 1H, PhH), 6.75-6.76 (q, *J* = 3.0 Hz, 1H, PhH), 3.75 (s, 3H, OCH3), 3.71 (s, 3H, OCH3), 2.50 (s, 1H, NH), 2.43-2.45 (t, 2H, CH2), 2.26-2.43 (t, 2H, CH2), 1.74-1.78 (m, 2H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 179.3, 173.2, 172.1, 162.0, 154.7, 153.1, 141.2, 135.1, 113.0, 107.2, 105.3, 90.0, 57.3, 35.1, 33.3, 22.1.

4.1.79 *5-((6-((3-Methoxyphenyl)amino)pyrimidin-4-yl)amino)-5-oxopentanoic acid (**Yfq75**)* Yield: 62.0 %; mp: 224-226 °C; ESI-MS [M+H]+: 330; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.13 (s, 1H, OH), 10.28 (s, 1H, H-2), 8.48 (s, 1H, H-5), 8.42 (s, 1H, NH), 7.35 (s, 1H, ArH), 7.27 (t, *J* = 3.6 Hz, 1H, ArH), 7.25 (s, 1H, NH), 7.15 (d, *J* = 7.8 Hz, 1H, PhH), 6.69 (dd, *J* = 6.6 Hz, 1H, ArH), 3.78 (s, 3H, CH3), 2.48 (q, *J* = 6.6 Hz, 2H, CH2), 2.27 (t, *J* = 7.2 Hz, 2H, CH2), 1.81 (t, *J* = 7.2 Hz, 2H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 179.4, 173.2, 172.3, 164.0, 161.7, 153.5, 144.3, 131.1, 113.0, 112.7, 103.3, 90.0, 57.4, 35.8, 33.5, 22.3.

4.1.80 *5-((6-((3-Bromophenyl)amino)pyrimidin-4-yl)amino)-5-oxopentanoic acid (**Yfq76**)* Yield: 79.3 %; mp: 212-213 °C; ESI-MS [M+H]+: 378; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.61 (s, 1H, COOH), 9.59 (s, 1H, H-2), 8.46 (s, 1H, NH), 7.91 (s, 1H, H-5), 7.54 (s, 1H, PhH), 7.34-7.35 (dd, *J* = 9.0 Hz, 1H, PhH), 6.57-6.58 (m, 1H, PhH), 6.54-6.56 (t, *J* = 9.5 Hz, 1H, PhH), 6.56 (s, 1H, NH), 2.49-2.51 (m, 2H, CH2), 2.44-2.45 (t, *J* = 3.0 Hz, 2H, CH2), 2.27-2.42 (t, *J* = 7.8 Hz, 2H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.8, 173.7, 170.4, 166.1, 160.3, 151.8, 144.6, 130.6, 123.9, 121.6, 116.6, 115.5, 88.6, 28.5.

4.1.81 *5-((6-Morpholinopyrimidin-4-yl)amino)-5-oxopentanoic acid (**Yfq77**)* Yield: 76.5 %; mp: 237-239 °C; ESI-MS [M+H]+: 294; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.44 (s, 1H, OH), 8.46 (s, 1H, NH), 8.28 (s, 1H, H-2), 7.42 (s, 1H, H-5), 3.66 (t, *J* = 4.2 Hz, 4H, CH2), 3.51 (t, *J* = 4.8 Hz, 4H, CH2), 2.42 (t, *J* = 7.2 Hz, 2H, CH2), 2.24 (t, *J* = 7.2 Hz, 2H,CH2), 1.76 (m, 2H, H2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 180.1, 173.2, 172.1, 162,5, 152,9, 89.0, 68.5, 50.2, 34.2, 33.6, 21.6.

4.1.82 *5-((6-(4-Methylpiperazin-1-yl)pyrimidin-4-yl)amino)-5-oxopentanoic acid (**Yfq78**)* Yield: 75.1 %; mp: 240-241 °C; ESI-MS [M+H]+: 307; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.40 (s, 1H, OH), 8.40 (s, 1H, NH), 8.25 (s, 1H, H-2), 7.42 (s, 1H, H-5), 3.44 (t, *J* = 6.6 Hz, 4H, CH2), 2.50 (t,

J = 1.8 Hz, 2H, CH2), 2.41 (t, *J* = 7.2 Hz, 4H, CH2), 2.36 (t, *J* = 4.8 Hz, 2H, CH2), 2.22 (s, 3H, CH3), 1.76 (m, 2H, CH2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 179.7, 172.4, 172.3, 162.3, 152.9, 90.2, 68.5, 59.2, 49.0, 47.2, 34.2, 33.5, 21.6.

4.1.83 *Ethyl-4-((6-((3-bromophenyl)amino)pyrimidin-4-yl)amino)-4-oxobutanoate (**Yfq79**)* Yield: 61.8 %; mp: 178-179 ℃; ESI-MS [M+H]+: 392; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.63 (s, 1H, CONH), 9.75 (s, 1H, NH), 8.45 (s, 1H, H-2), 8.11 (m, 1H, H-5), 7.14-7.56 (m, 4H, PhH), 4.03-4.07 (q, 2H, *J* = 7.2 Hz, OCH2CH3), 2.68-2.70 (t, 2H, *J* = 6.0 Hz, NHCOCH2CH2COOC2H5), 2.56-2.58 (t, 2H, *J* = 6.0 Hz, NHCOCH2CH2COOC2H5), 1.16-1.9 (t, 3H, *J* = 7.2 Hz, CH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.2, 170.2, 160.8, 151.5, 144.7, 130.9, 123.7, 116.9, 115.4, 88.6, 61.3, 28.8, 14.1.

4.1.84 *Ethyl-4-((6-((4-chlorophenyl)amino)pyrimidin-4-yl)amino)-4-oxobutanoate (**Yfq80**)* Yield: 60.3 %; mp: 187-189 ℃; ESI-MS [M+H]+: 348; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.61 (s, 1H, CONH), 9.74 (s, 1H, H-2), 8.39 (s, 1H, NH), 7.70-7.71 (d, *J* = 9.0 Hz, 2H, PhH), 7.55 (s, 1H, H-5), 7.33-7.34 (d, *J* = 9.0 Hz, 2H, PhH), 4.03-4.07 (q, 2H, *J* = 7.2 Hz, OCH2CH3), 2.68-2.70 (t, 2H, *J* = 6.0 Hz, NHCOCH2CH2COOC2H5), 2.56-2.58 (t, 2H, *J* = 6.0 Hz, NHCOCH2CH2COOC2H5), 1.16-1.19 (t, 3H, *J* = 7.2 Hz, CH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.2, 170.2, 165.9, 160.2, 150.8, 142.1, 133.4, 128.3, 123.5, 120.2, 114.7, 87.5, 28.4.

4.1.85 *Ethyl-4-((6-((3-methoxyphenyl) amino) pyrimidin-4-yl) amino)-4-oxobutanoate (**Yfq81**)* Yield: 57.8 %; mp: 211-212 ℃; ESI-MS [M+H]+: 344. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.57 (s, 1H, CONH), 9.55 (s, 1H, NH), 8.39 (s, 1H, H-2), 7.54 (s, 1H, H-5), 7.34 (s, 1H, 2'-PhH), 7.17-7.21 (m, 2H, ArH), 6.57-6.58 (m, 1H, ArH), 4.05 (q, 2H, *J* = 7.2 Hz, -COO-CH2CH3), 3.73 (s, 3H, OCH3), 2.67-2.69 (t, 2H, *J* = 6.6 Hz, COCH2CH2COOC2H5), 2.55-2.58 (t, 2H, *J* = 6.6 Hz, NHCOCH2CH2COOC2H5), 1.16-1.18 (t, 3H, *J* = 6.9 Hz, CH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 61.5, 157.6, 141.3, 129.5, 112.1, 107.4, 105.7, 96.8, 93.0, 85.7, 80.9, 59.9, 55.0, 30.9, 28.4, 18.0, 14.1.

4.1.86 *Ethyl-4-((6-((2,5-dimethoxyphenyl)amino)pyrimidin-4-yl)amino)-4-oxobutanoate (**Yfq82**)* Yield: 51.6 %; mp: 223-226 ℃; ESI-MS [M+H]+: 374; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.48 (s, 1H, CONH), 8.75 (s, 1H, H-2), 8.32 (s, 1H, H-5), 7.52-7.53 (m, 2H, PhH+NH), 6.95-6.96 (m, 1H, PhH), 6.63-6.64 (m, 1H, PhH), 4.04-4.05 (q, 2H, CH2CH3), 3.74 (s, 3H, OCH3), 3.70 (s, 3H, OCH3), 2.65-2.66 (m, 2H, CH2CH2COOC2H5), 2.55-2.56 (m, 2H, CH2CH2COOC2H5), 1.16-1.18 (t, 3H, CH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.2, 166.2, 162.0, 157.4, 156.7, 153.0, 145.3, 112.3, 110.0, 108.0, 92.8, 61.1, 53.8, 30.9, 28.4, 14.1.

4.1.87 *Ethyl-4-((6-(4-ethoxy-4-oxobutanamido)pyrimidin-4-yl)amino)benzoate (**Yfq83**)* Yield:

55.5 %; mp: 190-192 °C; ESI-MS [M+H]+: 386. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.67 (s, 1H, CONH), 9.99 (s, 1H, NH), 8.48 (s, 1H, H-2), 7.89-7.90 (d, 2H, *J* = 9.0 Hz, 2',6'-PhH), 7.83-7.85 (d, 2H, *J* = 9.0 Hz, 3',5'-PhH), 7.63 (s, 1H, H-5), 4.26-4.29 (q, 2H, *J* = 7.2 Hz, COOCH2CH3), 4.05-4.06 (q, 2H, *J* = 7.2 Hz, COOCH2CH3), 2.68-2.71 (t, 2H, *J* = 6.0 Hz, NHCOCH2CH2COOC2H5), 2.57-2.59 (t, 2H, *J* = 6.0 Hz, COCH2CH2COOC2H5), 1.29-1.32 (t, 3H, *J* = 7.2 Hz, COOCH2CH3), 1.16-1.19 (t, 3H, *J* = 7.2 Hz, COOCH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.7, 170.4, 165.9, 160.3, 151.8, 145.2, 130.2, 120.1, 111.2, 88.6, 61.3, 60.8, 28.9, 14.1.

4.1.88 *Ethyl-3-((6-(4-ethoxy-4-oxobutanamido)pyrimidin-4-yl)amino)benzoate* ***(Yfq84)*** Yield: 52.1 %; mp: 193-195 °C; ESI-MS [M+H]+: 386; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.61 (s, 1H, CONH), 9.80 (s, H, NH), 8.43 (s, 1H, H-2), 8.28 (s, 1H, H-5), 7.99-8.00 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz, 5'-PhH), 7.56-7.57 (d, 2H, *J* = 8.4 Hz, 4',6'-PhH), 7.43-7.46 (t, 1H, *J* = 8.4 Hz, 2'-PhH), 4.30-4.33 (q, 2H, *J* = 7.2 Hz, COOCH2CH3), 4.04-4.07 (q, 2H, *J* = 7.2 Hz, COOCH2CH3), 2.68-2.70 (t, 2H, *J* = 6.0 Hz, CH2CH2COOC2H5), 2.56-2.59 (t, 2H, *J* = 6.0 Hz, CH2CH2COOC2H5), 1.31-1.34 (t, 3H, *J* = 7.2 Hz, COOCH2CH3), 1.16-1.19 (t, 3H, *J* = 7.2 Hz, COOCH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.2, 170.5, 1657, 160.6, 151.6, 142.4, 133.7, 131.0, 122.4, 119.9, 114.3, 88.5, 61.0, 61.3, 28.9, 14.2.

4.1.89 *Ethyl-4-oxo-4-((6-((4-sulfamoylphenyl)amino)pyrimidin-4-yl)amino)butanoate* ***(Yfq85)*** Yield: 53.4 %; mp: 218-220 °C; ESI-MS [M+H]+: 393; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.56 (s, 1H, CONH), 9.95 (s, 1H, NH), 8.47 (s, 1H, H-2), 7.84-7.85 (d, 2H, *J* = 9.0 HZ, 2',6'-PhH), 7.73-7.74 (d, 2H, *J* = 9.0 HZ, 2',6'-PhH), 7.62 (s, 1H, H-5), 7.20 (s, 2H, SO2NH2), 4.04-4.07 (q, 2H, *J* = 7.2 Hz, COOCH2CH3), 2.68-2.71 (t, 2H, *J* = 6.0 Hz, CH2CH2COOC2H5), 2.50-2.59 (t, 2H, *J* = 6.0 Hz, CH2CH2COOC2H5), 1.16-1.19 (t, 3H, *J* = 7.2 Hz, COOCH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.2, 170.2, 160.8, 151.5, 144.7, 130.9, 130.0, 113.9, 88.5, 61.9, 28.8, 14.1.

4.1.90 *Ethyl-4-((6-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)amino)-4-oxobutano-ate* ***(Yfq86)*** Yield: 51.2 %; mp: 187-189 °C; ESI-MS [M+H]+: 412; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.46 (s, 1H, CONH), 9.26 (s, 1H, NH), 8.29 (s, 1H, H-2), 7.40 (s, 1H, H-5), 7.39-7.40 (d, 2H, *J* = 9.0 Hz, PhH), 6.89-6.91 (d, 2H, *J* = 9.0 Hz, PhH), 4.03-4.06 (q, 2H, *J* = 7.2 Hz, CH2CH3), 3.32-3.43 (m, 4H, piperazineH), 3.09-3.12 (m, 4H, piperazineH), 2.55-2.56 (m, 2H, CH2CH2COOC2H5), 2.52-2.54 (m, 2H, CH2CH2COOC2H5), 2.27 (s, 3H, N-CH3), 1.18-1.19 (t, 3H, *J* = 7.2 Hz, CH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.9, 173.8, 170.4, 160.3, 151.8, 139.7, 130.4, 118.5, 113.7, 88.5, 61.4, 57.3, 52.1, 46.7, 28.9, 14.1.

4.1.91 *Ethyl-4-oxo-4-((6-((2-(thiophen-2-yl)ethyl)amino)pyrimidin-4-yl)amino)butanoate* ***(Yfq87)***

Yield: 67.3 %; mp: 193-195 °C; ESI-MS [M+H]+: 348; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.35 (s, 1H, CONH), 8.20 (s, 1H, -NH), 7.50 (s, 1H, H-2), 7.32-7.33 (d, 1H, *J* = 5.4 Hz, Thiophene-H), 7.16 (s, 1H, H-5), 6.94-6.95 (t, 1H, *J* = 5.0 Hz, Thiophene-H), 6.89-6.90 (m, 1H, Thiophene-H), 4.02-4.06 (q, 2H, *J* = 7.2 Hz, COOCH2CH3), 3.48-3.51 (m, 2H, 1'-CH2), 3.01-3.04 (t, 2H, *J* = 7.2 Hz, 2'-CH2), 2.63-2.65 (t, 2H, *J* = 6.6 Hz, CH2CH2COOC2H5), 2.53-2.55 (t, 2H, *J* = 6.6 Hz, CH2CH2COOC2H5), 1.16-1.18 (t, 3H, *J* = 7.2 Hz, COOCH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.2, 163.0, 160.3, 151.5, 137.3, 128.2, 127.1, 125.6, 88.1, 61.6, 46.0, 28.8, 14.3.

4.1.92 *Ethyl-5-((6-((3-chlorophenyl)amino)pyrimidin-4-yl)amino)-5-oxopentanoate* ***(Yfq88)*** Yield: 74.2 %; mp: 197-198 °C; ESI-MS [M+H]+: 362; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.33 (s, 1H, CONH), 9.78 (s, 1H, H-2), 8.44 (s, 1H, NH), 7.99 (s, 1H, PhH), 7.55 (s, 1H, H-5), 7.33-7.35 (dd, *J* = 9.0 Hz, 1H, PhH), 6.57-6.58 (m, 1H, ArH), 6.55-6.57 (t, *J* = 9.5 Hz, 1H, PhH), 4.03-4.06 (q, 2H, *J* = 7.2 Hz, CH2CH3), 2.49-2.51 (m, 2H, CH2), 2.44-2.45 (t, *J* = 3.0 Hz, 2H,CH2), 2.27-2.42 (t, *J* = 7.8 Hz, 2H, CH2), 1.18-1.19 (t, 3H, *J* = 7.2 Hz, CH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.8, 170.4, 166.1, 160.3, 151.8, 144.6, 130.6, 123.9, 121.6, 116.6, 115.5, 88.5, 62.2, 28.7, 15.1.

4.1.93 *Ethyl-5-((6-((2,5-dimethoxyphenyl)amino)pyrimidin-4-yl)amino)-5-oxopentanoate* ***(Yfq89)*** Yield: 69.5 %; mp: 224-223 °C; ESI-MS [M+H]+: 388; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.97 (s, 1H, CONH), 9.56 (s, 1H, H-2), 8.40 (s, 1H, NH), 7.30 (s, 2H, PhH+H-5), 7.02 (d, *J* = 10.2 Hz, 1H, PhH), 6.75-6.76 (m, 1H, PhH), 4.12-4.22 (q, 2H, *J* = 7.2 Hz, OCH2CH3), 3.75 (s, 3H, OCH3), 3.71 (s, 3H, OCH3), 2.43-2.45 (t, 2H, COOCH2CH2CH2), 2.26-2.43 (t, 2H, COCH2), 1.74-1.78 (m, 2H, CH2CH2CH2), 1.18-1.19 (t, 3H, *J* = 7.2 Hz, OCH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 179.3, 173.2, 172.1, 162.0, 154.7, 153.1, 141.2, 135.1, 113.0, 107.2, 105.3, 90.0, 67.3, 35.1, 22.1.

4.1.94 *Pentan-2-yl-4-((6-((3-bromophenyl)amino)pyrimidin-4-yl)amino)-4-oxobutanoate* ***(Yfq90)*** Yield: 52.8 %; mp: 249-253 °C; ESI-MS [M+H]+: 434; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.62 (s, 1H, CONH), 9.76 (s, 1H, -NH), 8.45 (s, 1H, H-2), 8.11 (s, 1H, H-5), 7.55-7.57 (m, 2H, PhH), 7.25-7.26 (m, 1H, PhH), 7.14-7.15 (m, 1H, PhH), 4.79-4.80 (m, 1H, -OCH), 2.67-2.69 (m, 2H, COCH2CH2COO), 2.50 (m, 2H, COCH2CH2COO), 1.15-1.16 (m, 2H, CHCH2CH2CH3), 1.15 (m, 2H, CH2CH2CH3), 1.13-1.14 (d, 3H, *J* = 6.6 Hz, CHCH3), 0.82-0.84 (t, 3H, *J* = 7.2 Hz, CH2CH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.7, 170.4, 160.3, 151.8, 144.6, 130.6, 123.9, 121.6, 116.8, 115.5, 88.5, 71.5, 38.9, 32.4, 28.9, 20.2, 18.7, 14.1.

4.1.95 *Pentan-2-yl-3-((6-(4-oxo-4-(pentan-2-yloxy)butanamido)pyrimidin-4-yl)amino)benzoate* ***(Yfq91)*** Yield: 50.2 %; mp: 260-262 °C; ESI-MS [M+H]+: 470; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.60 (s, 1H, CONH), 9.81 (s, 1H, NH), 8.42 (s, 1H, H-2), 8.26 (s, 1H, H-5), 8.00-8.01 (m, 1H, PhH), 7.54-7.56 (m, 2H, PhH), 7.42-7.45 (m, 1H, PhH), 5.04-5.09 (m, 1H, COOCH(CH3)C3H7),

4.77-4.83 (m, 1H, COOCH(CH3)C3H7), 2.67-2.69 (t, 2H, *J* = 6.0 Hz, NHCOCH2CH2), 2.55-2.57 (t, 2H, *J* = 6.0 Hz, NHCOCH2CH2), 1.23-1.71 (m, 8H, CH(CH3)CH2CH2CH3), 1.29-1.30 (d, 3H, *J* = 6.6 Hz, CHCH3), 1.13-1.14 (d, 3H, *J* = 6.6 Hz, CHCH3), 0.90-0.92 (t, 3H, *J* = 7.2 Hz, CH2CH2CH3), 0.82-0.84 (t, 3H, *J* = 7.2 Hz, CH2CH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.2, 170.5, 165.7, 160.6, 151.6, 145.2, 130.7, 120.07, 111.3, 88.5, 71.6, 38.9, 28.9, 20.2, 18.7.

4.1.96 *Isopropyl-4-((6-(4-isopropoxy-4-oxobutanamido)pyrimidin-4-yl)amino)benzoate* ***(Yfq92)*** Yield: 54.8 %; mp: 252-253 °C; ESI-MS [M+H]+: 414; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.95 (s, 1H, CONH), 10.29 (s, 1H, NH), 8.51 (s, 1H, H-2), 7.82-7.90 (m, 4H, PhH), 7.55 (s, 1H, H-5), 5.09-5.11 (m, 1H, OCH(CH3)2), 4.87-4.89 (m, 1H, OCH(CH3)2), 2.69-2.71 (t, 2H, *J* = 6.6 Hz, CH2CH2COO), 2.54-2.56 (m, 2H, *J* = 6.6 Hz, CH2CH2COO), 1.30-1.31 (t, 6H, *J* = 6.0 Hz, OCHCH3×2), 1.16-1.17 (t, 6H, *J* = 6.0 Hz, OCHCH3×2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.7, 170.4, 165.9, 160.3, 151.2, 145.8, 130.8, 120.5, 111.2, 88.7, 69.8, 67.3, 28.8, 21.3.

4.1.97 *Isopropyl-4-((6-((4-fluorophenyl)amino)pyrimidin-4-yl)amino)-4-oxobutanoate* ***(Yfq93)*** Yield: 63.5 %; mp: 238-240 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.52 (s, 1H, CONH), 9.55 (s, 1H, NH), 8.35 (s, 1H, H-2), 7.46 (s, 1H, H-5), 7.62-7.64 (d, 2H, *J* = 9.0 Hz, PhH), 7.13-7.14 (d, 2H, *J* = 9.0 Hz, PhH), 4.89-4.91 (m, 1H, CH), 2.65-2.65 (m, 2H, CH2CH2COO), 2.50-2.52 (m, 2H, CH2CH2COO), 1.15-1.16 (m, 6H, CH2CH3×2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.9, 171.7, 161.4, 157.6, 156.6, 136.5, 121.6, 121.5, 115.3, 115.2, 92.6, 87.2, 31.0, 28.7, 21.6. HRMS (ESI) *m/z*: $[M+H]^+$ calc = 347.1519; found 347.1513 for $C_{17}H_{19}FN_4O_3$.

4.1.98*Ethyl-2-((6-(4-isopropoxy-4-oxobutanamido)pyrimidin-4-yl)amino)-4,5,6,7-tetrahydrobenzo [b] thiophene-3-carboxylate* ***(Yfq94)*** Yield: 52.8 %; mp: 268-269 °C; ESI-MS [M+H]+: 460; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.89 (s, 1H, CONH), 10.78 (s, 1H, NH), 8.54 (s, 1H, H-2), 7.60 (s, 1H, H-5), 4.87-4.90 (m, 1H, OCH), 4.27-4.28 (q, 2H, *J* = 6.0 Hz, CH2CH3), 2.68-2.69 (m, 4H, cyclohexaneH), 2.55-2.59 (m, 2H, CH2CH2COO), 2.50-2.54 (m, 2H, CH2CH2COO), 1.71-1.73 (m, 4H, cyclohexaneH), 1.31-1.33 (t, 3H, *J* = 6.0 Hz, CH2CH3), 1.28-1.31 (m, 6H, CH3×2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.7, 173.1, 170.1, 160.2, 152.1, 151.8, 148.9, 138.4, 128.8, 112.4, 88.5, 69.5, 60.9, 28.9, 25.0, 23.4, 21.6, 14.1.

4.1.99 *Ethyl-3-((6-((4-fluorophenyl)amino)pyrimidin-4-yl)amino)-3-oxopropanoate* ***(Yfq95)*** Yield: 72.7 %; mp: 223-225 °C; ESI-MS [M+H]+: 318; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.56 (s, 1H, NH), 8.97 (s, 1H, H-2), 7.54 (d, 2H, *J* = 7.2 Hz, PhH), 7.49 (d, 2H, *J* = 7.2 Hz, PhH), 7.34 (s, 1H, H-5), 6.56 (s, 1H, NH), 4.26 (q, 2H, *J* = 6.0 Hz, CH2CH3), 3.71 (s, 2H, OCH2CH3), 1.42 (t, 3H, *J* = 6.0 Hz, OCH2CH3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.1, 171.1, 166.1, 162.1, 159.8, 153.9, 138.4, 122.8, 118.4, 89.5, 62.9, 38.8, 16.2.

4.2 Biological Activities

4.2.1 Cell Lines and Reagents A431 (epidermoid carcinoma, EGFR overexpression) and PC-9 (NSCLC, EGFR del 19) cells were grown in DMEM (GIBCO) containing 10% FBS (GIBCO). HCC827 (NSCLC, EGFR del E746_A750), H3255 (NSCLC, EGFR L858R), H1975 (NSCLC, EGFRL858R/T790M) and BEAS-2B (human bronchial epithelial) cells were grown in RPMI-1640 (GIBCO) containing 10% FBS (GIBCO). The cell lines were obtained from ATCC maintained at 37 ℃ in a 5% CO_2 incubator.

4.2.2 Kinase Inhibition Assay The procedure was as follows: Prepare 2.5 × enzyme solution; add kinase in 1 kinase base buffer. Prepare 2.5 × peptide solution, add FAM-labeled (GL Biochem, Cat. No. 112396, Lot. No. P100804-XZ112396) peptide and ATP (Sigma, Cat. No. A7699-1G, CAS No. 987-65-5) in a 1× kinase base buffer. The assay plate already contains 5μl of compound in 10% DMSO (Sigma, Cat. No. D2650, Lot. No. 474382). Transfer 2.5× enzyme solution to the assay plate, add 10 μl of 2.5× enzyme solution to each well of the 384-well (Corning, Cat. No. 3573, Lot. No. 12608008) assay plate. Incubate at room temperature for 10 min. Transfer 2.5× peptide solution to the assay plate. Add 10 μl of 2.5× peptide solution to each well of the 384-well assay plate. Incubate at 28℃ for specified period of time. Add 25 μL of buffer to quench reaction. The data was collected using Caliper. Convert conversion values to inhibition values, percent inhibition = (max-conversion) / (max-min) ×100, that “max” stands for DMSO control; “min” stands for low control. Fit the data in XLfit excel add-in version 4.3.1 to obtain IC_{50} values. The equation used was Y = Bottom + (Top-Bottom)/(1+(LogIC50/X)×HillSlope)). Including EGFR (Carna, Cat. No. 08-115, Lot. No 13CBS-0005L), FGFR1 (Carna, Cat. No. 08-133, Lot. No 09CBS-0989), KDR (Carna, Cat. No. 08-191, Lot. No 07CBS-0540), EGFR L858R (Invitrogen, Cat.No PR7447A, Lot. 853375A) and EGFR (d746-750) (Carna , Cat.No 08-527, Lot. 11CBS-1129C) were tested. The same method was used to perform the kinase selectivity test of 27 tyrosine kinases. AURA, EGFR, FGFR1, FGFR2, FGFR4, FLT3, HER2, HER4, IKKb, JAK1, JAK2, MET, MAP4K2, MAP4K4, PAK2, PDGFRb, RET, SRC, TYK2, and TRK-A came from Carna. BRAF, FLT1 (VEGFR1), FLT4 (VEGFR3), IGF1R and PDGFRa were purchased from Invitrogen. AKT1 was purchased from BPS. cKIT was purchased from Millipore. Throughout experiments for testing the relationship between compounds **Yfq07** or **Yfq071** and ATP, the concentration of the substrate was constant, while the concentration of ATP was set at 81, 27, 9, 3, 1, 0.333, 0.111 and 0.037 μM. The global competitive inhibition fit for the compounds was performed based on a %-conversion = (Vmax_X)/{km_[(1 + I/Ki)n] + X}, where X is the ATP concentration and n is the Hill coefficient.

4.2.3 MTT Assay PC-9, HCC827, H3255, A431, H1975 and BEAS-2B were cultured in 10% FBS respective growth medium in 96-well plates (3000 cells/well) overnight. Nine concentrations

(0.458 to 30,000 nM) were set for the compounds. The cells were treated with various concentrations of each compound and cultured in 10% FBS medium for 72 h in triplicate. The control cells were treated with dimethyl sulfoxide (DMSO) only. During the last 4 h of incubation, tetrazolium dye (MTT) solution (5 mg/mL, 20 μL/well) was added to each well. After discarding the supernatant completely, the generated formazan crystals were dissolved in 150 μL of DMSO and the absorbance was read spectrophotometrically at a test wavelength of 490 nm using an enzyme-linked immunosorbent assay plate reader. The data were calculated using Graph Pad Prism version 5.0. The IC_{50} values were fitted using a non-linear regression model with a sigmodial dose response.

4.2.4 Cell Apoptosis Assay A431, HCC827, H3255, H1975 (2×10^5) cells were seeded into 6-well plates overnight. Fresh growth media with **Yfq07** (100, 250, 500 and 1000 nM) as well as positive drug AZD3759 (1000 nM) were used and medium with 1‰ DMSO was used as control. After 48 h, the growth medium was collected and cells were trypsined and collected with the corresponding medium. After centrifugation at 1500 × g at 4 ºC for 5 min, the supernatant was removed completely and the cells were washed twice with pre-cold PBS. 100 μL 1 × binding buffer, 5 μL PI (PI, BD) and 5 μL annexin-V (FITC-Annexin V, BD) were added. The cells were then gently vortex-mixed and incubated for 15 min at 25 °C in the dark and 1 × binding buffer was used for dilution to 500 μL. The cells were then stained with PI, Annexin-V alone as positive control. The samples were measured with a BD Accuri™ C6 flow cytometer (Becton Dickinson) and the data were processed using FlowJo 7.6.1.

4.2.5 Cell Cycle Assay A431, HCC827, H3255, H1975 (2×10^5) cells were seeded into 6-well plates overnight. Fresh growth media with **Yfq07** (50, 100, 250 and 500 nM) as well as positive drug AZD3759 (500 nM) were used and medium with 1‰ DMSO was used as control. After 24 h, the growth medium was collected and the cells were trypsined and collected in the corresponding medium. After centrifugation at 1500 × g at 4 ºC for 5 min, the supernatant was removed completely and the cells were washed twice with pre-cold PBS. The cells were fixed in 70% cold ethanol, incubated at −20 °C overnight, and then stained with PI/RNase staining buffer (BD Pharmingen). The samples were measured with a BD Accuri™ C6 flow cytometer (Becton Dickinson) and the data were processed using FlowJo 7.6.1.

4.2.6 Western Blot Assay The cells were harvested, homogenized in protein lysate buffer, and debris was removed by centrifugation at 12,000 g for 10 min at 4 °C. After addition of sample loading buffer, the protein samples (50 μg) were electrophoresed and transferred to poly-vinylidene difluoride transfer membranes. Equivalent amounts of proteins were loaded into 8% SDS−PAGE and transferred by nitrocellulose membranes. Using ECL kit (Bio-Rad, Hercules, CA) to visualize the immunoreactive bands, the results were analyzed using ImageJ computer software

(National Institute of Health, MD). EGF receptor, phospho-EGF receptor (Tyr1068), AKT (pan), phospho-AKT (Ser473), p44/42 MAPK (Erk1/2), phospho-p44/42 MAPK (Erk1/2) (Thr202/ Tyr204) antibody (cell signaling technology) were used for immunoblotting. The GAPDH antibody used was obtained from Bioword Technology.

4.2.7 Xenograft Tumor Growth in Vivo Six-week-old BALB/C-nu mice were obtained from Shanghai SLAC Laboratory Animal Co., Ltd.. The animal license number was SCXK (Shanghai) 2012-0002. All mice were housed at the Wenzhou Medical University Laboratory Animal Research Center. The animals were housed under controlled conditions (22 °C) with a natural light-dark cycle. All experimental procedures were conducted according to the Institutional Animal Care guidelines and approved by the Administration Committee of Experimental Animals, Laboratory Animal Center of Wenzhou Medical University. Injections were performed subcutaneously into the right flank at a total volume of 0.1 mL/mouse with about 2×10^6/0.1ml PC-9 cells. As the PC-9 tumors reached a size of ~0.2−0.4 cm^3, the mice were randomly divided into four groups (5 mice/group) for subsequent studies. Compound **Yfq07** and AZD3759 were dissolved in DMSO:Span-80:ddH_2O = 2:1:50 solution. Three groups of mice treated with compound **Yfq07,** AZD3759 and vehicle every day lasting for 19 days. Tumor volumes were calculated as follows: tumor volume (mm^3) = [($W^2 \times L$)/2]; L stands for length and W refers to width.

4.2.8 Pharmacokinetics Study in Mice *in vivo* Four female and four male Sprague-Dawley rats (weight: 200-220 g) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd.. The animal license number was SCXK (Shanghai) 2012-0002. All rats were housed at the Wenzhou Medical University Laboratory Animal Research Center. All animals were housed under controlled conditions (22 °C) with a natural light-dark cycle. All experimental procedures were conducted according to the Institutional Animal Care guidelines and approved by the Administration Committee of Experimental, Animals, Laboratory Animal Center of Wenzhou Medical University. The rats were fasted for 12 h before the beginning of all drug treatments via oral administration (0.5% CMC-Na suspension at 30 mg/kg dose). At time points 0.083 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h, and 24 h after administration, blood samples were collected from each animal and separated by centrifugation (4000 r/min for 10 min). Then, the samples were analyzed by UPLC−MS/MS and the acquired data were analyzed using Masslynx 4.0.

4.2.9 Immunohistochemical Assays Xenotransplanted tumor tissues were sectioned (5 μm thickness), deparaffinized and hydrated. Heat-induced antigen retrieval was performed with 10 mM sodium citrate buffer. Before blocking in 5% bovine serum albumin (BSA), all sections were subjected to 3% H_2O_2. The mixture was stored overnight at 4 °C in a 1:200 dilution of cleaved caspase 3 antibody (cell signaling technology), phospho-EGF receptor (Tyr1068), antibody (Cell signaling Technology) and a 1:100 dilution of phosphor-AKT antibody. The negative-control

sections were incubated with Phosphate Buffered Saline (PBS) instead. Goat anti-rabbit secondary antibodies were choosed and visualized with 3,3′-diaminobenzidine tetrahydrochloride (DAB) solution (Sigma-Aldrich). The sections were then analyzed the next day under the Nikon fluorescence microscope (400× magnification; Nikon, Japan).

4.2.10 Docking Simulations and Binding Free Energy Calculations Molecular simulation docking was performed using the Ligand Fit module from the Discovery Studio 2.5 software package developed by Accelrys. Compound **Yfq07** was calculated in the three-dimensional $EGFR^{L858R}$ complex structure (PDB code: 4LQM) http://www.rcsb.org/pdb/explore/explore.do?structureId=4LQM. The docked complex was used and the MD simulation was finished with the Amber 11 package. The compound was then solvated by adding 1.8 nm of a TIP3P spherical water layer to the outer layer of the composite. At the beginning of the simulation, the energy of the 50,000 steps was optimized by the conjugate gradient method. Then, the 0.5-position MD simulation was performed and the MD simulation was performed for 15.0 ns. In the MD simulation process, the step length is set to 2.0 fs, and the truncation radius of the non-bond interaction was set to 1.2 nm. The SHAKE method was used to constrain the bond length. Finally, a 0.5-1.0 ns MD simulation of the trajectory was performed for data analysis. After obtaining the kinetic trajectory of the complex model, the free energy (ΔG_{bind}) was calculated as follows:

$$\Delta G_{bind} = \Delta G_{gas} - \Delta G_{solv}^{A} - \Delta G_{solv}^{B} + \Delta G_{solv}^{AB} \quad (1)$$

$$\Delta G_{gas} = \Delta H - T\Delta S_{gas} \approx (\Delta E_{vdw} + \Delta E_{\text{int } ra} + \Delta E_{ele}) - T\Delta S_{gas} \quad (2)$$

$$\Delta G_{solv} = \Delta\Delta G_{PB} + \Delta\Delta G_{SA} \quad (3)$$

The combination of free energy was used to analyze the contribution of Van-der-Waals interaction and hydrophobic interaction energies and the main interaction residues. Finally, the Pymol software was used for analysis.

4.2.11 Statistical Analysis In addition to the kinase assay, all experiments were assayed in triplicates (n = 3), and the data are presented as mean ± SEM. All statistical analyses were performed using the GraphPad Pro Prism 5.0 software package (GraphPad, San Diego, CA). The student's t-test and two-way ANOVA were employed to analyze the differences between treatment groups. A p-value of < 0.05 was considered statistically significant.

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- A novel series of 4, 6-disubstituted pyrimidines derivatives were designed and synthesized.
- Compound **Yfq07** showed the best activity in a MTT assay.
- Compound **Yfq07** was selected for cell cycle analysis and apoptosis assay to study the inhibitory activity against phosphorylated **EGFR, AKT and ERK1/2**.
- Compound **Yfq07** showed potential inhibitory activity in a **PC-9** xenograft model *in vivo*.