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Design and synthesis of alkyl substituted pyridino[2,3-*D*]pyrimidine compounds as PI3Kα/mTOR dual inhibitors with improved pharmacokinetic properties and potent in vivo antitumor activity

Yinyin Liu[a], Qinhua Xia[a,*], Lei Fang[b,*]

[a] Nanjing University of Chinese Medicine, Nanjing 210046, China
[b] Jiangsu Province Hi-Tech Key Laboratory for Bio-medical Research, School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, China

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ABSTRACT

Using pyridino[2,3-*D*]pyrimidine as the core, total 13 pyridino[2,3-*D*]pyrimidine derivatives with different alkyl substituents at C2 site have been designed and synthesized to search for novel PI3Kα/mTOR dual inhibitors. Most of the target compounds showed potent mTOR inhibition activity with IC_{50} values ranging from single to double digit nanomole. Five target compounds exhibited pronounced PI3Kα inhibition activity. In vitro cellular assay indicated that most of the target compounds showed excellent antiproliferative activity, especially **3j** whose potency against SKOV3 was 8-fold higher than the positive control AZD8055. In vitro metabolic stability study found that **3j** had a comparable stability to that of AZD8055. More importantly, **3j** showed better antitumor activity and pharmacokinetic properties in vivo as compared with AZD8055.

1. Introduction

With the deepening insight on cancer genomics and biology, several key intracellular cancer-related signaling pathways have been discovered. One of the main pathways is the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway that has become the preferred target for anti-cancer drug development. The kinase mTOR, existed in two distinct multiprotein complexes (mTORC1 and mTORC2), has a high degree of active site similarity with PI3Ks.[1,2] AKT attenuates the inhibitory effect of tuberous sclerosis (TSC) protein on mTORC1 by phosphorylating the TSC protein, allowing mTORC1 to be activated by GTPase. Activation of mTOR further achieves a specific gene transcription and translation through the ribosomal protein kinase p70S6K and transcriptional regulator protein 4EBP1 etc. Then the conduction process is completed, which ultimately achieves cell response to extracellular signals.[3,4] Depending on this extracellular signal, tumor cells regulate their continued proliferation, invasion, metastasis and anti-apoptotic activity to maintain their malignant phenotypes.

Due to the central role of mTOR in the vital cellular processes, mTOR inhibitors have emerged as promising anticancer agents. Rapamycin analogs, known as the first generation of mTOR selective inhibitors, have shown to be effective in a series of preclinical models. However, this success is limited to several rare cancers. In the primary solid tumor, single dose treatment with rapamycin analogs can achieve only a moderate overall response rate.[5] The limited success of these single-hit agents may be due to the rapid acquired drug resistance and compensatory mechanisms. In addition, the cross talk and feedback of downstream members of the PI3K/AKT/mTOR pathway can also attenuate the inhibitory effect on the early components. Thereby, to improve the efficacy, PI3Kα/mTOR dual inhibitors have been developed. Since such inhibitors could target at two nodal points of the pathway, the PI3Kα/mTOR dual inhibitors appear to augment efficacy and lower the likelihood to induce drug resistance.[6] Pyridino[2,3-*d*] pyrimidine scaffold seems to be a promising template for the development of PI3Kα/mTOR dual inhibitors. In 2014, Routier's group disclosed a series of pyridopyrimidine-based PI3Kα/mTOR dual inhibitors which showed nanomolar enzymatic and cellular activities on both targets.[7] Later, Childers et al. reported another series of pyridopyrimidines with a phenylurea moiety at the C2 site.[8] Besides, AZD8055 (Fig. 1), a well-known mTORC1/mTORC2 dual inhibitor that containing a pyridino [2,3-*d*] pyrimidine core, has also been reported to have inhibitory effect on PI3Kα, though the potency is mild.[9] Referring to the structure-activity relationship of pyridino[2,3-*d*] pyrimidine compounds that summarized from Pike's work,[9] the structure of the substituent at the C4 and C7 site of the pyridino[2,3-*d*] pyrimidine scaffold is conserved.

Abbreviations: PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; TSC, tuberous sclerosis; DDQ, 2,3-dicyano-5,6-dichlorobenzoquinone; THF, tetrahydrofuran; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; MRT, mean retention time

* Corresponding authors.
E-mail addresses: xa-qh@163.com (Q. Xia), lei.fang@seu.edu.cn (L. Fang).

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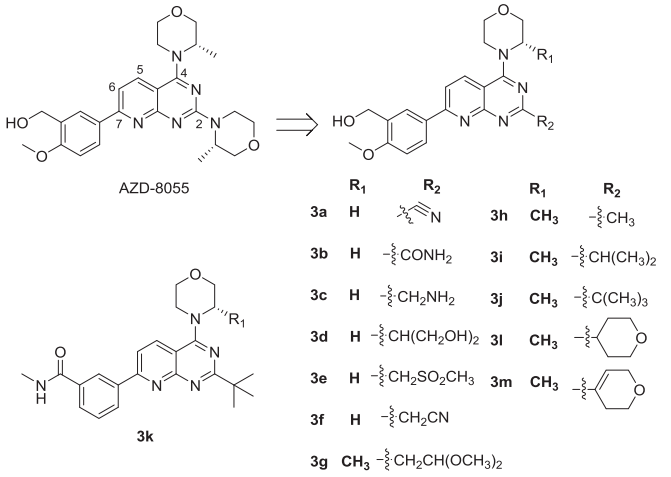


Fig. 1. The drug design strategy and the structures of the target compounds **3a–3m**.

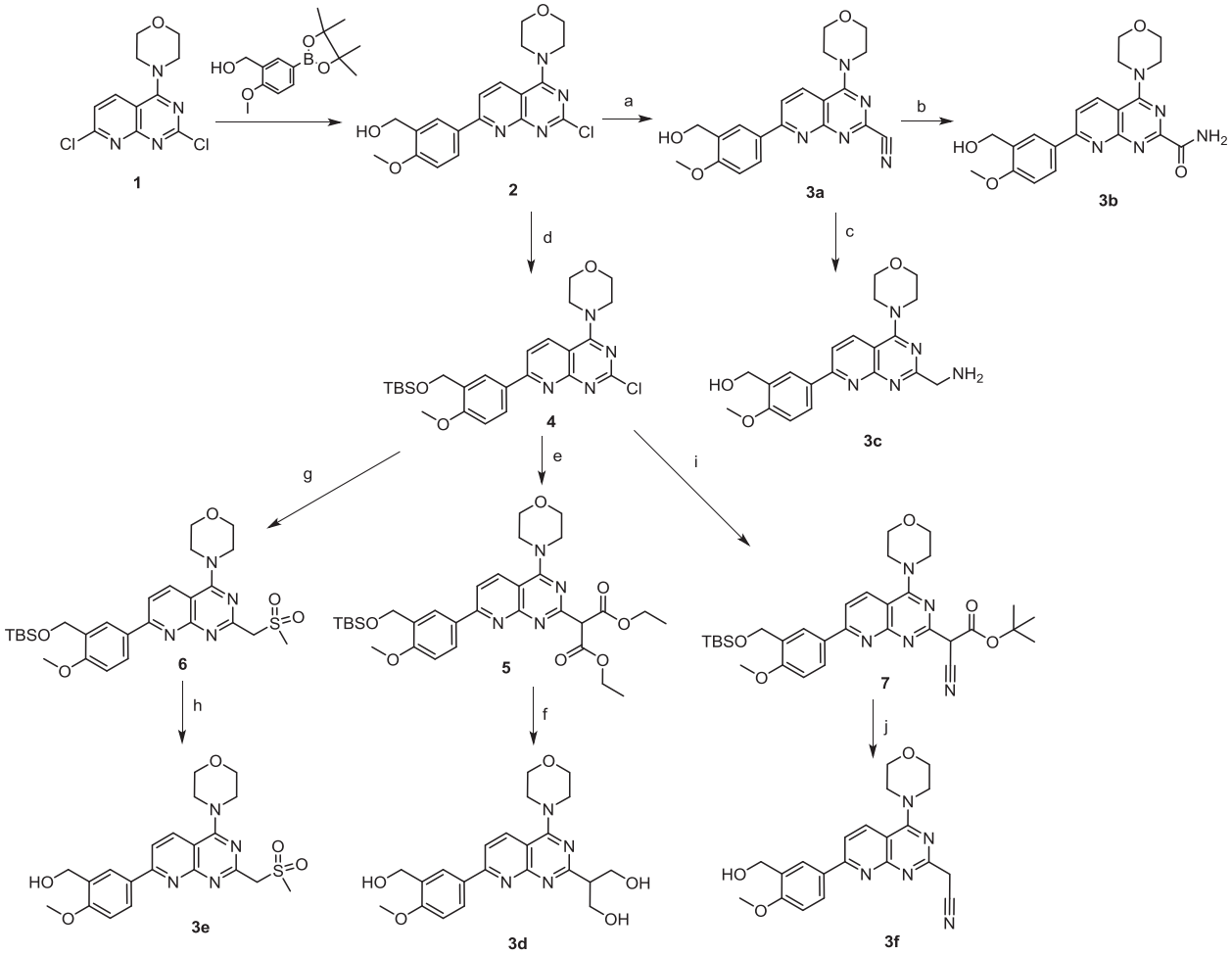
The optimal substituent at the C4 site is morpholine or 3*S*-methyl morpholine since the morpholine oxygen may form a critical hydrogen bond with amino acid residues.[10] As for the C4 site, 3,4-disubstituted phenyl group (e.g. 3-hydroxymethyl-4-methoxy phenyl group) seems the preferred substituent. Changing these substituents to the others would significantly decrease the activity. In contrast, the substituents at the C2 site may allow various structural modifications, leading to the change of potency, physicochemical and pharmacokinetic properties. A successful example is AZD8055 which is derived from Ku-0063794 by replacing the 2,6-dimethyl morpholine with 3-methyl morpholine at the C2 site. This modification significantly improved the potency of the enzyme inhibition, aqueous solubility and pharmacokinetic properties.

Thereby, using AZD8055 as the lead compound, we further explored the structural modifications on the pyridino[2,3-*d*] pyrimidine scaffold, paying emphasis on the C2 site. Different from the former studies which often employed heterocycle as the substituent, our modifications focus on small alkyl group which connected to C2 site via a C—C bond. In this work, a series of pyridino[2,3-*D*]pyrimidine derivatives with different small alkyl substituents at C2 site have been designed (Fig. 1). We comprehensively investigated the influence of the modifications on the PI3Kα/mTOR inhibition activity, antiproliferative activity, metabolic stability, pharmacokinetic property and the in vivo antitumor activity. We found a potent PI3Kα/mTOR dual inhibitor (i.e. **3j**) with remarkable antitumor activity both in vitro and in vivo, showing a potential for further exploration.

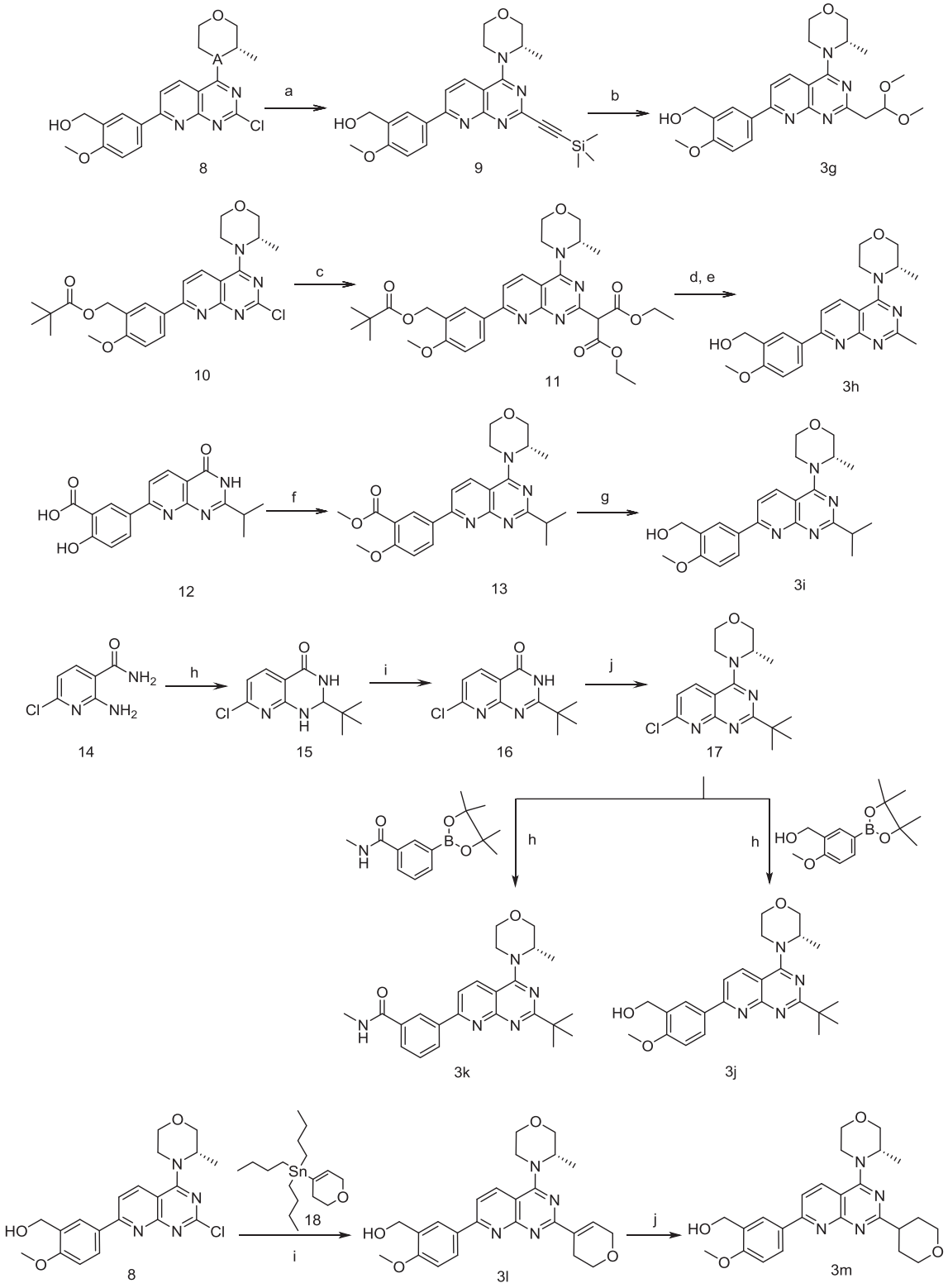
2. Results and discussion

2.1. Chemistry

The synthetic routes were depicted in Schemes 1 and 2. As shown in Scheme 1, the desired pyrido[2,3-*d*]pyrimidine derivatives (**3a–3f**)



Scheme 1. Synthesis of compounds **3a–3f**. Regents and condition: (a) NaCN, DMSO, 140 °C, 2 h; (b) H_2O_2, DMSO, K_2CO_3, rt, 12 h; (c) Pd/C, H_2, CH_3OH, rt, 1 h; (d) $(CH_3)_3CSi(CH_3)_2Cl$, NEt_3, THF, 60 °C, 12 h; (e) NaH, $CH_2(CO_2Et)_2$, CH_3CN, reflux, 3 h; (f) $LiBH_4$, THF, 60 °C, 2 h; (g) $CH_3SO_2CH_3$, *n*-BuLi, THF, rt, 12 h; (h) CH_3COCl, CH_3OH, 40 °C, 12 h; (i) *tert*-butyl cyanoacetate, *t*-BuOK, THF, rt, 12 h; (j) *p*-toluenesulfonic acid, toluene, reflux, 12 h.



Scheme 2. Synthesis of compounds **3g–3m**. Regents and condition: (a) trimethylsilylacetylene, CuI, $Pd(PPh_3)_4$, NEt_3, DMF, 100 °C, 12 h; (b) NaOH, CH_3OH, rt, 12 h; (c) NaH, $CH_2(CO_2Et)_2$, CH_3CN, reflux, 3 h; (d) LiCl, DMSO, 150 °C, 2 h; (e) LiOH, THF/H_2O, rt, 4 h; (f) $SOCl_2$, CH_3OH, then $POCl_3$, $CH_3CON(CH_3)_2$, DIPEA, 3*S*-methyl morpholine, THF, 120 °C, 12 h; (g) $LiAlH_4$, THF, 0 °C, 12 h; (h) $(CH_3)_3CHO$, $CuCl_2$, CH_3CH_2OH, 75 °C, 12 h; (i) DDQ, CH_2Cl_2, rt, 1 h; (j) $POCl_3$, 3*S*-methyl morpholine, CH_2Cl_2, rt, 1 h; (h) K_2CO_3, $Pd(PPh_3)_4$, dioxane, 80 °C, 12 h; (i) $Pd(PPh_3)_2Cl_2$, CuI, DIPEA, DMF, 130 °C, 0.5 h; (j) H_2, 10% Pd/C, methanol, rt, 1 h.

were prepared from the key intermediate **2** which was prepared according to a literature method.[9] Briefly, compound **2** was treated with NaCN to give **3a**, which was further reacted with H_2O_2 in the presence of K_2CO_3 to give **3b**. Compound **3c** was obtained from the catalytic hydrogenation of **3a**. For the synthesis of **3d**, the hydroxyl group of intermediate **2** was firstly protected by $(CH_3)_3CSi(CH_3)_2Cl$ to yield key intermediate **4**. Thereafter compound **5** was prepared via a condensation reaction of **4** and diethyl malonate. Then **3d** was successfully prepared by reducing **5** with $LiBH_4$. Intermediate **4** was treated with $CH_3SO_2CH_3$ and followed the remove of the protection group to offer

Table 1
IC_{50} values for enzymatic inhibition of mTOR and PI3Kα.

Compound	IC_{50} (μM)[a]		Compound	IC_{50} (μM)[a]	
	mTOR	PI3Kα		mTOR	PI3Kα
AZD8055	0.008 ± 0.001	3.6 ± 0.2	**3g**	0.029 ± 0.008	> 10
BEZ-235	–	0.03 ± 0.01	**3h**	0.095 ± 0.010	> 10
3a	0.032 ± 0.011	2.6 ± 0.5	**3i**	0.010 ± 0.002	3.0 ± 0.5
3b	0.048 ± 0.015	3.2 ± 0.4	**3j**	0.002 ± 0.001	0.4 ± 0.1
3c	0.146 ± 0.030	> 10	**3k**	0.072 ± 0.010	1.3 ± 0.2
3d	> 0.5	> 10	**3l**	0.030 ± 0.009	> 10
3e	> 0.5	> 10	**3m**	0.053 ± 0.015	> 10
3f	0.088 ± 0.018	> 10			

[a] The IC_{50} values are the mean of at least three experiments.

3e. Intermediate **4** was treated with *tert*-butyl cyanoacetate under basic condition to give **7** which was refluxed in toluene in the presence of p-toluenesulfonic acid to give **3f** (Scheme 1).

As for the synthesis of target compounds containing 3*S*-methyl morpholine group at the C4 site of the core (**3g–3m**), the synthetic route was outlined in Scheme 2. With CuI and $Pd(PPh_3)_4$ as catalysts, compound **8** reacted with trimethylsilylacetylene to give **9**, which was treated with NaOH in methanol to yield **3g**. Using the similar synthetic method of **5**, compound **11** was prepared from **10** and $CH_2(CO_2Et)_2$.[11] Compound **11** was treated with LiCl and thereafter the protection group was removed by treating with LiOH to yield **3h**. Compound **12**, prepared according to the former reported protocol,[12] was coupled to 3*S*-methyl morpholine to give **13**, which was reduced by $LiAlH_4$ to give **3i**. Compound **14** reacted with trimethyl acetaldehyde to give **15** which was further treated with DDQ to yield **16**. Using the similar synthetic method of **13**, 3*S*-methyl morpholine was coupled to the pyridino[2,3-*d*] pyrimidine core, resulting in compound **17**. Finally, **3j** and **3k** were prepared from **17** and corresponding borate ester via a Suzuki reaction, respectively. As for **3l** and **3m**, compound **8** reacted with **18** to give **3l** which was further reduced to offer **3m** (Scheme 2).

2.2. Enzymatic inhibition of PI3Kα and mTOR

All of the target compounds were screened for the enzymatic inhibition activity against PI3Kα and mTOR using Lance Ultra assay and Kinase-Glo Plus luminescent assay, respectively. BEZ-235 (a potent PI3Kα inhibitor) and AZD8055 were used as the positive controls. The results were shown in Table 1. Similar to the lead compound AZD8055, most of the target compounds, except **3c**, **3d** and **3e**, exhibited potent inhibitory activity towards mTOR with IC_{50} values ranging from single to double digit nM. In particular, the potency of compound **3j** was 4-fold higher than that of AZD8055. When PI3Kα was regarded, five target compounds showed positive inhibitory effect on PI3Kα with IC_{50} values at micromole level. The potency of the active target compounds was lower than the positive control BEZ-235, but superior to the lead compound AZD8055. Notably, **3j** again showed the best inhibition activity with an IC_{50} value of 0.4 μM, a tenth of that of AZD8055. Interestingly, the enzymatic inhibition potency of compound **3k**, which had a similar structure to **3j** but possessed a 3-(methylcarbamoyl)phenyl group (a moiety from mTOR inhibitor AZD2014) at C7 site instead of the 3-hydroxymethyl-4-methoxy phenyl group of **3j**, significantly decreased as compared with **3j**, indicating 3-hydroxymethyl-4-methoxy phenyl group was optimal for C7 site. This result is consistent with the Pike's findings.[9]

By analyzing the structure-activity relationship, it can be found that introduction of a sulfonyl group to the C2 site of the pyridino[2,3-*d*] pyrimidine core (e.g. **3e**) significantly decreased the inhibition activity towards both PI3Kα and mTOR. Meanwhile, the hydrophilic substituents at C2 site seemed also unfavorable for the inhibition activity. For example, when 1,3-propanediol was connected to the core resulting in **3d**, no inhibition activity was observed in the assay. Interestingly, when the hydroxyl group was converted to the ether form (e.g. **3g**), the inhibition activity came back. Among all of the employed substituents the hydrophobic alkyl groups like methyl, isopropyl and tertiary butyl groups seemed to be optimal. Moreover, the size of the substituents also had influence on the activity. It was found that the larger the size is, the better the activity is. As for the alkyl groups, the order of the activity is tertiary butyl > methyl > isopropyl. As for the substituent at C4 site, both morpholine and 3*S*-methyl morpholine were suitable for the enzyme inhibition activity. No obvious difference of the activity was observed between the two groups.

2.3. Antitumor activity in vitro

The antiproliferative activity of the target compounds was measured using the CCK-8 assay with AZD8055 as a positive control.[13] Since genetic aberrations of the PI3K pathway frequently occur in various human cancers, four different human cancer cell lines including U87MG human glioblastoma cell line, MCF-7 human breast cancer cell line, PC-3 human prostate cancer cell line, and SKOV-3 human ovarian cancer cell line, were employed in the assay. The results were shown in Table 2. Similar to the results of the enzymatic inhibition test, compounds **3h–3j** that containing hydrophobic alkyl groups at C2 site showed better activity than the others. In contrast, compounds **3b–3d** that containing hydrophilic substituents at C2 site showed very weak antiproliferative effect. The consistence of the enzymatic and cellular activity suggested that the antiproliferative effect mainly depended on the enzymatic inhibition of PI3Kα and mTOR. As far as the cancer cell lines were concerned, U87MG and SKOV-3 were generally more sensitive to the target compounds than the other two. Among all of the target compounds, **3j** exhibited best activity against all of the tested cell lines. In particular, the inhibition potency of **3j** against SKOV-3 cell line was even 8-fold higher than that of AZD8055, indicating a promising potential for further exploration.

2.4. Western blot analysis

To further confirm the intracellular PI3Kα and mTOR inhibitory activity of **3j**, we performed western blot analysis to investigate the activity of the two major kinases, Akt and mTORC1, after the treatment of U87MG cells with **3j**. The kinase activity was determined by monitoring the phosphorylation level of AKT and its downstream target S6 in U87MG cells. As shown from Fig. 2, **3j** could completely inhibit the phosphorylation of AKT at the dose of 200 and 20 nM. Even at the dose of 2 nM, a significant inhibition was also observed. As for S6, a dose-dependent inhibition of the phosphorylation of S6 was also found after the treatment of **3j**, though the potency was relatively lower than that to AKT. These results are in accordance with the results of enzymatic assays, which confirms the intracellular inhibition activity of **3j** against PI3K pathway.

Table 2
Antiproliferative activities of the target compounds.

Compound	IC_{50} (nM) ± SEM[a]			
	U87MG	MCF-7	PC-3	SKOV-3
AZD8055	21.2 ± 7.9	27.0 ± 8.5	36.7 ± 6.8	55.0 ± 9.0
3a	920.0 ± 25.8	> 1000	> 1000	660.0 ± 25.0
3b	821.0 ± 32.8	> 1000	> 1000	640.5 ± 12.0
3c	> 1000	> 1000	> 1000	> 1000
3d	> 1000	> 1000	> 1000	> 1000
3e	> 1000	> 1000	> 1000	> 1000
3f	90.3 ± 2.7	185.7 ± 5.2	66.6 ± 7.9	54.5 ± 4.2
3g	89.2 ± 7.2	72.0 ± 7.6	163.1 ± 17.0	52.4 ± 8.7
3h	91.9 ± 8.0	77.3 ± 2.3	66.0 ± 5.1	49.1 ± 7.7
3i	33.8 ± 2.9	54.0 ± 2.5	155.0 ± 12.0	44.7 ± 4.3
3j	3.2 ± 0.7	6.0 ± 2.2	23.0 ± 7.0	7.0 ± 1.1
3k	32.5 ± 4.0	31.0 ± 2.0	110 ± 7.0	58.6 ± 5.0
3l	85.5 ± 12.1	225 ± 6.5	212 ± 8.1	125 ± 11.0
3m	94.2 ± 14.0	443 ± 13.6	476.0 ± 15.0	118.5 ± 9.8

[a] Data is the mean value of triplicate measurements. The cell viability was measured after 48 h drug exposure.

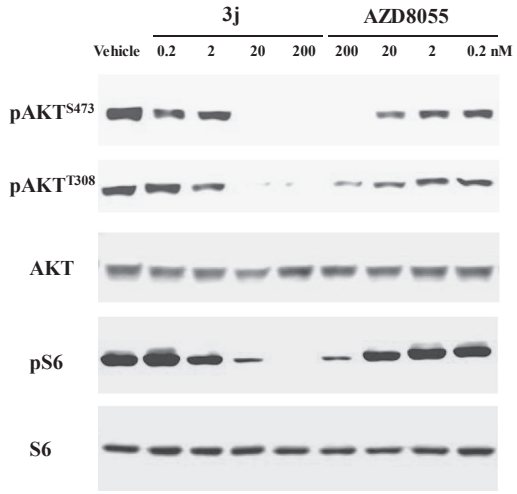


Fig. 2. Effects of **3j** and AZD8055 on the phosphorylation level of AKT and its downstream target S6 in U87MG cells after 24 h exposure.

2.5. Stability in human liver microsomes

The metabolic stability of compound **3j** and AZD8055 was examined in vitro using human liver microsomes. The results were summarized in Table 3. It was found that **3j** showed excellent stability in human liver microsomes with $t_{1/2}$ value of 30.9 min, much longer than that of AZD8055 ($t_{1/2}$ = 21.0 min). This results indicated that structural modification by replacing the 3-methyl morpholine moiety with small alkyl group could improve the stability, which conformed to our preconceived ideas. The clearance rate of **3j** and AZD8055 was at the same level.

2.6. In vivo antitumor activity

In view of the good results of the enzymatic and cellular tests in vitro, compounds **3j** (3, 10 and 30 mg/kg) was selected for further in vivo efficacy studies in U87MG tumor xenograft mouse model (Fig. 3). AZD8055 (10 mg/kg) was used as positive control. It was found that **3j** exhibited significant in vivo efficacy. At a high dose of 30 mg/kg, the tumor inhibition rate of **3j** reached 83%. At a middle dose of 10 mg/kg, **3j** also exhibited a good tumor inhibiting activity with an inhibition rate of 64%, higher than that of equal dose of AZD8055 (59%). Even at a low dose of 3 mg/kg, **3j** still showed a moderate tumor inhibiting activity with an inhibition rate of 28%. In addition, no body weight loss was observed after the administration of the tested compound (data not shown), indicating a good safety **3j** possessed. The results clearly indicated that **3j** could effectively inhibit tumor growth in vivo in a dose-dependent manner, and its potency was higher than AZD8055. Thus, on the basis of its potent in vivo efficacy, **3j** was selected as a candidate for further pharmacokinetic investigation.

2.7. Pharmacokinetic investigation

In order to investigate whether the structural modification has an effect on the pharmacokinetic properties or not, compounds **3j** was selected for single dose studies on the pharmacokinetics property. Beagle dogs were treated with **3j** at a dose of 1 mg/kg by gavage, and after 24 h the pharmacokinetic parameters of the half life ($t_{1/2}$), maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}), mean residence time (MRT) and area under the plasma concentration–time curve for 0–24 h after dosing (AUC_{0-24h}) were determined (Table 4).

Compared with AZD8055, **3j** demonstrated a general higher plasma concentration (288 ng/mL vs 228 ng/mL), longer half-life (5.4 h vs 4.5 h) and mean residence time (4.3 h vs 3.1 h), suggesting that the designed pyridino[2,3-*D*]pyrimidine derivative showed a better performance than AZD8055 in pharmacokinetic.

3. Conclusion

A series of pyridino[2,3-*D*]pyrimidine derivatives with different alkyl substituents at C2 site have been designed and synthesized to search for novel PI3Kα/mTOR dual inhibitors. In the enzyme assay, most of the target compounds showed potent mTOR inhibition activity with IC_{50} values ranging from single to double digit nM. Five target compounds exhibited pronounced PI3Kα inhibition activity. Particularly, compound **3j** showed most potent inhibitory activity towards both kinases among all of the target compounds. In vitro cellular assay indicated that most of the target compounds showed excellent antiproliferative activity, especially **3j** whose potency against SKOV3 was 8-fold higher than the positive control AZD8055. The consistence of the enzymatic and cellular results suggested that PI3Kα/mTOR probably were the targets of the designed compounds. Indeed, western blot analysis further confirmed the intracellular inhibition effect of **3j** on the phosphorylation level of AKT and its downstream target S6. In vitro metabolic stability study found that **3j** had a better stability than that of AZD8055. More importantly, **3j** showed better antitumor activity and pharmacokinetic properties in vivo as compared with AZD8055. All of the findings suggested a promising potential of **3j** for further exploration.

4. Experiment section

4.1. Chemistry

4.1.1. Materials and instruments

All chemicals were obtained from commercial purchase and solvents were purified and dried by standard procedures. Flash chromatography (FC): silica gel (SiO_2; 40 mm, 200–300 mesh). Melting points were uncorrected and were determined using a capillary apparatus (RDCSY-I). ^{1}H NMR and ^{13}C NMR Spectra: Bruker AVANCE-400 Digital NMR Spectrometer, ESI-MS: Thermo Finnigan LCQ advantage MAX. Elemental analysis was performed on a Vario EL III apparatus.

4.1.2. 7-(3-(Hydroxymethyl)-4-methoxyphenyl)-4-morpholinopyrido[2,3-d]pyrimidine-2-carbonitrile (**3a**)

Compound **2** (50 mg, 0.13 mmol) and NaCN (7 mg, 0.14 mmol) were dissolved in 2 mL of DMSO. The obtained solution was heated to 140 °C and kept stirring for 2 h. After cooling to room temperature, 20 mL of water was added and the solution was extracted with AcOEt

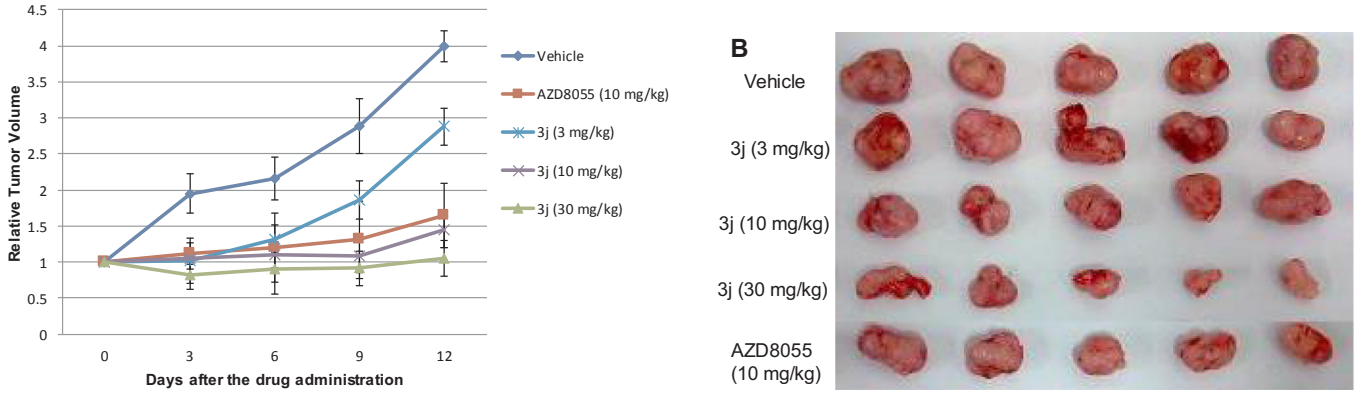


Fig. 3. (A) Relative volumes of U87MG tumors after the treatment of different dose of **3j** and AZD8055 in xenograft mouse models (n = 5). (B) Images of the tumors after treatments with different formulations.

Table 3
Metabolic stability of **3j** and AZD805 in human liver microsomes.

Compound	$T_{1/2}$ (min)	CLF (μL/min/mg)
AZD8055	21.0	28.6
3j	30.9	28.1

Table 4
Pharmacokinetic profile of **3j** and AZD8055.[a]

Compd.	Dose[a] (mg/kg)	C_{max} (ng/mL)	AUC (ng/mL * h)	$t_{1/2}$ (h)	MRT (h)
AZD8055	1	228 ± 39.2	787 ± 108.5	4.5 ± 0.7	3.1 ± 0.5
3j	1	288 ± 44.5	857 ± 98.4	5.4 ± 0.5	4.3 ± 0.4

[a] Dosed as a solution of stroke-physiological saline solution with 5% DMSO.

(20 mL × 2). The organic phase was successively washed by water (40 mL × 3) and saturated salt solution (40 mL × 3), and then dried over anhydrous Na_2SO_4, filtrated, and purified by column chromatography (CH_2Cl_2:EtOAc = 5:1) to give compound **3a** as yellow solid. Yield 21%; m.p. 98–99 °C. ^{1}HNMR (400 MHz, CD_3OD): δ 8.57 (d, 1H), 8.33 (s, 1H), 8.25 (d, 1H), 8.13 (d, 1H), 7.19 (d, 1H), 5.37 (s, 1H), 4.75 (s, 2H), 4.13–4.09 (m, 4H), 3.98 (s, 3H), 3.89–3.88 (m, 4H); ^{13}CNMR (100 MHz, CD_3OD): δ 174.8, 158.3, 156.8, 153.8, 151.6, 144.6, 141.2, 136.7, 134.6, 132.2, 126.8, 117.6, 114.4, 99.9, 66.5, 62.5, 55.3, 47.2; ESI-MS: 378.0 $[M+H]^+$; Element Analysis for $C_{20}H_{19}N_5O_3$ (%) C: 63.65%, H: 5.07%, N: 18.56% Found C: 63.56%, H: 5.01%, N: 18.53%.

*4.1.3. 7-(3-(Hydroxymethyl)-4-methoxyphenyl)-4-morpholinopyrido[2,3-d]pyrimidine-2-carboxamide (**3b**)*

Compound **3a** (130 mg, 0.33 mmol), 30% H_2O_2 aqueous solution (112 mg, 0.99 mmol) and K_2CO_3 (92 mg, 0.66 mmol) were added into 5 mL of DMSO and the obtained mixture was stirred for 12 h at room temperature. Then 20 mL of water was added and the solution was extracted with AcOEt (20 mL × 2). The organic phase was successively washed by water (40 mL × 3) and saturated salt solution (40 mL × 3), and then dried over anhydrous Na_2SO_4, filtrated, and purified by column chromatography (CH_2Cl_2:EtOAc = 5:1) to give compound **3b** as yellow solid. Yield 16%; m.p. 112–114 °C. ^{1}HNMR (400 MHz, CD_3OD): δ 8.32 (s, 1H), 7.92–7.90 (m, 2H), 7.56 (d, 1H), 7.04 (d, 1H), 5.39 (s, 1H), 4.86 (s, 2H), 4.00 (s, 3H), 3.90–3.85 (m, 8H); ^{13}CNMR (100 MHz, CD_3OD): δ 186.6, 168.1, 166.9, 156.8, 156.0, 149.4, 144.4, 143.4, 136.7, 134.6, 132.2, 126.6, 114.4, 104.1, 66.5, 62.8, 55.5, 47.8; ESI-MS: 396.2 $[M+H]^+$; Element Analysis for $C_{20}H_{21}N_5O_4$ (%) C: 60.75%, H: 5.35%, N: 17.71% Found C: 60.66%, H: 5.30%, N: 17.59%.

*4.1.4. (5-(2-(Aminomethyl)-4-morpholinopyrido[2,3-d]pyrimidin-7-yl)-2-methoxyphenyl)methanol (**3c**)*

Compound **2** (6 mg, 0.02 mmol) was dissolved in 2 mL of methanol. To the obtained solution ammonia solution (28%, 0.1 mL) and Pd/C (10%, 3 mg) were added and H_2 gas was charged. The mixture was stirred for 1 h at room temperature. The mixture was then filtrated and the filtrate was concentrated in vacuo and the crude product was purified by column chromatography (CH_2Cl_2:EtOAc = 5:1) to give compound **3c** as yellow solid. Yield 83%; m.p. 105–107 °C. ^{1}HNMR (400 MHz, CD_3OD): δ 8.32 (s, 1H), 7.99–7.97 (m, 2H), 7.56 (d, 1H), 7.04 (d, 1H), 5.39 (s, 2H), 4.85 (s, 1H), 4.72 (s, 2H), 4.04–3.93 (m, 4H), 3.89 (s, 3H), 3.77–3.72 (m, 4H), 2.28–2.26 (m, 2H); ^{13}CNMR (100 MHz, CD_3OD): δ 188.3, 180.2, 158.3, 156.8, 150.8, 145.7, 140.5, 136.7, 134.6, 132.8, 122.7, 114.9, 100.4, 66.0, 62.5, 55.3, 48.1, 47.2; ESI-MS: 382.2 $[M+H]^+$; Element Analysis for $C_{20}H_{23}N_5O_3$ (%) C: 62.98%, H: 6.08%, N: 18.36% Found C: 62.86%, H: 6.20%, N: 18.44%.

*4.1.5. 4-(7-(3-(((tert-Butyldimethylsilyl)oxy)methyl)-4-methoxyphenyl)-2-chloropyrido[2,3-d]pyrimidin-4-yl)morpholine (**4**)*

Compound **2** (300 mg, 0.78 mmol), $(CH_3)_3CSi(CH_3)_2Cl$ (140 mg, 0.93 mmol) and NEt_3 (0.2 mL, 1.55 mmol) were dissolved in 10 mL of THF. The solution was stirred for 12 h at 60 °C. The mixture was then filtrated and the filtrate was successively washed by saturated Na_2CO_3 solution (40 mL × 3) and saturated salt solution (40 mL × 3), and then the organic phase was dried over anhydrous Na_2SO_4, filtrated, and concentrated in vacuo to give the yellow crude product which was used for the next reaction without purification.

*4.1.6. Diethyl 2-(7-(3-(((tert-butyldimethylsilyl)oxy)methyl)-4-methoxyphenyl)-4-morpholinopyrido[2,3-d]pyrimidin-2-yl)malonate (**5**)*

NaH (28.80 mg, 1.20 mmol) and $CH_2(CO_2Et)_2$ (192 mg, 1.20 mmol) were dissolved in 10 mL of acetonitrile. The mixture was stirred for 0.5 h and then compound **4** (300 mg, 0.60 mmol) was added. The obtained mixture was refluxed for 3 h. After cooling to room temperature, the reaction mixture was poured into 30 mL of ice-water. The mixture was extracted with AcOEt (40 mL × 3). The organic phase was washed by saturated salt solution (50 mL × 2), and then dried over anhydrous Na_2SO_4, filtrated, concentrated in vacuo and purified by column chromatography (CH_2Cl_2:EtOAc = 5:1) to give compound **5** as brown solid. Yield 64%; m.p. 85–87 °C. ESI-MS: 382.2 $[M+H]^+$.

*4.1.7. 2-(7-(3-(Hydroxymethyl)-4-methoxyphenyl)-4-morpholinopyrido[2,3-d]pyrimidin-2-yl)propane-1,3-diol (**3d**)*

Compound **5** (120 mg, 0.20 mmol) and $LiBH_4$ (17 mg, 0.80 mmol) were added into 10 mL of THF. The mixture was stirred for 2 h at 60 °C. After cooling to room temperature, the reaction solution was concentrated in vacuo and purified by column chromatography (CH_2Cl_2:EtOAc = 5:1) to give compound **3d** as yellow solid. Yield 59%;

m.p. 89–90 °C. ^{1}HNMR (400 MHz, CD_3OD): δ 8.33 (s, 1H), 8.01–7.96 (m, 2H), 7.51 (d, 1H), 7.10 (d, 1H), 5.02 (s, 1H), 4.97 (s, 2H), 4.78 (s, 2H), 3.87–3.85 (m, 7H), 3.71–3.55 (m, 8H), 2.97–2.96 (m, 1H); ^{13}CNMR (100 MHz, CD_3OD): δ 174.1, 172.1, 156.8, 153.2, 147.4, 145.5, 140.7, 136.7, 134.0, 132.0, 122.7, 114.0, 92.4, 66.5, 62.5, 58.8, 55.1, 47.2, 45.9; ESI-MS: 427.1 $[M+H]^+$; Element Analysis for $C_{22}H_{26}N_4O_5$ (%) C: 61.96%, H: 6.15%, N: 13.14% Found C: 62.05%, H: 6.21%, N: 13.01%.

4.1.8. 4-(7-(3-(((tert-Butyldimethylsilyl)oxy)methyl)-4-methoxyphenyl)-2-((methylsulfonyl)methyl)pyrido [2,3-d]pyrimidin-4-yl)morpholine (6)

To a solution of $CH_3SO_2CH_3$ (282 mg, 3 mmol) in 5 mL of THF was added *n*-BuLi dropwise, and the obtained solution was stirred for 0.5 h. Then compound **4** (150 mg, 0.30 mmol) was added and the reaction solution was stirred for 12 h at room temperature. Thereafter, the solution was concentrated in vacuo and purified by column chromatography (CH_2Cl_2:EtOAc = 5:1) to give compound **6** as yellow solid. Yield 39%; m.p. 80–82 °C. ESI-MS: 599.2 $[M+H]^+$.

4.1.9. (2-Methoxy-5-(2-((methylsulfonyl)methyl)-4-morpholinopyrido [2,3-d]pyrimidin-7-yl)phenyl) methanol (3e)

Compound **6** (10 mg, 0.02 mmol) and acetyl chloride (5 mg, 0.05 mmol) were dissolved in 10 mL of methanol. The solution was stirred for 12 h at 40 °C. After cooling to room temperature, the reaction solution was concentrated in vacuo and purified by column chromatography (CH_2Cl_2:EtOAc = 5:1) to give compound **3e** as yellow solid. Yield 63%; m.p. 95–97 °C. ^{1}HNMR (400 MHz, CD_3OD): δ 8.55 (s, 1H), 8.45 (d, 1H), 8.15 (d, 1H), 7.67 (d, 1H), 7.05 (d, 1H), 5.39 (s, 1H), 4.86 (s, 2H), 4.83 (s, 2H), 4.14–4.10 (m, 4H), 3.97 (s, 3H), 3.88–3.83 (m, 4H), 3.15 (s, 3H); ^{13}CNMR (100 MHz, CD_3OD): δ 178.4, 167.1, 157.4, 156.8, 154.0, 143.7, 141.4, 136.7, 134.5, 132.2, 125.8, 114.4, 98.1, 68.0, 66.5, 62.5, 53.0, 47.2, 42.3; ESI-MS: 445.1 $[M+H]^+$; Element Analysis for $C_{21}H_{24}N_4O_5S$ (%) C: 56.74%, H: 5.44%, N: 12.60% Found C: 57.00%, H: 5.51%, N: 12.78%.

4.1.10. tert-Butyl 2-(7-(3-(((tert-butyldimethylsilyl)oxy)methyl)-4-methoxyphenyl)-4-morpholinopyrido [2,3-d]pyrimidin-2-yl)-2-cyanoacetate (7)

t-BuOK (40 mg, 0.35 mmol) and *tert*-butyl cyanoacetate (140 mg, 1.00 mmol) were dissolved in 5 mL of THF. The mixture was stirred for 0.5 h. Then compound **4** was added and the solution was stirred for 12 h. The solvent was then removed and the residue was added into 30 mL of water. The mixture was extracted with AcOEt (40 mL × 3). The organic phase was then successively washed by saturated Na_2CO_3 solution (40 mL × 3) and saturated salt solution (40 mL × 3), and then dried over anhydrous Na_2SO_4, filtrated, and concentrated in vacuo to give yellow product which was used for the next reaction without purification.

4.1.11. 2-(7-(3-(Hydroxymethyl)-4-methoxyphenyl)-4-morpholinopyrido [2,3-d]pyrimidin-2-yl)acetonitrile (3f)

Compound **7** (100 mg, 0.16 mmol) and *p*-toluenesulfonic acid (10 mg, 0.03 mmol) were dissolved in 10 mL of toluene. The solution was firstly stirred for 12 h at room temperature and then further stirred for 3 h at 65 °C. The solvent was then removed and the residue was added into 50 mL of AcOEt. The solution was washed by saturated NH_4Cl solution (15 mL × 3), and then dried over anhydrous Na_2SO_4, filtrated, concentrated in vacuo and purified by column chromatography (CH_2Cl_2:EtOAc = 5:1) to give compound **3f** as yellow solid. Yield 23%; m.p. 116–118 °C. ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.48 (s, 1H), 8.26 (d, 1H), 8.02 (d, 2H), 6.97 (d, 1H), 4.81 (s, 2H), 4.45 (s, 2H), 4.15–4.06 (m, 4H), 3.97 (s, 3H), 3.89 (m, 4H); ^{13}CNMR (100 MHz, $CDCl_3$): δ 175.2, 157.1, 156.8, 154.5, 150.4, 145.5, 142.0, 136.7, 134.6, 132.2, 127.7, 120.8, 114.4, 99.1, 66.5, 62.5, 55.3, 47.2, 27.0; ESI-MS: 392.1 $[M+H]^+$; Element Analysis for $C_{21}H_{21}N_5O_3$ (%) C: 64.44%, H: 5.41%, N: 17.89% Found C: 64.19%, H: 5.21%, N: 17.78%.

4.1.12. (S)-(2-Methoxy-5-(4-(3-methylmorpholino)-2-((trimethylsilyl) ethynyl)pyrido[2,3-d]pyrimidin-7-yl)phenyl)methanol (9)

Compound **8** (150 mg, 0.37 mmol), trimethylsilylacetylene (74 mg, 0.75 mmol), CuI (8 mg, 0.037 mmol), $Pd(PPh_3)_4$ (15 mg, 0.037 mmol) and NEt_3 (114 mg, 1.10 mmol) were dissolved in 5 mL of DMF. The solution was stirred for 12 h at 100 °C. The solution was then concentrated in vacuo and purified by column chromatography (CH_2Cl_2:EtOAc = 5:1) to give compound **9** as yellow solid. Yield 80%; m.p. 110–112 °C. ESI-MS: 463.2 $[M+H]^+$.

4.1.13. (S)-(5-(2-(2,2-Dimethoxyethyl)-4-(3-methylmorpholino)pyrido [2,3-d]pyrimidin-7-yl)-2-methoxy-phenyl)methanol (3g)

Compound **9** (100 mg, 0.21 mmol) and NaOH (17 mg, 0.42 mmol) were dissolved in 5 mL of methanol. The solution was stirred for 12 h at room temperature. The solvent was then removed and the residue was added into 50 mL of AcOEt. The solution was washed by saturated salt solution (15 mL × 3), and then dried over anhydrous Na_2SO_4, filtrated, concentrated in vacuo and purified by column chromatography (CH_2Cl_2:EtOAc = 5:1) to give compound **3g** as yellow solid. Yield 25%; m.p. 86–88 °C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 6.91 (d, 1H), 6.81 (s, 1H), 6.71 (d, 1H), 6.48 (d, 1H), 5.62 (d, 1H), 3.59–3.56 (m, 1H), 3.18 (s, 3H), 3.17 (m, 1H), 2.77–2.73 (m, 1H), 2.48–2.46 (m, 1H), 2.32 (s, 3H), 2.28–2.17 (m, 4H), 1.85 (s, 6H), 1.77 (m, 2H), 1.65 (m, 2H); ^{13}CNMR (100 MHz, DMSO-d_6): δ 173.0, 163.5, 156.8, 153.0, 152.1, 145.5, 142.0, 136.5, 134.6, 132.2, 127.9, 114.0, 105.4, 95.8, 72.1, 68.3, 62.5, 55.3, 54.9, 48.9, 47.1, 42.4, 13.2; ESI-MS: 455.2 $[M+H]^+$; Element Analysis for $C_{24}H_{30}N_4O_5$ (%) C: 63.42%, H: 6.65%, N: 12.33% Found C: 63.20%, H: 6.45%, N: 12.39%.

4.1.14. (S)-2-(7-(4-Methoxy-3-((pivaloyloxy)methyl)phenyl)-4-(3-methylmorpholino)pyrido[2,3-d] pyrimidin-2-yl)malonate diethyl (11)

NaH (133 mg, 3.27 mmol) was dissolved in 35 mL of THF. $CH_2(CO_2Et)_2$ (525 mg, 3.27 mmol) was added into the reaction solution dropwise. The obtained solution was stirred for 0.5 h at room temperature. Thereafter a solution of compound **10** (700 mg, 1.49 mmol) in 20 mL THF was added dropwise. The reaction solution was then refluxed for 12 h. The solvent was then removed and the residue was added into 50 mL of AcOEt. The solution was successively washed by water (15 mL × 2) and saturated salt solution (15 mL × 3), and then dried over anhydrous Na_2SO_4, filtrated, concentrated in vacuo to give compound **11** as yellow solid which was used for the next reaction without purification.

4.1.15. (S)-(2-Methoxy-5-(2-methyl-4-(3-methylmorpholino)pyrido[2,3-d]pyrimidin-7-yl)phenyl)methanol (3h)

Compound **11** (860 mg, 1.41 mmol) and LiCl (60 mg, 1.41 mmol) were dissolved in 15 mL of DMSO. The obtained solution was stirred for 2 h at 150 °C. After cooling to room temperature, 30 mL water and 30 mL AcOEt were added into the reaction solution. The organic phase was dried over anhydrous Na_2SO_4, filtrated, concentrated in vacuo to give pale yellow oil which was used for the next reaction without purification.

The obtained oil and LiOH (28 mg, 0.65 mmol) were dissolved in a mixture of 5 mL THF and 1 mL water. The solution was stirred for 4 h at room temperature. The solution was then concentrated in vacuo and purified by column chromatography (CH_2Cl_2:EtOAc = 5:1) to give compound **3h** as pale yellow solid. Yield 58%; m.p. 52–55 °C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 8.29 (d, 1H), 8.26 (s, 1H), 8.17 (d, 1H), 7.77 (d, 1H), 7.03 (d, 1H), 4.79 (s, 2H), 4.56–4.55 (m, 1H), 4.09–4.06 (m, 1H), 4.01–3.99 (m, 1H), 3.96 (s, 3H), 3.86–3.83 (m, 1H), 3.76–3.73 (m, 3H), 2.71 (s, 3H), 1.52 (d, 3H); ^{13}CNMR (100 MHz, DMSO-d_6): δ 175.4, 174.2, 156.8, 152.8, 152.0, 145.5, 139.9, 136.7, 134.6, 132.0, 127.5, 114.0, 97.5, 72.1, 68.0, 63.3, 55.3, 48.9, 47.1, 24.6, 13.2; ESI-MS: 455.2 $[M+H]^+$; Element Analysis for $C_{21}H_{24}N_4O_3$ (%) C: 66.30%, H: 6.36%, N: 14.73% Found C: 66.20%, H: 6.46%, N: 14.66%.

*4.1.16. Methyl 5-(2-isopropyl-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-7-yl)-2-methoxybenzoate (**13**)*

Compound **12** (200 mg, 0.60 mmol) and $SOCl_2$ (1 mL) were dissolved in 5 mL of methanol. The solution was stirred and refluxed for 12 h. The solvent was then removed and the residue was added into a mixture of 5 mL of CH_2Cl_2 and 5 mL of water. The organic phase was washed by saturated salt solution (15 mL × 3), and then dried over anhydrous Na_2SO_4, filtrated, concentrated in vacuo to give yellow solid which was used for the next reaction without purification.

The obtained yellow solid was added into 3 mL of $POCl_3$ and the solution was refluxed for 6 h. The solution was concentrated in vacuo. The residue was dissolved in a solution of 5 mL $CH_3CON(CH_3)_2$, 3*S*-methyl morpholine (100 mg, 1 mmol) and 1 mL DIPEA. The solution was stirred for 12 h at 120 °C. Then 20 mL of water was added into the reaction solution which was further extracted with EtOAc (25 mL × 2). The organic phase was washed by saturated salt solution (15 mL × 3), and then dried over anhydrous Na_2SO_4, filtrated, concentrated in vacuo to give crude product which was used for the next reaction without purification.

*4.1.17. (S)-(5-(2-Isopropyl-4-(3-methylmorpholino)pyrido[2,3-d] pyrimidin-7-yl)-2-methoxyphenyl) methanol (**3i**)*

Compound **13** (40 mg, 0.09 mmol) was dissolved in 5 mL of THF. The solution was cooled to 0 °C, and then $LiAlH_4$ (5 mg, 0.11 mmol) was added. The solution was stirred for 12 h at room temperature, then concentrated in vacuo and purified by column chromatography (CH_2Cl_2:EtOAc = 5:1) to give compound **3i** as yellow solid. Yield 21%; m.p. 81–83 °C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 8.30–8.25 (m, 2H), 8.17 (d, 1H), 7.78 (d, 1H), 7.03 (d, 1H), 4.79 (s, 2H), 4.52–4.50 (m, 1H), 4.10–4.02 (m, 1H), 3.96 (s, 3H), 3.78–3.73 (m, 2H), 3.44–3.38 (m, 2H), 2.91–2.89 (m, 2H), 1.69 (s, 6H), 1.53–1.51 (m, 3H); ^{13}CNMR (100 MHz, DMSO-d_6): δ 179.4, 174.1 156.8, 152.4, 150.7, 145.7, 139.5, 136.7, 134.6, 132.2, 127.9, 114.0, 99.7, 72.1, 68.3, 62.5, 55.3, 48.9, 47.1, 35.0, 20.8, 13.2; ESI-MS: 409.2 $[M+H]^+$; Element Analysis for $C_{23}H_{28}N_4O_3$ (%) C: 67.63%, H: 6.91%, N: 13.72% Found C: 67.48%, H: 6.85%, N: 13.79%.

*4.1.18. 2-(tert-Butyl)-7-chloro-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (**15**)*

Compound **14** (500 mg, 2.90 mmol), $(CH_3)_3CHO$ (754 mg, 8.80 mmol) and $CuCl_2$ (1.20 g, 8.80 mmol) were added into 5 mL of ethanol. The obtained mixture was stirred at 75 °C for 12 h. Then the mixture was filtrated and the filtrate was concentrated in vacuo and purified by column chromatography (CH_2Cl_2:CH_3OH = 20:1) to give compound **15** as white solid. Yield 29%; m.p. 121–123 °C. ESI-MS: 240.2 $[M+H]^+$.

*4.1.19. 2-(tert-Butyl)-7-chloropyrido[2,3-d]pyrimidin-4(3H)-one (**16**)*

Compound **15** (110 mg, 0.46 mmol) and DDQ (210 mg, 0.92 mmol) were dissolved in 5 mL of CH_2Cl_2. The obtained solution was stirred at room temperature for 1 h. Then 5 mL of water was added and the solution was extracted with CH_2Cl_2 (10 mL × 3), and the organic phase was washed by saturated $NaHCO_3$ solution (10 mL × 2), and then dried over anhydrous Na_2SO_4, filtrated, concentrated in vacuo and purified by column chromatography (CH_2Cl_2:CH_3OH = 20:1) to give compound **16** as gray solid. Yield 75%; m.p. 133–134 °C. ESI-MS: 238.2 $[M+H]^+$.

*4.1.20. (S)-4-(2-(tert-Butyl)-7-chloropyrido[2,3-d]pyrimidin-4-yl)-3-methylmorpholine (**17**)*

Compound **16** (80 mg, 0.33 mmol) was added into 2 mL of $POCl_3$ and the solution was refluxed for 0.5 h. The reaction solution was then concentrated in vacuo. The residue was added into a solution of 3*S*-methyl morpholine (100 mg, 1 mmol) in 5 mL CH_2Cl_2 and the obtained solution was stirred for 1 h at room temperature. The solvent was then removed in vacuo to give crude product which was used for the next reaction without purification.

*4.1.21. (S)-(5-(2-(tert-Butyl)-4-(3-methylmorpholino)pyrido[2,3-d] pyrimidin-7-yl)-2-methoxyphenyl) methanol (**3j**)*

Compound **17** (60 mg, 0.19 mmol), phenylborate ester (59 mg, 0.22 mmol), $Pd(PPh_3)_4$ (6 mg, 10%) and K_2CO_3 (78 mg, 0.56 mmol) were added into a mixture of dioxane/H_2O (V/V = 4:1). The reaction solution was stirred for 12 h at 80 °C. Thereafter the solution was concentrated in vacuo. The residue was added into a solution of 3*S*-methyl morpholine (100 mg, 1 mmol) in 5 mL CH_2Cl_2. And the obtained solution was stirred for 1 h at room temperature. The solution was then concentrated in vacuo and purified by column chromatography (CH_2Cl_2:CH_3OH = 20:1) to give compound **3j** as pale yellow solid. Yield 65%; m.p. 73–75 °C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 8.27–8.25 (m, 1H), 8.23–8.22 (m, 1H), 8.18–8.16 (m, 1H), 7.77–7.75 (m, 1H), 7.03–7.01 (m, 1H), 4.80 (s, 2H), 4.47–4.45 (m, 1H) 4.06–4.98 (m, 2H), 3.96 (s, 3H), 3.84–3.69 (m, 4H), 1.66–1.64 (m, 3H), 1.49 (s, 9H); ^{13}CNMR (100 MHz, DMSO-d_6): δ 189.9, 174.7, 157.4, 152.5, 151.5, 145.5, 138.6, 136.7, 134.0, 132.5, 127.5, 114.4, 97.6, 72.1, 68.5, 62.0, 55.3, 48.9, 47.1, 29.4, 26.1, 13.2; ESI-MS: 423.2 $[M+H]^+$; Element Analysis for $C_{24}H_{30}N_4O_3$ (%) C: 68.22%, H: 7.16%, N: 13.26% Found C: 68.28%, H: 7.25%, N: 13.39%.

*4.1.22. (S)-3-(2-(tert-Butyl)-4-(3-methylmorpholino)pyrido[2,3-d] pyrimidin-7-yl)-N-methylbenzamide (**3k**)*

Yield 65%; m.p. 73–75 °C. ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.80 (s, 1H), 8.27–8.22 (m, 2H), 8.03–8.01 (m, 1H), 7.86–7.84 (m, 1H), 7.59–7.55 (m, 1H), 6.55 (s, 1H), 4.57–4.56 (m, 1H), 4.30–4.20 (m, 1H), 4.05–4.01 (m, 1H), 3.85–3.77 (m, 4H), 3.09–3.08 (m, 3H), 1.60–1.56 (m, 3H), 1.51 (s, 9H); ^{13}CNMR (100 MHz, $CDCl_3$): δ 192.0, 174.7, 168.0, 154.3, 152.6, 143.0, 137.4, 135.8, 133.0, 132.0, 131.3, 129.7, 128.9, 97.0, 72.5, 68.3, 48.9, 47.5, 29.5, 26.1, 26.1, 13.1; ESI-MS: 420.4 $[M+H]^+$; Element Analysis for $C_{24}H_{29}N_5O_2$ (%) C: 68.71%, H: 6.97%, N: 16.69% Found C: 68.52%, H: 6.88%, N: 16.45%.

*4.1.23. (S)-(5-(2-(3,6-Dihydro-2H-pyran-4-yl)-4-(3-methylmorpholino) pyrido[2,3-d]pyrimidin-7-yl)-2-methoxyphenyl)methanol (**3l**)*

Compound **8** (250 mg, 0.67 mmol), **18** (300 mg, 0.80 mmol), Pd$(PPh_3)_2Cl_2$ (47 mg, 0.067 mmol), CuI (15 mg, 0.07 mmol) and DIPEA (0.23 mL, 1.34 mmol) were added into 5 mL DMF. The reaction solution was stirred for 0.5 h at 130 °C. Thereafter 25 mL water was added into the solution. The obtained solution was extracted with AcOEt (30 mL × 2), and the organic phase was washed by saturated salt solution (20 mL × 2), and then dried over anhydrous Na_2SO_4, filtrated, concentrated in vacuo and purified by column chromatography (CH_2Cl_2:CH_3OH = 20:1) to give compound **3l** as yellow solid. Yield 29%; m.p. 78–80 °C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 8.27–8.26 (m, 2H), 8.22–8.20 (d, 1H), 7.80 (d, 1H), 7.07–7.05 (d, 1H), 5.96–5.94 (m, 1H), 4.86 (s, 2H), 4.56–4.54 (m, 1H), 4.48–4.46 (m, 2H), 4.06–4.04 (m, 2H), 4.00 (s, 3H), 3.98–3.96 (m, 1H), 3.83–3.80 (m, 3H), 3.78–3.76 (m, 1H), 2.92–2.90 (m, 2H), 2.35 (s, 2H), 1.52 (d, 2H); ^{13}CNMR (100 MHz, DMSO-d_6): δ 174.2, 170.0, 156.8, 156.0, 151.6, 144.4, 143.1, 137.9, 136.7, 134.6, 132.2, 126.6, 124.0, 114.4, 101.8, 72.1, 68.3, 67.7, 67.1, 62.5, 55.3, 48.9, 47.1, 23.8, 13.2; ESI-MS: 449.2 $[M+H]^+$; Element Analysis for $C_{25}H_{28}N_4O_4$ (%) C: 66.95%, H: 6.29%, N: 12.49% Found C: 66.90%, H: 6.18%, N: 12.39%.

*4.1.24. (S)-(2-Methoxy-5-(4-(3-methylmorpholino)-2-(tetrahydro-2H-pyran-4-yl)pyrido[2,3-d]pyrimidin- 7-yl)phenyl)methanol (**3m**)*

Compound **3l** (25 mg, 0.05 mmol) and 10% Pd/C (10 mg) were added into 20 mL of methanol. After charging with H_2 gas, the reaction solution was stirred for 3 h at room temperature. Thereafter the reaction mixture was filtrated and the filtrate was concentrated in vacuo and purified by column chromatography (CH_2Cl_2:CH_3OH = 20:1) to give compound **3m** as pale yellow solid. Yield 79%; m.p. 92–95 °C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 8.29 (d, 1H), 8.25 (d, 1H), 8.18 (d, 1H), 7.78 (d, 1H), 7.04 (d, 1H), 4.80 (s, 2H), 4.56–4.54 (m, 1H), 4.17 (d, 2H), 3.99 (d, 1H), 3.96 (s, 3H), 3.82–3.80 (m, 1H), 3.75 (d, 3H),

3.58–3.56 (m, 2H), 3.22 (s, 1H), 2.14–2.12 (m, 3H), 2.02–2.00 (m, 2H), 1.56 (d, 3H); ^{13}CNMR (100 MHz, DMSO-d_6): δ 173.5, 173.2, 156.8, 152.8, 148.1, 145.5, 139.9, 136.7, 134.6, 132.2, 127.6, 114.4, 102.8, 72.1, 70.2, 68.3, 62.5, 55.3, 48.9, 47.1, 38.7, 26.9, 13.2; ESI-MS: 451.2 $[M+H]^+$; Element Analysis for $C_{25}H_{30}N_4O_4$ (%) C: 66.65%, H: 6.71%, N: 12.44% Found C: 66.54%, H: 6.59%, N: 12.31%.

4.2. Biological studies

4.2.1. Enzymatic inhibition

All of the compounds are tested for their activities against mTOR using Lance Ultra Assay. The kinase reaction is done in 384-well black plate by adding 2.5 mL of different concentration of compound solution, 2.5 mL mTOR (final 10 nM, control wells were added 2.5 mL kinase buffer) solution and 5 mL substrate solution of ULight-4E-BP1 peptide substrate (final 50 nM) and ATP (final 13 mM) in kinase buffer to each well in order. The assay plate was incubated at room temperature for 1 h. Then 10 mL detection solution buffer was added to each well of the assay plate to stop the reaction. The assay plate was read on a plate reader Envision program for luminescence (Ex320/Em665).

For the Kinase-Glo® Luminescent Kinase Assay, the compound, PI3Kα enzyme, the PIP2 (Life) substrate, and ATP (25 μM) were diluted in kinase buffer to the indicated concentrations. The assay plate was covered and incubated at room temperature for 1 h. Then, the Kinase-Glo reagent was added to the PI3Kα plate to stop the reaction and shaken for 15 min, followed by the addition of kinase detection reagent, shaken for 1 min, and equilibrated for 60 min. The data were collected on FlexStation and presented in Excel. The curves were fitted by Graphpad Prism 5.0.

4.2.2. Antitumor test in vitro

The cells were plated in 96-well culture plates at density of 5000 cells per well and incubated for 24 h at 37 °C in a water-atmosphere (5% CO_2). The compounds with desired concentration were obtained by dissolving in DMSO and diluting with culture medium (DMSO final concentration < 0.4%). Then the diluted solution of compounds was treated with the cells for 48 h at 37 °C in a 5% CO_2 incubator. After that, 10 μL of a freshly diluted CCK-8 solution (5 mg/mL in PBS) were added to each well and the plates were incubated for 1 h. The cell survival was evaluated by measuring the absorbance at 540 nm. IC_{50} values were determined from the chart of cell viability (%) against compound concentration (nM). All experiments were carried out in triplicate.

4.2.3. Western blot analysis

U87MG cells were treated with **3j** and AZD8055 at the indicated concentrations for 24 h at 37 °C, then the cells were harvested, washed in ice-cold PBS, and lysed with RIPA buffer, protease inhibitors, phosphatase cocktails A and B, and PMSF (1 mM). Protein concentration was determined by the BCA Protein Assay Kit (beyotime#p0012s). The samples were subjected to SDS–PAGE and then transferred onto PVDF membranes. The membranes were incubated overnight at 4 °C with the primary antibody in 5% BSA/TBST buffer with gentle shaking, then washed with 1 × TBS/T 3 times, followed by incubation for 1 h with a 1/5000 dilution of secondary HRP antibody in 5% nonfat milk/TBST. Primary antibodies used were anti-AKT, anti-pAKTser473, antipAKTthr308, anti-S6RP, and anti-pS6RP Ser235/236, and the target blots were detected with chemiluminescence system.

4.2.4. Stability in human liver microsomes

Commercial, pooled, human (adult male and female) liver microsomes (HLMs) were purchased from Sigma-Aldrich. The biotransformations were conducted using 1 mg/mL of HLMs in 200 μL of reaction buffer containing 0.1 M Tris–HCl (pH 7.4) and the respective test compound with a final volume of 50 μM. The reaction mixture was preincubated at 37 °C for 5 min, and then, the reaction was commenced by adding 50 μL of NADPH Regeneration System. The reaction was ended after 120 min by the addition of cold methanol (200 μL). The mixture was next centrifuged at 14,000 rpm for 15 min, and HPLC analysis was performed.

4.2.5. In vivo efficacy study

Total 25 BALB/c small nude mice (divided into five groups), female for 6–7 weeks and body weight 16–20 g, are purchased from Shanghai SLAC Laboratory Animal CO. LTD; feed the mice under grade SPF. U87MG cells (5×10^6) were inoculated subcutaneously into the BALB/c nude mice; when the tumor grows up to 100–150 mm^3, allocate the mice in groups randomly (D0). Respectively dose each group of mice by intragastric administration with indicated dosage of the tested compounds every day for 10 days; weigh the weight of mice twice every 3 days and record the data. Gross tumor volume (V): $V = 1/2ab^2$. In which, a means length; b means width.

4.2.6. Pharmacokinetic study

Three male Beagle dogs (9–10 months old, body weight 9–10 kg) were administrated with tested compounds at a daily dose of 1 mg/kg by oral gavage, respectively. Blood collection was performed at time points of 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 24 h following administration. Blood samples (~1 mL) from Beagle dogs were collected on ice until centrifugation was performed with in 1 h (1000 × *g* and 4 °C for 15 min). All plasma samples were stored at −20 °C before analysis. The pharmacokinetic analyses were performed using WinNonlin version 5.2.1 (Pharsight Corporation, Mountain View, CA, USA). The pharmacokinetic parameters included half-life ($t_{1/2}$), area under the plasma concentration versus time curve within the 24-h dosing intervals (AUC_{0-24}) were determined by standard model independent methods based on the plasma concentration-time data. C_{max}, MRT, and T_{max} were determined directly from the plasma concentration-time data.

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