Bioorganic Chemistry 92 (2019) 103232

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Discovery and optimization of 5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one derivatives as mTORC1/mTORC2 dual inhibitors

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ARTICLE INFO

Keywords:
mTOR
mTORC1/mTORC2 dual-target
5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one
Synthesis
Biological study
Theoretical calculation

ABSTRACT

New potent mTORC1/mTORC2 dual inhibitors, 5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one derivatives, were obtained by optimizing functional groups on our previously reported PI3Kα inhibitor. All the target compounds were synthesized and structural optimization on the structure of the lead compound based on cytotoxic activity. The results showed that some of the target compounds exhibited moderate to high cytotoxic activity against cell line U87MG and PC-3. The activities against mTOR kinase were investigated and the compound **12q** showed excellent activity with an IC_{50} value of 54 nM in the same level of the positive control BEZ235 with IC_{50} value of 55 nM under the same test conditions. The western blot and cell cycle results demonstrate that compound **12q** is a candidate as an mTORC1/mTORC2 dual-target inhibitor. The theoretical calculations were also performed to better understanding the binding modes of the compound **12q** in the mTOR active site.

1. Introduction

The PI3K-Akt-mTOR signaling pathway, regulated by growth factors, is one of the most important intracellular network pathways which regulating various cell life activities including survival, metabolism, proliferation and growth [1–4]. In mammalian cells, mammalian target of rapamycin (mTOR) is composed of two complexes respectively, mTORC1 and mTORC2, which regulate different cellular processes with a variety of related proteins. As a regulatory-associated protein of mTOR (Raptor), mammalian lethal with SEC13 protein 8 (mLST8) and the non-core components PRAS40 and DEPTOR, mTORC1 regulates cell growth and proliferation by controlling protein synthesis and regulation of ribosomal biosynthesis [5,6]. As a rapamycin-insensitive companion of mTOR (RICTOR), mLST8 and mammalian stress-activated protein kinase interacting protein 1 (mSIN1), mTORC2 regulates Akt phosphorylation upstream of Akt [7]. Abnormal activation of the mTOR signaling pathway may directly or indirectly lead to the occurrence, development, and metastasis of tumors [8]. Over-activation mainly characterized by excessive activation of mTOR signaling pathway may cause loss of PTEN function and obstruction of autophagy pathway [9,10]. Therefore, inhibiting the activity of mTOR can inhibit the proliferation of tumor cells, induce apoptosis of tumor cells and reverse the resistance of tumor cells to cytotoxic drugs.

Rapamycin (Fig. 1), a natural macrolide compound, was originally used as an antifungal and immunosuppressive agent [11,12]. It was discovered to have potent immunosuppressive and antiproliferative properties as an anti-cancer agent due to its ability to inhibit mTOR. The complex of Rapamycin and Rapamycin analogs (Rapalogs) with FK506-binding protein-12 (FKBP12) binds to mTOR via FKBP12-Rapamycin binding (FRB) domain. It is near to the tyrosine kinase domain of mTORC1 and consequently inhibits the phosphorylation of mTOR substrates such as S6K1 and 4EBP1 [13–15]. The US Food and Drug Administration (FDA) has approved several Rapalogs inhibitors, such as Everolimus [16,17] and Temsirolimus [18,19] for cancer treatments (Fig. 1). However, only limited benefits from Rapalogs therapy have been observed in clinical due to incomplete inhibition of mTORC1 and the inability to effectively inhibit mTORC2 [20,21].

The small-molecule inhibitors of mTOR under development are divided into two categories: PI3K/mTOR dual-target inhibitors and mTORC1/mTORC2 dual-target inhibitors [20,22]. The mTORC1/mTORC2 dual-target inhibitors, also known as ATP-competitive mTOR inhibitors, are completely inhibiting the phosphorylation of the Rapamycin-insensitive sites Thr37 and Thr46 in the mTORC1 substrate 4E-BP1 and S6K1 [23]. Moreover, it is inhibiting phosphorylation of mTORC2 and its substrate Akt at Ser473 [22,24]. For their smaller molecular weight than that of the FKBP-12 Rapamycin complex, mTORC1/mTORC2 dual-target inhibitors were easier to target the mTOR binding site and result in stronger inhibition of protein synthesis,

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https://doi.org/10.1016/j.bioorg.2019.103232
Received 18 March 2019; Received in revised form 26 August 2019; Accepted 28 August 2019
Available online 04 September 2019
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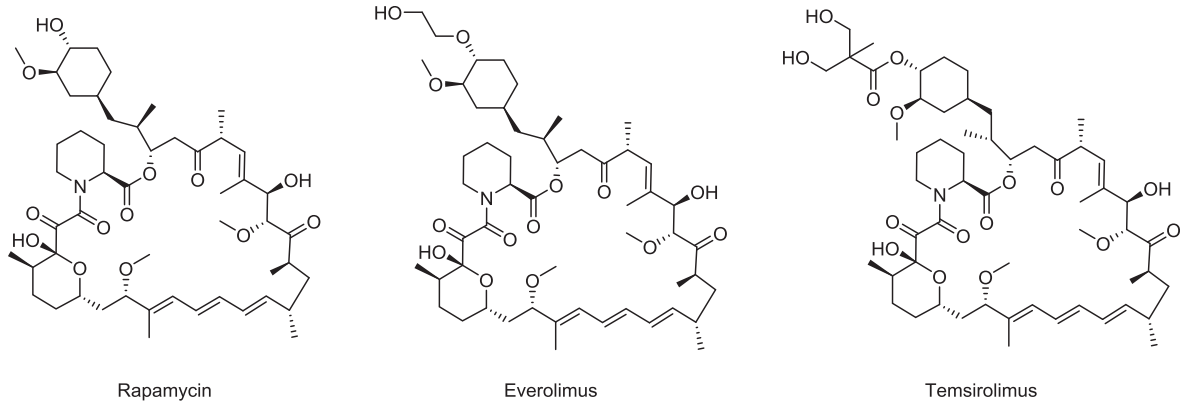


Fig. 1. Chemical structures of Rapamycin and Rapamycin analogs.

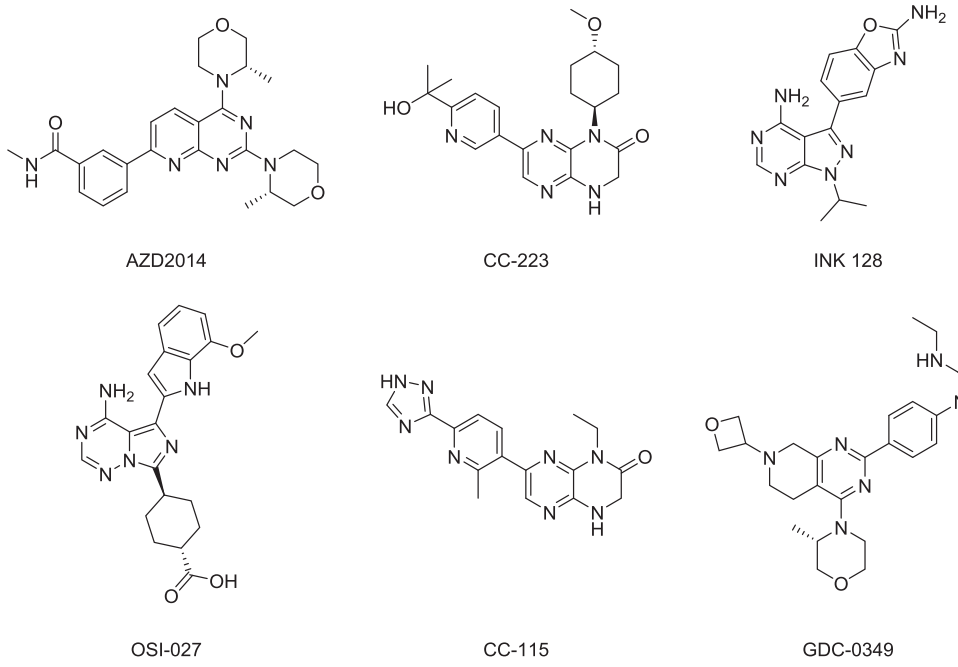


Fig. 2. Chemical structures of mTORC1/mTORC2 dual-target inhibitors.

cell growth and autophagy-inducing effect [25]. In addition, they have strong selectivity for mTOR and little or no inhibition of the PITOR kinase family with high mTOR homology (Fig. 2) [20,24].

In our previous work, the lead compound of 4-aryl-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one derivatives were designed as a PI3Kα inhibitor by molecular hybridization and scaffold hopping [26]. It showed a strong PI3Kα inhibitory activity with IC_{50} of 113 nM and mTOR inhibitory activity with IC_{50} of 1094 nM. Here, based on cytotoxic activities, the synthesis and structural optimization design on the three functional groups of the lead compound centered on pyrrolo[2,3-*d*]pyrimidin-6-one were investigated (Fig. 3). All the target compounds were evaluated in vitro for their anti-mTORC1/mTORC2 activity against cell lines U87MG and PC-3. The enzyme inhibitory activity against mTOR kinase of compounds was evaluated inferior to the positive control of BEZ235. The intracellular mTOR pathway inhibitory activities of the promising compound was evaluated by western blot and cell cycle. Their preliminary structure-activity relationships (SARs) and theoretical calculations were described to better understanding the possible binding modes in the mTOR active site.

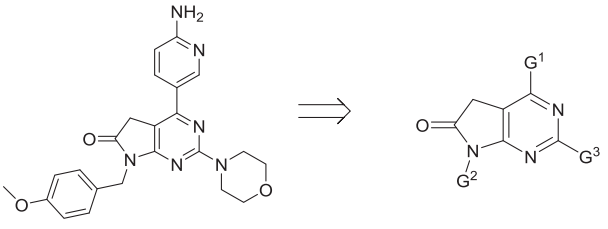
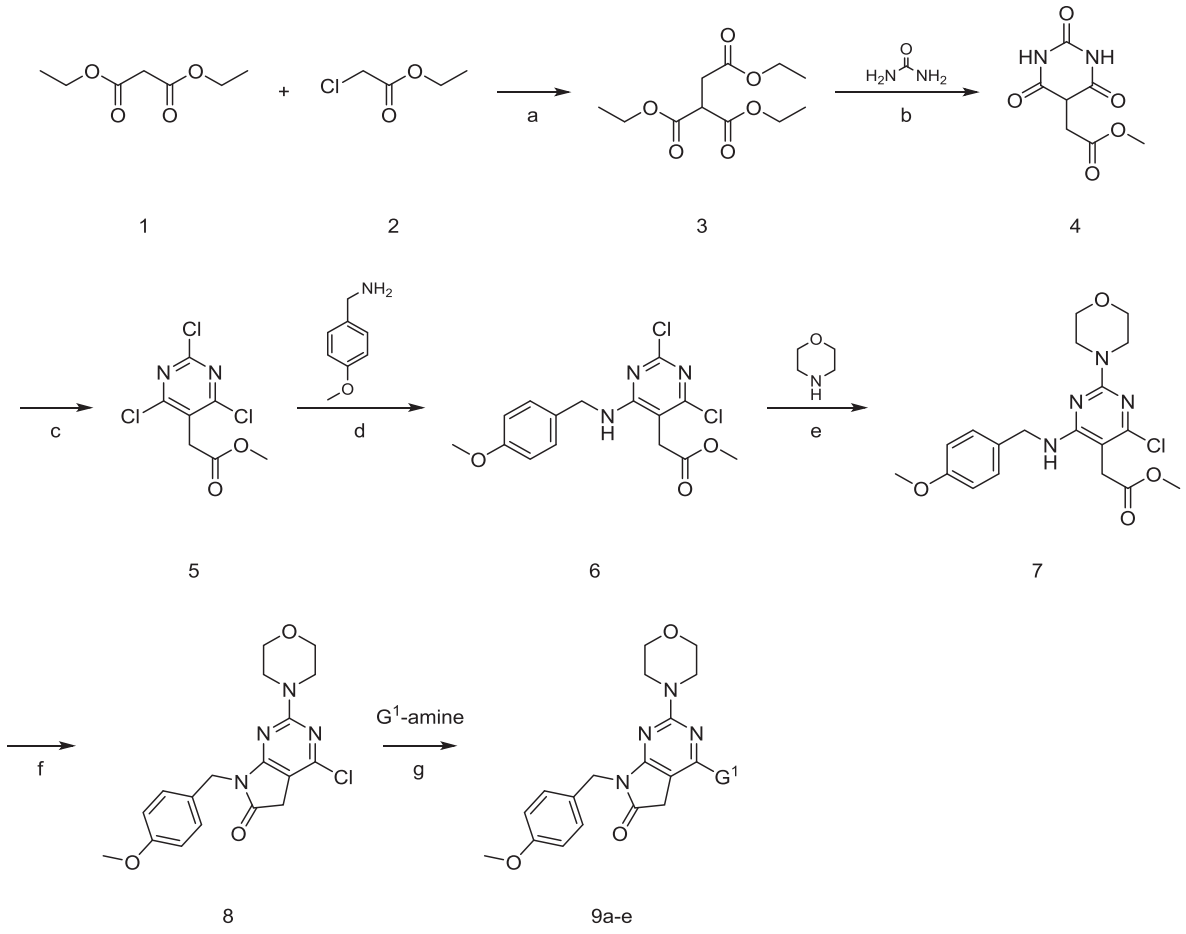


Fig. 3. Optimization design of 5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one derivatives.

2. Result and discussion

2.1. Synthesis and structural optimization based on cytotoxic activity

The initial optimization design was the dearylation of the C-4 position substituents of the lead compound. The target compounds **9a-e**



Scheme 1. Reagents and conditions: (a) EtONa, EtOH; (b) MeONa, MeOH; (c) DIPEA, $POCl_3$; (d) DIPEA, DMF; (e) 4-DMAP, DMF; (f) TsOH, Toluene; (g) DIPEA, DMA.

were synthesized with the diethyl malonate **(1)** and ethyl chloroacetate **(2)** as starting materials (Scheme 1). The intermediate **3** was prepared by the nucleophilic substitution of **1** and **2** under basic conditions. The intermediate **3** was reacted with urea in a solution of sodium methoxide to give the corresponding barbituric acid **4**. Intermediate **5** was obtained by the chlorinated of **4** with phosphorus oxychloride. The intermediate **6** was obtained by the substitution of **5** with 4-methoxybenzylamine. The intermediate **7** was obtained by the non-nucleophilic substitution reactions of **6** with morpholine. The intermediate **7** was intraamidation under acidic conditions to form the corresponding product **8**. Intermediate **8** reacts with the corresponding secondary amine under the basic condition to give the target compounds **9a-e** respectively.

Cytotoxic activities of **9a-e** were carried out as described previously in vitro detected on mTOR-driven cancer cell lines human malignant glioblastoma U87MG and human prostate cancer PC-3 by CCK-8 [27,28]. The multi-targeted pyridinylfuranopyrimidine inhibitor PI-103 served as the reference compound. The inhibition rates of compounds (50 μM) and positive control (50 μM) were presented in Table 1, which calculated by Formula (1) calculate for inhibition rates.

$$\text{Inhibition rates} = \frac{OD^{Negative} - OD^{Treatment}}{OD^{Negative}} \times 100\% \quad (1)$$

As can be seen from Table 1, the compound **9a-e** shown no significant cytotoxicity, all the inhibition rates were far less than that of the positive control. These results led us to abandon the idea of dearylation of the C-4 position substituents and reserve original 6-aminopyridin-3-yl at the C-4 position of lead compound.

The further optimization design was derivatization on the N-7 position of the lead compound by the various substituted benzyl or aliphatic groups. The target compounds **12a-p** were synthesized with the methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** as starting materials (Scheme 2). The **10a-p** were synthesized by a one-pot synthesis strategy in two substitutions of the **5** by the G^2-amine and morpholine under alkaline conditions. The **11a-p** were obtained by the intraamidation of **10a-p** described above. Moreover, the **11a-p** reacted with the (6-aminopyridin-3-yl) boronic acid to give the target compounds **12a-p** under the palladium catalysis.

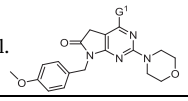
The results of cytotoxic activities shown that the **12a-p** activities were improved but not obvious. The activities of **12k**, **12l** and **12m** were close to that of the lead compound with cyclohexyl, cyclopropyl and isopropyl groups as G^2 (Table 2). That means the derivatization on the N-7 position does not improved significantly the cytotoxic activity of target compounds.

Since the optimization design on the dearylation of the C-4 position and the derivatization at the N-7 position does not improved significantly the cytotoxic activity, the 6-aminopyridin-3-yl substitute at C-4 position may be one of the best choices. Therefore, the **12q-y** were designed as target compounds while the G^2 was cyclohexyl, cyclopropyl, and isopropyl, the G^1 was a pyrimidinylamine or arylamine. The **12q-y** were synthesized by the substitution of **11k-m** with the aromatic amine, the **13q** was the acetylation of **12q** (Scheme 3). The cytotoxic activities of **12q-y** and **13q** shown that the compound **12q** displayed the highest inhibition with the inhibition rate of 71.60% on U87MG and 61.98% on PC-3 (Table 3). The compound **12q** and **13q** have higher inhibition than the PI-103 positive control on both cancer cell lines with the inhibition rate of 62.34% and 53.36%. These two compounds achieve the same level of activity as the lead compound with an inhibition rate of 70.84% and 67.03%.

The finally optimization design was the analogues of morpholine on

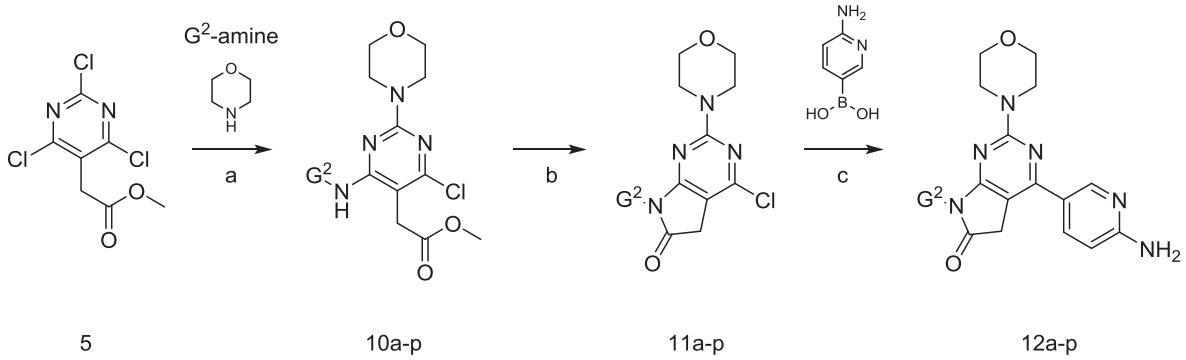
Table 1

Cytotoxic activities of compound **9a-e** on U87MG and PC-3 cancer cell.



Entry	Comp. No.	G^1	Log K_{ow} [a]	U87MG		PC-3	
				OD[b]	Inhibition Rate (%)	OD[b]	Inhibition Rate (%)
1	**9a**		2.33	1.356 ± 0.034	2.80	1.261 ± 0.030	2.52
2	**9b**		4.08	1.303 ± 0.019	6.86	1.267 ± 0.023	2.79
3	**9c**		3.18	1.384 ± 0.024	2.74	1.237 ± 0.016	3.96
4	**9d**		2.30	1.360 ± 0.028	2.79	1.267 ± 0.019	2.79
5	**9e**		2.54	1.350 ± 0.011	2.06	1.062 ± 0.055	16.62
Lead compound			2.84	0.407 ± 0.037	70.84	0.427 ± 0.025	67.03
Positive control	**PI-103**		1.88	0.530 ± 0.037	62.34	0.603 ± 0.024	53.36

[a] The value predicted by KOWWIN.
[b] The mean value of five times measurements.



Scheme 2. Reagents and conditions: (a) I DIPEA, DMF, II 4-DMAP, DMF; (b) TsOH, Toluene; (c) $(dppf)_2PdCl_2$, Na_2CO_3, Dioxane/H_2O.

the C-2 position substituents of the lead compound. The **16a-f** were designed as target compounds while the G^1 was 3-aminophenyl and G^2 was 4-methoxybenzyl. The **17a-f** was the acetylated products of **16a-f**. The **16a-f** and **17a-f** were synthesized by the method previously described (Scheme 4). The cytotoxic activities of **16a-f** and **17a-f** show that compound **16d** displayed the highest inhibition with the inhibition rate of 65.40% of U87MG and 68.07% of PC-3 (Table 4). The **17d** also displayed strong inhibition with the inhibition rate of 65.21% of U87MG and 14.66% of PC-3. The piperidinyl and 4-methylpiperidinyl on C-2 position may have the same effect as morpholinyl and be key powerful pharmacophore.

Through the structural optimization design on the C-4, N-7 and C-2 position of the lead compound, the compounds (**12q**, **13q**, **16d** and **17d**) were found with the similar inhibition with the lead compound against U87MG and PC-3. The dearylation of the C-4 position of lead compound was lowering the inhibition rates. The derivatization on the N-7 position by the substituted benzyl or aliphatic groups does not improved the cytotoxic activity significantly. The 6-aminopyridin-3-yl substitute at C-4 position may be one of the best choices. The piperidinyl and 4-methyl-piperidinyl on the C-2 position have the same effect as morpholinyl. They share the features that the C-2 position was the six-membered heterocycly, the C-4 position was the 3-aminophenyl or corresponding acetylation product, and the N-7 position was the cyclohexyl or 4-methoxybenzyl substitutes.

2.2. Biological study

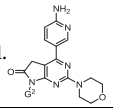
For the compounds **12q**, **13q**, **16d** and **17d** with higher inhibition, the further investigations were carried out to determine their IC_{50} values on cell line U87MG and cell line PC-3 by CCK-8. As can be seen from Table 5, compound **12q**, **13q**, **16d** and **17d** exhibit stronger activity than the lead compound. The IC_{50} value against the PC-3 cell line was between 10 and 20 μM and the IC_{50} value against the U87MG cell line was between 13 and 38 μM. The compound **12q** has significantly inhibited the proliferation of mTOR-driven cancer cell lines with an IC_{50} value of 12.71 μM against the cell line U87MG and 10.31 μM against the cell line PC-3. It was at the same level as the positive control PI-103 with an IC_{50} value of 8.49 μM of U87MG and 7.55 μM of PC-3 under the same test conditions.

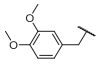
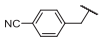
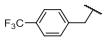
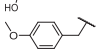
The enzyme inhibitory activities were carried out as described previously [28]. As shown in Table 5, the compound **12q** has higher mTOR inhibitory activity with IC_{50} of 54 nM than the lead compound with IC_{50} of 1094 nM. It is also at the same level to the positive control BEZ235with IC_{50} of 55 nM (reported IC_{50} of 9 nM) under the same test conditions. All these indicated that structural optimization was effective based on cytotoxic activity.

In order to investigate the intracellular mTOR pathway inhibitory activities of compound **12q,** the western blot was carried on [29]. Cellular efficacy of mTORC1/mTORC2 inhibition by inhibitor compounds was measured by changes in phosphorylation of P-S6K1 and p-

Table 2

Cytotoxic activities of compound **12a-p** on U87MG and PC-3 cancer cell.

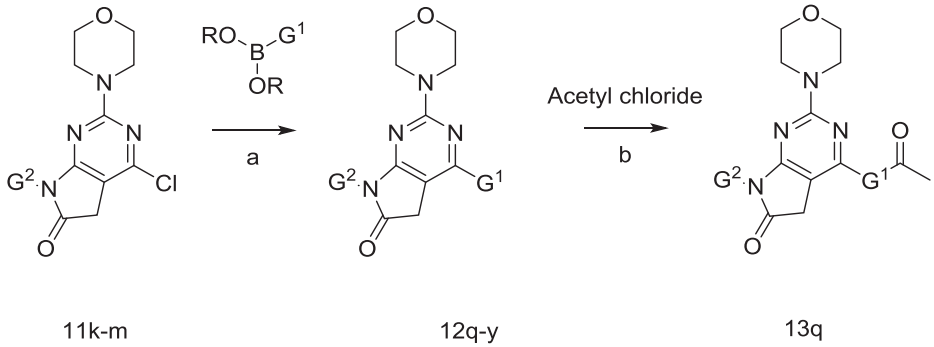


Entry	Comp. No	G^2	Log Kow[a]	U87MG		PC-3	
				OD[b]	Inhibition Rate (%)	OD[b]	Inhibition Rate (%)
6	**12a**		2.76	1.288 ± 0.012	8.26	0.980 ± 0.015	22.22
7	**12b**		3.16	1.317 ± 0.034	6.18	0.991 ± 0.033	21.35
8	**12c**		3.18	0.997 ± 0.039	27.70	0.780 ± 0.018	40.64
9	**12d**		2.40	1.186 ± 0.039	14.00	1.208 ± 0.018	8.07
10	**12e**		2.96	1.252 ± 0.054	9.21	1.194 ± 0.056	9.13
11	**12f**		2.30	1.243 ± 0.043	9.88	1.235 ± 0.066	6.01
12	**12g**		3.40	1.244 ± 0.052	9.82	1.184 ± 0.102	9.86
13	**12h**		3.72	1.309 ± 0.031	5.08	1.239 ± 0.053	5.72
14	**12i**		3.30	1.304 ± 0.034	5.42	1.227 ± 0.013	6.62
15	**12j**		2.96	1.315 ± 0.014	4.61	1.081 ± 0.046	17.70
16	**12k**		3.32	1.316 ± 0.022	6.28	0.790 ± 0.042	37.30
17	**12l**		1.85	0.906 ± 0.052	35.44	0.658 ± 0.049	47.78
18	**12m**		1.96	0.924 ± 0.044	34.22	0.684 ± 0.014	45.68
19	**12n**		1.01	0.993 ± 0.019	28.01	0.991 ± 0.020	24.57
20	**12o**		1.01	1.292 ± 0.026	6.31	0.777 ± 0.014	40.87
21	**12p**		0.08	0.986 ± 0.015	29.76	0.700 ± 0.025	44.43
Lead compound			2.84	0.407 ± 0.037	70.84	0.427 ± 0.025	67.03
Positive control	**PI-103**		1.88	0.530 ± 0.037	62.34	0.603 ± 0.024	53.36

[a] The value predicted by KOWWIN.
[b] The mean value of five times measurements.

Akt, respectively. U87MG cells were treated with **12q** at the indicated concentrations and subjected to western blot analysis. It was observed that compound **12q** almost completely inhibits the phosphorylation of S6K1 at 10 μM in U87MG cell lines, demonstrating the potent efficacy of **12q** in inhibiting the enzymatic activity of mTORC1 (Fig. 4). The dose-dependent decrease of phosphorylation of Akt at Ser 473 indicates that compound **12q** has a certain inhibition of mTORC2, which correlates well with the cells and kinase inhibition data.

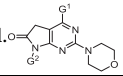
The cell cycle is the significant regulatory mechanisms of cell growth, development, and differentiation. The effect of compound **12q** treatment on breast cancer cell lines MCF-7 was investigated by flow cytometry (Fig. 5) [30]. Cells were treated with compound **12q** with varying concentrations and vehicle (DMSO) for 36 h. In the vehicle, the percentage in the cells at G1-phase is 71.9%. Compared to the vehicle, the treatment of compound **12q** led to an increase of 2.5% for 1 μM, 4.6% for 3 μM and 23.8% for 10 μM in the cells at G1-phase. The concomitant concentration of compound **12q** increase, a loss of S-phase accompanies with an increase in the percentage of cells in G1-phase demonstrated that compound **12q** blocked the tumor cells in G1-phase progression which resulted in decreased S-phase populations. This is consistent with the reported effect of Rapamycin [31].



Scheme 3. Reagents and conditions: (a) $(dppf)_2PdCl_2$, Na_2CO_3, Dioxane/H_2O; (b), DIPEA, DCM.

Table 3

Cytotoxic activities of compound **12q-y** and **13q** on U87MG and PC-3 cancer cell.



Entry	Comp. No	G^2	G^1	Log Kow[a]	U87MG		PC-3	
					OD[b]	Inhibition rate	OD[b]	Inhibition rate
22	**12q**			3.87	0.391 ± 0.039	71.60	0.484 ± 0.013	61.98
23	**12r**			3.87	1.189 ± 0.015	13.73	0.788 ± 0.027	38.15
24	**12s**			2.67	0.881 ± 0.017	36.10	0.794 ± 0.031	37.68
16	**12k**			3.32	1.316 ± 0.022	6.28	0.790 ± 0.042	37.30
25	**13q**			3.89	0.388 ± 0.011	71.81	0.475 ± 0.022	62.72
26	**12t**			2.39	1.328 ± 0.031	5.40	1.211 ± 0.043	3.86
27	**12u**			2.39	1.314 ± 0.041	6.44	0.919 ± 0.025	27.06
28	**12v**			1.20	0.919 ± 0.022	33.28	0.675 ± 0.053	47.03
17	**12l**			1.85	0.906 ± 0.052	35.44	0.658 ± 0.049	47.78
29	**12w**			2.50	1.022 ± 0.026	27.44	0.602 ± 0.025	54.53
30	**12x**			2.50	0.995 ± 0.034	29.38	0.872 ± 0.056	34.12
31	**12y**			1.31	1.015 ± 0.010	27.95	0.691 ± 0.038	47.78
18	**12m**			1.96	0.924 ± 0.044	34.22	0.684 ± 0.014	45.68
Lead compound				2.84	0.407 ± 0.037	70.84	0.427 ± 0.025	67.03
Positive control	**PI-103**			1.88	0.530 ± 0.037	62.34	0.603 ± 0.024	53.36

[a] The value predicted by KOWWIN.
[b] The mean value of five times measurements.

2.3. Theoretical calculation

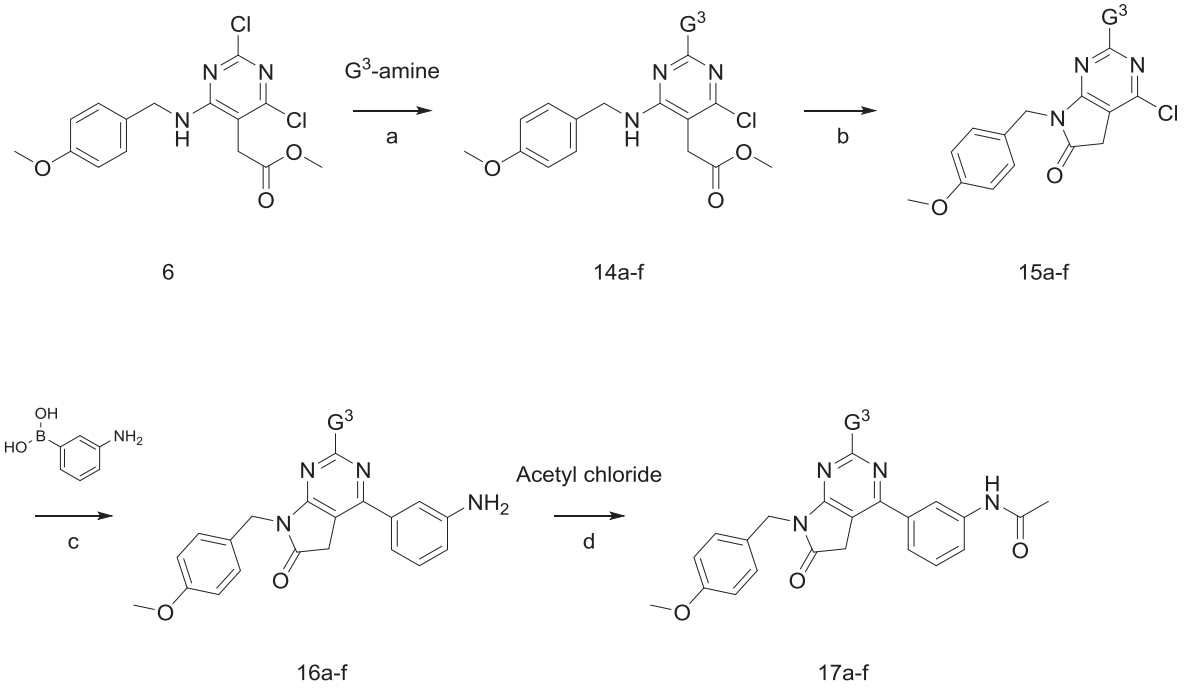
For the study, the physicochemical properties and better understanding the possible binding modes of the target compounds in the mTOR active site, the theoretical calculation were also performed. The Log Kow values of the target compounds were calculated using KOWWIN, a part of the EPI suite environmental modeling program, which was developed and was maintained by the US Environmental Protection Agency (EPA) and the Syracuse Research Corporation (SRC) [32]. The molecular docking analysis was carried out using the AutoDock software package as implemented through the graphical user interface AutoDockTools (ADT 1.5.2). The protein crystal structure of mTOR (PDB 4jt6) was chosen as a receptor protein [4].

The range of predicted Log K_{ow} value of the target compounds was between 0.08 and 5.16 with a molecular weight between 351 and 489 g/mol (shown in Tables 1–5). The number of hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen-hydrogen bonds) or hydrogen bond acceptors (all nitrogen or oxygen atoms) are also satisfied Lipinski's rule of five which is a rule of thumb to evaluate drug-likeness [33]. These results indicate that the synthesized target compound has good drug-like properties.

Molecular polar surface area (PSA) is a concept that describes the passive transport of molecules through membranes and predicts intestinal absorption, Caco-2 monolayer permeability, and blood-brain barrier penetration. A fast calculation of the PSA called the topological PSA (TPSA) assumes that the contribution of each of the same atom or group type in a molecule to a polar surface area is similar, and the TPSA of the entire molecule is the sum of the contributions of all atoms or groups. Molecules with a TPSA greater than 140 $Å^2$ are often difficult to penetrate the cell membrane. Oral absorption of the drug TPSA is no higher than 120 $Å^2$. For drugs acting on the central nervous system, the best TPSA for the molecule to penetrate the blood-brain barrier is between 60 and 70 $Å^2$. The maximum cannot exceed 90 $Å^2$.

The TPSA values of the synthesized target compounds were predicted using the SwissADME online database to evaluate their bioavailability properties. The specific results are shown in Table 5, The TPSA of the compounds were less than 100 $Å^2$, indicating that the compound is well absorbed orally.

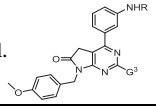
To understand the binding modes of the target compounds in the mTOR active site, the PI-103 was firstly re-dock to the active site of mTOR to verify the reliability of the docking method and parameters (Fig. 6). The stacking map showed that the dominant conformation of re-docked PI-103 and the original ligand conformation almost completely coincide. The morpholine ring of re-dock PI-103 (blue) binds to the adenine pocket and makes hydrogen bond to the hinge, whereas the phenol group binds to the inner pocket and makes two hydrogen bonds to the Tyr 2225 and Asp 2195 side chains at the back of the cleft. These hydrogen bonds are likely to be important for the high affinity of PI-103 for mTOR. The same interaction also occurs in the original ligand indicating that the docking method is reliable and effective.



Scheme 4. Reagents and conditions: (a) 4-DMAP, DMF; (b) TsOH, Toluene; (c) $(dppf)_2PdCl_2$, Na_2CO_3, Dioxane/H_2O; (d) DIPEA, DCM.

Table 4

Cytotoxic activities of compound **16a-f** and **17a-f** on U87MG and PC-3 cancer cell.

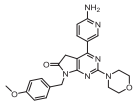
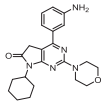
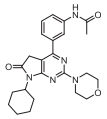
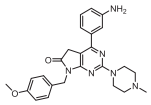
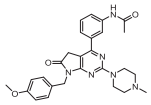
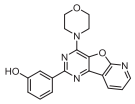
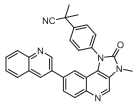


Entry	Comp. No	R	G^3	Log Kow[a]	U87MG		PC-3	
					OD[b]	Inhibition rate	OD[b]	Inhibition rate
32	16a	H		4.64	1.386 ± 0.023	2.63	1.253 ± 0.023	2.69
33	**17a**	Ac		4.67	1.292 ± 0.035	9.21	1.012 ± 0.030	21.44
34	**16b**	H		4.24	1.354 ± 0.030	2.98	1.214 ± 0.028	5.58
35	**17b**	Ac		4.26	1.366 ± 0.023	2.12	1.138 ± 0.067	11.48
36	**16c**	H		5.13	1.366 ± 0.009	2.34	1.269 ± 0.021	2.59
37	**17c**	Ac		5.16	1.394 ± 0.034	2.01	0.502 ± 0.030	61.01
38	**16d**	H		3.36	0.492 ± 0.032	65.40	0.411 ± 0.030	68.07
39	**17d**	Ac		3.38	0.495 ± 0.018	65.21	1.099 ± 0.034	14.66
40	**16e**	H		3.59	1.383 ± 0.014	2.83	0.98 ± 0.019	23.93
41	**17e**	Ac		3.62	1.388 ± 0.036	2.46	1.204 ± 0.040	6.49
42	**16f**	H		3.59	1.381 ± 0.042	2.95	1.255 ± 0.025	2.53
43	**17f**	Ac		3.62	1.375 ± 0.003	3.36	1.254 ± 0.021	2.61
Leadcompound				2.84	0.407 ± 0.037	70.84	0.427 ± 0.025	67.03
Positivecontrol	PI-103			1.88	0.530 ± 0.037	62.34	0.603 ± 0.024	53.36

[a] The value predicted by KOWWIN.
[b] The mean value of five times measurements.

The same method was used to dock compound **12q** to the active site of mTOR (Fig. 7). The results showed that the morpholine ring of compound **12q** binds to the adenine pocket and makes hydrogen bond to Val 2240. The aniline group binds to the inner pocket. Although, the amino of aniline did not form a hydrogen bond with Tyr 2225 and Asp 2195, hydrogen bonding with Glu 2190 compensated for this loss. There are some interactions, such as pi-sigma at Ile 2356, Ile 2237, alkyl or pi-alkyl at Ieu 2185, Trp 2239 and Van der Waals at Asp 2357, Lys 2187, Met 2345, Pro 2169, Leu 2354, Gly 2238, also found in the original ligand. The cyclohexyl group at the N-7 position is at the edge of the active pocket, so it does not contribute much to the activity. When these two hydrogen bond positions are fixed, most of their

Table 5
The TPSAs and IC_{50} of compounds against U87MG, PC-3, PI3Kα and mTOR.

Comp. No.	Chemical Structure	TPSA(Å^2)[a]	cell IC_{50} (μM)[b]		enzyme IC_{50} (nM)[c]	
			U87MG	PC-3	PI3Kα	mTOR
Lead compound		106.70	28.39	50.42	113	1094
12q		84.58	12.71	10.31	1057	54
13q		87.66	15.20	20.43	NT	NT
16d		87.82	20.74	10.95	63,000	ND
17d		90.90	37.71	10.35	NT	NT
PI-103		NT	8.49	7.55	NT	NT
BEZ235		NT	NT	NT	45	55

NT: not tested.
ND: not detected.
[a] TPSA was calculated by SwissADME.
[b] Ten concentrations calculated results, the mean value of five times measurements.
[c] Ten concentrations calculated results, the mean value of twice measurements.

conformations overlap with the original ligand. Compared to the docking results of the PI-103, the higher inhibition on mTOR of **12q** was due to the effective combination model to the active site of mTOR. In addition, it is consistent with the test data of enzyme inhibitory activity (**12q** with IC_{50} of 54 nM and PI-103with NT).

In the molecular docking study, it was found that compared to **12q**, the C-2 aliphatic ring of **16d** (N-methylpiperazinyl) did not penetrate the adenine pocket to form a hydrogen bond with Val 2240, which is the key to the inhibition of mTOR (Fig. 8). This may be the cause of **16d** inactivity in the mTOR kinase activity test. The cytotoxicity of compound **16d** on cell lines U87MG and PC-3 may act on other signaling pathways or mechanisms, pending further study.

3. Conclusion

Based on a novel PI3Kα inhibitor as a lead compound, 5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one derivatives were synthesized and structural optimization on the structures of lead compound based on cytotoxic activity. All the synthesized target compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS. Through the structural optimization design on the C-4, N-7 and C-2 position of lead compound, the compounds (**12q, 13q**, **16d** and **17d**) were found with the similar inhibition to the lead compound on U87MG and PC-3. They share the features that the C-2 position was replaced by six-membered heterocycly, the C-4 position was substituted by 3-aminophenyl or corresponding acetylation product, and the N-7 position was substituted by cyclohexyl or 4-methoxybenzyl substitutes. It is noteworthy that the IC_{50} values of the typical compounds (**12q**, **13q**, **16d** and **17d**) against cancer cell lines were obtained in the range from 10 to 50 μM and at the same level as the positive control of PI-103 with IC_{50} of 8.49 μM. Moreover, the IC_{50} of the compound **12q** inhibited mTOR at 54 nM and the lead compound at 1094 nM, which equal to the positive control of BEZ235 with IC_{50} of 55 nM. By the western blot analysis, the compound **12q** was observed that it almost completely inhibit the mTORC1 at 10 μM in U87MG cell lines, and has a certain inhibition of mTORC2 by the dose-dependent phosphorylation of Akt at Ser 473. The results of compound **12q** treatment on breast cancer cell lines MCF-7 show that it arrests the cells in G1-phase of the cell cycle as well. The binding modes of the compound **12q** in the mTOR active site indicate that the higher inhibition on mTOR of **12q** was due to the similarity effective combination model to the active site of mTOR with that of the PI-103. These results show that the compound **12q** is a hopeful candidate as an mTORC1/mTORC2 dual-target inhibitor and provides valuable information for the further investigation of mTOR inhibitors.

4. Experimental section

Unless noted otherwise, all reagents were commercially available and used without further purification. All air and moisture sensitive reactions were carried out under an inert atmosphere of dry nitrogen. Purification of the crude products was performed using flash column chromatography on silica gel (300–400 mesh). Reactions were monitored by TLC carried out on 0.25 mm SDS silica gel coated glass plates (60F254) and compounds were detected with UV light and/or with

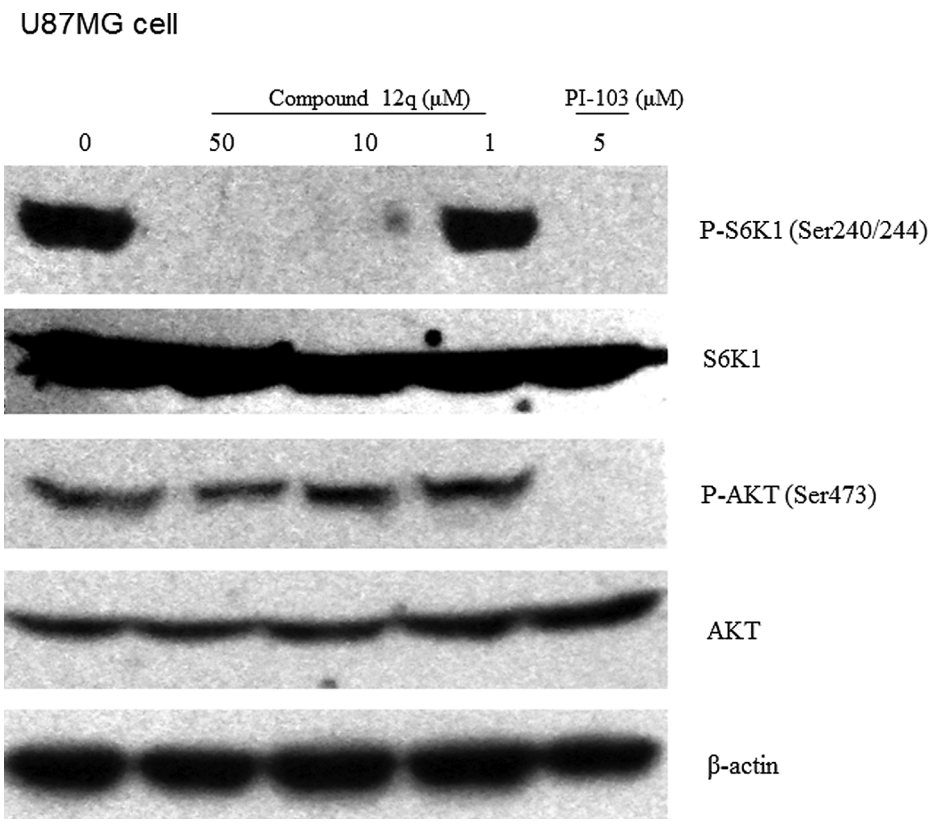


Fig. 4. Effects of compound **12q** on p-Akt, Akt, p-S6K1 and S6K1.

iodide. The melting point is determined by X-5 precision micro melting point tester produced by Beijing Fukai Instrument Co., Ltd. NMR spectra were recorded on Bruker DRX 400 instrument. The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintuplet, m = multiplet, br = broad. High-resolution mass spectra (HRMS) were performed on Thermo Scientific™ Exactive™ Plus Orbitrap system. Tumor cells were cultured in MCO-15AC carbon dioxide incubator produced by Japan Sanyo Co., Ltd. The Thermo MK3 microplate reader produced by American Thermoelectric Corporation measured the OD. Abbreviations of solvents are used as follows: DCC, carbodicyclohexylimide; 4-DMAP, 4-(dimethylamino)-pyridine; ACN, acetonitrile; DCM, dichloromethane; EA, ethyl acetate; PE, petroleum ether; TFA, trifluoroacetic acid; MeOH, methanol; DMSO-d_6, dimethyl sulfoxide-d6; $CDCl_3$, deuterated chloroform; TEA, triethylamine; THF, tetrahydrofuran.

4.1. General experimental procedure

4.1.1. Chemistry

Triethyl ethane-1,1,2-tricarboxylate (3): Diethyl malonate **(1)** (80 g, 0.5 mol) was added dropwise to a solution of sodium ethoxide (34 g, 0.5 mol) in EtOH (600 mL) at 0 °C and stirred for 30 min. Ethyl chloroacetate **(2)** (62 g, 0.5 mol) was added dropwise; the mixture was heated to reflux for 6 h and then cooled to room temperature. All volatiles were removed by rotary evaporation, the residue was partitioned between EtOAc (600 mL) and water (300 mL), and the layers were separated. The organic layer was washed with brine, dried over Na_2SO_4, filtered and evaporating solvent under reduced pressure to afford the title compound **3** (119 g, 97% crude yield) as colorless oil which used for next step without purification.

Methyl 2-(2,4,6-trioxohexahydropyrimidin-5-yl)acetate (4): Triethyl ethane-1,1,2-tricarboxylate **(3)** (123 g, 0.5 mol) and urea (30 g, 0.5 mol) was added to a solution of sodium ethoxide (51 g, 0.75 mol) in MeOH (500 mL), and the mixture was heated to reflux for 10 h, then cooled to room temperature. After filter, the filter cake was dissolved in water (300 mL). Large amount of solid production was generated when the pH adjusted to 1 with 2N hydrochloric acid. After filter, the filter cake was dried in vacuum drying oven to afford the title compound **4** (30 g, 30% yield) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 2H), 4.00 (t, J = 4.2 Hz, 1H), 3.59 (s, 3H), 3.00 (d, J = 4.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.78, 169.57, 150.81, 52.01, 44.60, 30.32.

Methyl 2-(2,4,6-trichloropyrimidin-5-yl)acetate (5): DIPEA (10.1 mL, 0.1 mol) was added dropwise to a mixture of methyl 2-(2,4,6-trioxohexahydropyrimidin-5-yl)acetate **(4)** (10 g, 0.05 mol) in phosphorous(V) oxychloride (50 mL), and the mixture was heated to reflux for 4 h, then cooled to room temperature. All volatiles were removed by rotary evaporation; the residue was carefully poured into ice water (200 g). Large amount of dark solid production was collected by filtering; the filter cake was dissolved in EtOAc (300 mL) and dried over Na_2SO_4. Filtered and evaporating solvent under reduced pressure to afford the title compound **5** (7.3 g, 57% crude yield) as brown solid which used for next step without purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.02 (s, 2H), 3.69 (d, J = 1.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.03, 162.82, 156.50, 126.06, 52.59, 34.92.

Methyl 2-(2,4-dichloro-6-(4-methoxybenzylamino)pyrimidin-5-yl)acetate (6): DIPEA (0.5 mL) and 4-methoxybenzylamine (137 mg, 1 mmol) was added to a solution of methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (255 mg, 1 mmol) in DMF (5 mL). The resulting mixture was stirred at room temperature for 1 h, quenched by water (50 mL) and then extracted with EtOAc (2 × 50 mL). The organic phases were combined, washed by brine, dried over Na_2SO_4, evaporated and purified by chromatography on silica gel to afford the title compound **6** (295 mg, 83% yield) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56–8.33 (m, 1H), 7.33–7.13 (m, 2H), 6.98–6.81 (m, 2H), 4.49 (d, J = 5.8 Hz, 2H), 3.75 (s, 2H), 3.72 (s, 3H), 3.65 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.86, 162.38, 158.11, 157.56, 156.64, 129.99, 128.27, 113.50, 107.43, 54.82, 51.92, 43.55, 31.42.

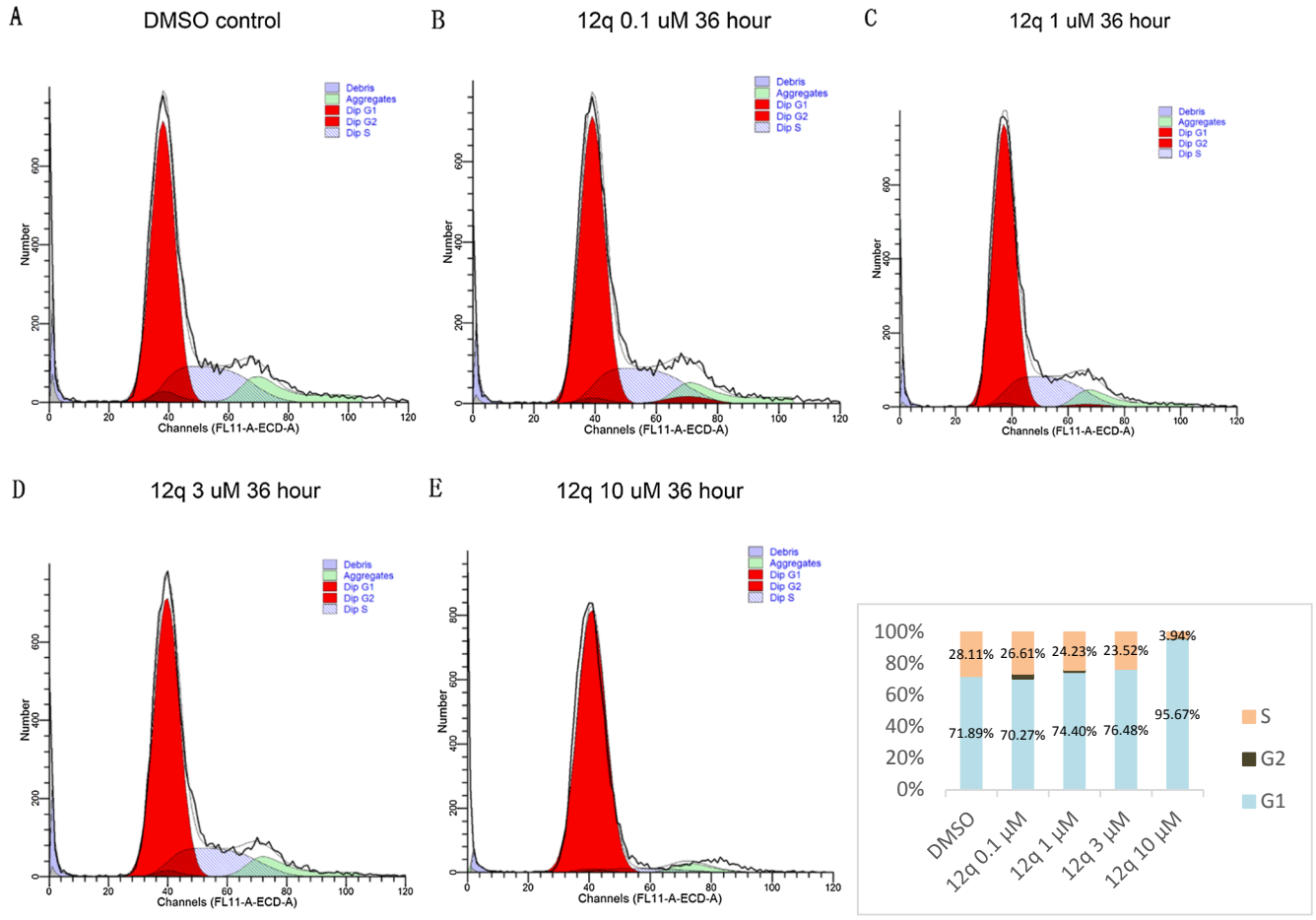


Fig. 5. Compound **12q** induces cell cycle arrest in breast cancer MCF-7. A: DMSO; B: **12q** at 0.1 μM; C: **12q** at 1 μM; D: **12q** at 3 μM; E: **12q** at 10 μM.

Methyl 2-(4-chloro-6-(4-methoxybenzylamino)-2-morpholinopyrimidin-5-yl)acetate (7): DMAP (244 mg, 2 mmol) and morpholine (348 mg, 4 mmol) was added to a solution of methyl 2-(2,4-dichloro-6-((4-methoxybenzyl)amino)pyrimidin-5-yl)acetate **(6)** (712 mg, 2 mmol) in DMF (10 mL). The resulting mixture was stirred at room temperature overnight; more morpholine was added until the methyl acetate complete consumption. Water (100 mL) was added to the reaction mixture then extracted with EtOAc (2 × 100 mL). The organic phases were combined, washed by brine, dried over Na_2SO_4, evaporated and purified by chromatography on silica gel to afford the title compound **7** (577 mg, 71% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.64 (t, J = 5.9 Hz, 1H), 7.22 (d, J = 8.3 Hz, 2H), 6.93–6.81 (m, 2H), 4.44 (d, J = 5.7 Hz, 2H), 3.71 (s, 3H), 3.63 (s, 3H), 3.61 (s, 2H), 3.56 (s, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.39, 161.63, 158.93, 158.29, 158.04, 131.85, 128.42, 113.51, 97.09, 65.89, 55.00, 51.79, 43.86, 43.53, 31.36.

4-chloro-7-(4-methoxybenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (8): p-toluenesulfonic acid monohydrate (570 mg, 3 mmol) was added to a solution of methyl 2-(4-chloro-6-((4-methoxybenzyl)amino)-2-morpholinopyrimidin-5-yl)acetate **(7)** (1.21 g, 3 mmol) in toluene (30 mL). The resulting mixture was heated at reflux until the reaction was complete. Water (200 mL) was added to the reaction mixture then extracted with EtOAc (2 × 200 mL). The organic phases were combined, washed by brine, dried over Na_2SO_4, evaporated and purified by chromatography on silica gel to afford the title compound **8** (731 mg, 65% yield) as a pink solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28 (d, J = 8.6 Hz, 2H), 6.91–6.81 (m, 2H), 4.71 (s, 2H), 3.74–3.60 (m, 11*H*), 3.55 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.72, 165.74, 160.18, 158.66, 151.57, 129.48, 128.31, 113.79, 102.00, 65.75, 55.04, 44.20, 41.84, 32.34.

7-(4-methoxybenzyl)-2,4-dimorpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (9a): A solution of 4-chloro-7-(4-methoxybenzyl)-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*] pyrimidin-6-one **(8)** (100 mg, 0.27 mmol), DIPEA (70 mg, 0.54 mmol), morpholine (47 mg, 0.54 mmol) and DMA (3 mL) was heated to 80 °C until complete consumption of raw materials. Water (50 mL) was added to the reaction mixture then extracted with DCM (2 × 50 mL). The organic phases were combined, washed by brine, dried over Na_2SO_4, evaporated and purified by chromatography on silica gel to afford the title compound **9a** (36 mg, 31% yield) as a light yellow solid. Melting point: 209–212 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28–7.25 (m, 2H), 6.85 (d, J = 8.5 Hz, 2H), 4.68 (s, 2H), 3.71 (d, J = 3.3 Hz, 3H), 3.67 (s, 2H), 3.62 (s, 13H), 3.55 (d, J = 4.6 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.31, 164.90, 159.80, 158.46, 157.12, 129.32, 129.27, 113.67, 84.02, 65.97, 65.91, 55.01, 44.91, 44.12, 41.25, 35.06. HRMS Calcd for $C_{22}H_{28}O_4N_5$, 426.21358, found m/z 426.21307 $[(M+H)^+]$.

7-(4-methoxybenzyl)-2-morpholino-4-(piperidin-1-yl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (9b): The title compound **9b** (27 mg) was prepared from 4-chloro-7-(4-methoxybenzyl)-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*] pyrimidin-6-one **(8)** (100 mg, 0.27 mmol) and piperidine (46 mg, 0.54 mmol) using the procedure described for compound **9a** in 23% yield as a light yellow solid. Melting point: 183–185 °C, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.34 (d, J = 8.2 Hz, 2H), 6.82 (d, J = 8.2 Hz, 2H), 4.82 (s, 2H), 4.09 (s, 1H), 3.97 (d, J = 4.9 Hz, 2H), 3.87 (s, 2H), 3.76 (d, J = 8.2 Hz, 10*H*), 1.63 (t, J = 19.0 Hz, 8H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 173.02, 172.01, 161.89, 160.96, 159.31, 156.82, 130.15, 128.91, 114.01, 86.85, 77.37, 66.95, 66.75, 55.42, 44.84, 42.18, 24.82. HRMS Calcd for $C_{23}H_{30}O_3N_5$, 424.23432, found m/z 424.23410 $[(M+H)^+]$.

7-(4-methoxybenzyl)-2-morpholino-4-thiomorpholino-5,7-

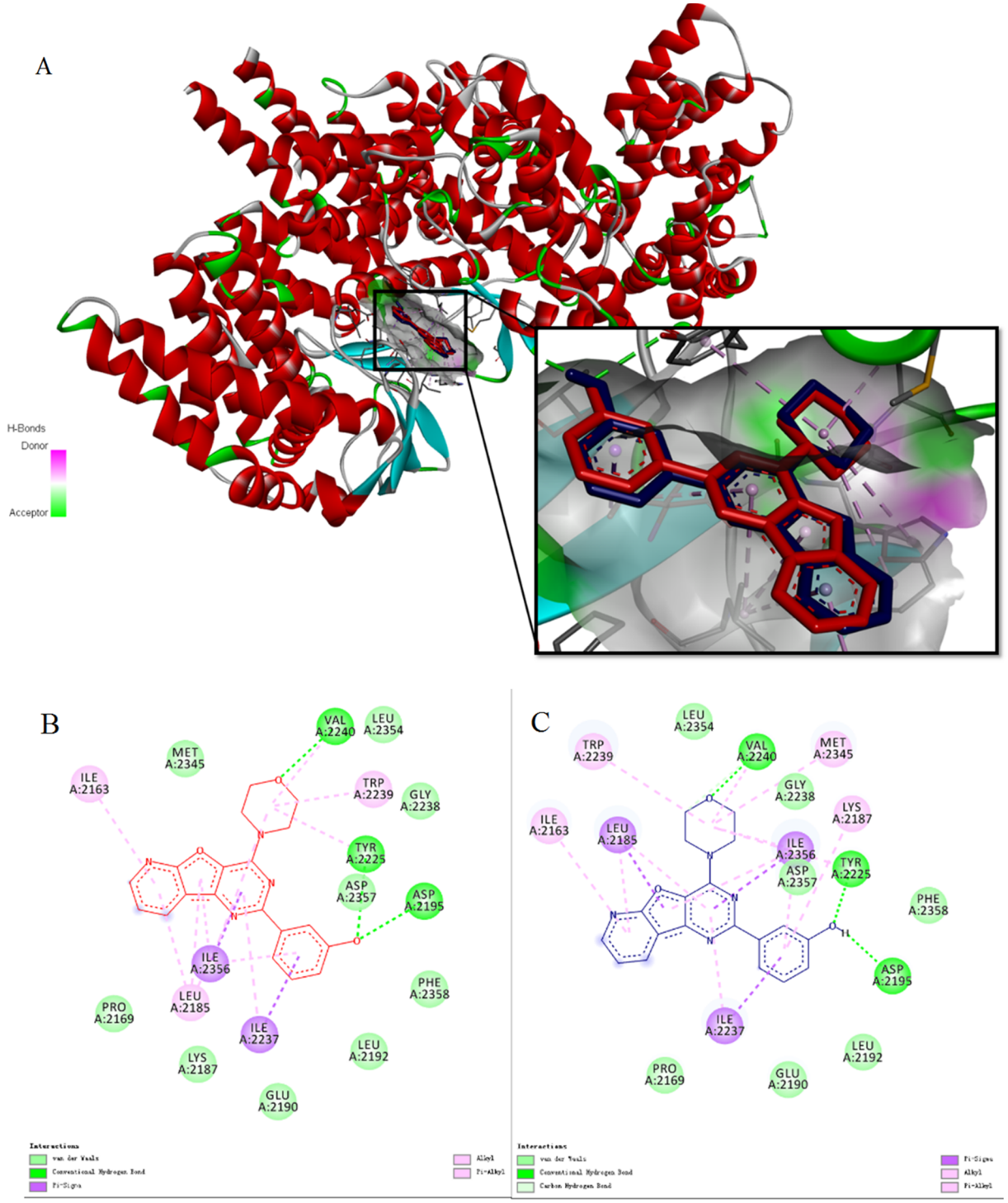


Fig. 6. A: Stacking map of re-docked PI-103 (blue) and the original ligand (red); B: the binding mode of original ligand (red) with mTOR (PDB:4jt6); C: the binding mode of re-docked PI-103 (blue) with mTOR (PDB:4jt6).

dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (9c): The title compound **9c** (45 mg) was prepared from 4-chloro-7-(4-methoxybenzyl)-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*] pyrimidin-6-one **(8)** (100 mg, 0.27 mmol) and thiomorpholine (70 mg, 0.54 mmol) using the procedure described for compound **9a** in 37% yield as a black solid. Melting point: 202–205 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28 (d, J = 8.2 Hz, 2H), 6.85 (d, J = 8.0 Hz, 2H), 4.68 (s, 2H), 3.93–3.77 (m, 4H), 3.71 (s, 3H), 3.62 (s, 8H), 3.30 (s, 2H), 2.58 (t, J = 4.8 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.24, 164.94, 159.91, 158.48, 156.21, 129.42, 129.27, 113.66, 83.95, 65.90, 55.01, 47.56, 44.11, 41.27, 35.13, 25.78. HRMS Calcd for $C_{22}H_{28}O_3N_5S$, 442.19074, found m/z 442.19031 $[(M+H)^+]$.

7-(4-methoxybenzyl)-4-(4-methylpiperazin-1-yl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (9d): The title compound **9d** (35 mg) was prepared from 4-chloro-7-(4-methoxybenzyl)-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*] pyrimidin-6-one **(8)** (100 mg, 0.27 mmol) and 1-methylpiperazine (54 mg, 0.54 mmol) using the procedure described for compound **9a** in 30% yield as a yellow solid. Melting point: 183–185 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26 (d, J = 8.3 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 4.67 (s, 2H), 3.70 (s, 3H),

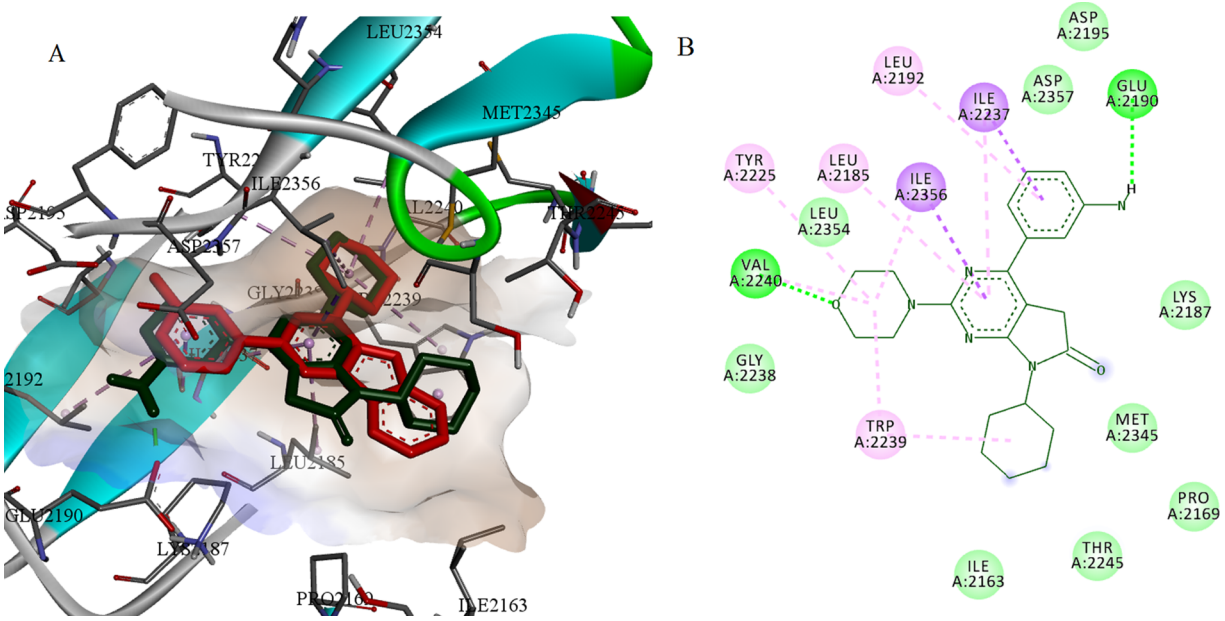


Fig. 7. A: Stacking map of **12q** (green) and the original ligand (red); B: the binding mode of **12q** (green) with mTOR (PDB:4jt6).

3.63 (d, J = 15.1 Hz, 10*H*), 3.55 (t, J = 4.9 Hz, 4H), 2.31 (t, J = 4.9 Hz, 4H), 2.17 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.34, 164.86, 159.86, 158.48, 156.96, 129.36, 129.33, 113.70, 83.91, 65.95, 55.04, 54.46, 45.68, 44.40, 44.15, 41.27, 35.26. HRMS Calcd for $C_{23}H_{30}O_3N_6$, 439.24576, found *m/z* 439.24509 [(M + H)$^+$].

4-(4-hydroxypiperidin-1-yl)-7-(4-methoxybenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (9e): The title compound **9e** (15 mg) was prepared from 4-chloro-7-(4-methoxybenzyl)-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*] pyrimidin-6-one **(8)** (100 mg, 0.27 mmol) and 4-hydroxypiperidine (55 mg, 0.54 mmol) using the procedure described for compound **9a** in 12% yield as a yellow solid. Melting point:181-183 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27 (d, J = 8.3 Hz, 2H), 6.84 (d, J = 8.8 Hz, 2H), 4.82–4.62 (m, 3H), 4.21 (dd, J = 12.4, 5.4 Hz, 2H), 3.96 (s, 1H), 3.71 (d, J = 2.9 Hz, 3H), 3.69–3.42 (m, 10*H*), 3.14 (ddd, J = 13.3, 10.0, 3.0 Hz, 2H), 1.73 (dd, J = 12.8, 4.1 Hz, 2H), 1.42–1.21 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.36, 164.97, 159.58, 158.47, 157.30, 129.39, 129.37, 113.65, 83.32, 66.34, 66.01, 55.03, 44.93, 41.42, 41.26, 35.10, 33.88. HRMS Calcd for $C_{23}H_{30}O_4N_5$, 440.22923, found *m/z* 440.22900 [(M + H)$^+$].

Methyl 2-(4-benzylamino-6-chloro-2-morpholinopyrimidin-5-yl) acetate (10a): DIPEA (1.0 mL) and benzylamine (620 mg, 5.8 mmol) was added to a solution of methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) in DMF (10 mL). The resulting mixture was stirred at room temperature for 1 h, then DMAP (708 mg, 5.8 mmol) and morpholine (870 mg, 10 mmol) was added to the solution. The resulting mixture was stirred at room temperature overnight; more morpholine was added until the intermediate complete consumption. Water (200 mL) was added to the reaction mixture then extracted with EtOAc (2 × 200 mL). The organic phases were combined, washed by brine, dried over Na_2SO_4, evaporated and purified by chromatography on silica gel to afford the title compound **10a** (911 mg, 42% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (t, J = 5.8 Hz, 1H), 7.29 (d, J = 4.4 Hz, 4H), 7.26–7.17 (m, 1H), 4.53 (d, J = 5.8 Hz, 2H), 3.64 (s, 5H), 3.53 (s, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.43, 161.72, 158.94, 158.36, 140.02, 128.11, 127.08, 126.52, 97.14, 65.88, 51.83, 44.14, 43.86, 31.40.

Methyl 2-(4-chloro-6-(3,4-difluorobenzylamino)-2-morpholino-pyrimidin-5-yl)acetate (10b): The title compound **10b** (790 mg) was

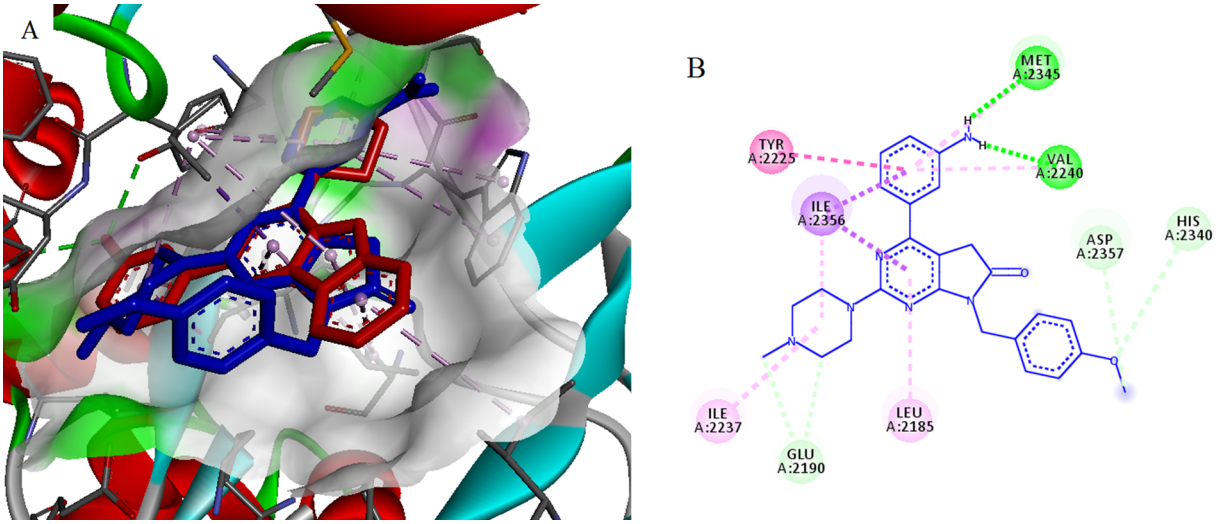


Fig. 8. A: Stacking map of **16d** (blue) and the original ligand (red); B: the binding mode of **16d** (blue) with mTOR (PDB:4jt6).

prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) and 3,4-difluorobenzylamine (829 mg, 5.8 mmol) using the procedure described for compound **10a** in 33% yield as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.70 (t, J = 5.7 Hz, 1H), 7.35 (td, J = 8.7, 6.7 Hz, 1H), 7.18 (ddd, J = 10.5, 9.3, 2.6 Hz, 1H), 7.03 (tdd, J = 8.5, 2.6, 1.0 Hz, 1H), 4.50 (d, J = 5.6 Hz, 2H), 3.63 (d, J = 5.0 Hz, 5H), 3.53 (p, J = 2.2 Hz, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.37, 162.42, 162.30, 161.59, 161.26, 161.14, 159.99, 159.87, 158.87, 158.81, 158.69, 158.43, 130.43, 130.37, 130.34, 130.28, 123.00, 122.96, 122.85, 122.81, 111.33, 111.29, 111.12, 111.08, 103.66, 103.40, 103.14, 97.26, 65.85, 51.86, 43.78, 37.35, 37.31, 31.38.

Methyl (R)-2-(4-chloro-2-morpholino-6-(1-phenylethylamino) pyrimidin-5-yl)acetate (10c): The title compound **10c** (1.1 g) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) and (*R*)-α-methylbenzylamine (702 mg, 5.8 mmol) using the procedure described for compound **10a** in 46% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39–7.32 (m, 3H), 7.28 (dd, J = 8.5, 6.8 Hz, 2H), 7.20–7.13 (m, 1H), 5.19 (t, J = 7.1 Hz, 1H), 3.72 (s, 2H), 3.66 (s, 3H), 3.51 (td, J = 8.7, 5.1 Hz, 8H), 1.45 (d, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.59, 161.05, 158.79, 158.51, 145.61, 128.07, 126.32, 125.91, 97.20, 65.89, 51.79, 50.47, 43.87, 31.48, 22.65.

Methyl 2-(4-chloro-6-(3,4-dimethoxybenzylamino)-2-morpholinopyrimidin-5-yl)acetate (10d): The title compound **10d** (1.2 g) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) and veratrylamine (969 mg, 5.8 mmol) using the procedure described for compound **10a** in 47% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (t, J = 5.8 Hz, 1H), 6.93 (d, J = 1.9 Hz, 1H), 6.90–6.79 (m, 2H), 4.44 (d, J = 5.8 Hz, 2H), 3.71 (d, J = 4.5 Hz, 6H), 3.63 (d, J = 3.1 Hz, 5H), 3.57 (s, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.46, 161.67, 158.97, 158.31, 148.53, 147.59, 132.44, 119.31, 111.63, 111.36, 97.15, 65.92, 55.54, 55.35, 51.80, 43.88, 43.88, 31.36.

Methyl 2-(4-chloro-6-(4-fluorobenzylamino)-2-morpholinopyrimidin-5-yl)acetate (10e): The title compound **10e** (955 mg) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) and 4-fluorobenzylamine (725 mg, 5.8 mmol) using the procedure described for compound **10a** in 42% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (t, J = 5.9 Hz, 1H), 7.37–7.28 (m, 2H), 7.19–7.08 (m, 2H), 4.49 (d, J = 5.7 Hz, 2H), 3.63 (d, J = 5.8 Hz, 5H), 3.59–3.47 (m, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.40, 162.23, 161.64, 159.83, 158.92, 158.36, 136.19, 136.16, 129.01, 128.93, 114.92, 114.71, 97.15, 65.88, 51.85, 43.85, 43.43, 31.39.

Methyl 2-(4-chloro-6-(4-isocyanobenzylamino)-2-morpholinopyrimidin-5-yl)acetate (10f): The title compound **10f** (630 mg) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) and 4-(aminomethyl)benzonitrile (766 mg, 5.8 mmol) using the procedure described for compound **10a** in 27% yield as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.84 (s, 1H), 7.80–7.75 (m, 2H), 7.46 (d, J = 8.3 Hz, 2H), 4.57 (d, J = 5.8 Hz, 2H), 3.65 (s, 3H), 3.64 (s, 2H), 3.49 (dd, J = 18.8, 5.1 Hz, 8H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.82, 162.08, 159.29, 158.88, 146.67, 132.57, 128.26, 119.42, 109.71, 97.69, 66.28, 52.36, 44.27, 34.36, 31.86.

Methyl 2-(4-chloro-6-(3-chlorobenzylamino)-2-morpholinopyrimidin-5-yl)acetate (10g): The title compound **10g** (1.0 g) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) and 3-chlorobenzylamine (824 mg, 5.8 mmol) using the procedure described for compound **10a** in 42% yield as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (t, J = 5.8 Hz, 1H), 7.36–7.30 (m, 2H), 7.28–7.23 (m, 2H), 4.51 (d, J = 5.8 Hz, 2H), 3.64 (d, J = 5.2 Hz, 5H), 3.53 (s, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.39, 161.64, 158.89, 158.42, 142.78, 132.86, 129.97, 126.99, 126.46, 125.77, 97.25, 65.89, 51.87, 43.88, 43.69, 31.44.

Methyl 2-(4-chloro-2-morpholino-6-(4-trifluoromethylbenzylamino) pyrimidin-5-yl)acetate (10h): The title compound **10h** (741 mg) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) and 4-(trifluoromethyl)benzylamine (1.0 g, 5.8 mmol) using the procedure described for compound **10a** in 29% yield as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (t, J = 5.9 Hz, 1H), 7.66 (d, J = 7.9 Hz, 2H), 7.49 (d, J = 8.0 Hz, 2H), 4.58 (d, J = 5.6 Hz, 2H), 3.64 (d, J = 3.6 Hz, 5H), 3.49 (s, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.40, 161.67, 158.87, 158.43, 145.11, 128.22, 127.95, 127.68, 127.41, 127.10, 125.04, 125.00, 97.23, 65.84, 51.91, 43.90, 43.83, 31.42.

Methyl 2-(4-chloro-6-(4-methylbenzylamino)-2-morpholinopyrimidin-5-yl)acetate (10i): The title compound **10i** (1.0 g) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) and 4-methylbenzylamine (702 mg, 5.8 mmol) using the procedure described for compound **10a** in 44% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.68 (t, J = 5.8 Hz, 1H), 7.20–7.15 (m, 2H), 7.09 (d, J = 7.8 Hz, 2H), 4.47 (d, J = 5.4 Hz, 2H), 3.69–3.60 (m, 5H), 3.60–3.48 (m, 8H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.41, 161.70, 158.94, 158.33, 136.93, 135.53, 128.65, 127.12, 97.12, 65.90, 51.81, 43.86, 31.39, 20.65.

Methyl 2-(4-chloro-6-(3-fluorobenzylamino)-2-morpholinopyrimidin-5-yl)acetate (10j): The title compound **10j** (730 mg) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) and 3-fluorobenzylamine (725 mg, 5.8 mmol) using the procedure described for compound **10a** in 32% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (t, J = 5.9 Hz, 1H), 7.33 (td, J = 7.9, 6.1 Hz, 1H), 7.17–7.07 (m, 2H), 7.01 (td, J = 8.6, 2.7 Hz, 1H), 4.54 (d, J = 5.7 Hz, 2H), 3.65 (s, 5H), 3.53 (s, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.43, 163.45, 161.69, 161.04, 158.91, 158.42, 143.29, 143.22, 130.02, 129.94, 123.03, 123.00, 113.70, 113.49, 113.31, 113.10, 97.24, 65.88, 51.83, 43.86, 43.69, 31.43.

Methyl 2-(4-chloro-6-(cyclohexylamino)-2-morpholinopyrimidin-5-yl)acetate (10k): The title compound **10k** (1.9 g) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (2.5 g, 10 mmol) and cyclohexylamine (1.0 g, 10 mmol) using the procedure described for compound **10a** in 51% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.63 (d, J = 7.4 Hz, 1H), 3.89–3.78 (m, 1H), 3.60 (dt, J = 11.8, 4.8 Hz, 13H), 1.84 (d, J = 10.0 Hz, 2H), 1.78–1.68 (m, 2H), 1.59 (d, J = 12.7 Hz, 1H), 1.28–1.21 (m, 4H), 1.16–1.08 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.48, 161.05, 159.05, 158.39, 97.01, 65.94, 51.63, 49.94, 43.84, 32.04, 31.35, 25.38, 24.88.

Methyl 2-(4-chloro-6-(cyclopropylamino)-2-morpholinopyrimidin-5-yl)acetate (10l): The title compound **10l** (1.3 g) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (2.5 g, 10 mmol) and cyclopropylamine (570 mg, 10 mmol) using the procedure described for compound **10a** in 40% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.09 (s, 1H), 3.58 (d, J = 34.7 Hz, 13H), 2.79 (s, 1H), 0.67 (s, 2H), 0.47 (s, 2H).

Methyl 2-(4-chloro-6-(isopropylamino)-2-morpholinopyrimidin-5-yl)acetate (10m): The title compound **10m** (1.4 g) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (2.5 g, 10 mmol) and isopropylamine (590 mg, 10 mmol) using the procedure described for compound **10a** in 43% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.75 (s, 1H), 4.49 (p, J = 7.0 Hz, 1H), 3.66 (s, 8H), 3.47 (s, 5H), 1.40 (d, J = 6.9 Hz, 6H).

Methyl (6-chloro-5-(2-methoxy-2-oxoethyl)-2-morpholinopyrimidin-4-yl)-L-alaninate (10n): The title compound **10n** (779 mg) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) and L-alanine methyl ester hydrochloride (812 mg, 5.8 mmol) using the procedure described for compound **10a** in 36% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37 (d, J = 5.6 Hz, 1H), 4.33–4.25 (m, 1H), 3.66 (d, J = 8.8 Hz, 2H), 3.64 (s, 3H), 3.61–3.57 (m, 7H), 3.56–3.51 (m, 4H), 1.38 (d, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.88, 170.44, 161.19, 158.95, 158.53, 97.26, 65.91, 51.80, 51.59, 50.43, 43.73, 31.26, 16.50.

Methyl (6-chloro-5-(2-methoxy-2-oxoethyl)-2-morpholinopyrimidin-4-yl)-D-alaninate (10o): The title compound **10o** (907 mg) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) and D-alanine methyl ester hydrochloride (812 mg,

5.8 mmol) using the procedure described for compound **10a** in 42% yield as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.35 (d, J = 5.7 Hz, 1H), 4.34–4.26 (m, 1H), 3.65 (d, J = 6.8 Hz, 5H), 3.60 (q, J = 5.2, 3.8 Hz, 7H), 3.55 (q, J = 5.1, 4.4 Hz, 4H), 1.38 (d, J = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.92, 170.47, 161.24, 159.00, 158.58, 97.30, 65.96, 51.80, 51.58, 50.47, 43.77, 31.30, 16.52.

7-benzyl-4-chloro-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d] pyrimidin-6-one (11a): The title compound **11a** (511 mg) was prepared from methyl 2-(4-(benzylamino)-6-chloro-2-morpholinopyrimidin-5-yl)acetate **(10a)** (890 mg, 2.4 mmol) using the procedure described for compound **8** in 62% yield as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.34–7.32 (m, 2H), 7.30 (dd, J = 7.9, 0.9 Hz, 2H), 7.28 (s, 1H), 4.78 (s, 2H), 3.68–3.61 (m, 8H), 3.52 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.87, 165.79, 160.20, 151.64, 136.32, 128.46, 127.92, 127.49, 102.09, 65.78, 44.20, 42.41, 32.40.

4-chloro-7-(3,4-difluorobenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (11b): The title compound **11b** (325 mg) was prepared from methyl 2-(4-chloro-6-((3,4-difluorobenzyl) amino)-2-morpholinopyrimidin-5-yl)acetate **(10b)** (750 mg, 1.8 mmol) using the procedure described for compound **8** in 47% yield as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43 (td, J = 8.7, 6.6 Hz, 1H), 7.22 (ddd, J = 10.5, 9.4, 2.6 Hz, 1H), 7.04 (tdd, J = 8.6, 2.6, 1.0 Hz, 1H), 4.80 (s, 2H), 3.72–3.52 (m, 10*H*). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.67, 165.57, 163.06, 162.94, 161.45, 161.45, 161.33, 161.32, 160.61, 160.49, 160.13, 158.98, 158.86, 151.64, 131.84, 131.79, 131.74, 131.69, 119.37, 119.22, 111.62, 111.59, 111.41, 111.38, 104.09, 103.84, 103.58, 102.07, 65.76, 44.17, 35.83, 35.79, 32.40.

(R)-4-chloro-2-morpholino-7-(1-phenylethyl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (11c): The title compound **11c** (673 mg) was prepared from methyl (*R*)-2-(4-chloro-2-morpholino-6-((1-phenylethyl)amino)pyrimidin-5-yl)acetate **(10c)** (1.0 g, 2.6 mmol) using the procedure described for compound **8** in 74% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43–7.37 (m, 2H), 7.34–7.29 (m, 2H), 7.28–7.22 (m, 1H), 5.56 (q, J = 7.2 Hz, 1H), 3.59 (ddd, J = 13.8, 5.2, 2.5 Hz, 10*H*), 1.81 (d, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.73, 165.78, 160.04, 151.70, 140.06, 128.25, 127.30, 126.91, 102.05, 65.75, 49.69, 44.25, 32.49, 16.70.

4-chloro-7-(3,4-dimethoxybenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (11d): The title compound **11d** (851 mg) was prepared from methyl 2-(4-chloro-6-((3,4-dimethoxybenzyl)amino)-2-morpholinopyrimidin-5-yl)acetate **(10d)** (1.2 g, 2.7 mmol) using the procedure described for compound **8** in 78% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.99 (s, 1H), 6.87 (d, J = 1.1 Hz, 2H), 4.70 (s, 2H), 3.75–3.67 (m, 10*H*), 3.64 (dd, J = 5.7, 3.7 Hz, 4H), 3.56 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.79, 165.80, 160.17, 151.57, 148.52, 148.29, 128.74, 120.48, 112.19, 111.69, 102.08, 65.80, 55.50, 55.36, 44.20, 42.22, 32.37.

4-chloro-7-(4-fluorobenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (11e): The title compound **11e** (569 mg) was prepared from methyl 2-(4-chloro-6-((4-fluorobenzyl)amino)-2-morpholinopyrimidin-5-yl)acetate **(10e)** (900 mg, 2.3 mmol) using the procedure described for compound **8** in 68% yield as a light yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.45–7.36 (m, 2H), 7.14 (t, J = 8.9 Hz, 2H), 4.77 (s, 2H), 3.67 (t, J = 4.9 Hz, 4H), 3.64–3.61 (m, 4H), 3.57 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.81, 165.67, 162.31, 160.70, 160.17, 151.62, 132.54, 132.52, 130.13, 130.08, 115.27, 115.13, 102.09, 65.76, 44.19, 41.66, 32.39.

4-((4-chloro-2-morpholino-6-oxo-5,6-dihydro-7H-pyrrolo[2,3-d] pyrimidin-7-yl)methyl)benzonitrile (11f): The title compound **11f** (209 mg) was prepared from methyl 2-(4-chloro-6-((4-isocyanobenzyl) amino)-2-morpholinopyrimidin-5-yl)acetate **(10f)** (610 mg, 1.5 mmol) using the procedure described for compound **8** in 37% yield as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.79 (d, J = 8.3 Hz, 2H), 7.52 (d, J = 8.3 Hz, 2H), 4.88 (s, 2H), 3.65–3.58 (m, 10*H*). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.94, 165.60, 160.14, 151.70, 141.88, 132.43, 128.63, 110.29, 102.19, 65.76, 44.17, 42.09, 32.48.

4-chloro-7-(3-chlorobenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (11g): The title compound **11g** (433 mg) was prepared from methyl 2-(4-chloro-6-((3-chlorobenzyl)amino)-2-morpholinopyrimidin-5-yl)acetate **(10g)** (980 mg, 2.4 mmol) using the procedure described for compound **8** in 48% yield as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.43 (d, J = 1.9 Hz, 1H), 7.37–7.32 (m, 2H), 7.30 (dt, J = 7.1, 1.8 Hz, 1H), 4.79 (s, 2H), 3.67 (q, J = 3.8, 3.0 Hz, 4H), 3.62 (dd, J = 5.4, 3.6 Hz, 4H), 3.59 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.87, 165.64, 160.12, 151.64, 138.67, 132.99, 130.37, 127.91, 127.49, 126.59, 102.18, 65.76, 44.20, 41.84, 32.44.

4-chloro-2-morpholino-7-(4-(trifluoromethyl)benzyl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (11h): The title compound **11h** (271 mg) was prepared from methyl 2-(4-chloro-2-morpholino-6-((4-(trifluoromethyl)benzyl)amino)pyrimidin-5-yl)acetate **(10h)** (720 mg, 1.6 mmol) using the procedure described for compound **8** in 41% yield as a yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.69 (d, J = 8.0 Hz, 2H), 7.56 (d, J = 8.0 Hz, 2H), 4.89 (s, 2H), 3.65 (dd, J = 5.8, 3.9 Hz, 4H), 3.63–3.57 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.86, 165.62, 160.14, 151.67, 140.97, 128.60, 128.21, 128.00, 125.36, 125.34, 125.31, 125.29, 125.06, 123.26, 102.12, 65.73, 44.16, 41.97, 32.43.

4-chloro-7-(4-methylbenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (11i): The title compound **11i** (519 mg) was prepared from methyl 2-(4-chloro-6-((4-methylbenzyl)amino)-2-morpholinopyrimidin-5-yl)acetate **(10i)** (980 mg, 2.5 mmol) using the procedure described for compound **8** in 58% yield as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.22 (d, J = 8.0 Hz, 2H), 7.11 (d, J = 7.8 Hz, 2H), 4.74 (s, 2H), 3.67 (d, J = 4.8 Hz, 4H), 3.62 (td, J = 5.7, 3.2 Hz, 6H), 2.25 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.83, 165.81, 160.20, 151.60, 136.71, 133.32, 128.98, 127.93, 102.08, 65.78, 44.20, 42.15, 32.38, 20.66.

4-chloro-7-(3-fluorobenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (11j): The title compound **11j** (259 mg) was prepared from methyl 2-(4-chloro-6-((3-fluorobenzyl)amino)-2-morpholinopyrimidin-5-yl)acetate **(10j)** (700 mg, 1.8 mmol) using the procedure described for compound **8** in 40% yield as a light yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.36 (td, J = 8.1, 6.2 Hz, 1H), 7.22–7.14 (m, 2H), 7.09 (ddd, J = 10.6, 8.2, 2.6 Hz, 1H), 4.81 (s, 2H), 3.69–3.64 (m, 4H), 3.61 (dd, J = 5.6, 3.7 Hz, 4H), 3.59 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.88, 165.65, 162.89, 161.27, 160.14, 151.63, 139.07, 139.02, 130.47, 130.42, 123.82, 123.80, 114.65, 114.51, 114.35, 114.21, 102.17, 65.74, 44.18, 41.86, 32.42.

4-chloro-7-cyclohexyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (11k): The title compound **11k** (1.1 g) was prepared from methyl 2-(4-chloro-6-(cyclohexylamino)-2-morpholinopyrimidin-5-yl)acetate **(10k)** (1.8 g, 4.9 mmol) using the procedure described for compound **8** in 67% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.08 (td, J = 12.2, 10.4, 6.0 Hz, 1H), 3.66 (s, 8H), 3.47 (s, 2H), 2.27–2.10 (m, 2H), 1.79 (d, J = 13.0 Hz, 2H), 1.60 (d, J = 13.5 Hz, 3H), 1.29 (t, J = 13.4 Hz, 2H), 1.15 (q, J = 12.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.45, 166.09, 160.14, 151.57, 101.95, 65.77, 51.22, 44.19, 32.35, 28.55, 25.39, 24.99.

4-chloro-7-cyclopropyl-2-morpholino-5,7-dihydro-6H-pyrrolo [2,3-d]pyrimidin-6-one (11l): The title compound **11l** (750 mg) was prepared from methyl 2-(4-chloro-6-(cyclopropylamino)-2-morpholinopyrimidin-5-yl)acetate **(10l)** (1.3 g, 3.9 mmol) using the procedure described for compound **8** in 64% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.66 (q, J = 4.0 Hz, 8H), 3.40 (s, 2H), 2.71 (dq, J = 7.3, 4.9, 3.7 Hz, 1H), 0.92 (d, J = 3.8 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.40, 166.93, 160.32, 151.38, 101.84, 65.85, 44.19, 32.29, 22.33, 5.03.

4-chloro-7-isopropyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (11m): The title compound **11m** (939 mg) was prepared from methyl 2-(4-chloro-6-(isopropylamino)-2-morpholinopyrimidin-5-yl)acetate **(10m)** (1.3 g, 3.9 mmol) using the procedure

described for compound **8** in 80% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.49 (p, J = 6.9 Hz, 1H), 3.66 (q, J = 2.4 Hz, 8H), 3.46 (s, 2H), 1.39 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.50, 166.09, 160.17, 151.55, 102.09, 65.80, 44.20, 43.40, 32.46, 19.13.

Methyl (S)-2-(4-chloro-2-morpholino-6-oxo-5,6-dihydro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)propanoate (11n): The title compound **11n** (333 mg) was prepared from methyl (6-chloro-5-(2-methoxy-2-oxoethyl)-2-morpholinopyrimidin-4-yl)-L-alaninate **(10n)** (750 mg, 2.0 mmol) using the procedure described for compound **8** in 49% yield as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 5.07 (q, J = 7.1 Hz, 1H), 3.62 (dd, J = 10.8, 8.7 Hz, 13H), 1.49 (d, J = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.04, 169.76, 164.84, 159.96, 151.99, 101.69, 65.75, 52.42, 47.88, 44.13, 32.30, 14.26.

Methyl (R)-2-(4-chloro-2-morpholino-6-oxo-5,6-dihydro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)propanoate (11o): The title compound **11o** (309 mg) was prepared from methyl (6-chloro-5-(2-methoxy-2-oxoethyl)-2-morpholinopyrimidin-4-yl)-D-alaninate **(10o)** (800 mg, 2.1 mmol) using the procedure described for compound **8** in 43% yield as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 5.07 (q, J = 7.1 Hz, 1H), 3.65–3.59 (m, 13H), 1.49 (dd, J = 7.2, 1.7 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.06, 169.78, 164.85, 159.97, 152.02, 101.69, 65.77, 52.44, 47.90, 44.15, 32.31, 14.26.

4-chloro-7-(2-hydroxyethyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (11p): The title compound **11p** (300 mg) was prepared from methyl (2,4,6-trichloropyrimidin-5-yl) acetate **(5)** (1.5 g, 5.8 mmol) and ethanolamine (354 mg, 5.8 mmol) directly using the procedure described for compound **10a** in 17% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.75 (t, J = 5.9 Hz, 1H), 3.65 (dt, J = 18.8, 9.5 Hz, 13H), 3.49 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.95, 166.33, 160.23, 151.33, 102.05, 65.78, 57.14, 44.13, 41.67, 32.33.

4-(6-aminopyridin-3-yl)-7-benzyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12a): A solution of 7-benzyl-4-chloro-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11a)** (100 mg, 0.29 mmol), (6-aminopyridin-3-yl)boronic acid (48 mg, 0.35 mmol), Pd(dppf)$_2$Cl$_2$ (10 mg, 0.014 mmol), 2 N Na_2CO_3 aqueous solution (1.5 mL) and 1,4-dioxane (5 mL) was heated under 100 W of microwave radiation for 30 min under nitrogen protection. Water (50 mL) was added to the reaction mixture then extracted with DCM (2 × 50 mL). The organic phases were combined, washed by brine, dried over Na_2SO_4, evaporated and purified by chromatography on silica gel to afford the title compound **12a** (35 mg, 30% yield) as a light yellow solid. Melting point: 278–280 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (dd, J = 2.4, 0.7 Hz, 1H), 8.02 (dd, J = 8.8, 2.5 Hz, 1H), 7.39–7.23 (m, 5H), 6.51 (dd, J = 8.8, 0.8 Hz, 1H), 6.46 (s, 2H), 4.82 (s, 2H), 3.86 (s, 2H), 3.73 (dd, J = 5.7, 3.5 Hz, 4H), 3.65 (dd, J = 5.5, 3.6 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.78, 165.39, 160.69, 160.23, 154.46, 148.77, 136.86, 136.42, 128.44, 127.92, 127.38, 120.83, 107.42, 99.09, 65.99, 44.20, 41.99, 34.20. HRMS Calcd for $C_{22}H_{23}O_2N_6$, 403.18770, found m/z 403.18692 [$(M+H)^+$].

4-(6-aminopyridin-3-yl)-7-(3,4-difluorobenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12b): The title compound **12b** (42 mg) was prepared from 4-chloro-7-(3,4-difluorobenzyl)-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11b)** (100 mg, 0.26 mmol) and (6-aminopyridin-3-yl)boronic acid (43 mg, 0.31 mmol) using the procedure described for compound **12a** in 37% yield as a yellow solid. Melting point: 267–269 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (dd, J = 2.4, 0.7 Hz, 1H), 8.01 (dd, J = 8.8, 2.5 Hz, 1H), 7.42 (td, J = 8.7, 6.6 Hz, 1H), 7.23 (ddd, J = 10.4, 9.3, 2.6 Hz, 1H), 7.04 (tdd, J = 8.6, 2.6, 1.0 Hz, 1H), 6.51 (dd, J = 8.8, 0.7 Hz, 1H), 6.47 (s, 2H), 4.84 (s, 2H), 3.86 (s, 2H), 3.70 (dd, J = 5.7, 3.6 Hz, 4H), 3.64 (dt, J = 5.8, 2.6 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.61, 165.14, 160.71, 160.16, 154.50, 148.78, 136.42, 131.66, 131.60, 131.56, 131.51, 120.79, 119.95, 119.91, 119.80, 119.76, 111.63, 111.60, 111.42, 111.38, 107.44, 103.82, 99.01, 65.97, 44.15, 35.34, 34.21. HRMS Calcd for $C_{22}H_{21}O_2N_6F_2$, 439.16886, found m/z 439.16818 [$(M+H)^+$].

(R)-4-(6-aminopyridin-3-yl)-2-morpholino-7-(1-phenylethyl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12c): The title compound **12c** (28 mg) was prepared from (R)-4-chloro-2-morpholino-7-(1-phenylethyl)-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11c)** (100 mg, 0.28 mmol) and (6-aminopyridin-3-yl)boronic acid (46 mg, 0.33 mmol) using the procedure described for compound **12a** in 24% yield as a light green solid. Melting point: 255–257 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (d, J = 2.4 Hz, 1H), 8.00 (dd, J = 8.8, 2.5 Hz, 1H), 7.42 (d, J = 7.3 Hz, 2H), 7.31 (dd, J = 8.3, 6.6 Hz, 2H), 7.28–7.20 (m, 1H), 6.51 (d, J = 8.8 Hz, 1H), 6.45 (s, 2H), 5.63 (q, J = 7.2 Hz, 1H), 3.83 (s, 2H), 3.74–3.66 (m, 4H), 3.62 (dd, J = 8.1, 3.5 Hz, 4H), 1.85 (d, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.63, 165.46, 160.65, 160.07, 154.48, 148.70, 140.58, 136.38, 128.18, 127.13, 126.90, 120.91, 107.39, 99.02, 65.94, 49.23, 44.23, 34.27, 16.84. HRMS Calcd for $C_{23}H_{25}O_2N_6$, 417.20335, found m/z 417.20273 [$(M+H)^+$].

4-(6-aminopyridin-3-yl)-7-(3,4-dimethoxybenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12d): The title compound **12d** (40 mg) was prepared from 4-chloro-7-(3,4-dimethoxybenzyl)-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11d)** (100 mg, 0.25 mmol) and (6-aminopyridin-3-yl)boronic acid (41 mg, 0.30 mmol) using the procedure described for compound **12a** in 35% yield as a light yellow solid. Melting point: 266–268 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (dd, J = 2.4, 0.7 Hz, 1H), 8.02 (dd, J = 8.8, 2.5 Hz, 1H), 7.03 (d, J = 1.5 Hz, 1H), 6.88 (t, J = 1.3 Hz, 2H), 6.51 (dd, J = 8.7, 0.8 Hz, 1H), 6.46 (s, 2H), 4.74 (s, 2H), 3.83 (s, 2H), 3.77 (dd, J = 5.7, 3.7 Hz, 4H), 3.71 (d, J = 3.4 Hz, 6H), 3.67 (dd, J = 5.7, 3.8 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.70, 165.40, 160.68, 160.20, 154.38, 148.76, 148.49, 148.22, 136.41, 129.31, 120.83, 120.48, 112.29, 111.75, 107.40, 99.10, 66.02, 55.52, 55.37, 44.21, 41.80, 34.19. HRMS Calcd for $C_{24}H_{27}O_4N_6$, 463.20883, found m/z 463.20786 [$(M+H)^+$].

4-(6-aminopyridin-3-yl)-7-(4-fluorobenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12e): The title compound **12e** (23 mg) was prepared from 4-chloro-7-(4-fluorobenzyl)-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11e)** (100 mg, 0.28 mmol) and (6-aminopyridin-3-yl)boronic acid (46 mg, 0.33 mmol) using the procedure described for compound **12a** in 20% yield as a light yellow solid. Melting point: 281–283 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60–8.54 (m, 1H), 8.02 (dd, J = 8.8, 2.5 Hz, 1H), 7.47–7.36 (m, 2H), 7.21–7.11 (m, 2H), 6.51 (dd, J = 8.8, 0.8 Hz, 1H), 6.46 (s, 2H), 4.81 (s, 2H), 3.85 (s, 2H), 3.74 (dd, J = 5.5, 3.5 Hz, 4H), 3.66 (dd, J = 5.3, 3.4 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.76, 165.27, 160.70, 160.22, 154.50, 148.77, 136.42, 133.11, 130.16, 130.08, 120.80, 115.33, 115.12, 107.42, 99.08, 66.00, 44.20, 41.26, 34.21. HRMS Calcd for $C_{22}H_{22}O_2N_6F$, 421.17828, found m/z 421.17749 [$(M+H)^+$].

4-((4-(6-aminopyridin-3-yl)-2-morpholino-6-oxo-5,6-dihydro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)methyl)benzonitrile (12f): The title compound **12f** (19 mg) was prepared from 4-((4-chloro-2-morpholino-6-oxo-5,6-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl)benzonitrile **(11f)** (100 mg, 0.27 mmol) and (6-aminopyridin-3-yl)boronic acid (45 mg, 0.32 mmol) using the procedure described for compound **12a** in 16% yield as a light yellow solid. Melting point: 272–279 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62–8.54 (m, 1H), 8.02 (dd, J = 8.8, 2.5 Hz, 1H), 7.84–7.77 (m, 2H), 7.58–7.50 (m, 2H), 6.52 (dd, J = 8.7, 0.7 Hz, 1H), 6.48 (s, 2H), 4.91 (s, 2H), 3.88 (s, 2H), 3.70 (t, J = 4.8 Hz, 4H), 3.64 (dd, J = 5.6, 3.8 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.81, 165.11, 160.72, 160.15, 154.56, 148.79, 142.43, 136.40, 132.43, 128.63, 120.74, 118.70, 110.17, 107.42, 99.07, 65.95, 44.15, 41.69, 34.25. HRMS Calcd for $C_{23}H_{22}O_2N_7$, 428.18295, found m/z 428.18237 [$(M+H)^+$].

4-(6-aminopyridin-3-yl)-7-(3-chlorobenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12g): The title compound **12g** (47 mg) was prepared from 4-chloro-7-(3-chlorobenzyl)-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11g)** (100 mg,

0.26 mmol) and (6-aminopyridin-3-yl)boronic acid (44 mg, 0.31 mmol) using the procedure described for compound **12a** in 41% yield as a yellow solid. Melting point: 286–288 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (dd, J = 2.5, 0.7 Hz, 1H), 8.02 (dd, J = 8.8, 2.5 Hz, 1H), 7.44 (d, J = 2.0 Hz, 1H), 7.38–7.29 (m, 3H), 6.51 (dd, J = 8.8, 0.7 Hz, 1H), 6.47 (s, 2H), 4.83 (s, 2H), 3.87 (s, 2H), 3.74 (dd, J = 5.6, 3.6 Hz, 4H), 3.65 (dd, J = 5.5, 3.6 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.79, 165.20, 160.71, 160.16, 154.54, 148.79, 139.24, 136.42, 132.97, 130.41, 127.96, 127.42, 126.64, 120.79, 107.42, 99.12, 65.99, 44.21, 41.47, 34.23. HRMS Calcd for $C_{22}H_{22}O_2N_6Cl$, 437.14873, found m/z 437.14853 [$(M+H)^+$].

4-(6-aminopyridin-3-yl)-2-morpholino-7-(4-(trifluoromethyl) benzyl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12h): The title compound **12h** (25 mg) was prepared from 4-chloro-2-morpholino-7-(4-(trifluoromethyl)benzyl)-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11h)** (100 mg, 0.24 mmol) and (6-aminopyridin-3-yl)boronic acid (40 mg, 0.29 mmol) using the procedure described for compound **12a** in 22% yield as a yellow solid. Melting point: 244–246 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (dd, J = 2.5, 0.7 Hz, 1H), 8.03 (dd, J = 8.8, 2.5 Hz, 1H), 7.72–7.68 (m, 2H), 7.57 (d, J = 8.1 Hz, 2H), 6.52 (dd, J = 8.8, 0.9 Hz, 1H), 6.47 (s, 2H), 4.92 (s, 2H), 3.88 (s, 2H), 3.71 (q, J = 3.7, 2.9 Hz, 4H), 3.64 (dd, J = 5.2, 3.4 Hz, 4H). HRMS Calcd for $C_{23}H_{22}O_2N_6F$, 471.17509, found m/z 471.17496 [$(M+H)^+$].

4-(6-aminopyridin-3-yl)-7-(4-methylbenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12i): The title compound **12i** (44 mg) was prepared from 4-chloro-7-(4-methylbenzyl)-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11i)** (100 mg, 0.28 mmol) and (6-aminopyridin-3-yl)boronic acid (46 mg, 0.33 mmol) using the procedure described for compound **12a** in 37% yield as a yellow solid. Melting point: 288–290 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (t, J = 1.6 Hz, 1H), 8.01 (dt, J = 8.9, 1.8 Hz, 1H), 7.29–7.22 (m, 2H), 7.11 (d, J = 7.7 Hz, 2H), 6.51 (d, J = 8.7 Hz, 1H), 6.45 (s, 2H), 4.77 (s, 2H), 3.84 (s, 2H), 3.74 (t, J = 4.5 Hz, 4H), 3.69–3.64 (m, 4H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.66, 165.36, 160.63, 160.20, 154.41, 148.70, 136.51, 136.36, 133.81, 128.90, 127.89, 120.84, 107.36, 99.04, 65.95, 44.18, 41.69, 34.14, 20.61. HRMS Calcd for $C_{23}H_{25}O_2N_6$, 417.20335, found m/z 417.20297 [$(M+H)^+$].

4-(6-aminopyridin-3-yl)-7-(3-fluorobenzyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12j): The title compound **12j** (30 mg) was prepared from 4-chloro-7-(3-fluorobenzyl)-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11j)** (100 mg, 0.28 mmol) and (6-aminopyridin-3-yl)boronic acid (46 mg, 0.33 mmol) using the procedure described for compound **12a** in 26% yield as a light yellow solid. Melting point: 270–272 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62–8.56 (m, 1H), 8.02 (dd, J = 8.8, 2.5 Hz, 1H), 7.37 (td, J = 8.0, 6.0 Hz, 1H), 7.21–7.15 (m, 2H), 7.10 (tt, J = 8.8, 1.8 Hz, 1H), 6.52 (dd, J = 8.8, 0.7 Hz, 1H), 6.47 (s, 2H), 4.85 (s, 2H), 3.87 (s, 2H), 3.73 (dd, J = 5.6, 3.6 Hz, 4H), 3.65 (dd, J = 5.6, 3.5 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.74, 165.19, 163.26, 160.83, 160.65, 160.16, 154.51, 148.71, 139.60, 139.53, 136.36, 130.44, 130.36, 123.81, 123.78, 120.80, 114.67, 114.45, 114.25, 114.04, 107.38, 99.09, 65.92, 44.16, 41.44, 34.18. HRMS Calcd for $C_{22}H_{22}O_2N_6F$, 421.17828, found m/z 421.17789 [$(M+H)^+$].

4-(6-aminopyridin-3-yl)-7-cyclohexyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12k): The title compound **12k** (19 mg) was prepared from 4-chloro-7-cyclohexyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11k)** (100 mg, 0.28 mmol) and (6-aminopyridin-3-yl)boronic acid (46 mg, 0.33 mmol) using the procedure described for compound **12a** in 16% yield as a light green solid. Melting point: 250–252 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (d, J = 2.4 Hz, 1H), 8.00 (dd, J = 8.8, 2.5 Hz, 1H), 6.51 (d, J = 8.8 Hz, 1H), 6.43 (s, 2H), 4.13 (ddt, J = 12.1, 7.4, 3.8 Hz, 1H), 3.80–3.66 (m, 10*H*), 2.35–2.20 (m, 2H), 1.81 (d, J = 12.9 Hz, 2H), 1.62 (dd, J = 23.6, 12.3 Hz, 3H), 1.31 (dd, J = 9.9, 6.7 Hz, 2H), 1.16 (d, J = 12.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.56, 165.90, 160.66, 160.26, 154.34, 148.69, 136.46, 121.06, 107.48, 99.15, 66.07, 50.89, 44.29, 34.25, 28.76, 25.61, 25.18. HRMS Calcd for $C_{21}H_{27}O_2N_6$, 395.21900, found m/z 395.21811 [$(M+H)^+$].

4-(6-aminopyridin-3-yl)-7-cyclopropyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12l): The title compound **12l** (44 mg) was prepared from 4-chloro-7-cyclopropyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11l)** (100 mg, 0.34 mmol) and (6-aminopyridin-3-yl)boronic acid (57 mg, 0.41 mmol) using the procedure described for compound **12a** in 37% yield as a light green solid. Melting point: 276–278 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (d, J = 2.4 Hz, 1H), 8.00 (dd, J = 8.8, 2.4 Hz, 1H), 6.51 (d, J = 8.8 Hz, 1H), 6.44 (s, 2H), 3.75 (dd, J = 5.9, 3.9 Hz, 4H), 3.72–3.65 (m, 6H), 2.80–2.73 (m, 1H), 0.99–0.90 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 175.27, 166.56, 160.60, 160.35, 154.05, 148.66, 136.37, 120.95, 107.38, 98.84, 66.03, 44.19, 34.15, 22.16, 4.98. HRMS Calcd for $C_{18}H_{21}O_2N_6$, 353.17205, found m/z 353.17133 [$(M+H)^+$].

4-(6-aminopyridin-3-yl)-7-isopropyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12m): The title compound **12m** (32 mg) was prepared from 4-chloro-7-isopropyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11m)** (100 mg, 0.34 mmol) and (6-aminopyridin-3-yl)boronic acid (57 mg, 0.41 mmol) using the procedure described for compound **12a** in 27% yield as a light yellow solid. Melting point: 243–245 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.00 (dd, J = 8.8, 2.4 Hz, 1H), 6.52 (d, J = 8.8 Hz, 1H), 6.44 (s, 2H), 4.55 (p, J = 6.9 Hz, 1H), 3.73 (s, 2H), 3.72 (d, J = 4.0 Hz, 4H), 3.68 (dd, J = 5.7, 3.6 Hz, 4H), 1.42 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.42, 165.78, 160.58, 160.19, 154.26, 148.58, 136.38, 121.05, 107.44, 99.15, 65.99, 44.22, 42.91, 34.26, 19.25. HRMS Calcd for $C_{18}H_{23}O_2N_6$, 355.18770, found m/z 355.18713 [$(M+H)^+$].

Methyl (S)-2-(4-(6-aminopyridin-3-yl)-2-morpholino-6-oxo-5,6-dihydro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)propanoate (12n): The title compound **12n** (40 mg) was prepared from methyl (*S*)-2-(4-chloro-2-morpholino-6-oxo-5,6-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)propanoate **(11n)** (100 mg, 0.29 mmol) and (6-aminopyridin-3-yl)boronic acid (49 mg, 0.35 mmol) using the procedure described for compound **12a** in 35% yield as a light green solid. Melting point: 184–186 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, J = 2.4 Hz, 1H), 8.03 (dd, J = 8.8, 2.5 Hz, 1H), 6.55–6.51 (m, 1H), 6.48 (s, 2H), 5.09 (q, J = 7.1 Hz, 1H), 3.88 (d, J = 1.9 Hz, 2H), 3.68 (h, J = 3.9 Hz, 8H), 3.61 (s, 3H), 1.50 (d, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.93, 170.08, 164.42, 160.69, 159.93, 154.85, 148.76, 136.40, 120.77, 107.43, 98.66, 65.92, 52.28, 47.42, 44.08, 34.08, 14.43. HRMS Calcd for $C_{19}H_{23}O_4N_6$, 399.17753, found m/z 399.17706 [$(M+H)^+$].

Methyl (R)-2-(4-(6-aminopyridin-3-yl)-2-morpholino-6-oxo-5,6-dihydro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)propanoate (12o): The title compound **12o** (27 mg) was prepared from methyl (*R*)-2-(4-chloro-2-morpholino-6-oxo-5,6-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)propanoate **(11o)** (100 mg, 0.29 mmol) and (6-aminopyridin-3-yl)boronic acid (49 mg, 0.35 mmol) using the procedure described for compound **12a** in 23% yield as a light black solid. Melting point: 167–169 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62–8.55 (m, 1H), 8.03 (dd, J = 8.8, 2.5 Hz, 1H), 6.53 (dd, J = 8.8, 0.7 Hz, 1H), 6.48 (s, 2H), 5.09 (q, J = 7.1 Hz, 1H), 3.88 (d, J = 1.9 Hz, 2H), 3.69 (d, J = 3.1 Hz, 4H), 3.66 (d, J = 3.2 Hz, 4H), 3.61 (s, 3H), 1.50 (d, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.93, 170.08, 164.42, 160.69, 159.93, 154.85, 148.75, 136.40, 120.77, 107.43, 98.66, 65.92, 52.28, 47.43, 44.08, 34.08, 14.43. HRMS Calcd for $C_{19}H_{23}O_4N_6$, 399.17753, found m/z 399.17734 [$(M+H)^+$].

4-(6-aminopyridin-3-yl)-7-(2-hydroxyethyl)-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12p): The title compound **12p** (25 mg) was prepared from 4-chloro-7-(2-hydroxyethyl)-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11p)** (100 mg, 0.34 mmol) and (6-aminopyridin-3-yl)boronic acid (57 mg, 0.41 mmol) using the procedure described for compound **12a** in 21% yield as a light green solid. Melting point: 246–248 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (d, J = 2.3 Hz, 1H), 8.02 (dd, J = 8.8, 2.5 Hz, 1H), 6.52 (d,

J = 8.7 Hz, 1H), 6.45 (s, 2H), 4.80 (t, J = 5.8 Hz, 1H), 3.75 (s, 2H), 3.73 (d, J = 5.3 Hz, 4H), 3.71 (s, 2H), 3.69–3.66 (m, 4H), 3.63 (t, J = 5.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.85, 165.87, 160.55, 160.23, 154.05, 148.55, 136.37, 120.92, 107.42, 99.09, 65.98, 57.35, 44.14, 41.19, 34.15. HRMS Calcd for $C_{17}H_{21}O_3N_6$, 357.16697, found m/z 357.16620 [$(M+H)^+$].

4-(3-aminophenyl)-7-cyclohexyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12q): The title compound **12q** (28 mg) was prepared from 4-chloro-7-cyclohexyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11k)** (100 mg, 0.30 mmol) and 3-aminobenzeneboronic acid (49 mg, 0.36 mmol) using the procedure described for compound **12a** in 24% yield as a light yellow solid. Melting point: 167–169 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.30–7.18 (m, 1H), 7.11 (d, J = 5.8 Hz, 2H), 6.65 (dt, J = 5.9, 2.7 Hz, 1H), 5.20 (s, 2H), 4.14 (td, J = 10.2, 8.3, 6.0 Hz, 1H), 3.73 (tt, J = 9.3, 4.3 Hz, 10*H*), 2.29 (tt, J = 12.8, 7.1 Hz, 2H), 1.82 (d, J = 13.0 Hz, 2H), 1.73–1.55 (m, 3H), 1.39–1.25 (m, 2H), 1.19 (t, J = 12.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.41, 166.07, 160.23, 156.33, 148.75, 137.70, 128.84, 115.51, 115.42, 113.25, 100.95, 65.97, 50.84, 44.22, 34.34, 28.63, 25.51, 25.09. HRMS Calcd for $C_{22}H_{28}O_2N_5$, 394.22375, found m/z 394.22360 [$(M+H)^+$].

4-(4-aminophenyl)-7-cyclohexyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12r): The title compound **12r** (39 mg) was prepared from 4-chloro-7-cyclohexyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11k)** (100 mg, 0.30 mmol) and 4-aminophenylboronic acid pinacol ester (79 mg, 0.36 mmol) using the procedure described for compound **12a** in 33% yield as a white solid. Melting point: 292–295 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (d, J = 8.7 Hz, 2H), 6.62 (d, J = 8.7 Hz, 2H), 5.62 (s, 2H), 4.13 (ddd, J = 12.2, 8.4, 3.8 Hz, 1H), 3.71 (dd, J = 10.8, 4.6 Hz, 10*H*), 2.29 (qd, J = 12.6, 11.5, 2.9 Hz, 2H), 1.81 (d, J = 13.0 Hz, 2H), 1.63 (dd, J = 24.3, 12.3 Hz, 3H), 1.35–1.11 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.62, 165.84, 160.21, 155.94, 150.88, 129.38, 124.05, 113.25, 98.80, 66.08, 50.81, 44.30, 34.61, 28.78, 25.61, 25.18. HRMS Calcd for $C_{22}H_{28}O_2N_5$, 394.22375, found m/z 394.22336 [$(M+H)^+$].

4-(2-aminopyrimidin-5-yl)-7-cyclohexyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12s): The title compound **12s** (41 mg) was prepared from 4-chloro-7-cyclohexyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11k)** (100 mg, 0.30 mmol) and (2-Aminopyrimidin-5-yl)boronic acid pinacol ester (80 mg, 0.36 mmol) using the procedure described for compound **12a** in 35% yield as a light yellow solid. Melting point: 280–282 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 2H), 7.16 (s, 2H), 4.13 (ddd, J = 12.2, 8.4, 3.7 Hz, 1H), 3.81 (s, 2H), 3.78–3.59 (m, 8H), 2.27 (qd, J = 12.8, 3.6 Hz, 2H), 1.81 (d, J = 12.8 Hz, 2H), 1.70–1.56 (m, 3H), 1.36–1.13 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.43, 165.89, 163.65, 160.20, 157.78, 152.22, 119.34, 99.37, 66.00, 50.86, 44.20, 33.75, 28.66, 25.52, 25.10. HRMS Calcd for $C_{20}H_{26}O_2N_7$, 396.21425, found m/z 396.21381 [$(M+H)^+$].

4-(3-aminophenyl)-7-cyclopropyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12t): The title compound **12 t** (36 mg) was prepared from 4-chloro-7-cyclopropyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11l)** (100 mg, 0.34 mmol) and 3-aminobenzeneboronic acid (56 mg, 0.41 mmol) using the procedure described for compound **12a** in 30% yield as a yellow solid. Melting point: 212–215 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.22 (q, J = 1.4 Hz, 1H), 7.15–7.07 (m, 2H), 6.65 (dt, J = 6.3, 2.4 Hz, 1H), 5.23 (s, 2H), 3.77 (dd, J = 5.6, 3.5 Hz, 4H), 3.73–3.66 (m, 6H), 2.76 (tt, J = 7.4, 3.6 Hz, 1H), 0.99–0.90 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 175.31, 166.87, 160.43, 156.14, 148.86, 137.69, 128.90, 115.51, 115.45, 113.31, 100.80, 66.04, 44.22, 34.36, 22.18, 5.00. HRMS Calcd for $C_{19}H_{22}O_2N_5$, 352.17680, found m/z 352.17621 [$(M+H)^+$].

4-(4-aminophenyl)-7-cyclopropyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12u): The title compound **12u** (28 mg) was prepared from 4-chloro-7-cyclopropyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11l)** (100 mg, 0.34 mmol) and 4-aminophenylboronic acid pinacol ester (90 mg, 0.41 mmol) using the procedure described for compound **12a** in 23% yield as a light yellow solid. Melting point: 265–268 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (d, J = 8.7 Hz, 2H), 6.62 (d, J = 8.7 Hz, 2H), 5.62 (s, 2H), 3.75 (dd, J = 6.0, 4.0 Hz, 4H), 3.71–3.62 (m, 6H), 2.75 (ddd, J = 7.9, 6.5, 3.6 Hz, 1H), 1.00–0.89 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 175.37, 166.52, 160.33, 155.68, 150.83, 129.34, 123.98, 113.20, 98.52, 66.06, 44.22, 34.52, 22.17, 4.97. HRMS Calcd for $C_{19}H_{22}O_2N_5$, 352.17680, found m/z 352.17648 [$(M+H)^+$].

4-(2-aminopyrimidin-5-yl)-7-cyclopropyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12v): The title compound **12v** (34 mg) was prepared from 4-chloro-7-cyclopropyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11l)** (100 mg, 0.34 mmol) and (2-Aminopyrimidin-5-yl)boronic acid pinacol ester (91 mg, 0.41 mmol) using the procedure described for compound **12a** in 28% yield as a light yellow solid. Melting point: 281–283 °C, ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.82 (s, 2H), 7.17 (s, 2H), 3.75 (d, J = 8.2 Hz, 6H), 3.68 (t, J = 4.8 Hz, 4H), 2.76 (tt, J = 7.2, 4.1 Hz, 1H), 0.97–0.90 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 175.21, 166.63, 163.61, 160.36, 157.79, 151.97, 119.31, 99.14, 66.02, 44.17, 33.69, 22.17, 4.98. HRMS Calcd for $C_{17}H_{20}O_2N_7$, 354.16730, found m/z 354.16660 [$(M+H)^+$].

4-(3-aminophenyl)-7-isopropyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12w): The title compound **12w** (18 mg) was prepared from 4-chloro-7-isopropyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11m)** (100 mg, 0.34 mmol) and 3-aminobenzeneboronic acid (56 mg, 0.41 mmol) using the procedure described for compound **12a** in 15% yield as a green solid. Melting point: 178–180 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25–7.21 (m, 1H), 7.15–7.08 (m, 2H), 6.67–6.64 (m, 1H), 5.21 (s, 2H), 4.58 (q, J = 6.8 Hz, 1H), 3.75 (dd, J = 5.6, 3.5 Hz, 4H), 3.73–3.68 (m, 6H), 1.44 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.40, 166.03, 160.25, 156.33, 148.76, 137.73, 128.87, 124.86, 115.56, 113.28, 101.04, 65.97, 44.23, 42.93, 34.43, 19.22. HRMS Calcd for $C_{19}H_{24}O_2N_5$, 354.19245, found m/z 354.19226 [$(M+H)^+$].

4-(4-aminophenyl)-7-isopropyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12x): The title compound **12x** (35 mg) was prepared from 4-chloro-7-isopropyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11m)** (100 mg, 0.34 mmol) and 4-aminophenylboronic acid pinacol ester (90 mg, 0.41 mmol) using the procedure described for compound **12a** in 29% yield as a yellow solid. Melting point: 260–262 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.74 (d, J = 8.7 Hz, 2H), 6.62 (d, J = 8.7 Hz, 2H), 5.62 (s, 2H), 4.56 (p, J = 6.9 Hz, 1H), 3.73 (d, J = 5.4 Hz, 6H), 3.69 (dd, J = 5.3, 3.4 Hz, 4H), 1.43 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.46, 165.71, 160.14, 155.87, 150.78, 129.28, 124.04, 113.20, 98.79, 65.98, 44.22, 42.79, 34.60, 19.27. HRMS Calcd for $C_{19}H_{24}O_2N_5$, 354.19245, found m/z 354.19223 [$(M+H)^+$].

4-(2-aminopyrimidin-5-yl)-7-isopropyl-2-morpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (12y): The title compound **12y** (38 mg) was prepared from 4-chloro-7-isopropyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(11m)** (100 mg, 0.34 mmol) and (2-Aminopyrimidin-5-yl)boronic acid pinacol ester (91 mg, 0.41 mmol) using the procedure described for compound **12a** in 31% yield as a white solid. Melting point: 297–299 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 2H), 7.16 (s, 2H), 4.60–4.52 (m, 1H), 3.80 (s, 2H), 3.74 (dd, J = 5.9, 3.6 Hz, 4H), 3.68 (dd, J = 5.7, 3.7 Hz, 4H), 1.43 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.32, 165.80, 163.62, 160.17, 157.73, 152.18, 119.33, 99.41, 65.94, 44.17, 42.93, 33.78, 19.20. HRMS Calcd for $C_{17}H_{22}O_2N_7$, 356.18295, found m/z 356.18250 [$(M+H)^+$].

N-(3-(7-cyclohexyl-2-morpholino-6-oxo-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)acetamide (13q): Acetyl chloride (5 mg, 0.06 mmol) was added to a solution of 4-(3-aminophenyl)-7-cyclohexyl-2-morpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(12q)** (20 mg, 0.05 mmol) and DIPEA (8 mg, 0.06 mmol) in DCM (5 mL) in an

ice bath. The resulting mixture was stirred at room temperature until complete consumption of raw materials. Water (50 mL) was added to the reaction mixture then extracted with DCM (2 × 30 mL). The organic phases were combined, washed by brine, dried over Na_2SO_4, evaporated and purified by chromatography on silica gel to afford the title compound **13** (10 mg, 46% yield) as a green solid. Melting point: 183–185 °C, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.13 (d, J = 2.0 Hz, 1H), 7.78 (s, 1H), 7.65–7.54 (m, 2H), 7.38 (t, J = 7.9 Hz, 1H), 4.30–4.19 (m, 1H), 3.93–3.74 (m, 8H), 3.64 (s, 2H), 2.36 (dd, J = 12.5, 3.6 Hz, 2H), 2.19 (s, 3H), 1.90–1.83 (m, 2H), 1.74–1.64 (m, 3H), 1.37 (d, J = 13.4 Hz, 3H). HRMS Calcd for $C_{24}H_{30}O_3N_5$, 436.23432, found m/z 436.23407 [$(M+H)^+$].

Methyl 2-(4-chloro-6-(4-methoxybenzy)amino)-2-(pyrrolidin-1-yl)pyrimidin-5-yl)acetate (14a): The title compound **14a** (231 mg) was prepared from methyl 2-(2,4-dichloro-6-((4-methoxybenzyl) amino)pyrimidin-5-yl)acetate **(6)** (250 mg, 0.70 mmol) and tetrahydro pyrrole (100 mg, 1.40 mmol) using the procedure described for compound **7** in 85% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.51 (t, J = 5.9 Hz, 1H), 7.26–7.20 (m, 2H), 6.87–6.82 (m, 2H), 4.44 (d, J = 5.8 Hz, 2H), 3.71 (s, 3H), 3.62 (s, 3H), 3.58 (s, 2H), 3.35 (s, 4H), 1.88–1.80 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.56, 161.46, 158.10, 157.99, 157.75, 132.17, 128.56, 113.45, 95.82, 54.99, 51.72, 46.09, 43.43, 31.35, 24.89.

Methyl 2-(4-chloro-6-(4-methoxybenzylamino)-2-thiomorpholinopyrimidin-5-yl)acetate (14b): The title compound **14b** (222 mg) was prepared from methyl 2-(2,4-dichloro-6-((4-methoxybenzyl)amino) pyrimidin-5-yl)acetate **(6)** (250 mg, 0.70 mmol) and thiomorpholine (144 mg, 1.40 mmol) using the procedure described for compound **7** in 75% yield as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (s, 1H), 7.23 (d, J = 8.1 Hz, 2H), 6.86 (d, J = 8.1 Hz, 2H), 4.34 (d, J = 6.1 Hz, 2H), 3.71 (s, 3H), 3.64 (s, 3H), 3.50 (s, 2H), 3.41 (d, J = 4.9 Hz, 4H), 2.66 (d, J = 6.1 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.15, 161.43, 159.43, 158.06, 131.98, 128.46, 128.30, 113.53, 113.53, 54.96, 51.91, 51.14, 43.60, 33.92, 26.23.

Methyl 2-(4-chloro-6-(4-methoxybenzylamino)-2-(piperidin-1-yl) pyrimidin-5-yl)acetate (14c): The title compound **14c** (181 mg) was prepared from methyl 2-(2,4-dichloro-6-((4-methoxybenzyl)amino) pyrimidin-5-yl)acetate **(6)** (250 mg, 0.70 mmol) and piperidine (120 mg, 1.40 mmol) using the procedure described for compound **7** in 64% yield as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (s, 1H), 7.25–7.16 (m, 2H), 6.90–6.82 (m, 2H), 4.42 (d, J = 5.7 Hz, 2H), 3.71 (s, 3H), 3.62 (s, 3H), 3.59 (d, J = 5.2 Hz, 6H), 1.56 (d, J = 5.5 Hz, 2H), 1.43 (d, J = 10.0 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.50, 161.61, 158.65, 158.35, 157.99, 132.00, 128.37, 113.48, 96.01, 55.00, 51.72, 44.12, 43.53, 31.33, 25.17, 24.25.

Methyl 2-(4-chloro-6-(4-methoxybenzylamino)-2-(4-methylpiperazin-1-yl)pyrimidin-5-yl)acetate (14d): The title compound **14d** (232 mg) was prepared from methyl 2-(2,4-dichloro-6-((4-methoxybenzyl)amino)pyrimidin-5-yl)acetate **(6)** (250 mg, 0.70 mmol) and 1-methylpiperazine (140 mg, 1.40 mmol) using the procedure described for compound **7** in 79% yield as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60 (s, 1H), 7.21 (d, J = 8.5 Hz, 2H), 6.93–6.77 (m, 2H), 4.43 (d, J = 5.8 Hz, 2H), 3.71 (s, 3H), 3.62 (s, 3H), 3.58 (dd, J = 9.6, 4.9 Hz, 6H), 2.25 (t, J = 5.0 Hz, 4H), 2.16 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.41, 161.61, 158.82, 158.28, 158.02, 131.89, 128.40, 113.49, 96.68, 54.99, 54.28, 51.75, 45.75, 43.54, 43.21, 31.34.

4-chloro-7-(4-methoxybenzyl)-2-(pyrrolidin-1-yl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (15a): The title compound **15a** (139 mg) was prepared from methyl 2-(4-chloro-6-((4-methoxybenzyl) amino)-2-(pyrrolidin-1-yl)pyrimidin-5-yl)acetate **(14a)** (215 mg, 0.55 mmol) using the procedure described for compound **8** in 70% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37–7.25 (m, 2H), 6.87 (d, J = 8.6 Hz, 2H), 4.70 (s, 2H), 3.70 (d, J = 3.2 Hz, 3H), 3.53 (s, 2H), 3.42 (s, 4H), 1.91 (d, J = 6.9 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.74, 165.38, 158.80, 158.65, 151.47, 129.64, 128.41, 113.78, 100.47, 55.03, 46.59, 41.79, 32.33, 24.79.

4-chloro-7-(4-methoxybenzyl)-2-thiomorpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (15b): The title compound **15b** (115 mg) was prepared from methyl 2-(4-chloro-6-((4-methoxybenzyl) amino)-2-thiomorpholinopyrimidin-5-yl)acetate **(14b)** (210 mg, 0.50 mmol) using the procedure described for compound **8** in 59% yield as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29 (d, J = 8.2 Hz, 2H), 6.87 (d, J = 8.2 Hz, 2H), 4.70 (s, 2H), 4.02 (t, J = 4.9 Hz, 4H), 3.71 (s, 4H), 3.54 (s, 2H), 2.62–2.55 (m, 4H).

4-chloro-7-(4-methoxybenzyl)-2-(piperidin-1-yl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (15c): The title compound **15c** (90 mg) was prepared from methyl 2-(4-chloro-6-((4-methoxybenzyl)amino)-2-(piperidin-1-yl)pyrimidin-5-yl)acetate **(14c)** (160 mg, 0.40 mmol) using the procedure described for compound **8** in 60% yield as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32–7.24 (m, 2H), 6.86 (d, J = 8.5 Hz, 2H), 4.70 (s, 2H), 3.71 (d, J = 5.9 Hz, 7H), 3.52 (s, 2H), 1.62 (t, J = 6.0 Hz, 2H), 1.50 (q, J = 5.6 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.78, 165.71, 159.90, 158.66, 151.66, 129.51, 128.42, 113.78, 100.84, 55.06, 44.67, 41.81, 32.35, 25.13, 24.14.

4-chloro-7-(4-methoxybenzyl)-2-(4-methylpiperazin-1-yl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (15d): The title compound **15d** (113 mg) was prepared from methyl 2-(4-chloro-6-((4-methoxybenzyl)amino)-2-(4-methylpiperazin-1-yl)pyrimidin-5-yl)acetate **(14d)** (210 mg, 0.50 mmol) using the procedure described for compound **8** in 58% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28 (d, J = 8.2 Hz, 2H), 6.90–6.84 (m, 2H), 4.70 (s, 2H), 3.70 (d, J = 3.7 Hz, 7H), 3.53 (s, 2H), 2.33 (t, J = 5.1 Hz, 4H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.74, 165.72, 160.05, 158.65, 151.57, 129.48, 128.34, 113.77, 101.60, 55.04, 54.12, 45.67, 43.62, 41.82, 32.34.

4-chloro-2-(3-hydroxypiperidin-1-yl)-7-(4-methoxybenzyl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (15e): The title compound **15e** (98 mg) was prepared from methyl 2-(2,4-dichloro-6-((4-methoxybenzyl)amino)pyrimidin-5-yl)acetate **(6)** (250 mg, 0.70 mmol) and 3-hydroxypiperidine (141 mg, 1.40 mmol) directly using the procedure described for compound **7** in 35% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.30 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 4.94 (s, 1H), 4.70 (s, 2H), 4.38–4.30 (m, 1H), 4.20 (d, J = 12.4 Hz, 1H), 3.71 (s, 3H), 3.51 (s, 2H), 3.46 (s, 1H), 3.14–3.04 (m, 1H), 2.95 (t, J = 10.9 Hz, 1H), 1.89 (d, J = 9.9 Hz, 1H), 1.73 (q, J = 6.3, 4.2 Hz, 1H), 1.43–1.31 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.74, 165.67, 160.05, 158.68, 151.62, 129.58, 128.40, 113.78, 100.98, 65.01, 55.04, 50.86, 43.76, 41.82, 33.12, 32.34, 22.51.

4-chloro-2-(4-hydroxypiperidin-1-yl)-7-(4-methoxybenzyl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (15f): The title compound **15f** (107 mg) was prepared from methyl 2-(2,4-dichloro-6-((4-methoxybenzyl)amino)pyrimidin-5-yl)acetate **(6)** (250 mg, 0.70 mmol) and 4-hydroxypiperidine (141 mg, 1.40 mmol) directly using the procedure described for compound **7** in 39% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31–7.26 (m, 2H), 6.86 (d, J = 8.6 Hz, 2H), 4.74 (d, J = 4.1 Hz, 1H), 4.70 (s, 2H), 4.17 (d, J = 13.2 Hz, 2H), 3.71 (s, 4H), 3.53 (s, 2H), 1.80–1.72 (m, 2H), 1.31 (dtd, J = 12.8, 9.1, 3.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.74, 165.73, 159.90, 158.64, 151.63, 129.49, 128.39, 113.76, 101.06, 65.73, 55.04, 41.80, 41.43, 33.70, 32.34.

4-(3-aminophenyl)-7-(4-methoxybenzyl)-2-(pyrrolidin-1-yl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (16a): The title compound **16a** (59 mg) was prepared from 4-chloro-7-(4-methoxybenzyl)-2-(pyrrolidin-1-yl)-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(15a)** (115 mg, 0.32 mmol) and 3-aminobenzeneboronic acid (48 mg, 0.35 mmol) using the procedure described for compound **12a** in 44% yield as a purple solid. Melting point: 210–212 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39–7.33 (m, 2H), 7.24 (d, J = 2.4 Hz, 1H), 7.11 (d, J = 6.7 Hz, 2H), 6.90–6.85 (m, 2H), 6.65 (dd, J = 6.8, 2.1 Hz, 1H), 5.17 (s, 2H), 4.75 (s, 2H), 3.76 (s, 2H), 3.71 (s, 3H), 3.57 (d, J = 6.5 Hz, 4H), 1.97–1.91 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.63, 165.27, 158.99, 158.57, 156.62, 148.65, 137.85, 129.67, 128.91, 128.76,

115.56, 115.34, 113.75, 113.34, 99.43, 55.02, 46.33, 41.39, 34.32, 24.93. HRMS Calcd for $C_{24}H_{26}O_2N_5$, 416.20810, found *m/z* 416.20792 $[(M+H)^+]$.

4-(3-aminophenyl)-7-(4-methoxybenzyl)-2-thiomorpholino-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (16b): The title compound **16b** (41 mg) was prepared from 4-chloro-7-(4-methoxybenzyl)-2-thiomorpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(15b)** (100 mg, 0.26 mmol) and 3-aminobenzeneboronic acid (39 mg, 0.28 mmol) using the procedure described for compound **12a** in 35% yield as a green solid. Melting point: 201–203 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.33 (d, J = 8.2 Hz, 2H), 7.21 (s, 1H), 7.11 (d, J = 6.9 Hz, 2H), 6.88 (d, J = 8.2 Hz, 2H), 6.66 (d, J = 6.7 Hz, 1H), 5.20 (s, 2H), 4.84–4.71 (m, 2H), 4.14 (d, J = 5.0 Hz, 4H), 3.78 (s, 2H), 3.71 (s, 3H), 2.62 (d, J = 4.9 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.58, 165.69, 159.61, 158.59, 156.61, 148.76, 137.60, 129.58, 128.87, 128.78, 115.52, 115.51, 113.76, 113.27, 100.56, 55.05, 46.24, 41.48, 34.30, 25.78. HRMS Calcd for $C_{24}H_{26}O_2N_5S$, 448.18017, found *m/z* 448.17953 $[(M+H)^+]$.

4-(3-aminophenyl)-7-(4-methoxybenzyl)-2-(piperidin-1-yl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (16c): The title compound **16c** (35 mg) was prepared from 4-chloro-7-(4-methoxybenzyl)-2-(piperidin-1-yl)-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(15c)** (80 mg, 0.22 mmol) and 3-aminobenzeneboronic acid (32 mg, 0.24 mmol) using the procedure described for compound **12a** in 37% yield as a purple solid. Melting point: 197–200 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.36–7.30 (m, 2H), 7.26–7.18 (m, 1H), 7.10 (d, J = 6.6 Hz, 2H), 6.90–6.85 (m, 2H), 6.65 (dd, J = 6.6, 2.5 Hz, 1H), 5.20 (s, 2H), 4.75 (s, 2H), 3.81 (t, J = 5.3 Hz, 4H), 3.77 (s, 2H), 3.71 (s, 3H), 1.64 (s, 2H), 1.53 (d, J = 7.2 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.65, 165.55, 160.10, 158.57, 156.56, 148.74, 137.81, 129.50, 128.89, 128.83, 115.52, 115.41, 113.75, 113.24, 99.84, 55.04, 44.51, 41.41, 34.33, 25.21, 24.40. HRMS Calcd for $C_{25}H_{28}O_2N_5$, 430.22375, found *m/z* 430.22366 $[(M+H)^+]$.

4-(3-aminophenyl)-7-(4-methoxybenzyl)-2-(4-methylpiperazin-1-yl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (16d): The title compound **16d** (48 mg) was prepared from 4-chloro-7-(4-methoxybenzyl)-2-(4-methylpiperazin-1-yl)-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(15d)** (100 mg, 0.25 mmol) and 3-aminobenzeneboronic acid (39 mg, 0.28 mmol) using the procedure described for compound **12a** in 43% yield as a white solid. Melting point: 221–223 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.38–7.30 (m, 2H), 7.23 (t, J = 1.6 Hz, 1H), 7.11 (d, J = 5.9 Hz, 2H), 6.90–6.84 (m, 2H), 6.66 (dt, J = 5.9, 2.6 Hz, 1H), 5.19 (s, 2H), 4.76 (s, 2H), 3.83–3.76 (m, 6H), 3.71 (s, 3H), 2.37 (t, J = 5.0 Hz, 4H), 2.22 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.64, 165.58, 160.20, 158.58, 156.49, 148.76, 137.65, 129.48, 128.86, 128.83, 115.54, 115.48, 113.77, 113.26, 100.56, 55.04, 54.37, 45.82, 43.57, 41.44, 34.33. HRMS Calcd for $C_{25}H_{29}O_2N_6$, 445.23465, found *m/z* 445.23407 $[(M+H)^+]$.

4-(3-aminophenyl)-2-(3-hydroxypiperidin-1-yl)-7-(4-methoxybenzyl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (16e): The title compound **16e** (42 mg) was prepared from 4-chloro-2-(3-hydroxypiperidin-1-yl)-7-(4-methoxybenzyl)-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(15e)** (100 mg, 0.25 mmol) and 3-aminobenzeneboronic acid (39 mg, 0.28 mmol) using the procedure described for compound **12a** in 37% yield as a yellow solid. Melting point: 204–206 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.40–7.31 (m, 2H), 7.26–7.18 (m, 1H), 7.17–7.05 (m, 2H), 6.92–6.84 (m, 2H), 6.66 (dt, J = 6.1, 2.6 Hz, 1H), 5.20 (s, 2H), 4.90 (d, J = 4.5 Hz, 1H), 4.76 (s, 2H), 4.65–4.55 (m, 1H), 4.45 (d, J = 12.9 Hz, 1H), 3.76 (d, J = 2.0 Hz, 2H), 3.72 (s, 3H), 3.52–3.44 (m, 1H), 3.02 (d, J = 10.4 Hz, 1H), 2.89 (dd, J = 12.5, 9.1 Hz, 1H), 1.93 (s, 1H), 1.75 (s, 1H), 1.45–1.35 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 175.14, 166.03, 160.65, 159.08, 157.11, 149.22, 138.29, 130.07, 129.37, 129.33, 116.04, 115.92, 114.26, 113.76, 100.50, 65.74, 55.53, 51.42, 44.04, 41.91, 34.80, 34.05, 23.34. HRMS Calcd for $C_{25}H_{28}O_3N_5$, 446.21867, found *m/z* 446.21817 $[(M+H)^+]$.

4-(3-aminophenyl)-2-(4-hydroxypiperidin-1-yl)-7-(4-methoxybenzyl)-5,7-dihydro-6H-pyrrolo[2,3-d]pyrimidin-6-one (16f): The title compound **16f** (35 mg) was prepared from 4-chloro-2-(4-hydroxypiperidin-1-yl)-7-(4-methoxybenzyl)-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(15f)** (100 mg, 0.25 mmol) and 3-aminobenzeneboronic acid (39 mg, 0.28 mmol) using the procedure described for compound **12a** in 31% yield as a yellow solid. Melting point: 190–192 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37–7.30 (m, 2H), 7.22 (d, J = 2.4 Hz, 1H), 7.10 (d, J = 6.9 Hz, 2H), 6.90–6.84 (m, 2H), 6.65 (dt, J = 7.1, 2.2 Hz, 1H), 5.20 (s, 2H), 4.75 (s, 2H), 4.71 (d, J = 4.3 Hz, 1H), 4.37 (d, J = 13.1 Hz, 2H), 3.74 (d, J = 23.4 Hz, 6H), 3.29 (d, J = 13.6 Hz, 2H), 1.80 (d, J = 12.5 Hz, 2H), 1.33 (d, J = 9.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.63, 165.59, 160.09, 158.57, 156.57, 148.74, 137.76, 129.52, 128.88, 128.84, 115.52, 115.43, 113.75, 113.25, 100.05, 66.27, 55.04, 41.45, 34.32, 33.91, 26.30. HRMS Calcd for $C_{25}H_{28}O_3N_5$, 446.21867, found *m/z* 446.21820 $[(M+H)^+]$.

N-(3-(7-(4-methoxybenzyl)-6-oxo-2-(pyrrolidin-1-yl)-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)acetamide (17a): The title compound 17a (17 mg) was prepared from 4-(3-aminophenyl)-7-(4-methoxybenzyl)-2-(pyrrolidin-1-yl)-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(16a)** (30 mg, 0.07 mmol) using the procedure described for compound **13** in 53% yield as a brown solid. Melting point: 131–133 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.24–8.13 (m, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.67 (d, J = 7.8 Hz, 1H), 7.45–7.31 (m, 3H), 6.87 (d, J = 8.2 Hz, 2H), 4.74 (s, 2H), 3.78 (s, 2H), 3.70 (d, J = 1.4 Hz, 3H), 3.58 (d, J = 6.7 Hz, 4H), 2.07 (s, 3H), 1.94 (d, J = 6.9 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.52, 168.37, 165.47, 158.97, 158.59, 155.42, 139.46, 137.55, 129.69, 128.83, 128.70, 122.34, 120.17, 118.44, 113.75, 99.66, 55.01, 46.34, 41.45, 34.23, 24.92, 24.01. HRMS Calcd for $C_{26}H_{28}O_3N_5$, 458.21867, found *m/z* 458.21817 $[(M+H)^+]$.

N-(3-(7-(4-methoxybenzyl)-6-oxo-2-thiomorpholino-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)acetamide (17b): The title compound **17b** (10 mg) was prepared from 4-(3-aminophenyl)-7-(4-methoxybenzyl)-2-thiomorpholino-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(16b)** (20 mg, 0.04 mmol) using the procedure described for compound **13** in 46% yield as a yellow solid. Melting point: 137–139 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.16 (s, 1H), 7.76 (d, J = 8.1 Hz, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.40 (t, J = 7.9 Hz, 1H), 7.33 (d, J = 8.2 Hz, 2H), 6.88 (d, J = 8.2 Hz, 2H), 4.76 (s, 2H), 4.15 (t, J = 4.7 Hz, 4H), 3.81 (s, 2H), 3.71 (s, 3H), 2.62 (t, J = 4.8 Hz, 4H), 2.07 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.46, 168.41, 165.88, 159.64, 158.61, 155.46, 139.52, 137.31, 129.59, 128.82, 128.70, 122.34, 120.32, 118.37, 113.76, 100.83, 55.04, 46.27, 41.54, 34.20, 25.78, 24.02. HRMS Calcd for $C_{26}H_{28}O_3N_5S$, 490.19074, found *m/z* 490.19009 $[(M+H)^+]$.

N-(3-(7-(4-methoxybenzyl)-6-oxo-2-(piperidin-1-yl)-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)acetamide (17c): The title compound **17c** (14 mg) was prepared from 4-(3-aminophenyl)-7-(4-methoxybenzyl)-2-(piperidin-1-yl)-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(16c)** (20 mg, 0.05 mmol) using the procedure described for compound **13** in 64% yield as a light yellow solid. Melting point: 166–168 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.16 (s, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.39 (t, J = 7.9 Hz, 1H), 7.33 (d, J = 8.2 Hz, 2H), 6.87 (d, J = 8.2 Hz, 2H), 4.76 (s, 2H), 3.82 (dd, J = 11.1, 5.7 Hz, 6H), 3.71 (s, 3H), 2.07 (s, 3H), 1.64 (d, J = 6.0 Hz, 2H), 1.54 (d, J = 7.5 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.57, 168.41, 165.76, 160.12, 158.59, 155.42, 139.52, 137.54, 129.52, 128.82, 128.82, 122.35, 120.25, 118.35, 113.76, 100.13, 55.04, 44.53, 41.48, 34.23, 25.21, 24.39, 24.04. HRMS Calcd for $C_{27}H_{30}O_3N_5$, 472.23432, found *m/z* 472.23389 $[(M+H)^+]$.

N-(3-(7-(4-methoxybenzyl)-2-(4-methylpiperazin-1-yl)-6-oxo-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)acetamide (17d): The title compound **17d** (18 mg) was prepared from 4-(3-aminophenyl)-7-(4-methoxybenzyl)-2-(4-methylpiperazin-1-yl)-5,7-

dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(16d)** (30 mg, 0.07 mmol) using the procedure described for compound **13** in 55% yield as a green solid. Melting point: 159–161 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H), 8.18 (t, J = 2.0 Hz, 1H), 7.78 (dd, J = 8.0, 2.1 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.39 (t, J = 7.9 Hz, 1H), 7.36–7.27 (m, 2H), 6.91–6.82 (m, 2H), 4.76 (s, 2H), 3.86 (s, 4H), 3.81 (s, 2H), 3.70 (s, 3H), 2.54 (s, 4H), 2.32 (s, 3H), 2.07 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.54, 168.49, 165.85, 160.12, 158.62, 155.40, 139.57, 137.29, 129.55, 128.85, 128.74, 122.40, 120.38, 118.41, 113.79, 101.13, 55.06, 53.82, 45.03, 43.01, 41.54, 34.23, 24.05. HRMS Calcd for $C_{27}H_{31}O_3N_6$, 487.24576, found m/z 487.24506 [$(M+H)^+$].

N-(3-(2-(3-hydroxypiperidin-1-yl)-7-(4-methoxybenzyl)-6-oxo-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)acetamide (17e): The title compound **17e** (12 mg) was prepared from 4-(3-aminophenyl)-2-(3-hydroxypiperidin-1-yl)-7-(4-methoxybenzyl)-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(16e)** (20 mg, 0.04 mmol) using the procedure described for compound **13** in 56% yield as a yellow solid. Melting point: 168–170 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.13 (s, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.35 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 4.91 (d, J = 4.5 Hz, 1H), 4.76 (s, 2H), 4.59 (d, J = 12.0 Hz, 1H), 4.44 (d, J = 13.0 Hz, 1H), 3.79 (d, J = 2.4 Hz, 2H), 3.71 (s, 3H), 3.48 (d, J = 4.4 Hz, 1H), 3.06 (t, J = 11.2 Hz, 1H), 2.92 (dd, J = 12.5, 9.1 Hz, 1H), 2.07 (s, 3H), 1.93 (s, 1H), 1.75 (s, 1H), 1.40 (d, J = 8.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.54, 168.49, 165.85, 160.12, 158.62, 155.40, 139.57, 137.29, 129.55, 128.85, 128.74, 122.40, 120.38, 118.41, 113.79, 101.13, 55.06, 53.82, 53.82, 45.03, 45.02, 43.01, 41.54, 34.23, 24.05. HRMS Calcd for $C_{27}H_{30}O_4N_5$, 488.22923, found m/z 488.22876 [$(M+H)^+$].

N-(3-(2-(4-hydroxypiperidin-1-yl)-7-(4-methoxybenzyl)-6-oxo-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)acetamide (17f): The title compound **17f** (10 mg) was prepared from 4-(3-aminophenyl)-2-(4-hydroxypiperidin-1-yl)-7-(4-methoxybenzyl)-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one **(16f)** (20 mg, 0.04 mmol) using the procedure described for compound **13** in 47% yield as a yellow solid. Melting point: 181–183 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.16 (s, 1H), 7.84–7.73 (m, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.40 (t, J = 7.9 Hz, 1H), 7.33 (d, J = 8.3 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 4.76 (s, 2H), 4.72 (d, J = 4.3 Hz, 1H), 4.38 (d, J = 12.8 Hz, 2H), 3.80 (s, 2H), 3.71 (s, 4H), 3.29 (s, 2H), 2.07 (s, 3H), 1.80 (d, J = 12.2 Hz, 2H), 1.34 (d, J = 9.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.55, 168.42, 165.81, 160.13, 158.59, 155.45, 139.52, 137.49, 129.54, 128.81, 128.81, 122.36, 120.28, 118.37, 113.76, 100.35, 66.23, 55.05, 41.47, 41.46, 34.22, 33.90, 24.04. HRMS Calcd for $C_{27}H_{30}O_4N_5$, 488.22923, found m/z 488.22867 [$(M+H)^+$].

4.2. Biological assay

4.2.1. Cytotoxicity assay

The tests were carried out under the condition that the ratio of living cells was greater than 90%. The cell proliferation inhibition assay was performed using the EnoGeneCell™ Counting Kit-8 (CCK-8) cell viability assay kit. The cells were digested, counted, and prepared into a cell suspension at a concentration of 1×10^5 cells/mL. 100 μL of cell suspension (1×10^4 cells per well) was added to each well of a 96-well plate. The 96-well plates were incubated at 37 °C for 24 h under 5% CO_2. 100 μL of the corresponding drug-containing medium was added to each well at a concentration of 50 μM and set up a negative control group, vehicle control group, positive control group, 5 replicate wells per group. The 96 well plates were incubated at 37 °C for 72 h under 5% CO_2. 10 μL of CCK-8 solution was added to each well, and the 96 well plates were incubated in an incubator for 4 h. The OD value at 450 nm was measured with a microplate reader. The inhibition rates of 28 compounds and positive control against human malignant glioblastoma cell line U87MG and human prostate cancer cell line PC-3 were calculated.

4.2.2. Kinase enzyme assay

These assays were carried out as described previously. All of the enzymatic reactions were conducted at 30 °C for 40 min. The 50 μL reaction mixture contains 40 mM Tris, pH 7.4, 10 mM $MgCl_2$, 0.1 mg/mL BSA, 1 mM DTT, 10 μM ATP, 0.2 ug/mL PI3 Kinase and 100 uM lipid substrate. The compounds were diluted in 10% DMSO and 5 μL of reactions. The assay was performed using Kinase-Glo Plus luminescence kinase assay kit. It measures kinase activity by quantitating the amount of ATP remaining in solution following a kinase reaction. The luminescenct signal from the assay was correlated with the amount of ATP present and is inversely correlated with the amount of kinase activity. The IC_{50} values were calculated using nonlinear regression with normalized dose-response fit using Prism GraphPad software.

4.2.3. Western blotting

After drug treatment, cells were washed twice with ice-cold PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4, pH 7.4) and lysed in SDS sample buffer. Cell lysates containing equal amounts of protein were separated by SDS polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. After being blocked in 5% nonfat milk in Tris-buffered saline with 0.1% Tween 20, pH 7.6, the membranes were incubated with the appropriate primary antibodies at 4 °C overnight and then exposed to secondary antibodies for 2 h at room temperature. Immunoreactive proteins were visualized using the enhanced chemiluminescence system from Pierce Chemical.

4.2.4. Cell cycle assay

MCF-7 cells (5×10^5 cells/mL) were seeded in six-well plates and treated with compounds at different concentrations for 24 h. The cells were then harvested by trypsinization and washed twice with cold PBS. After centrifugation and removal of the supernatants, cells were re-suspended in 400 μL of 1 × PBS buffer. After adding 10 μL of PI the cells were incubated at room temperature for 15 min in the dark. The stained cells were analyzed by a flow cytometer (BD Accuri C6).

Acknowledgment

This study was supported by Beijing Natural Science Foundation (No. 2192004). Thanks to Xiuqing Song for the NMR test. The services of cytotoxic activity and cell IC_{50} were kindly provided by Nanjing Ogpharmaceutical Co., Ltd. (Nanjing, China) The service of enzyme IC_{50}, WB and cell cycle was kindly provided by Huawei Pharmaceutical Co., Ltd. (Shandong, China)

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103232.

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