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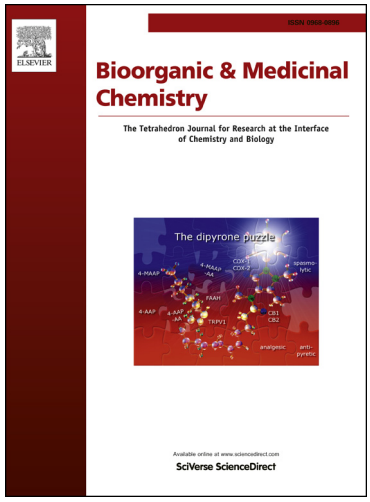

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PII: S0968-0896(15)00680-X
DOI: http://dx.doi.org/10.1016/j.bmc.2015.08.013
Reference: BMC 12517

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 13 July 2015
Revised Date: 12 August 2015
Accepted Date: 12 August 2015

Please cite this article as: Xie, X-X., Li, H., Wang, J., Mao, S., Xin, M-H., Lu, S-M., Mei, Q-B., Zhang, S-Q., Synthesis and anticancer effects evaluation of 1-alkyl-3-(6-(2-methoxy-3-sulfonylaminopyridin-5-yl)benzo[*d*] thiazol-2-yl)urea as anticancer agents with low toxicity, *Bioorganic & Medicinal Chemistry* (2015), doi: http://dx.doi.org/10.1016/j.bmc.2015.08.013

Synthesis and anticancer effects evaluation of 1-alkyl-3-(6-(2-methoxy-3-sulfonylaminopyridin-5-yl)benzo[*d*]thiazol-2-yl)urea as anticancer agents with low toxicity

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Abstract

As a PI3K and mTOR dual inhibitor, *N*-(2-chloro-5-(2-acetylaminobenzo[*d*]thiazol-6-yl)pyridin-3-yl)-4-fluorophenylsulfonamide displays toxicity when orally administrated. In the present study, alkylurea moiety replaced the acetamide group in the compound and a series of 1-alkyl-3-(6-(2,3-disubstituted pyridin-5-yl)benzo[*d*]thiazol-2-yl)urea derivatives were synthesized. The antiproliferative activities of the synthesized compounds *in vitro* were evaluated against HCT116, MCF-7, U87 MG and A549 cell lines. The compounds with potent antiproliferative activity were tested for their acute oral toxicity and inhibitory activity against PI3Ks and mTORC1. The results indicate that the compound attached a 2-(dialkylamino)ethylurea moiety at the 2-position of benzothiazole can retain the antiproliferative activity and inhibitory activity against PI3K and mTOR. In addition, their acute oral toxicity reduced dramatically. Moreover, compound **2f** can effectively inhibit tumor growth in a mice S180 homograft model. These findings suggest that 1-(2-dialkylaminoethyl)-3-(6-(2-methoxy-3-sulfonylaminopyridin-5-yl)benzo[*d*]thiazol-2-yl)urea derivatives can serve as potent PI3K inhibitors and anticancer agents with low toxicity.

Key Words benzo[*d*]thiazole ∗ urea ∗ PI3K inhibitor ∗ anticancer ∗ acute toxicity

1. Introduction

Benzothiazole derivatives exhibit a broad spectrum of biological activities such as antitumor, antimicrobial, anti-inflammatory, anticonvulsant and antidiabetic activity. The study progress of benzothiazole derivatives in medicinal chemistry has been reviewed.[1-3] There are hydrogen-bond acceptors and hydrogen-bond donor in the 2-aminobenzothiazole. Thus it is considered as a privileged drug scaffold in drug discovery.

Chemotherapy is one of the major approaches in cancer treatment. Traditional cytotoxic agents clinically used have been increasingly limited due to their high risk of toxicity, drug resistance and lack of selectivity. At present, the molecularly targeted therapies aiming at special targets have become effective approaches in cancer therapies. Kinases play an important role in tumor cell proliferation, survival and metastasis. Therefore, inhibitors against key kinases have emerged as novel targeted anticancer agents. PI3K (phosphoinositide 3-kinase) and mTOR (mammalian target of rapamycin) are critical nodes of PI3K/Akt/mTOR pathway, which are abnormally active in many tumor cells. For example, PIK3CA, the gene encoding for p110α (PI3Kα), is often over-expressed or mutate in a wide variety of cancer cell lines. Thus, targeting PI3K, especially PI3Kα, and / or mTOR has become an appealing strategy for cancer therapies.[4-6]

In recent years, a remarkable progress has been made in the design, synthesis and evaluation of PI3K and mTOR dual inhibitors,[7-12] and thereupon the pharmacophore of the dual inhibitors has been put forward.[13] Among the reported PI3K/mTOR dual inhibitors, *N*-(5-(quinilin-6-yl)-pyridin-3-yl)phenylsulfonamide is an important class of active compounds. GlaxoSmithKline identified GSK2126458 as a potent, orally bioavailable inhibitor of PI3Kα and mTOR.[14] Amgen designed, synthesized and evaluated several classes of *N*-(2,5-disubstitutedpyridin-3-yl)phenylsulfonamides. Therefore, they discovered that *N*-(2-chloro-5-(4-morpholinoquinilin-6-yl)pyridin-3-yl)-4-fluorophenylsulfonamide,[15] *N*-(2-chloro-5-(2-acetyl aminobenzo[*d*]thiazol-6-yl) pyridin-3-yl)-4-fluorophenylsulfonamide (compound **A**, Fig. 1),[16] *N*-(2-chloro-5-(2-

acetylaminoimidazo[1,2-*b*]pyridazin-6-yl)pyridin-3-yl)-4-fluorophenylsulfonamide[17] and AMG 511[18] are excellent PI3Kα/mTOR dual inhibitors or selective PI3Kα inhibitor, and orally bioavailable anticancer agents as well. The pharmacophore of above compounds consists of the two ring nitrogen atoms in pyridine and quinoline. Another active compound, *N*-(2-methoxy-5-(acetylamino[1,2,4]triazolo[1,5-*a*] pyridin-6-yl)pyridin-3-yl)-4-fluorophenylsulfonlyamide, having a pharmacophore similar to that of compound **A**, displays a potent anticancer effect.[19] Later, QSAR and pharmacophore of analogues of compound **A** were studied.[20] BEZ235[21] and PF-04979064[22] also possess a pharmacophore similar to compound **A**. As PI3Kα and mTOR dual inhibitors, they are in phase I/II clinical trials for treating solid tumors. Recently, it has been reported that VS-5584, a PI3K/mTOR dual inhibitor, can preferentially targets cancer stem cells.[23] This discovery may potentially bring a breakthrough to the treatment of cancer with small molecules. Thus, it is necessary to develop some new PI3K/mTOR dual inhibitors.

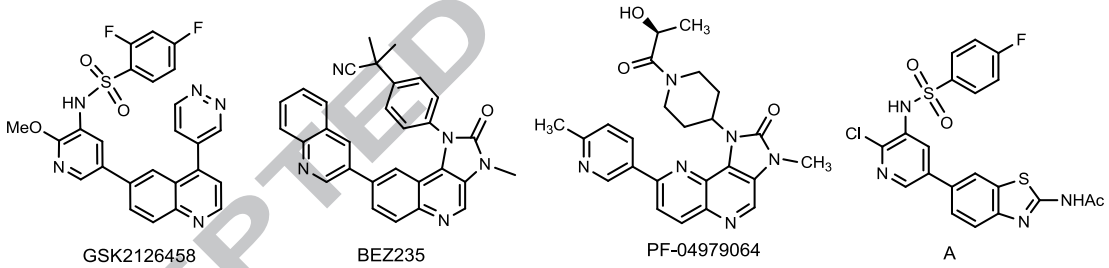


Figure 1. The structures of PI3K and mTOR dual inhibitors

As a potent PI3K/mTOR dual inhibitor, compound **A** can inhibit tumor growth against a wide range of tumors with different genetic backgrounds. Its EC_{50} ranges from 0.26 mg/kg to 0.53 mg/kg against three established nude mice human cancer cell xenograft models. However, compound **A** displays significant peroral toxicity.[16] On the basis of the result, we suspect that the toxicity of compound A is unfavorable to its development into clinical trials. To overcome this problem, it is of vital importance to treat seriously the toxicity of compound **A**.

According to the co-crystal structure of compound A with PI3Kγ,[16] we proposed that the structure of an amide group may take the place of the water molecule bridge. Thereupon, we synthesized a series of 2-substituted-3-phenylsulfonylamino-5-

(quinazolin-6-yl or quinolin-6-yl)benzamides, and discovered that the designed compounds are novel PI3K inhibitors and anticancer agents.[24] In our previous work, we combined the benzamide moiety with 2-aminobenzothiazole to discover novel anticancer agents (strategy **A** in Figure 2.).[25] Recently, we discovered that 1-alkyl-3-(6-(2-methoxy-3-(4-fluorophenylsulfonylamino)pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)urea derivatives can serve as potent PI3K inhibitors and anticancer agents with low toxicity.[26] In this work, we intend to replace the 2-acetylamino moiety in compound **A** with alkylamino or alkylurea moiety to search for the novel anticancer agents with low toxicity (strategy **B** in Fig. 2). Herein, we report our studies on the synthesis, biological activities and acute toxicity of designed compounds.

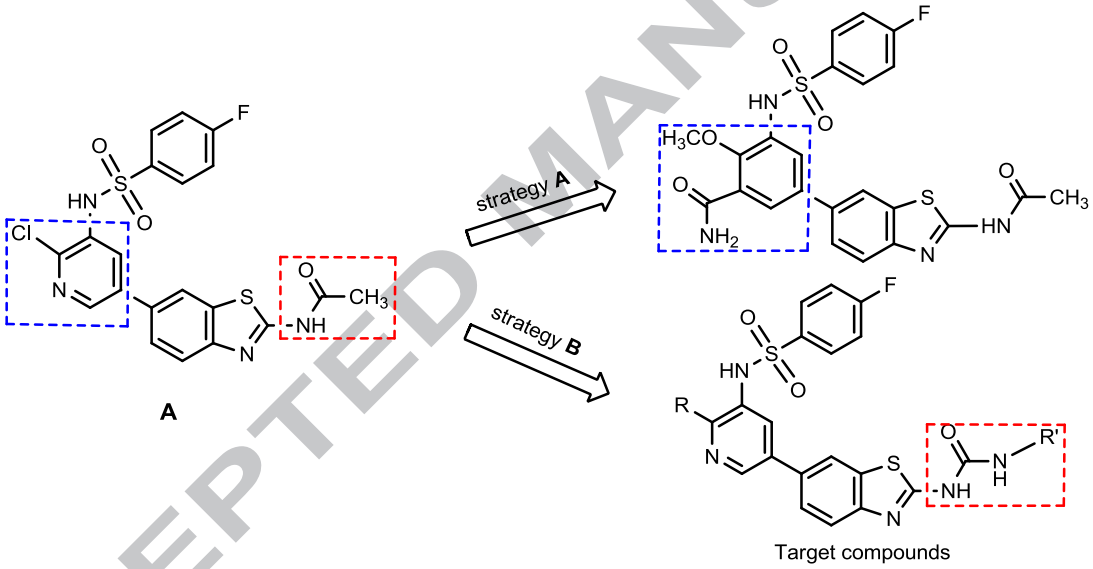


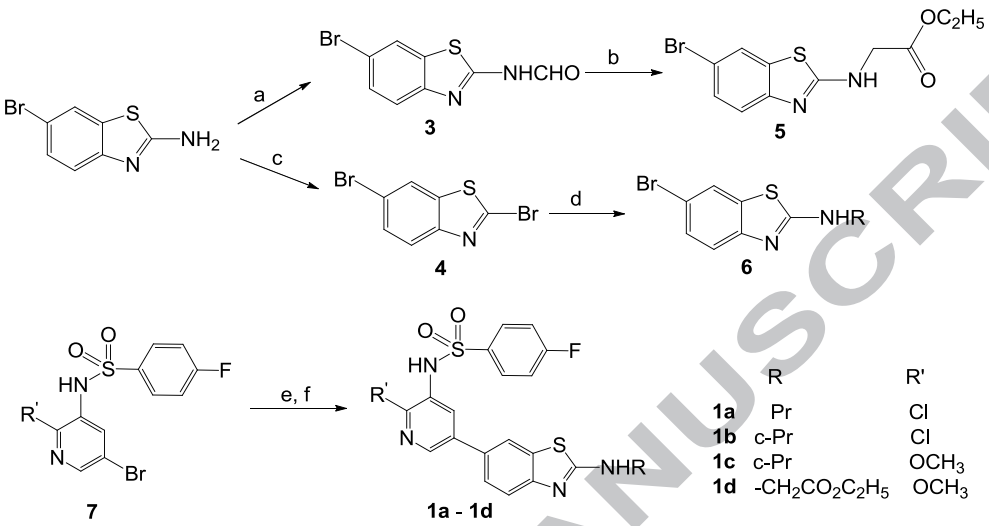
Figure 2. optimizing strategy

2. Results and discussion

2.1 Synthesis of designed compounds

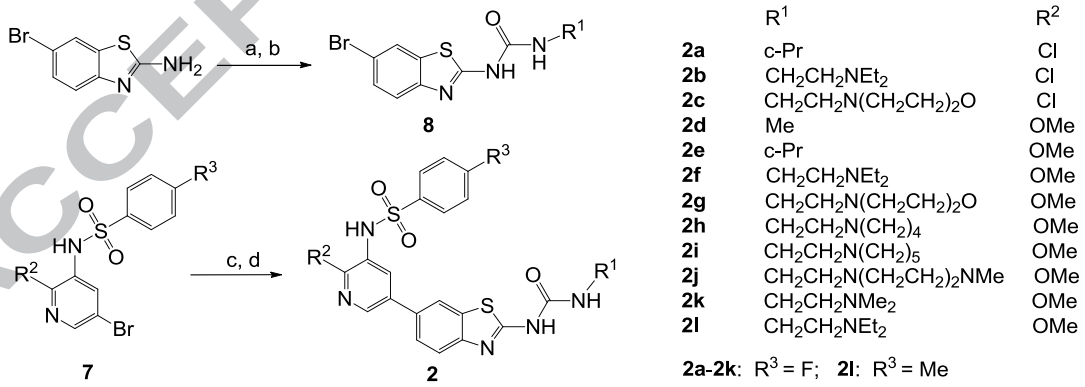
The synthetic route of compounds **1** is outlined in Scheme 1. Commercially available 2-amino-6-bromobenzo[*d*]thiazole was used as starting material to prepare intermediates **3**, **4**, **5** and **6**. The details were previously described in our work.[25] The sulfonamides **7** were prepared from 5-bromopyridine derivatives according to the synthetic route reported in our previous work.[19] Catalyzed by $PdCl_2$(dppf), intermediate **7** was reacted with bis(pinacolato)diboron to produce corresponding arylboronic esters. Without isolation of arylboronic esters, intermediate **5**, or **6**,

$PdCl_2$(dppf), water and potassium carbonate as well were added to the above reaction mixture. The resultant mixture was refluxed to produce compounds **1a-1d**. The preparation of arylboronic esters and Suzuki coupling were completed in one pot.



Scheme 1 Reagents and conditions: (a) AcOCHO, rt, ether, overnight; (b) $BrCH_2CO_2Et$, NaH, DMF, rt, 4 h; (c) H_3PO_4, $NaNO_2$, -10°C, 1 h, then CuBr, 40% HBr, rt, 1 h, 40°C, 2h; (d) n-$PrNH_2$ or c-$PrNH_2$, 1,4-dioxane, 45°C, 4 h; (e) bis(pinacolato)diboron, AcOK, $PdCl_2$(dppf), 1,4-dioxane, reflux, N_2, 3 h; (f) **5** or **6**, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/water (5:1), reflux, N_2, 2 h.

In Scheme 2, alkylurea moiety replaced the acetylamino group in compound A and compounds **2** were synthesized to probe the structure-activity relationship.

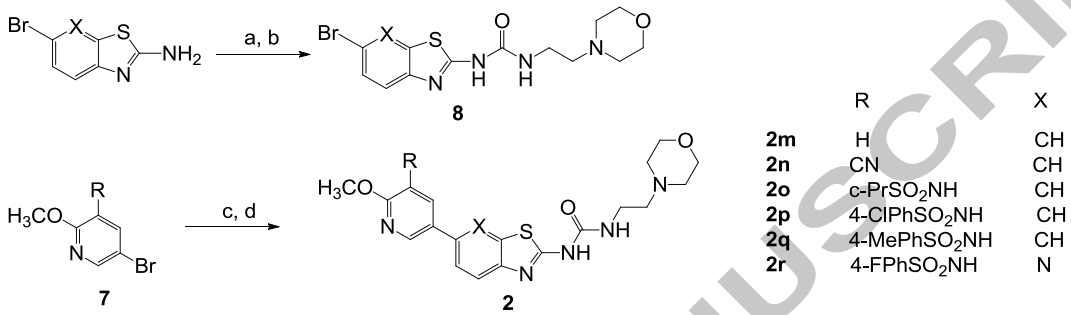


Scheme 2 Reagents and conditions: (a) CDI, DMF; (b) H_2NR^1; (c): bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, 1,4-dioxane, N_2, reflux 3 h; (d) **8**, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/water (5:1), N_2, reflux, 2 h.

2-Amino-6-bromobenzo[*d*]thiazole was reacted successively with carbonyldimidazole (CDI) and alkylamine to yield compounds **8**. In the same way with the preparation of compounds **1**, intermediate **7** was reacted successively with

bis(pinacolato)diboron and intermediate **8**, catalyzed by $PdCl_2$(dppf), to yield compounds **2a-2l**.

To compare the activities of compounds with different substituents at the 3-position of pyridine ring, compounds **2m-2q** were synthesized. Meanwhile, compound **2r** with a thiazolo[5,4-*b*]pyridine core was also synthesized (Scheme 3).



Scheme 3 Reagents and conditions: (a) CDI, DMF; (b) $H_2NCH_2CH_2N(CH_2CH_2)_2O$; (c): bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, 1,4-dioxane, N_2, reflux 3 h; (d) **8**, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/water (5:1), N_2, reflux, 2 h.

2.2. Biological evaluations

2.2.1 Antiproliferative assays *in vitro*

The antiproliferative activities of synthesized compounds were evaluated against human colon carcinoma cell line (HCT-116, PI3CA mutant: H1047R), human breast adenocarcinoma carcinoma cell line (MCF-7, PI3CA mutant: E545K), glioma cell line (U87 MG, PTEN null) and lung adenocarcinoma epithelial cell line (A549, KRAS mutant) by applying the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl- *2H*-tetrazolium bromide (MTT) colorimetric assay. The PI3K/mTOR dual inhibitors compound **A** and BEZ235 were used as the positive controls. The results are summarized in Table 1.

Table 1 Antiproliferative activities of compounds **1**, **2** ($\bar{x} \pm s$, n = 3)

Compds	IC_{50} (μM)			
	HCT-116	MCF-7	U87 MG	A549
1a	>20	nt	nt	nt
1b	5.75 ± 1.76	nt	nt	nt
1c	1.56 ± 0.58	0.45 ± 0.08	1.50 ± 0.26	1.33 ± 0.25
1d	>20	nt	nt	nt
2a	1.75±0.26	1.09±0.13	1.72±0.36	1.03±0.33
2b	0.92±0.13	0.71±0.16	1.39±0.39	5.26±0.59
2c	2.96±0.07	0.95±0.25	2.39±0.61	6.69±0.29
2d	0.87±0.15	1.19±0.22	1.23±0.28	1.56±0.38
2e	0.47±0.05	1.19±0.32	0.52±0.07	0.90±0.15
2f	0.30±0.10	0.32±0.10	0.39±0.01	0.45±0.04
2g	0.52±0.07	0.75±0.06	0.49±0.07	0.43±0.12
2h	0.31±0.08	0.41±0.04	0.36±0.04	0.30±0.07
2i	0.61±0.03	0.45±0.14	0.75±0.04	0.33±0.07
2j	0.54±0.12	0.47±0.15	0.44±0.08	0.55±0.09
2k	0.24±0.05	0.50±0.06	1.02±0.04	0.47±0.01
2l	0.18±0.02	0.17±0.04	0.36±0.07	0.31±0.05
2m	>20	>20	7.23±0.62	5.16±0.71
2n	4.55±0.34	2.30±0.42	8.38±2.13	4.71±1.97
2o	0.64±0.14	1.80±0.32	1.67±0.12	1.92±0.32
2p	0.54±0.15	0.65±0.06	1.05±0.29	0.53±0.02
2q	0.57±0.05	0.21±0.06	0.52±0.15	0.38±0.03
2r	0.25±0.02	0.26±0.04	0.48±0.13	0.49±0.04
A	0.44±0.05	0.43±0.07	0.44±0.08	0.41±0.08
BEZ235	0.29±0.03	0.48±0.10	1.41±0.32	0.76±0.08

nt: not tested.

Firstly, the replacement of 2-acetylamino in compound **A** with akylamino produced compounds **1a**-**1d**, which showed a significant drop in the cell-based activity. These results suggest that alkylamino is not a suitable substituent at the 2-position of benzothiazole core. Secondly, compounds **2a**-**2l** displayed potent antiproliferative activities against the four cancer cell lines and the activities of most compounds **2** were close to that of positive controls. These results suggest that

alkylurea moiety was tolerable at the 2-position of benzothiazole. The data in Table 1 reveal that the activity of compounds **2** is related to the substituent at the 2- and 3-positions of pyridine ring and the 1-position of urea moiety as well. The fact that compound **2f** was more potent than compound **2b** against four cancer lines indicates that the title compound with a methoxy at the 2-position pyridine ring may improve the antiproliferative activity. Thirdly, the compounds with the arylsulfonamino group at the 3-position of pyridine ring, such as 4-methylphenylsulfonamino (**2l**), exhibited more potent activity than the compounds with the cyclopropylsulfonamino group (**2o**) and the cyano group (**2n**) at the 3-position of pyridine ring. In the case where there was no a substituent at the 3-position of pyridine ring (**2m**), the activity dramatically dropped. Fourthly, to further investigate the structure-activity relationship (SAR) of compound **2**, a diverse range of amines were selected to attach to the 1-position of urea moiety. We synthesized compounds **2** by modifying substituent R^1 (Scheme 2). As the data in Table 1 indicate, the simple methyl urea **2d** and cyclopropyl urea **2e** showed a drop in their cell-based activity against four cancer lines compared with compound **A**. However, 2-(*N*,*N*-disubstituted amino)ethylureas **2f**-**2l** displayed similar or improved antiproliferative activity against the four cancer lines compared with the positive controls. Fifthly, the replacement of benzothiazole core in compound **2g** with thiazolo[5,4-*b*]pyridine moiety afforded compound **2r**, which displayed an improved activity against HCT-116 and MCF-7 cells and a close activity against U87 MG and A549 cells. These results reveal that compound **2r** is sensitive to PI3K mutant cells. In the cell-based activity, the IC_{50} of compound **2f** are comparable to that of PI3K and mTOR dual inhibitor compound **A** and **BEZ235**. In view of this, we further investigated compound **2f**.

2.2.2 PI3K and mTOR enzymatic activity assay

The selected compound **2f** was evaluated for its PI3K and mTOR enzymatic activity using an ATP depletion assay.[27] **BEZ235** was used as the positive drug. The results are listed in Table 2.

Table 2 Inhibitory enzymatic activities of compounds (n = 2)

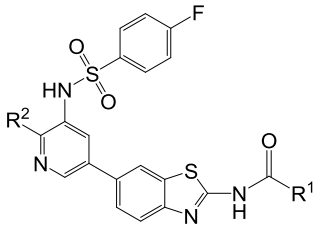
Compds	IC_{50} (nM)				
	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ	mTORC1
2f	13	90	11	148	78
BEZ235	49	478	72	138	44

Compound **2f** exhibited significant activities against PI3K and mTORC1. The inhibitory activities against PI3Kα, PI3Kβ and PI3Kγ are higher than **BEZ235**. The benzothiazole derivatives linked a methylurea moiety to C-2 were reported as effective inhibitors against the wild-type and T315I mutant of Bcr-Abl kinase.[28] In this work, 1-alkyl-3-(2,3-disubstituted pyridin-5-ylbenzo[*d*]thiazol-2-yl)urea were identified as PI3K and mTOR dual inhibitors.

2.2.3 Acute oral toxicity *in vivo*

Compounds **A**, **2d**, **2f**, **2h** and **2j** with potent antiproliferative activity *in vitro* were tested for their acute oral toxicity in male mice by employing an "up-and-down procedure".[29] The mice were administrated orally the prepared drug solution. The acute oral toxicity of the tested compounds is listed in Table 3.

Table 3. The acute oral toxicity of tested compounds



Compds	R^1	R^2	LD_{50} (mg/kg)	95% CI (mg/kg)*
A	$-CH_3$	Cl	20	19-22
2d	$-NHCH_3$	OCH_3	40	38-42
2f	$-NHCH_2CH_2NEt_2$	OCH_3	320	304-336
2h	$-NHCH_2CH_2N(CH_2)_4$	OCH_3	300	285-315
2j	$-NHCH_2CH_2N(CH_2CH_2)_2NMe$	OCH_3	300	285-315

* CI: confidence interval

The data in Table 3 indicate that the sequence of acute oral toxicity for tested

compounds is compound **A** > **2d** > **2h** > **2j** > **2f** and the toxicity is closely related to the structure of substitute at the 2-posotion of benzo[*d*]thiazole moiety. To our surprise, the acute oral toxicity of compounds **2f**, **2h** and **2j**, produced by the replacement of 2-acetylamino moiety in compound **A** with a 2-(*N*,*N*-disubstituted amino)ethylurea moiety, reduced dramatically. Hitherto, we have discovered new PI3K and mTOR dual inhibitors with low toxicity by optimizing compound **A**.

2.2.4 Anticancer effect on the mice S180 homograft models *in vivo*

Lastly, we tested the anticancer effect of compound **2f** in the homograft mice models established for this study. Mice bearing sarcoma (S180) were treated orally with compound **2f** at 1.0 mg/kg, 3.0 mg/kg and 10 mg/kg once a day for 8 days. As it was difficult to measure the volume of S180 tumor, tumor weights were used as evaluating indicators as illustrated in Figure 3A. The inhibitory ratios of compound **2f** at the dosage of 1.0 mg/kg, 3.0 mg/kg and 10.0 mg/kg were 27.2%, 44.3% and 56.2%, respectively. Additionally, the body weights of the tested mice increased during the treatment (Figure 3B). These results suggest that **2f** might be an effective anticancer agent with low toxicity.

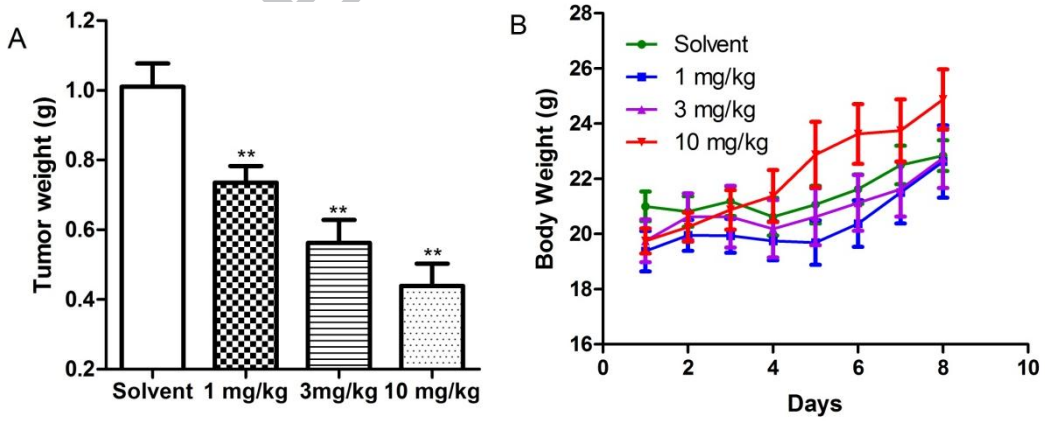


Figure 3. The anticancer effect of compound **2f** in S180 homograft model (**A**) and the change of tested mice body weights (**B**). Mice bearing subcutaneous tumors were orally administered vehicle, compound **2f (**1.0, 3.0 and 10.0 mg/kg doses) once daily for 8 days. $^{**}P < 0.01$.

2.3 Docking studies

In order to further understand the potency of compounds **2** in Table 1, we performed a docking analysis utilizing the C-DOCKER Program within Discovery Studio 2.5 software package. Docking simulations were carried out on human PI3Kγ (PDB code

3QK0)[18] with compounds **2f** and **2g**. The results are depicted in Figure 4.

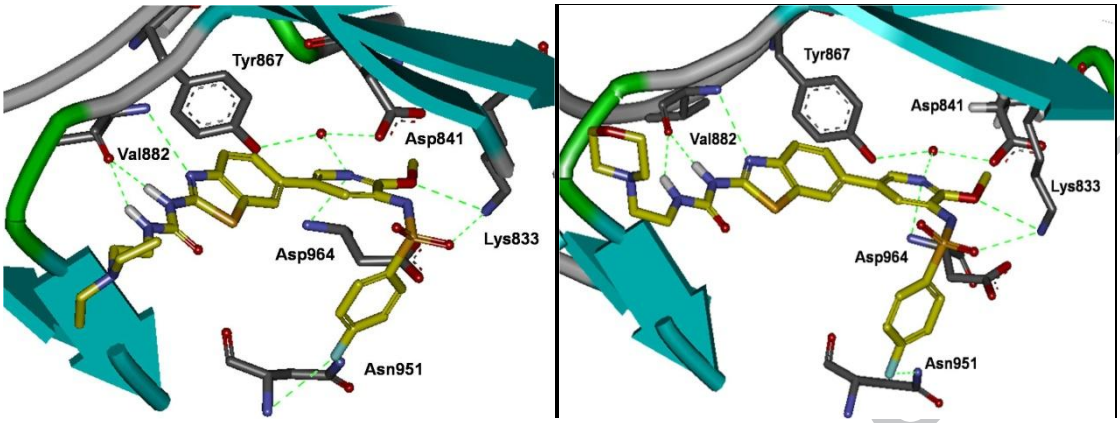


Figure 4. Docking mode of compound **2f** and **2g** with PI3Kγ. Selected residues Val882, Ala885, Tyr867, Lys833 are shown. Green dashed lines indicate hydrogen bond.

By the analysis of the binding mode of compounds **2f** (Figure 4, left) and **2g** (Figure 4, right) with PI3Kγ, we observed that the interaction of **2f** or **2g** with PI3Kγ is similar to that of compound **A** with PI3Kγ. The pyridine ring nitrogen atom can form two hydrogen bonds via an ordered water molecule located between the central and the Tyr867 and Asp841 residues. The methoxy at the 2-position of pyridine ring can form a hydrogen bond with Lys833, which may explain that the compound with a methoxy at the 2-position of pyridine ring is more potent than the compound with a chloride at the 2-position of pyridine ring in cell-based activity. As observed in Figure 4, the urea moiety in two compounds forms three hydrogen bonds with Val882. The binding mode is similar to benzothiazole-based Bcr-Abl inhibitors.[27]

3. Conclusion

In the present study, a series of 1-alkyl-3-(6-(2,3-disubstitutedpyridin-5-yl) benzo[*d*]thiazol-2-yl)urea derivatives were synthesized and characterized. Their antiproliferative activities *in vitro* were evaluated via MTT assay against four human cancer cell lines. Compound **2f** was tested for its inhibitory activity against PI3Ks and mTORC1. In addition, the acute oral toxicity of four compounds was tested by oral administration. The results indicate that the compound, produced by replacing the 2-acetylamino moiety in compound **A** with a 2-(dialkylamino)ethylurea moiety, can retain the antiproliferative activity and inhibitory PI3K and mTOR activity. In addition, their acute oral toxicity reduced dramatically. Moreover, compound **2f** can

effectively inhibit tumor growth in a mice S180 homograft model. These findings suggest that 1-(2-dialkylamino)ethyl-3-(6-(2-methoxy-3-sulfonylaminopyridin-5-yl) benzo[*d*]thiazol-2-yl)urea derivatives can serve as potent PI3K inhibitors and anticancer agents with low toxicity.

4. Experimental

4.1. Chemistry and chemical methods

Unless specified otherwise, all starting materials, reagents and solvents were commercially available. All reactions were monitored by thin-layer chromatography on silica gel plates (GF-254) and visualized with UV light. All the melting points were determined on a Beijing micro melting-point apparatus and thermometer was uncorrected. NMR spectra were recorded on a 400 Bruker NMR spectrometer with tetramethylsilane (TMS) as an internal reference. All chemical shifts are reported in parts per million (ppm). High-resolution exact mass measurements were performed using electrospray ionization (positive mode) on a quadrupole time-of-flight (QTOF) mass spectrometer (Maxis Q-TOF, Bruker Inc.).

4.1.1 General procedure for the synthesis of intermediates ***8***

*4.1.1.1 1-(6-Bromobenzo[d]thiazol-2-yl)-3-cyclopropylurea (**8a**)*

The mixture of 6-bromobenzo[*d*]thiazol-2-amine (2.0 g, 8.73 mmol), carbonyldimidazole (4.24 g, 26.2 mmol) and dried DMF (20 ml) was stirred at room temperature for 8 h, added cyclopropanamine (0.76 g, 13.2 mmol), then stirred at room temperature for another 8 h. The volatile was removed under reduced pressure. Water (30 ml) was added to the residue. The resulting suspension was stirred, standed. The solid was collected by filtration, dried to produce **8a** (1.77 g, 65%) as white solid. mp: >250°C; ^{1}H NMR(DMSO-d_6) 8.15 (s, 1H, Ar-H), 7.54 (d, J = 8.6 Hz, 1H, Ar-H), 7.48 (d, J = 8.6 Hz, 1H, Ar-H), 2.61 (dd, J = 6.8 Hz, 3.5 Hz, 1H, CH), 0.72-0.65 (m, 2H, CH_2), 0.51-0.44 (m, 2H, CH_2). MS (ESI, m/z): calcd for $[M+H]^+$ $C_{11}H_{11}BrN_3OS$: 312, 314, found 312, 314.

Intermediates **8b**-**8i** were synthesized according to the procedure described in **8a**.

*4.1.1.2 1-(6-Bromobenzo[d]thiazol-2-yl)-3-(2-(diethylamino)ethyl)urea (**8b**)*

White solid; Yield 76%; mp: 57-58°C; ^{1}H NMR ($CDCl_3$) δ 7.87 (s, 1H, Ar-H), 7.56 (d, 1H *J* = 8.6 Hz, 1H, Ar-H), 7.48 (d, *J* = 8.6 Hz, 1H, Ar-H), 3.46 (b, 2H, CH_2), 2.71 (m, 6H, CH_2×3), 1.12 (s, 6H, CH_3×2). MS (ESI, m/z): calcd for $[M+H]^+$ $C_{14}H_{20}BrN_4OS$: 314, 316, found 314, 316.

*4.1.1.3 1-(6-Bromobenzo[d]thiazol-2-yl)-3-(2-morpholinoethyl)urea (**8c**)*

White solid; Yield 90%; mp: 99-101°C; ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H, Ar-H), 7.54 (d, *J* = 8.6 Hz, 1H Ar-H), 7.48 (d, *J* = 8.6 Hz, 1H, Ar-H), 3.77 (s, 2H, CH_2), 3.51 (s, 4H, CH_2×2), 2.63 (m, 6H, CH_2×3). MS (ESI, m/z): calcd for $[M+H]^+$ $C_{14}H_{18}BrN_4O_2S$ 385, 387, found 385, 387.

*4.1.1.4 1-(6-Bromobenzo[d]thiazol-2-yl)-3-methylurea(**8d**)*

White solid; Yield 74%; mp: >250°C; ^{1}H NMR (DMSO-d_6) δ 10.94 (s, 1H, NH), 8.14 (s, 1H, Ar-H), 7.54 (d, J = 8.4 Hz, 1H, Ar-H), 7.48 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.63 (s, 1H, NH), 2.72 (d, J = 3.7 Hz, 3H, CH_3). MS (ESI, m/z): calcd for $[M+H]^+$ $C_9H_9BrN_3OS$: 286, 288, found 286, 288.

*4.1.1.5 1-(6-Bromobenzo[d]thiazol-2-yl)-3-(2-(pyrrolidin-1-yl)ethyl)urea (**8e**)*

White solid; Yield 85%; mp: 79-81°C; ^{1}H NMR ($CDCl_3$) δ 7.74 (s, 1H, Ar-H), 7.52 (d, 1H *J* = 8.6 Hz, 1H, Ar-H), 7.40 (d, *J* = 8.6 Hz, 1H, Ar-H), 3.56 (s, 2H, CH_2), 2.87 (m, 6H, CH_2×3), 1.88 (s, 4H, CH_2×2). MS (ESI, m/z): calcd for $[M+H]^+$ $C_{14}H_{18}BrN_4OS$: 369, 371, found 369, 371.

*4.1.1.6 1-(6-Bromobenzo[d]thiazol-2-yl)-3-(2-(piperidin-1-yl)ethyl)urea (**8f**)*

White solid; Yield 79%; mp: 84-86°C; ^{1}H NMR ($CDCl_3$) δ 7.84 (s, 1H, Ar-H), 7.50 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.45 (d, *J* = 8.5 Hz, 1H, Ar-H), 3.48 (s, 2H, CH_2), 2.67 - 2.49 (m, 6H, CH_2×3), 1.65 - 1.44 (m, 6H, CH_2×3). MS (ESI, m/z): calcd for $[M+H]^+$ $C_{15}H_{20}BrN_4OS$: 383, 385, found 383, 385.

*4.1.1.7 1-(6-Bromobenzo[d]thiazol-2-yl)-3-(2-(4-methylpiperazin-1-yl)ethyl)urea (**8g**)*

White solid; Yield 64%; mp: 97-99°C; ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H, Ar-H), 7.52 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.47 (d, *J* = 8.6 Hz, 1H, Ar-H), 3.49 (s, 2H, CH_2), 2.78 - 2.41 (m, 10H, CH_2 CH_2×5), 2.35 (s, 3H, CH_3). MS (ESI, m/z): calcd for $[M+H]^+$ $C_{15}H_{21}BrN_5OS$: 398, 400, found 398, 400.

*4.1.1.8 1-(6-Bromobenzo[d]thiazol-2-yl)-3-(2-(dimethylamino)ethyl)urea (**8h**)*

White solid; Yield 77%; mp: 176-178°C; ^{1}H NMR ($CDCl_3$) δ 7.84 (s, 1H, Ar-H), 7.50 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.45 (d, *J* = 8.5 Hz, 1H, Ar-H), 3.52 (s, 2H, CH_2), 2.65 (s, 2H, CH_2), 2.39 (s, 6H, CH_3 CH_2×3). MS (ESI, m/z): calcd for $[M+H]^+$ $C_{14}H_{20}BrN_4O_S$: 343, 345, found 343, 345.

*4.1.1.9 1-(6-Bromothiazolo[5,4-b]pyridin-2-yl)-3-(2-(morpholinoethyl)urea (**8i**)*

White solid; Yield 86%; mp: 152-154°C; ^{1}H NMR (DMSO-d_6) δ 11.13 (s, 1H, NH), 8.01 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.48 (d, *J* = 8.4 Hz, 1H, Ar-H), 6.81 (s, 1H, NH), 3.60 (s, 4H, OCH_2×2), 3.29 (d, *J* = 5.2 Hz, 2H, CH_2), 2.41 (s, 6H, NCH_2×3). MS (ESI, m/z): calcd for $[M+H]^+$ $C_{13}H_{17}BrN_5O_2S$: 386, 388, found 386, 388.

4.1.2 *General procedure for the synthesis of compounds **1a-1d, 2a-2r***

*4.1.2.1 N-(2-Chloro-5-(2-(propylamino)benzo[d]thiazol-6-yl-pyridin-3-yl)-4-fluoro phenylsulfonamide (**1a**)*

The preparation of intermediate **6a**, *N*-propyl-6-bromobenzo[*d*] thiazolyl-2-amine, was described in our previous work.[27] The preparation of intermediate **7a**, *N*-(2-chloro (or methoxy)-5-bromopyridin-3-yl)-4-fluorophenylsulfonamide, was described in our previously work.[21] The mixture containing **7a** (0.20 g, 0.55 mmol), bis(pinacolato)diboron (0.17 g, 0.66 mmol), KOAc (0.16 g, 1.65 mmol), $PdCl_2$(dppf) (30 mg, 0.04 mmol) and 1,4-dioxane (10 ml) was reflux for 2 h under nitrogen atmosphere, cooled to room temperature. To the resulted mixture were added **6a** (0.15 g, 0.55 mmol), Na_2CO_3 (0.64 g, 4.65 mmol), $PdCl_2$(dppf) (30 mg, 0.04 mmol) and water (2 ml). The mixture was refluxed under nitrogen atmosphere for 2 h. The volatiles were removed in vacuum and the residue was purified through a column chromatography on silica with chloroform/methanol (V:V = 50:1) as eluent to produce **1a** (0.12 g, 46%) as a white foam. mp: 214-215℃; ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H, NH), 8.58 (d, *J* = 2.4 Hz, 1H, Ar-H), 8.24 (t, *J* = 5.6 Hz, 1H, NH), 8.04 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.97 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.83-7.79 (m, 2H, Ar-H), 7.53 (dd, *J*=8.4, 2.0Hz, 1H, Ar-H), 7.47 (d, *J* = 8.4 Hz, 1H. Ar-H), 7.41-7.46 (m, 2H, Ar-H), 2.50 (m, 2H, CH_2), 1.62 (m, 2H, CH_2), 0.95 (t, *J* = 7.2 Hz, 3H, CH_3); ESI-HRMS *m/z*: calc'd for $C_{21}H_{20}ClFN_4O_2S_2$ $[M + H]^+$: 477.0622; found 477.0628.

Compounds **1b-1d**, **2a-2l** were synthesized according to the procedure described in **1a**.

*4.1.2.2 N-(2-Chloro-5-(2-(cyclopropylamino)benzo[d]thiazol-6-yl-pyridin-3-yl)-4-fluorophenylsulfonamide (**1b**)*

White solid; Yield 24%; mp: 213-215°C; ^{1}H NMR (DMSO-d_6) δ 10.48 (s, 1H, NH), 8.59 (s, 1H, Ar-H), 8.56 (s, 1H, NH), 8.11 (s, 1H, Ar-H), 7.97(s, 1H, Ar-H), 7.79-7.83 (m, 2H, Ar-H), 7.55 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.51 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.44 (m, 1H, Ar-H), 2.74 (m, 1H, CH), 0.80 (m, 2H, CH_2), 0.60 (m, 2H, CH_2); ESI-HRMS *m/z*: calc'd for $C_{21}H_{18}ClFN_4O_2S_2$ $[M + H]^+$: 475.0465; found 475.0472.

*4.1.2.3 N-(2-Methoxy-5-(2-(cyclopropylamino)benzo[d]thiazol-6-yl-pyridin-3-yl)-4-fluorophenylsulfonamide (**1c**)*

White solid; Yield 57%; mp: 194-195°C; ^{1}H NMR (DMSO-d_6) δ 10.03 (s, 1H, NH), 8.46 (s, 1H, NH), 8.27 (s, 1H, Ar-H), 8.00 (s, 1H, Ar-H), 7.84 (s, 1H, Ar-H), 7.82 (m, 2H, Ar-H), 7.81 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.47 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.42 (m, 2H, Ar-H), 3.64 (s, 3H, OCH_3), 2.72 (m, 1H, CH), 0.79 (m, 2H, CH_2), 0.59 (m, 2H, CH_2); ESI-HRMS *m/z*: calc'd for $C_{22}H_{21}FN_4O_3S_2$ $[M + H]^+$: 471.0961; found 471.0976.

*4.1.2.4 2-((6-(5-(4-fluorophenylsulfonamido)-6-mothoxypyridin-3-yl)benzo[d] thiazol-2-yl)amino)acetate (**1d**)*

White solid; Yield 20%; mp: 214-215°C; ^{1}H NMR (DMSO-d_6) δ 10.07 (s, 1H, NH), 8.99 (s, 1H, Ar-H), 8.35 (s, 1H, Ar-H), 7.94 (s, 1H, Ar-H), 7.81 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.73 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.51 (d, *J* = 9.2 Hz, 1H, Ar-H), 7.41(t, *J* = 8.8 Hz, 2H, Ar-H), 7.12 (t, *J* = 6.4 Hz, 1H, NH), 4.11 (q, *J* = 7.2 Hz, 2H, OCH_2), 4.01(d, *J* = 6.4 Hz, 2H, CH_2), 3.63(s, 3H, OCH_3), 1.20 (t, *J* = 6.8 Hz, 3H, CH_3); ESI-HRMS *m/z*: calc'd for $C_{23}H_{23}FN_4O_5S_2$ $[M + H]^+$: 517.1016; found 517.1026.

4.1.2.5 1-(6-(2-Chloro-3-(4-fluorophenylsulfonamino)pyridin-3-yl)benzo[d] thiazol-2-yl)-3-cyclopropylurea (2a)

White solid; Yield 39%; mp: >250°C; ^{1}H NMR (DMSO-d_6) δ 10.71 (s, 1H, NH), 10.51 (s, 1H, NH), 8.62 (s, 1H, Ar-H), 8.28 (s, 1H, Ar-H), 8.02 (s, 1H, Ar-H), 7.82 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.73 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.66 (d, *J* = 8.4 Hz, 1H, Ar-H),

7.44 (t, *J* = 8.8 Hz, 2H, Ar-H), 6.98 (s, 1H, NH), 2.63 (m, 1H, CH), 0.70 (m, 2H, CH_2), 0.50 (m, 2H, CH_2); ESI-HRMS *m/z*: calc'd for $C_{22}H_{17}ClFN_5NaO_3S_2$ $[M+Na]^+$: 540.0343; found 540.0338.

*4.1.2.6 1-(6-(2-Chloro-3-(4-fluorophenylsulfonamino)pyridin-3-yl)benzo[d] thiazol-2-yl)-3-(2-diethylamino)ethylurea (**2b**)*

White solid; Yield 36%; mp: 164-166°C; ^{1}H NMR (DMSO-d_6) δ 11.23 (s, 1H, NH), 9.49 (s, 1H, NH), 8.04 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.82 (s, 1H, Ar-H), 7.79 (d, J = 5.5 Hz, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.69 (d, J = 8.4 Hz, 1H, Ar-H), 7.48 (d, J = 8.4 Hz, 1H, Ar-H), 7.29 (t, J = 8.8 Hz, 2H, Ar-H), 6.92 (s, 2H s, 1H, NH), 3.45 (d, *J* = 5.6 Hz, 2H, CH_2), 3.00-3.05 (m, 6H, CH_2×3), 1.16 (t, *J* = 7.1 Hz, 6H, CH_3×2); ESI-HRMS *m/z*: calc'd for $C_{25}H_{27}ClFN_6O_3S_2$ $[M + H]^+$: 577.1259; found 577.1253.

*4.1.2.7 1-(6-(2-Chloro-3-(4-fluorophenylsulfonamino)pyridin-3-yl)benzo[d]thiazol-2-yl)-3-(2-morpholinoethyl)ethyl)urea (**2c**)*

White solid; Yield 38%; mp: 208-210°C; ^{1}H NMR(DMSO-d_6) δ 11.01 (s, 1H, NH), 10.22 (s, 1H, NH), 8.50 (s, 1H, Ar-H), 8.22 (s, 1H, Ar-H), 7.97 (s, 1H, Ar-H), 7.82 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.72 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.62 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.41 (t, *J* = 8.7 Hz, 2H, Ar-H), 6.86 (s, 1H, NH), 3.66-3.63 (m, 4H, OCH_2×2), 3.32-3.30 (m, 2H, NCH_2), 2.56- 2.51 (m, 6H, NCH_2×3); ESI-HRMS *m/z*: calc'd for $C_{25}H_{26}ClFN_6O_4S_2$ $[M+H]^+$: 591.1051; found 591.1046.

*4.1.2.8 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonamino)pyridin-3-yl)benzo[d] thiazol-2-yl)-3-methylurea (**2d**)*

White solid; Yield 31%; mp: >250°C; ^{1}H NMR (DMSO-d_6) δ 10.89 (s, 1H, NH), 10.05 (s, 1H, NH), 8.31 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.82 (dd, *J* = 8.8, 5.2 Hz, 2H, Ar-H), 7.69 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.57 (d, *J* = 8.4, 1H, Ar-H), 7.42 (t, *J* = 8.8 Hz, 2H, Ar-H), 6.65 (s, 1H, NH), 3.65 (s, 3H, OCH_3), 2.74 (d, *J* = 4.2 Hz, 3H, CH_3). ESI-HRMS *m/z*: calc'd for $C_{21}H_{20}FN_5O_4S_2$ $[M+H]^+$: 488.0862; found 488.0857.

*4.1.2.9 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonamino)pyridin-3-yl)benzo[d] thiazol-2-yl)-3-cyclopropylurea (**2e**)*

White solid; Yield 46%; mp: >250°C; ^{1}H NMR(DMSO-d_6) δ 10.64 (s, 1H, NH),

10.05 (s, 1H, NH) , 8.31 (s, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 7.83 (d, *J* = 4.5 Hz, 1H, Ar-H), 7.81 (d, *J* = 4.6 Hz, H, Ar-H), 7.69 (d, J = 8.2 Hz, 1H, Ar-H), 7.58 (d, J = 8.1 Hz, 1H, Ar-H), 7.42 (t, *J* = 8.5 Hz, 2H, Ar-H), 6.98 (s, 1H, NH), 3.65 (s, 3H, OCH_3), 2.64 (m, 1H, CH), 0.71 (s, 2H, CH_2), 0.50 (s, 2H, CH_2); ESI-HRMS *m/z*: calc'd for $C_{23}H_{20}FN_5O_4S_2$ $[M+H]^+$: 536.0838, found 536.0833.

*4.1.2.10 1-(2-Diethylamino)ethyl-3-(6-(2-methoxy-3-(4-fluorophenylsulfonamino) pyridin-3-yl)benzo[d]thiazol-2-yl)urea (**2f**)*

Off-White solid; Yield 52%; mp: 193-195°C; ^{1}H NMR(DMSO-d_6) δ 10.96 (s, 1H, NH), 9.93 (s, 1H, NH), 8.26 (s, 1H, Ar-H), 8.13 (s, 2H, Ar-H), 7.85 (s, 1H, Ar-H), 7.84-7.79 (m, 2H, Ar-H), 7.68 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.56 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.40 (t, *J* = 8.8 Hz, 2H, Ar-H), 6.80 (s, 1H), 3.66 (s, 3H, OCH_3), 3.26 (d, *J* = 5.7 Hz, 2H, NCH_2), 2.57 (m, 4H, CH_2×2), 1.01 (t, *J* = 7.0 Hz, 6H, CH_3×2). ESI-HRMS *m/z*: calc'd for $C_{26}H_{31}FN_6O_4S_2$ $[M+H]^+$: 573.1754; found 573.1748.

*4.1.2.11 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonamino)pyridin-3-yl)benzo[d]thiazol-2-yl)-1-(2-morpholino)ethylurea (**2g**)*

White solid; Yield 42%; mp: 209-211°C; ^{1}H NMR (DMSO-d_6) δ 10.90 (s, 1H, NH), 10.04 (s, 1H, NH), 8.30 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.82 (dd, *J* = 8.8, 5.2 Hz, 2H, Ar-H), 7.69 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.57 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.42 (t, *J* = 8.8 Hz, 2H, Ar-H), 6.83 (s, 1H, NH), 3.65 (s, 3H, OCH_3), 3.61 (s, 4H, OCH_2×2), 3.31 (b, 2H, NCH_2), 2.44 (m, 6H, NCH_2×3). ESI-HRMS *m/z*: calc'd for $C_{26}H_{29}FN_6O_5S_2$ $[M+H]^+$ 587.1547; found 587.1540.

*4.1.2.12 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonamino)pyridin-3-yl)benzo[d] thiazol-2-yl)-1-(2-pyrrolidin-1-yl)ethylurea (**2h**)*

White solid; Yield 34%; mp: 144-146°C; ^{1}H NMR(DMSO-d_6) δ 11.21 (s, 1H, NH), 10.12 (s, 1H, NH), 8.31 (d, *J* = 1.8 Hz, 1H, Ar-H), 8.16 (s, 1H Ar-H), 7.88 (s, 1H, Ar-H), 7.82 (dd, *J* = 8.5, 5.2 Hz, 2H, Ar-H), 7.70 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.59 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.42 (t, *J* = 8.7 Hz, 2H, Ar-H), 7.22 (s, 1H, NH), 3.65 (s, 3H, OCH_3), 3.49 (s, 2H, NCH_2), 3.13 (s, 6H, NCH_2×3), 1.89 (s, 4H, CH_2×2). ESI-HRMS *m/z*: calc'd for $C_{26}H_{29}FN_6O_4S_2$ $[M+H]^+$: 571.1597; found 571.1592.

4.1.2.13 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonamino)pyridin-3-yl)benzo[d]

*thiazol-2-yl)-1-(2-piperidin-1-yl)ethylurea (**2i**)*

Pale yellow solid; Yield 37%; mp: 160-162°C; ^{1}H NMR(DMSO-d_6) δ 11.15 (s, 1H, NH), 9.79 (s, 1H, NH), 8.31 (d, *J* = 2.2 Hz, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 7.88 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.82 (dd, *J* = 8.8, 5.2 Hz, 2H, Ar-H), 7.70 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.58 (dd, 1H, Ar-H), 7.42 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.04 (s, 1H, NH), 3.65 (s, 3H, OCH_3), 3.45 (s, 2H, NCH_2), 2.87 (m, 6H, NCH_2×3), 1.67 (s, 4H, CH_2×2), 1.49 (s, 2H, CH_2). ESI-HRMS *m/z*: calc'd for $C_{27}H_{31}FN_6O_4S_2$ $[M+H]^+$: 585.1754; found 585.1748.

*4.1.2.14 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonamino)pyridin-3-yl)benzo[d] thiazol-2-yl)-1-(2-(4-methylpiperazin-1-yl))ethylurea (**2j**)*

White solid; Yield 34%; mp: 214-216°C; ^{1}H NMR(DMSO-d_6) δ 11.06 (s, 1H, NH), 8.24 (d, J = 2.1 Hz, 1H, Ar-H), 8.11 (d, *J* = 1.5 Hz, 1H, Ar-H), 7.83(d, *J* = 2.2 Hz, 1H, Ar-H), 7.81 (d, *J* = 5.2 Hz, 1H), 7.68 (d, *J* = 8.3 Hz, 2H), 7.54 (dd, 1H, Ar-H), 7.40 (t, *J* = 8.9 Hz, 2H, Ar-H), 7.08 (s, 1H, NH), 3.66 (s, 3H, OCH_3), 3.27 (d, *J* = 5.8 Hz, 2H, CH_2), 2.48-2.38 (m, 10H, NCH_2×5), 2.25 (s, 3H, CH_3). ESI-HRMS *m/z*: calc'd for $C_{27}H_{32}FN_7O_4S_2$ $[M+H]^+$: 600.1863; found 600.1857.

*4.1.2.15 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonamino)pyridin-3-yl)benzo[d] thiazol-2-yl)-3-(2-dimethylamino)ethylurea (**2k**)*

White solid; Yield 33%; mp: 136-138°C; ^{1}H NMR(DMSO-d_6) δ 10.84 (s, 1H, NH), 8.29 (s, 1H, Ar-H), 8.14 (s, 1H, Ar-H), 7.87 (s, 1H, Ar-H), 7.84-7.80 (dd, *J* = 7.8, 5.4 Hz, 2H, Ar-H), 7.69 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.57 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.41 (t, *J* = 8.6 Hz, 2H, Ar-H), 6.86 (s, 1H, NH), 3.65 (s, 2H, OCH_3), 3.30-3.26 (m, 2H, NCH_2), 2.29 (s, 6H, CH_3×2). ESI-HRMS *m/z*: calc'd for $[M+H]^+$ $C_{24}H_{26}FN_6O_4S_2$: 545.1441, found 545.1435.

*4.1.2.16 1-(2-Diethylamino)ethyl-3-(6-(2-methoxy-3-(4-methylphenylsulfonamino) pyridin-5-yl)benzo[d]thiazol-2-yl)urea (**2l**)*

White solid; Yield 51%; mp: 115-117°C; ^{1}H NMR($CDCl_3$) δ 8.02 (s, 1H, Ar-H), 7.97 (s, 1H, Ar-H), 7.75 (s, 1H, Ar-H), 7.70 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.67 (s, 1H, Ar-H), 7.43 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.23 (d, *J* = 8.0 Hz, 2H, Ar-H), 3.84 (s, 3H, OCH_3), 3.79 (s, 2H, CH_2), 3.20 (d, *J* = 3.4 Hz, 6H, NCH_2×3), 2.36 (s, 3H, CH_3), 1.33

(t, *J* = 6.6 Hz, 6H, CH_3×2). ESI-HRMS *m/z*: calc'd for $C_{27}H_{33}N_6O_4S_2$ $[M+H]^+$: 569.2005; found 569.1999.

*4.1.2.17 1-(6-(6-methoxypyridin-3-yl)benzo[d]thiazol-2-yl)-3-(2-morpholino) ethylurea (**2m**)*

White solid; Yield 47%; mp: 100-102°C; ^{1}H NMR(DMSO-d_6) δ 10.83 (s, 1H, NH), 8.47 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 8.01 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.63 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.61 (s, 1H, Ar-H), 6.87 (d, *J* = 8.4 Hz, 1H, Ar-H), 6.79 (s, 1H, NH), 3.85 (s, 3H, OCH_3), 3.56 (s, 4H, OCH_2×2), 3.25 (d, *J* = 4.8 Hz, 2H, NCH_2), 2.38 (m, 6H, NCH_2×3). ESI-HRMS *m/z*: calc'd for $C_{20}H_{24}N_5O_3S$ $[M+H]^+$: 414.1600; found 414.1594

*4.1.2.18 1-(6-(3-Cyano-2-methoxypyridin-5-yl)benzo[d]thiazol-2-yl)-3-(2-morpholino) ethylurea (**2n**)*

White solid; Yield 42%; mp: 200-202°C; ^{1}H NMR(DMSO-d_6) δ 10.93 (s, 1H, NH), 8.83 (s, 1H, Ar-H), 8.65 (s, 1H, Ar-H), 8.28 (s, 1H, Ar-H), 7.74 (dd, J = 8.5, 1.5 Hz, 1H, Ar-H), 7.68 (s, 1H, Ar-H), 6.82 (s, 1H, NH), 4.05 (s, 3H, OCH_3), 3.61 (s, 4H, OCH_2×2), 3.320 (m, 2H, NCH_2), 2.43 (d, J = 5.6 Hz, 6H, NCH_2×3). ESI-HRMS *m/z*: calc'd for $C_{21}H_{23}N_6O_3S$ $[M+H]^+$: 439.1552; found 439.1547.

*4.1.2.19 1-(6-(3-cyclopropanylsulfonamino-2-methoxypyridin-5-yl)benzo[d]thiazol-2-yl)-3-(2-morpholino)ethylurea (**2o**)*

White solid; Yield 40%; mp: 191-193°C; ^{1}H NMR (DMSO-d_6) δ 10.89 (s, 1H, NH), 9.38 (s, 1H, NH), 8.34 (s, 1H, Ar-H), 8.20 (s, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 7.69 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.62 (dd, *J* = 8.4, 1.8 Hz, 1H, Ar-H), 6.83 (s, 1H, NH), 3.97 (s, 3H, OCH_3), 3.61 (m, 4H, OCH_2×2), 3.30 (dd, *J* = 11.7, 6.0 Hz, 2H, NCH_2), 2.78 (m, 1H, CH), 2.44 (m, 6H, NCH_2×3), 0.99-0.89 (m, 4H, CH_2×2). ESI-HRMS *m/z*: calc'd for $C_{23}H_{29}N_6O_5S_2$ $[M+H]^+$: 533.1641; found 533.1635.

*4.1.2.20 1-(6-(3-chlorophenylsulfonamino-2-methoxypyridin-5-yl)benzo[d]thiazol-2-yl)-3-(2-morpholino)ethylurea (**2p**)*

Pale yellow solid; Yield 41%; mp: 153-155°C; ^{1}H NMR(DMSO-d_6) δ 10.92 (s, 1H, NH), 10.12 (s, 1H, NH), 8.32 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.75 (s, 1H, Ar-H), 7.69 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.66 (d,

J = 8.6 Hz, 2H, Ar-H), 7.58 (d, *J* = 8.4 Hz, 1H, Ar-H), 6.84 (s, 1H, NH), 3.64 (s, 3H, OCH_3), 3.62 (s, 4H, OCH_2×2), 3.30 (m, 2H, NCH_2), 2.45 (m, 6H, NCH_2×3). ESI-HRMS *m/z*: calc'd for $C_{26}H_{28}ClN_6O_5S_2$ $[M+H]^+$: 603.1251; found 603.1246.

*4.1.2.21 1-(6-(2-methoxy-3-(4-methylphenylsulfonamino)pyridin-5-yl)benzo[d] thiazol-2-yl)-3-(2-morpholino)ethylurea (**2q**)*

White solid; Yield 31%; mp: 195-197°C; ^{1}H NMR(DMSO-d_6) δ 10.90 (s, 1H, NH), 9.90 (s, 1H, NH), 8.26 (s, 1H, Ar-H), 8.11 (s, 1H, Ar-H), 7.83 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.69 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.65 (s, 1H, Ar-H), 7.54 (d, *J* = 8.4, 1H, Ar-H), 7.37 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.83 (s, 1H, NH), 3.68 (s, 3H, OCH_3), 3.62 (s, 4H, OCH_2×3), 3.30 (m, 2H, NCH_2), 2.44 (m, 6H, NCH_2×3), 2.36 (s, 3H, CH_3). ESI-HRMS *m/z*: calc'd for $C_{27}H_{31}N_6O_5S_2$ $[M+H]^+$: 583.1797; found 583.1792.

4.1.2.22 1-(6-(3-(4-fluorophenylsulfonamino)-2-methoxypyridin-5-yl)thiazolo[5,4-b] pyridin-2-yl)-3-(2-morpholino)ethylurea (2r)

White solid; Yield 58%; mp: 192-194°C; ^{1}H NMR(DMSO-d_6) δ 11.05 (s, 1H, NH), 10.06 (s, 1H, NH), 8.67 (s, 1H, Ar-H), 8.28 (s, 1H, Ar-H), 8.02 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.95 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.82 (d, *J* = 5.2 Hz, 1H, Ar-H), 7.80 (d, *J* = 5.2 Hz, 1H, Ar-H), 7.42 (t, *J* = 8.8 Hz, 2H, Ar-H), 6.84 (s, 1H, NH), 3.68 (s, 3H, OCH_3), 3.61 (s, 4H, OCH_2×2), 3.32 (d, *J* = 5.7 Hz, 2H, NCH_2), 2.45 (d, *J* = 6.5 Hz, 6H, NCH_2×3). ESI-HRMS *m/z*: calc'd for $C_{25}H_{27}N_7O_5S_2$ $[M+H]^+$: 588.1499; found 588.1494.

4.2. Biology

4.2.1. Cell Culture

The human cell lines HCT-116, MCF-7and U-87 MG were maintained as a monolayer culture in DMEM, supplemented with 10% FBS, while A549 was cultured in RPMI-1640 with 10% calf serum in a humidified atmosphere (5% CO_2) at 37 °C, respectively.

4.2.2 Antiproliferative assays

Compound **A** was synthesized in our laboratory (Purity: 96%, HPLC). BEZ235 was purchased from Shanghai Biochempartner Company (Purity: 99%, HPLC). 3-[4,

5-dimethylthiazol-2-yl]-2, 5-diphenyl-*2H*-tetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO, USA). Cellular chemosensitivity was determined by using a modified MTT method assay in vitro. In brief, HCT-116, MCF-7, U-87 MG or A549 cells in 200 μl culture medium were seeded into 96-well microplates at 3000-5000 cells per well respectively and cultured in DMEM with 10% FBS or RPMI-1640 with 10% calf serum, incubated at 37 °C for 24 h prior to drug exposure. Cell numbers were titrated to keep control cells growing in the exponential phase throughout the 72 h incubation period. Cells were treated with final concentrations of 100.0, 10.0, 1.0, 0.1 and 0.01 μM of tested compounds simultaneously and incubated for 72 h and then 20 μl of MTT solution (5 mg/ml in PBS) was added to each well at lucifugal condition and incubated for 4 h at 37°C. The formed purple formazan crystals were pelleted at the bottom of the well, separated from the supernatant, and dissolved in 200 μl of DMSO. The optical density at 570 nm was determined by Varioskan Flash Multimode Reader (Thermo scientific). Three separate experiments with triplicate data were performed to obtain mean cell viability. The IC_{50} value, that is, the concentration (μM) of a compound was able to cause 50% cell death with respect to the control culture, was calculated according to the inhibition ratios.

PI3K and mTOR enzymatic activity assay

PI3K and mTORC1 enzymatic activity assay was performed according to process described in reference.[28] Briefly, compounds 2f and BEZ235 were dissolved in DMSO and diluted to a series of concentrations. Different concentrations of compounds were added to the enzyme reaction buffer containing 40 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 0.1 mg/mL BSA, 1 mM DTT, 2.5 μM ATP, PI3K (p110α/p85α, p110β/p85α, p110γ/PIK3R5 or p110δ/p85α), mTORC1 and L-α-phosphatidylinositol. The final reaction volume was 50 μL. After incubation for 40 min at 30 °C, the reaction was terminated by addition of stop solution. The amount of ADP was then detected via luciferase assay. After incubation for 5 min, the luminescence signal was determined by the multimode reader (MD-SpectraMax M5). The signal intensity is proportional to the enzyme activity.

The percentage of inhibition was calculated based on the following equation

% inhibition rate = [1 – ($Lu_{compound}$ – Lu_{min})/(Lu_{max} – Lu_{min})] × 100%,

Where $Lu_{compound}$ is the signal at a given compound concentration, Lu_{max} is the signal of PI3Ks without compound and Lu_{min} is the signal of background in the absence of enzyme and compound. The IC_{50} values were calculated according to the fit of the dose -response curves by using GraphPad Prism5.

4.2.3 Acute oral toxicity in vivo

Mice (20.1 ± 1.9 g) were purchased from Experiment Animal Center of Xi′an Jiaotong University and fed in the same place. The experimental protocol was approved by Ethic Committee of Xi′an Jiaotong University.

The tested compounds were, respectively, dissolved in NMP, diluted with PEG400 and H_2O to prepare proper solutions with different concentrations (NMP/PEG400/H_2O is 1:6:3 in volume ratio). A mouse was dosed orally once a day, and then observed for 7 days. The dosage was started at 10 mg/kg at a volume of 20 ml/kg. If the mouse exercised normally, the dosage was increased to 1.5-fold up to a mouse died in a single dose. The software AOT425StatPgm was employed to estimate LD_{50}.

4.2.4 In vivo anticancer effect on established mice S180 homograft models

Mice (19.6 ± 1.9 g) were purchased from Experiment Animal Center of Xi′an Jiaotong University Health Science Center and fed in the same place. The experimental protocol was approved by Ethic Committee of Xi′an Jiaotong University.

3×10^6 S180 cells were injected subcutaneously into the flank of the mice. All tumor-bearing mice were randomly divided into four groups, with 8 mice in each group. The next day, compound **2f** was dissolved in NMP/PEG400/H_2O (1:6:3) and dosed orally at 1.0 mg/kg, 3.0 mg/kg and 10.0 mg/kg for the low, middle and high dosage groups once a day for 8 days, respectively. In the solvent group, the same volume of solvent was administered orally. Body weights were recorded per day. The mice were anesthetized and sacrificed on Day 9. The weights of the body and the neoplasm were measured and inhibitory ratios of tumor weight were calculated.

4.2.5 Molecular modeling

The protein-ligand complex crystal structure of compound **A** bound to PI3Kγ was chosen as the template to compare the docking mode between compound **2f** bound to PI3Kγ and **2g** bound to PI3Kγ. The molecular docking procedure was performed by using C-DOCKER protocol within Discovery Studio 2.5. For enzyme preparation, the hydrogen atoms were added. The whole PI3Kγ enzyme was defined as a receptor and the site sphere was selected on the basis of the ligand binding location of compound **A**. Compound **A** was removed and compound **2f** or **2g** was placed. After end of molecular docking, ten docking poses was scored and selected based on calculated C-DOCKER energy.

Acknowledgment: Financial support from National Natural Science Foundation of China (Grant No. 81272448) is gratefully acknowledged.

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Figure and Scheme Legend

Figure 1. The structures of PI3K and mTOR dual inhibitors

Figure 2. optimizing strategy

Figure 3. The anticancer effect of compound **2f** in S180 homograft model (**A**) and the change of tested mice body weights (**B**). Mice bearing subcutaneous tumors were orally administered vehicle, compound **2f (**1.0, 3.0 and 10.0 mg/kg doses) once daily for 8 days. $^{**}P < 0.01$.

Figure 4. Docking mode of compound **2f** and **2g** with PI3Kγ. Selected residues Val882, Ala885, Tyr867, Lys833 are shown. Green dashed lines indicate hydrogen bond.

Scheme 1 Reagents and conditions: (a) AcOCHO, rt, ether, overnight; (b) $BrCH_2CO_2Et$, NaH, DMF, rt, 4 h; (c) H_3PO_4, $NaNO_2$, -10°C, 1 h, then CuBr, 40% HBr, rt, 1 h, 40°C, 2h; (d) n-$PrNH_2$ or c-$PrNH_2$, 1,4-dioxane, 45°C, 4 h; (e) bis(pinacolato)diboron, AcOK, $PdCl_2$(dppf), 1,4-dioxane, reflux, N_2, 3 h; (f) **5** or **6**, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/water (5:1), reflux, N_2, 2 h.

Scheme 2 Reagents and conditions: (a) CDI, DMF; (b) H_2NR^1; (c): bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, 1,4-dioxane, N_2, reflux 3 h; (d) **8**, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/water (5:1), N_2, reflux, 2 h.

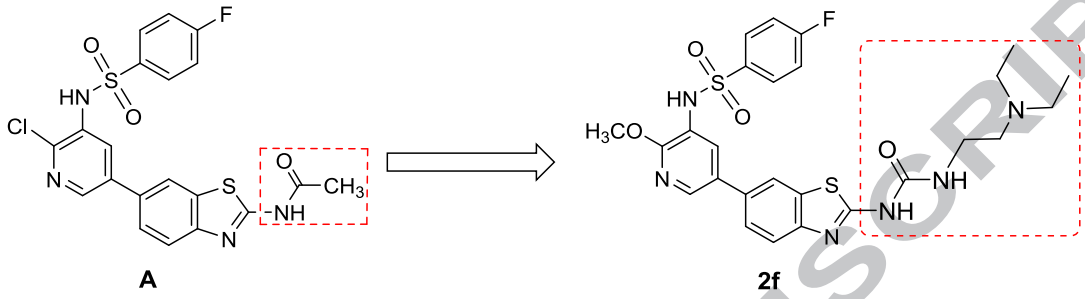
Scheme 3 Reagents and conditions: (a) CDI, DMF; (b) $H_2NCH_2CH_2N(CH_2CH_2)_2O$; (c): bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, 1,4-dioxane, N_2, reflux 3 h; (d) **8**, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/water (5:1), N_2, reflux, 2 h.

Table 1 Antiproliferative activities of compounds **1**, **2** ($\bar{x} \pm s$, n = 3)

Table 2 Inhibitory enzymatic activities of compounds (n = 2)

Table 3. The acute oral toxicity of tested compounds

Graphical abstract



LD_{50}: 20 mg/kg

LD_{50}: 320 mg/kg

IC_{50} against PI3Kα: 13 nM