Bioorganic & Medicinal Chemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Modification of *N*-(6-(2-methoxy-3-(4-fluorophenylsulfonamido) pyridin-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)acetamide as PI3Ks inhibitor by replacement of the acetamide group with alkylurea

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ARTICLE INFO

Article history:
Received 10 May 2015
Revised 9 July 2015
Accepted 10 July 2015
Available online xxxx

Keywords:
[1,2,4]Triazolo[1,5-*a*]pyridine
Urea
PI3K inhibitors
Anticancer agent
Acute toxicity

ABSTRACT

N-(6-(2-Methoxy-3-(4-fluorophenylsulfonamido)pyridin-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)acetamide exhibits remarkable anticancer effects and toxicity when orally administrated. In present study, alkylurea moiety replaced the acetamide group in the compound and a series of 1-alkyl-3-(6-(2-methoxy-3-sulfonylaminopyridin-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)urea derivatives were synthesized. The antiproliferative activities of the synthesized compounds in vitro were evaluated against four human cancer cell lines. Several compounds with potent antiproliferative activities were tested for their acute oral toxicity and their inhibitory activity against PI3Ks and mTOR. The results indicate that the compound attached a alkylurea or 2-(dialkylamino)ethylurea moiety at the 2-position of [1,2,4]triazolo[1,5-*a*]pyridine can retain the antiproliferative activity and the inhibitory activity against PI3Ks and mTOR. In addition, their acute oral toxicity reduced dramatically. Moreover, the results also indicate that compound **1e** can efficaciously inhibit tumor growth in a mice S180 model. These findings suggest that title compounds can serve as potent PI3K inhibitors and effective anticancer agents with low toxicity.

1. Introduction

The chemical and biological study of heterocyclic compounds has been an interesting field in medicinal chemistry for a long time. [1,2,4]triazolo[1,5-*a*]pyridine consists of a triazole ring fused with a pyridine ring. There are hydrogen bond acceptors and hydrogen bond donor in the structure of 2-acetylmino-[1,2,4]triazolo[1,5-*a*]pyridine. Thus, the structure is considered as an ideal fragment or a scaffold in drug design. The derivatives of [1,2,4]triazolo[1,5-*a*]pyridine exhibit a broad spectrum of biological activities such as anticancer, anti-inflammatory, antimicrobial and antidiabetic activity. CEP-33779, possessing the scaffold of 1,2,4-triazolo[1,5-*a*]pyridine, was discovered as a novel, selective, and orally bioavailable JAK2 inhibitor and can be used for cancer therapy or rheumatoid arthritis treatment.[1–3] 2-Acylamino-6-aryl-[1,2,4]triazolo[1,5-*a*]pyridines were identified as inhibitors of the leishmania cdc 2-related protein kinase CRK3 and may be used to treat tropical parasitic diseases such as leishmaniasis and human African trypanosomiasis (HAT).[4] PI3Kγ plays a key role in innate immune responses such as immune cell migration.[5,6] CZC19945 and CZC24832, derived from 6-aryl-[1,2,4]triazolo[1,5-*a*]pyridine, were identified as PI3Kγ inhibitors with good in vivo PK profile and efficacy in vitro and in vivo models of inflammation.[7] Further, CZC24832 was proved to be efficacious in regulate interleukin-17-producing T helper cell (T_H17) differentiation and may be of use for the treatment of autoimmune and inflammatory disorders.[8] Recently, a series of 2-ureido-[1,2,4]triazolo[1,5-*a*]pyridine derivatives were synthesized and two compounds exhibited excellent PI3Kγ/δ potency with high selectivity over the other isoforms and the general kinome.[9] In addition, multisubstituted [1,2,4]triazolo[1,5-*a*]pyridines were discovered as inhibitors of β-1,6-glucan synthesis with potent antifungal activity.[10] 2-Ureido-5,7-disubstituted-[1,2,4]triazolo[1,5-*a*]pyridine was reported as good antibacterial agent with potent Gram-positive antibacterial activity.[11] Early, 6-substituted [1,2,4]triazolo[1,5-*a*] pyridine was reported as a potent DPP-4 inhibitor and may be used to treat type 2 diabetes.[12]

Both PI3K (a family of lipid kinase) and mTOR (mammalian target of rapamycin) have been found to play key regulatory roles in

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http://dx.doi.org/10.1016/j.bmc.2015.07.017
0968-0896/

many cellular processes, including cell growth, proliferation, differentiation, motility and survival.[13] Accumulation evidence supports the notion that The PI3K/AKT/mTOR signal transduction pathway is dysregulated expression in many cancers, contributing to cellular transformation and tumor growth.[14] Therefore, PI3Kα and mTOR, as key nodes of the PI3K/AKT/mTOR pathway, have been identified as promising kinase targets for cancer therapy.[15,16] Recently, several PI3K inhibitors and PI3K/mTOR dual inhibitors have been in clinical development.[17,18] Among the reported PI3K/mTOR dual inhibitors, *N*-(5-(quinilin-6-yl)-pyridin-3-yl)phenylsulfonamide derivatives are a class of PI3K/mTOR dual inhibitors with potent anticancer activity in vitro and in vivo. Two ring nitrogen atoms in pyridine and quinoline are the main components of pharmacophore. GlaxoSmithKline discovered GSK2126458[19] (Fig. 1) as a potent, orally bioavailable PI3Kα and mTOR dual inhibitor. Amgen Inc. designed, synthesized and evaluated several classes of *N*-(2,5-disubstituted-pyridin-3-yl)phenylsulfonamides, and discovered that *N*-(2-chloro-5-(4-morpholino quinilin-6-yl)pyridin-3-yl)-4-fluorophenylsulfonamide,[20] *N*-(2-chloro-5-(2-acetylaminobenzo[*d*]thiazol-6-yl)pyridin-3-yl)-4-fluorophenylsulfonamide[21] (compound **A**, Fig. 1) and *N*-(2-chloro-5-(2-acetylamonoimidazo[1,2-*b*]pyridazin-6-yl)pyridin-3-yl)-4-fluoro phenylsulfonamide[22] are excellent PI3K/mTOR dual inhibitors and anticancer agents. According to the X-ray cocrystal structure of PI3Kγ with GSK2126458,[19] 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.[23] Thereafter, we combined the benzamide moiety with 2-aminobenzothiazole to discover novel anticancer agents.[24] Recently, it has been reported that PI3K/mTOR dual inhibitor VS-5584 can preferentially targets cancer stem cells.[25] This discovery may potentially bring a breakthrough to the treatment of cancer. Thus, developing new PI3K/mTOR dual inhibitors is still needed.

In an attempt to develop novel anticancer agents, we combined 2-acetylamino-[1,2,4]triazolo[1,5-*a*]pyridine with *N*-pyridin-3-ylphenylsulfonamide to synthesize a series of [1,2,4]triazolo[1,5-*a*]pyridinylpyridines and the anticancer effects of the synthesized compounds were evaluated in vitro and in vivo. Therefore we discovered that compound **B** (Fig. 1) displayed potent antiproliferative activities in vitro and remarkable anticancer effects in vivo.[26] However, the body weight of mice dropped below 90% of the starting body weight over the course of oral administration at 5 mg/kg, which indicated that compound **B** displayed some toxicity. Therefore, the toxicity of compound **B** caught our attention.

To reduce the toxicity and retain the anticancer effect of compound **B**, we intend to replace the 2-acetylamide in compound **B** with chain-extended urea to search for the novel anticancer agents with low toxicity (Fig. 2). In this paper, we reported our studies on the synthesis, biological activities evaluation of a series of 1-alkyl-3-(6-(2-methoxy-3-sulfonylaminopyridin-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)urea derivatives.

2. Chemistry

The synthetic route of compounds **1a**–**1q** is outlined in Scheme 1. 6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridine-2-ylamine was reacted successively with CDI (*N,N'*-carbonyldiimidazole) and amine to yield intermediates **2a**–**2l**. In the case of preparing **2l**, glycine ethyl ester was used as an amine. The ester group in intermediate **2l** was hydrolyzed to afford the carboxylic acid **2m**, which was subsequently condensed with pyrrolidine, piperidine or morpholine to produce intermediates **2n**–**2p**. The phenylsulfonamides **3a**–**3d** were prepared from 2-amino-5-bromopyridine according to the synthetic route reported in our previous work.[26] Catalyzed by $PdCl_2$(dppf), intermediates **3a**–**3d** were reacted with bis(pinacolato)diboron to produce the corresponding arylboronic ester. Without further isolation and purification, the produced arylboronic ester was reacted with intermediates **2a**–**2k** or **2n**–**2p** to afford compounds **1a**–**1q** by Suzuki coupling. The preparation of arylboronic esters and Suzuki coupling were completed in one pot. All the structures of compounds **1a**–**1q** were characterized by ^{1}H NMR, ^{13}C NMR and HRMS.

3. Results and discussion

3.1. Antiproliferative activities in vitro

We first evaluated the antiproliferative activities of the synthesized compounds 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 MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay. The PI3K and mTOR dual inhibitor **BEZ235** was used as the positive control. The results are summarized in Table 1.

As the data shown in Table 1, the designed compounds displayed significant antiproliferative activities against four human cancer cell lines, some of which were comparable to that of BEZ235. Moreover, most of compounds showed more potent activities against HCT-116 than the other cells. Firstly, compounds with a small alkyl urea moiety (**1a**, **1b** and **1c**) displayed potent antiproliferative activities against four cancer cell lines and compound **1c** exhibited 3-fold activity against HCT-116 than that of BEZ235. These results indicate that alkylureido is a suitable substituent at the 2-position of 1,2,4-triazolo[1,5-*a*]pyridine core. Secondly, compounds **1d**–**1i**, produced by the replacement of acetylamino in compound **B** with a 2-(dialkylamino)ethylureido, retained the potency against HCT-116 and MCF-7. However, their activity against U87 MG and A549 dropped. Among compounds **1d**–**1i**, compounds **1e** and **1f** exhibited the most potent antiproliferative activities against the four cancer cell lines, which is comparable to BEZ235. These results suggest that 2-(dialkylamino)ethylureido moiety is tolerated at the 2-position of 1,2,4-triazolo[1,5-*a*]pyridine core. Replacing the 4-fluorophenyl in compound **1e** with 4-chlorophenyl, 4-methylphenyl or 2,4-difluorophenyl gave compounds **1j**–**1l**, which displayed the similar

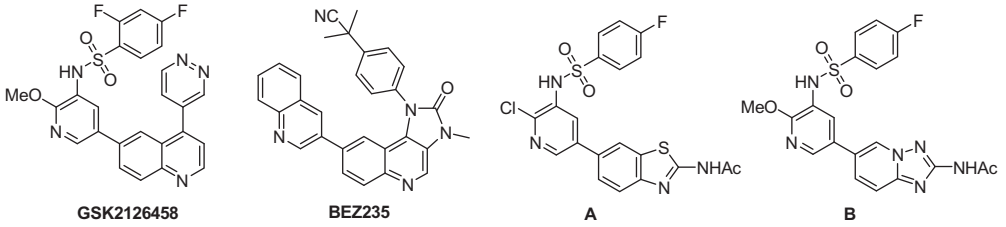


Figure 1. The structures of PI3K and mTOR dual inhibitors.

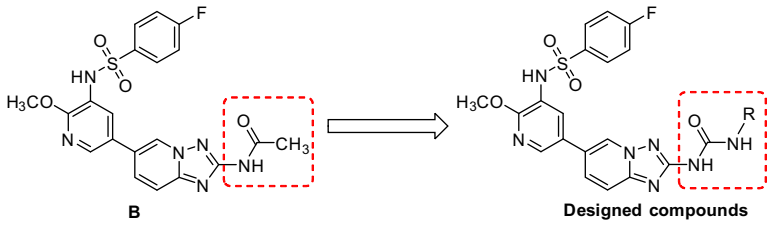
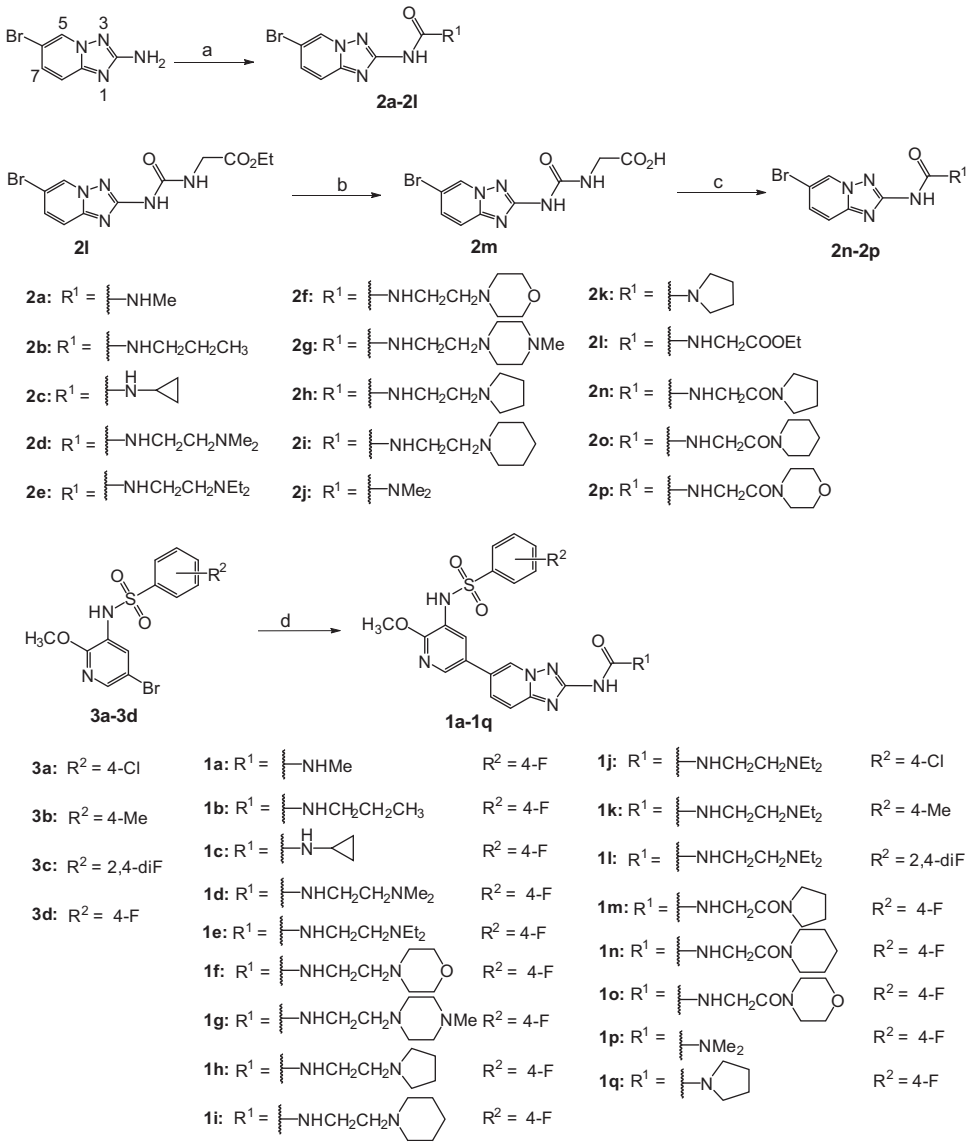


Figure 2. The design strategy of title compounds.



Scheme 1. Reagents and conditions: (a) (i) *N*,*N*′-carbonyldiimidazole (CDI), NaH, DMF, 60 °C; (ii) R^1H, DMF, 60 °C, 6 h, 68.0–93.7%; (b) 2 mol/L NaOH, MeOH/H_2O (1: 1), rt, 4 h, 90.2%; (c) morpholine or piperidine or pyrrolidine, EDCI, HOBt, DMF, rt, 8 h, 85.2–91.0%; (d) (i) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, 1,4-dioxane, reflux, 2 h; (ii) **2a**–**2k** or **2n**–**2p**, $PdCl_2$(dppf), Na_2CO_3, DME/EtOH/H_2O (7:3:2), reflux, 2 h, 41.5–73.7%.

potency with compound **1e**. These results are consistent with the observation in our previous work.[26] Thirdly, the replacement of acetylamino in compound B with an aminocarbonylmethylureido moiety gave compounds **1m**–**1o**, which displayed a drop in cell-based activity, especially against U87 MG and A549 cells. Fourthly, To probe the 1,1-dialkyl substituted urea moiety on activity, compounds **1p** and **1q** were synthesized. The antiproliferative activity of **1p** and **1q** displayed proximately a 5-fold loss in potency, which further verified that the two hydrogen atoms in the urea is indispensable. The results reveal that 2-methoxy-3-(4-fluorophenylsulfoamino)pyridine moiety linked at the 6-position of 1,2,4-triazolo[1,5-*a*]pyridine is beneficial for antiproliferative activity in vitro.

From the above results, we can conclude that the alkyl urea or the 2-(dialkylamino)ethyl urea linked to the 2-position and 2-methoxy-3-(4-fluorophenylsulfoamino)pyridine moiety attached

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

Compds	IC_{50} (μM)			
	HCT-116	MCF-7	U87 MG	A549
1a	0.18 ± 0.02	0.25 ± 0.02	0.64 ± 0.13	0.23 ± 0.03
1b	0.15 ± 0.04	0.36 ± 0.08	0.42 ± 0.11	0.22 ± 0.02
1c	0.09 ± 0.03	0.30 ± 0.06	0.54 ± 0.12	0.21 ± 0.02
1d	0.22 ± 0.01	0.56 ± 0.03	0.98 ± 0.03	0.80 ± 0.08
1e	0.18 ± 0.07	0.37 ± 0.04	0.72 ± 0.10	0.76 ± 0.06
1f	0.13 ± 0.02	0.21 ± 0.02	0.56 ± 0.05	0.42 ± 0.02
1g	0.26 ± 0.04	0.50 ± 0.09	0.76 ± 0.07	1.05 ± 0.10
1h	0.23 ± 0.01	0.38 ± 0.13	1.12 ± 0.14	0.96 ± 0.16
1i	0.15 ± 0.02	0.20 ± 0.05	1.08 ± 0.18	0.61 ± 0.10
1j	0.12 ± 0.03	0.68 ± 0.05	0.76 ± 0.03	0.68 ± 0.12
1k	0.17 ± 0.01	0.41 ± 0.05	0.67 ± 0.13	0.73 ± 0.04
1l	0.14 ± 0.02	0.74 ± 0.13	1.06 ± 0.08	0.98 ± 0.16
1m	0.14 ± 0.01	2.13 ± 0.04	1.00 ± 0.20	2.19 ± 0.12
1n	0.13 ± 0.02	0.57 ± 0.06	0.74 ± 0.03	2.06 ± 0.31
1o	0.49 ± 0.07	0.63 ± 0.03	3.26 ± 0.34	2.57 ± 0.34
1p	0.76 ± 0.06	1.56 ± 0.45	1.78 ± 0.17	1.19 ± 0.19
1q	1.29 ± 0.31	1.66 ± 0.44	1.69 ± 0.24	3.57 ± 0.23
BEZ235	0.29 ± 0.03	0.26 ± 0.08	0.77 ± 0.04	0.28 ± 0.10

at the 6-position of 1,2,4-triazolo[1,5-*a*]pyridine core are beneficial for the antiproliferative activities of title compounds.

3.2. PI3K and mTOR enzymatic activity

Next, to elucidate the mechanism of antiproliferative activities of title compounds, compounds **1c** and **1e** were selected to evaluate their inhibitory activity against PI3Ks and mTOR by performing an ATP depletion assay.[27,28] **BEZ235** was used as the positive control. the results of the PI3Ks and mTOR enzymatic activity are summarized in Table 2.

Compounds **1c** and **1e** displayed remarkable potency against PI3K and mTOR, especially against PI3K. Compared with **BEZ235**, compounds **1c** and **1e** displayed potent activities against PI3K and weaker activities against mTOR. The inhibitory activities of compounds **1c** and **1e** against PI3Kα were 7 nM and 14 nM, which are approximately 8-fold and 3-fold more potent than that of **BEZ235**, respectively. PIK3CA is one of the most frequently mutations in human cancer. Considering PIK3CA mutations in both HCT-116 and MCF-7, the excellent potency of compounds **1c** and **1e** on PI3Kα further explained the significant antiproliferative activities of compounds **1c** and **1e** against HCT-116 and MCF-7. These data suggest that compounds **1c** and **1e** are potent PI3K inhibitors.

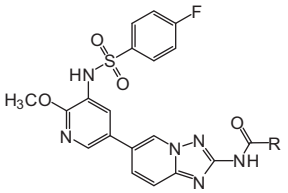
3.3. Acute oral toxicity in vivo

Thirdly, we picked out compounds **B**, **1b**, **1c** and **1e** to explore their acute oral toxicities in mice by employing an 'up-and-down procedure' (UDP).[29,30] Mice were treated orally the solution of tested drugs and the LD_{50s} (median lethal doses) of tested compounds are listed in Table 3. The LD_{50} values of compounds **1b**, **1c** and **1e** were about 5-fold, 4-fold and 18-fold higher than that of compound **B**. These data reveal that the acute oral toxicity is closely related to the structure of R. To our surprise, the acute oral toxicity of compound **1e**, produced by the replacement of 2-acetylamino moiety in compound **B** with a 2-(diethylamino)ethylurea moiety, reduced dramatically. In summary, we have identified the new PI3K inhibitor with low toxicity by optimizing compound **B**.

Table 2
Inhibitory activity of **1c** and **1e** against PI3K and mTOR ($n = 2$)

Compds	IC_{50} (nM)				
	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ	mTOR
1c	7	47	14	16	275
1e	14	41	22	12	123
BEZ235	54	399	95	164	89

Table 3
The acute oral toxicity of tested compounds



Compds	R	LD_{50} (mg/kg)	95% confidence interval (mg/kg)
B	$-CH_3$	28	25–33
1b	$-NHCH_2CH_2CH_3$	156	133–175
1c		118	101–133
1e	$-NHCH_2CH_2NEt_2$	513	400–530

3.4. Anticancer effect in vivo

Lastly, we evaluated whether compound **1e** could inhibit tumor growth in established mice homograft model. A study using mice bearing sarcoma S-180 was performed. Compound **1e** was dosed orally at 5 mg/kg, 10 mg/kg or 30 mg/kg once a day for 8 days. Considering that it is difficult to measure the volumes of S-180 tumor, tumor weights were used as evaluating indicators. The tumor weights and daily body weights were depicted in Fig. 3. The inhibitory ratios of compound **1e** at 5 mg/kg, 10 mg/kg and 30 mg/kg were 37.3%, 49.0% and 53.6%, respectively, which showed a dose-dependent effect. In addition, the body weight of all groups increased during the treatment. These results suggest that compound **1e** is an effective anticancer agent with low toxicity.

3.5. Docking study

To further explain the potent activities of compounds **1c** and **1e**, 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)[21] with compounds **1c** and **1e** and the results were depicted in Fig. 4. From the docking results of compounds **1c** and **1e** with PI3Kγ, we observed that: (1) 2-substituted ureido[1,2,4]triazolo[1,5-*a*]pyridine formed hydrogen bonds with Val882 and Val885; (2) the nitrogen atom of pyridine formed hydrogen bonds directly with Tyr867 and Asp864 or interacted with Asp841 via an ordered water molecule; the oxygen atom at 2-position and 3-sulfonamido of pyridine simultaneously formed hydrogen bonds with Lys833. In addition, the fluorine atom in phenyl of compound **1c** formed a hydrogen bond with Asn951, which may be related to the antiproliferative activity of **1c** and **1e**.

4. Conclusions

In present study, a series of 1-alkyl-3-(6-(5,6-disubstituted-pyridin-3-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)urea derivatives were synthesized and characterized. Their antiproliferative activities in vitro were evaluated via MTT assay against four cancer cell

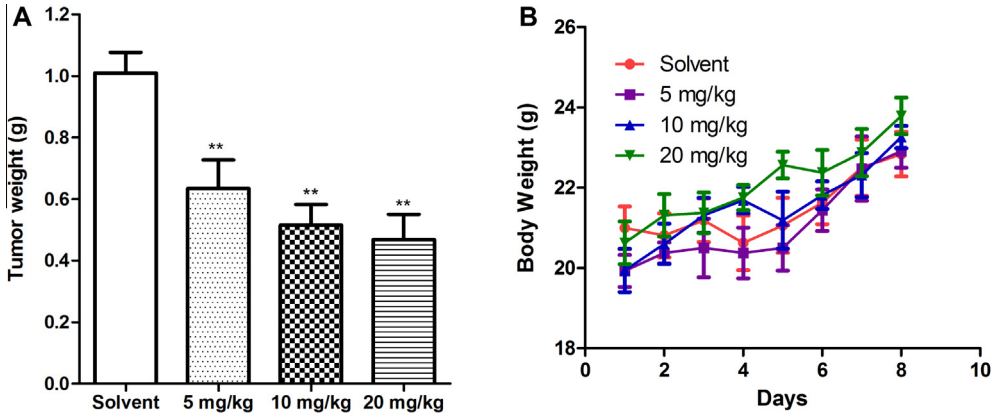


Figure 3. (A) The anticancer effect of compound **1e** in establishing mice S180 homograft model. (B) The change of tested mice body weights. Mice bearing subcutaneous cancers were treated orally vehicle, compound **1e** (5 mg/kg, 10 mg/kg or 20 mg/kg) once daily for 8 days. $^{**}P$ <0.01.

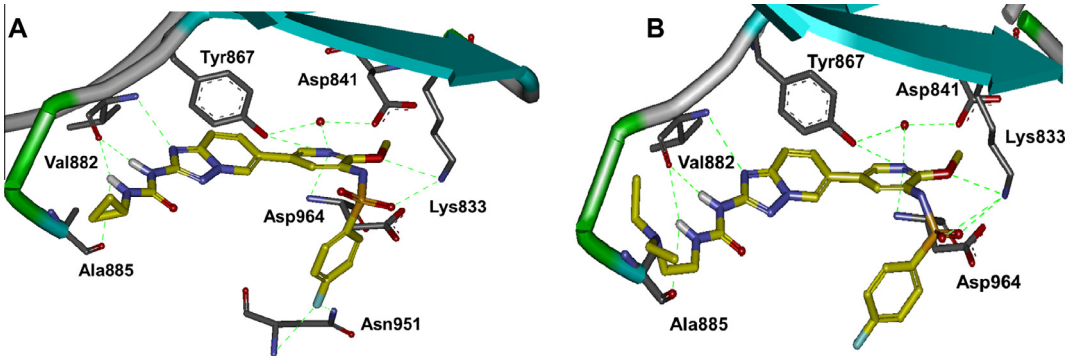


Figure 4. The comparison of docking mode between **1c** and **1e**. (A) Docking mode of **1c** with PI3Kγ and (B) Docking mode of **1e** with PI3Kγ. Selected residues Ala885, Val882, Tyr867, Asp841, Lys833, Asp964 and Asn951 are shown. Green dashed lines indicate hydrogen bond.

lines including HCT-116, MCF-7, U87 MG and A549. The SAR of the title compounds was discussed. Compounds **1c** and **1e** were tested for their inhibitory activity against PI3Ks and mTOR. Meanwhile, the acute oral toxicity of four compounds was investigated by oral administration. The results indicate that the compound with a alkyl urea or a 2-(dialkylamino)ethylurea moiety at the 2-position of [1,2,4]triazolo[1,5-*a*]pyridine moiety can retain the antiproliferative activity and the inhibitory activity against PI3K and mTOR. In addition, their acute oral toxicity reduced dramatically. Moreover, compound **1e** can effectively inhibit tumor growth in a mice S180 homograft model. These findings suggest that designed compounds can serve as potent PI3K inhibitors and anticancer agents with low toxicity.

5. Experimental

5.1. Chemistry

Unless specified otherwise, all the starting materials, reagents and solvents were commercially available. All the 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.).

5.1.1. General procedure for synthesis of compounds 2a–2l

To the suspension of sodium hydride (60%, 0.23 g, 5.66 mmol) in dried dimethyl formamide (15 mL) was added 6-bromo (or 7-bromo)-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine (0.60 g, 2.82 mmol), stirred for 10 min at room temperature, added *N,N′*-carbonyldimidazole (CDI, 1.37 g, 8.46 mmol), stirred at 60 °C until the starting material consumed, then added amine (9.87 mmol) and stirred at 60 °C for 6 h. The volatile was removed under reduced pressure to give a white pale yellow residue. Water (30 mL) was added to the residue. The mixture was stirred. The precipitate was collected by filtration, dried to afford the expected product as a white solid.

5.1.1.1. 1-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-methylurea (2a). White solid; yield: 79.6%; mp: 237–239 °C; ^{1}H NMR (DMSO-d_6): δ 10.05 (s, 1H, NH), 9.22 (d, J = 1.2 Hz, 1H, Ar-H), 8.04 (d, J = 4.4 Hz, 1H, NH), 7.80 (dd, J_1 = 1.6 Hz, J_2 = 9.6 Hz, 1H, Ar-H), 7.64 (d, J = 9.6 Hz, 1H, Ar-H), 2.78 (m, 6H, $CH_2 \times 3$), 1.50 (d, J = 4.8 Hz, 3H, CH_3). MS m/z (ESI): 270.0 $[M+H]^+$.

5.1.1.2. 1-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-propylurea (2b). White solid; yield: 91.4%; mp: 188–190 °C; ^{1}H NMR ($CDCl_3$): δ 9.67 (s, 1H, NH), 8.80 (s, 1H, Ar-H), 8.16 (m, 1H, NH), 7.63 (m, 2H, Ar-H), 3.42 (m, 2H, CH_2), 1.70 (m, 2H, CH_2), 1.03 (m, 3H, CH_3). MS m/z (ESI): 298.1 $[M+H]^+$.

5.1.1.3. 1-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-cyclopropylurea (2c). White solid; yield: 89.0%; mp: 204–206 °C; ^{1}H NMR ($CDCl_3$): δ 9.88 (s, 1H, NH), 8.80 (s, 1H, Ar-H), 8.25 (m, 1H, NH), 7.71 (d, J = 9.2 Hz, 1H, Ar-H), 7.61 (dd, J_1 = 1.6 Hz, J_2 = 9.6 Hz, 1H, Ar-H), 2.84 (m, 1H, CH), 0.87 (m, 2H, CH_2), 0.70 (m, 2H, CH_2). MS m/z (ESI): 296.0 $[M+H]^+$.

5.1.1.4. 1-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-(dimethylamino)ethyl)urea (2d). White solid; yield: 77.7%; mp: 195–197 °C; ^{1}H NMR ($CDCl_3$): δ 9.56 (s, 1H, NH), 8.76 (s, 1H, Ar-H), 8.31 (s, 1H, NH), 7.61 (m, 2H, Ar-H), 3.55 (m, 2H, CH_2), 2.59 (m, 2H, CH_2), 2.36 (s, 6H, $CH_3 \times 2$). MS m/z (ESI): 327.0 $[M+H]^+$.

5.1.1.5. 1-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-(diethylamino)ethyl)urea (2e). White solid; yield: 68.0%; mp: 148–150 °C; ^{1}H NMR ($CDCl_3$): δ 9.51 (s, 1H, NH), 8.75 (s, 1H, Ar-H), 8.40 (s, 1H, NH), 7.60 (m, 2H, Ar-H), 3.51 (m, 2H, CH_2), 2.71 (m, 2H, CH_2), 2.64 (m, 4H, $CH_2 \times 2$), 1.11 (m, 6H, $CH_3 \times 2$). MS m/z (ESI): 355.1 $[M+H]^+$.

5.1.1.6. 1-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-morpholinoethyl)urea (2f). White solid; yield: 92.3%; mp: 218–220 °C; ^{1}H NMR ($CDCl_3$): δ 9.51 (s, 1H, NH), 8.78 (s, 1H, Ar-H), 8.49 (s, 1H, NH), 7.63 (m, 2H, Ar-H), 3.81 (m, 4H, $CH_2 \times 2$), 3.58 (m, 2H, CH_2), 2.63 (m, 6H, $CH_2 \times 3$). MS m/z (ESI): 369.0 $[M+H]^+$.

5.1.1.7. 1-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-(4-methylpiperazin-1-yl)ethyl)urea (2g). White solid; yield: 81.0%; mp: 182–184 °C; ^{1}H NMR ($CDCl_3$): δ 9.60 (s, 1H, NH), 8.80 (s, 1H, Ar-H), 8.46 (s, 1H, NH), 7.63 (m, 2H, Ar-H), 2.60 (m, 10H, $CH_2 \times 5$), 2.36 (s, 3H, CH_3). MS m/z (ESI): 382.1 $[M+H]^+$.

5.1.1.8. 1-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-(pyrrolidin-1-yl)ethyl)urea (2h). White solid; yield: 79.2%; mp: 172–174 °C; ^{1}H NMR ($CDCl_3$): δ 9.51 (s, 1H, NH), 8.75 (s, 1H, Ar-H), 8.39 (s, 1H, NH), 7.61 (m, 2H, Ar-H), 3.61 (m, 2H, CH_2), 2.80 (m, 2H, CH_2), 2.68 (s, 4H, $CH_2 \times 2$), 1.86 (s, 4H, $CH_2 \times 2$). MS m/z (ESI): 353.1 $[M+H]^+$.

5.1.1.9. 1-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-(piperidin-1-yl)ethyl)urea (2i). White solid; yield: 71.1%; mp: 180–181 °C; ^{1}H NMR (DMSO-d_6): δ 10.03 (s, 1H, NH), 9.19 (d, J = 1.2 Hz, 1H, Ar-H), 8.38 (m, 1H, NH), 7.80 (dd, J_1 = 1.6 Hz, J_2 = 9.2 Hz, 1H, Ar-H), 7.57 (d, J = 9.2 Hz, 1H, Ar-H), 3.30 (m, 2H, CH_2), 2.41 (m, 6H, $CH_2 \times 3$), 1.50 (m, 6H, $CH_2 \times 3$). MS m/z (ESI): 367.1 $[M+H]^+$.

5.1.1.10. 3-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-1,1-dimethylurea (2j). White solid; yield: 75.7%; mp: 154–156 °C; ^{1}H NMR ($CDCl_3$): δ 8.67 (d, J = 1.2 Hz, 1H, Ar-H), 7.57 (dd, J_1 = 2.0 Hz, J_2 = 9.2 Hz, 1H, Ar-H), 7.52 (s, 1H, NH), 7.45 (d, J = 9.2 Hz, 1H, Ar-H), 3.09 (s, 6H, $CH_3 \times 2$). MS m/z (ESI): 284.0 $[M+H]^+$.

5.1.1.11. N-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)pyrrolidine-1-carboxamide (2k). White solid; yield: 72.8%; mp: 231–233 °C; ^{1}H NMR ($CDCl_3$): δ 8.67 (d, J = 1.2 Hz, 1H, Ar-H), 7.56 (dd, J_1 = 2.0 Hz, J_2 = 9.2 Hz, 1H, Ar-H), 7.44 (d, J = 9.6 Hz, 1H, Ar-H), 7.26 (s, 1H, NH), 3.53 (s, 4H, $CH_2 \times 2$), 2.00 (s, 4H, $CH_2 \times 2$). MS m/z (ESI): 310.0 $[M+H]^+$.

5.1.1.12. Ethyl 2-(3-(6-bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)ureido)acetate (2l). White solid; yield: 93.7%; mp: 186–188 °C; ^{1}H NMR ($CDCl_3$): δ 9.59 (s, 1H, NH), 8.79 (s, 1H, Ar-H), 8,72 (m, 1H, NH), 7.63 (d, J = 1.2 Hz, 2H, Ar-H), 4.28 (m, 2H, OCH_2), 4.02 (s, 2H, CH_2), 1.34 (m, 3H, CH_3). MS m/z (ESI): 342.0 $[M+H]^+$.

5.1.2. 2-(3-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)ureido)-acetic acid (2m)

To the solution of compound **2l** (0.68 g, 2 mmol) in methanol (10 mL) was added NaOH (2 mol/L) in water (10 mL) and the mixture was stirred for 4 h at room temperature. The solvent was evaporated under reduced pressure to afford a white residue. The residue was dissolved in water (20 mL) and the solution was adjusted to pH 4–5 with 6 mol/L HCl whereupon a white precipitate formed. The precipitate was collected by filtration under reduced pressure, washed with water and dried to afford compound **2m** as a white solid. Yield: 90.2%; mp: 233–235 °C; ^{1}H NMR (DMSO-d_6): δ 9.59 (s, 1H, NH), 8.79 (s, 1H, Ar-H), 8.72 (m, 1H, NH), 7.63 (d, J = 1.2 Hz, 2 H, Ar-H), 4.28 (m, 2H, OCH_2), 4.02 (s, 2H, CH_2), 1.34 (m, 3H, CH_3). MS m/z (ESI): 314.0 $[M+H]^+$.

5.1.3. General procedure for synthesis of compounds 2n–2p

A mixture of compound **2m** (0.63 g, 2 mmol), alkyl amine (8 mmol), *N*-hydroxybenzotrizole (HOBt, 0.68 g, 5 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 0.76 g, 4 mmol) and DMF (8 mL) was stirred at room temperature for 8 h and the solvent was removed under reduced pressure to give a yellow residue. The residue was suspended into water (20 mL) whereupon a precipitate formed. The precipitate was collected by suction, washed with water and dried to afford the compound as a white solid.

5.1.3.1. 1-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-oxo-2-(pyrrolidin-1-yl)ethyl)urea (2n). White solid; yield: 91.0%; mp: 248–250 °C; ^{1}H NMR ($CDCl_3$): δ 9.68 (s, 1H, NH), 9.02 (s, 1H, NH), 8.82 (s, 1H, Ar-H), 7.69 (d, J = 9.2 Hz, 1H, Ar-H), 7.61 (d, J = 9.2 Hz, 1H, Ar-H), 4.22 (d, J = 3.6 Hz, 2H, CH_2), 3.53 (m, 4H, $CH_2 \times 2$), 1.98 (m, 4H, $CH_2 \times 2$). MS m/z (ESI): 367.0 $[M+H]^+$.

5.1.3.2. 1-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-oxo-2-(piperidin-1-yl)ethyl)urea (2o). White solid; yield: 85.2%; mp: 247–249 °C; ^{1}H NMR ($CDCl_3$): δ 9.56 (s, 1H, NH), 9.04 (s, 1H, NH), 8.82 (s, 1H, Ar-H), 7.69 (d, J = 9.2 Hz, 1H, Ar-H), 7.61 (dd, J_1 = 1.2 Hz, J_2 = 9.2 Hz, 1H, Ar-H), 4.29 (d, J = 3.6 Hz, 2H, CH_2), 3.66 (m, 2H, CH_2), 3.43 (m, 2H, CH_2), 1.66 (m, 6H, $CH_2 \times 3$). MS m/z (ESI): 381.1 $[M+H]^+$.

5.1.3.3. 1-(6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-(morpholino-2-oxoethyl)urea (2p). White solid; yield: 88.2%; mp: >250 °C; ^{1}H NMR (DMSO-d_6): δ 10.16 (s, 1H, NH), 9.23 (s, 1H, Ar-H), 8.54 (m, 1H, NH), 7.80 (dd, J_1 = 1.6 Hz, J_2 = 9.2 Hz, 1H, Ar-H), 7.63 (d, J = 9.2 Hz, 1H, Ar-H), 4.14 (d, J = 4.8 Hz, 2H, CH_2), 3.52 (m, 8H, $CH_2 \times 4$). MS m/z (ESI): 383.0 $[M+H]^+$.

5.1.4. General procedure for synthesis of compounds 1a–1q

The mixture of compounds **3a**–**3d** (0.4 mmol), bis(pinacolato)diboron (0.11 g, 0.44 mmol), $PdCl_2$(dppf) (0.02 g, 0.03 mmol), potassium acetate (0.12 g, 1.2 mmol) and 1,4-dioxane (10 mL) was refluxed for 2 h under N_2 atmosphere. The solvent was evaporated off under reduced pressure to afford a dark brown residue. To the residue was added compounds **2a**–**2k** or **2n**–**2p** (0.32 mmol), $PdCl_2$(dppf) (0.02 g, 0.03 mmol), sodium carbonate (0.13 g, 1.2 mmol), 1,2-dimethoxyethane (10.5 mL), ethanol (4.5 mL) water (3 mL) and the mixture was refluxed for 2 h under N_2 atmosphere. The volatile was removed under reduced pressure to afford a dark brown residue and the residue was purified by

silica gel column chromatography using chloroform/methanol = 15: 1 as the eluent to produce the title compound as a white solid.

5.1.4.1. 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonylamino) pyridin-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-methylurea (1a). White solid; yield: 43.2%; mp: 237–239 °C; ^{1}H NMR (DMSO-d_6): δ 10.11 (s, 1H, NH), 10.05 (s, 1H, NH), 9.16 (s, 1H, Ar-H), 8.39 (s, 1H, Ar-H), 8.15 (d, J = 3.6 Hz, 1H, NH), 8.00 (s, 1H, Ar-H), 7.92 (d, J = 9.2 Hz, 1H, Ar-H), 7.80 (m, 2H, Ar-H), 7.75 (d, J = 9.2 Hz, 1H, Ar-H), 7.41 (m, 2H, Ar-H), 3.63 (s, 3H, OCH_3), 2.80 (d, J = 4.0 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 168.2 (C), 166.6 (C), 163.3 (C), 160.0 (CH), 157.4 (C), 157.3 (C), 151.4 (C), 144.8 (C), 139.8 (C), 135.4 (CH), 132.9 (CH), 132.8 (CH), 128.6 (CH), 126.1 (CH), 123.6 (C), 119.3 (CH), 119.2 (CH), 117.1 (CH), 56.6 (CH_3), 29.4 (CH_3). ESI-HRMS m/z: calcd for $C_{20}H_{19}FN_7O_4S$ $[M+H]^+$: 472.1203; found: 472.1198.

5.1.4.2. 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonylamino)pyridin-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-propylurea (1b). White solid; yield: 44.7%; mp: 221–223 °C; ^{1}H NMR (DMSO-d_6): δ 10.11 (s, 1H, NH), 10.00 (s, 1H, NH), 9.17 (s, 1H, Ar-H), 8.40 (d, J = 2.4 Hz, 1H, Ar-H), 8.30 (m, 1H, NH), 8.00 (d, J = 2.4 Hz, 1H, Ar-H), 7.92 (dd, J_1 = 9.2 Hz, J_2 = 1.6 Hz, 1H, Ar-H), 7.81 (m, 2H, Ar-H), 7.76 (d, J = 9.2 Hz, 1H, Ar-H), 7.41 (m, 2H, Ar-H), 3.63 (s, 3H, OCH_3), 3.20 (m, 2H, CH_2), 1.53 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 168.2 (C), 166.6 (C), 163.4 (C), 160.0 (CH), 156.8 (C), 151.4 (C), 144.9 (C), 139.7 (C), 135.6 (CH), 132.9 (CH), 132.8 (CH), 128.6 (CH), 128.6 (C), 126.1 (CH), 123.5 (C), 119.3 (CH), 119.1 (CH), 117.2 (CH), 56.6 (CH_3), 44.2 (CH_2), 26.0 (CH_2), 14.5 (CH_3). ESI-HRMS m/z: calcd for $C_{22}H_{23}FN_7O_4S$ $[M+H]^+$: 500.1516; found: 500.1511.

5.1.4.3. 1-Cyclopropyl-3-(6-(2-methoxy-3-(4-fluorophenylsulfonylamino)pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)urea (1c). White solid; yield: 49.2%; mp: 236–238 °C; ^{1}H NMR (DMSO-d_6): δ 10.11 (s, 1H, NH), 10.04 (s, 1H, NH), 9.17 (s, 1H, Ar-H), 8.39 (d, J = 2.0 Hz, 1H, Ar-H), 8.34 (s, 1H, NH), 8.00 (d, J = 2.4 Hz, 1H, Ar-H), 7.92 (dd, J_1 = 9.2 Hz, J_2 = 1.6 Hz, 1H, Ar-H), 7.80 (m, 2H, Ar-H), 7.75 (d, J = 9.2 Hz, 1H, Ar-H), 7.41 (m, 2H, Ar-H), 3.63 (s, 3H, OCH_3), 2.66 (m, 1H, CH), 0.72 (m, 2H, CH_2), 0.52 (m, 2H, CH_2). MS m/z (ESI): $[M+H]^+$. ^{13}C NMR (DMSO-d_6): δ 168.2 (C), 166.6 (C), 163.2 (C), 160.0 (CH), 157.6 (C), 151.4 (C), 144.9 (C), 139.7 (C), 135.6 (CH), 132.9 (CH), 132.8 (CH), 128.7 (CH), 128.5 (C), 126.1 (CH), 123.4 (C), 119.3 (CH), 119.1 (CH), 117.2 (CH), 56.5 (CH_3), 25.6 (CH), 9.5 ($CH_2 \times 2$). ESI-HRMS m/z: calcd for $C_{22}H_{20}FN_7NaO_4S$ $[M+Na]^+$: 520.1179; found: 520.1174.

5.1.4.4. 1-(2-(Dimethylamino)ethyl)-3-(6-(2-methoxy-3-(4-fluorophenylsulfonyl amino)pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)urea (1d). White solid; yield: 73.7%; mp: 241–243 °C; ^{1}H NMR (DMSO-d_6): δ 9.98 (bm, 2H, NH), 9.09 (s, 1H, Ar-H), 8.29 (d, J = 6.4 Hz, 2H, NH, Ar-H), 7.92 (s, 1H, Ar-H), 7.87 (d, J = 8.8 Hz, 1H, Ar-H), 7.80 (m, 2H, Ar-H), 7.73 (d, J = 9.2 Hz, 1H, Ar-H), 7.38 (m, 2H, Ar-H), 3.66 (s, 3H, OCH_3), 3.37 (d, J = 5.2 Hz, 2H, CH_2), 2.54 (d, J = 8.8 Hz, 2H, CH_2), 2.31 (s, 6H, $CH_3 \times 2$). ^{13}C NMR (DMSO-d_6): δ 168.0 (C), 166.3 (C), 163.2 (C), 160.0 (CH), 156.9 (C), 151.4 (C), 144.9 (C), 140.6 (C), 135.4 (C), 132.9 (CH), 132.8 (CH), 132.7 (CH), 128.5 (CH), 126.5 (CH), 123.4 (C), 119.1 (CH), 119.0 (CH), 117.2 (CH), 61.3 (CH_2), 56.5 (CH_3), 47.9 ($CH_3 \times 2$), 40.2 (CH_2). ESI-HRMS m/z: calcd for $C_{23}H_{26}FN_8O_4S$ $[M+H]^+$: 529.1776; found: 529.1782.

5.1.4.5. 1-(2-(Diethylamino)ethyl)-(6-(2-methoxy-3-(4-fluorophenylsulfonyl amino)pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)urea (1e). White solid; yield: 49.0%; mp: 228–230 °C; ^{1}H NMR (DMSO-d_6): δ 10.03 (s, 2H, NH), 9.06 (s, 1H, Ar-H), 8.43 (s, 1H, NH), 8.27 (s, 1H, Ar-H), 7.88 (m, 2H, Ar-H), 7.80 (m, 2H, Ar-H), 7.79 (d, J = 9.2 Hz, 1H, Ar-H), 7.38 (m, 2H, Ar-H), 3.66 (s, 3H, OCH_3), 3.32 (m, 2H, CH_2), 2.63 (m, 6H, $CH_2 \times 3$), 1.03 (m, 6H, $CH_3 \times 2$). ^{13}C NMR (DMSO-d_6): δ 168.2 (C), 166.6 (C), 163.1 (C), 160.0 (CH), 157.3 (C), 151.4 (C), 144.8 (C), 139.8 (C), 135.4 (C), 133.0 (CH), 132.8 (CH), 132.7 (CH), 128.5 (CH), 126.3 (CH), 123.7 (C), 119.3 (CH), 119.2 (CH), 117.2 (CH), 56.5 (CH_3), 53.8 (CH_2), 50.0 ($CH_2 \times 2$), 38.5 (CH_2), 12.4 ($CH_3 \times 2$). ESI-HRMS m/z: calcd for $C_{25}H_{30}FN_8O_4S$ $[M+H]^+$: 557.2095; found: 557.2089.

5.1.4.6. 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonylamino) pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-morpholinoethyl)urea (1f). White solid; yield: 70.4%; mp: >250 °C; ^{1}H NMR (DMSO-d_6): δ 10.06 (bm, 2H, NH), 9.12 (s, 1H, Ar-H), 8.40 (s, 1H, NH), 8.38 (s, 1H, Ar-H), 7.85 (m, 5H, Ar-H), 7.41 (m, 2H, Ar-H), 3.64 (s, 7H, OCH_3, $CH_2 \times 2$), 3.36 (s, 2H, CH_2), 2.47 (d, J = 3.6 Hz, 6H, $CH_2 \times 3$). ^{13}C NMR (DMSO-d_6): δ 168.2 (C), 166.6 (C), 163.4 (C), 160.0 (CH), 157.0 (C), 151.4 (C), 144.7 (C), 139.8 (C), 135.3 (C), 132.9 (CH × 2), 132.8 (CH), 128.6 (CH), 126.2 (CH), 123.7 (C), 119.3 (CH), 119.2 (CH), 117.2 (CH), 69.5 ($CH_2 \times 2$), 60.3 (CH_2), 56.6 (CH_3), 56.2 ($CH_2 \times 2$), 39.5 (CH_2). ESI-HRMS m/z: calcd for $C_{25}H_{28}FN_8O_5S$ $[M+H]^+$: 571.1887; found: 571.1882.

5.1.4.7. 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonylamino) pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-(4-methylpipe razin-1-yl)ethyl)urea (1g). White solid; yield: 62.5%; mp: 237–239 °C; ^{1}H NMR (DMSO-d_6): δ 10.02 (s, 2H, NH), 9.09 (s, 1H, Ar-H), 8.48 (m, 1H, NH), 8.32 (d, J = 2.0 Hz, 1H, Ar-H), 7.95 (d, J = 2.4 Hz, 1H, Ar-H), 7.91 (dd, J_1 = 9.2 Hz, J_2 = 1.6 Hz, 1H, Ar-H), 7.80 (m, 2H, Ar-H), 7.71 (d, J = 9.2 Hz, 1H, Ar-H), 7.39 (m, 2H, Ar-H), 3.64 (s, 3H, OCH_3), 3.35 (m, 2H, CH_2), 2.48 (m, 10H, $CH_2 \times 5$), 2.25 (s, 3H, NCH_3). ESI-HRMS m/z: calcd for $C_{26}H_{31}FN_9O_4S$ $[M+H]^+$: 584.2204; found: 584.2198.

5.1.4.8. 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonylamino) pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-(pyrrolidin-1-yl)ethyl)urea (1h). White solid; yield: 45.8%; mp: 225–227 °C; ^{1}H NMR (DMSO-d_6): δ 10.00 (m, 2H, NH), 9.06 (s, 1H, Ar-H), 8.40 (m, 1H, NH), 8.25 (d, J = 2.0 Hz, 1H, Ar-H), 7.90 (d, J = 2.4 Hz, 1H, Ar-H), 7.86 (dd, J_1 = 9.2 Hz, J_2 = 1.6 Hz, 1H, Ar-H), 7.80 (m, 2H, Ar-H), 7.71 (d, J = 9.2 Hz, 1H, Ar-H), 7.37 (m, 2H, Ar-H), 3.66 (s, 3H, OCH_3), 3.39 (m, 2H, CH_2), 2.73 (m, 2H, CH_2), 2.68 (s, 4H, $CH_2 \times 2$), 1.76 (s, 4H, $CH_2 \times 2$). ^{13}C NMR (DMSO-d_6): δ 168.0 (C), 166.3 (C), 163.2 (C), 160.0 (CH), 156.9 (C), 151.4 (C), 143.0 (C), 140.7 (C), 133.8 (C), 132.9 (CH), 132.7 (CH), 132.6 (CH), 128.4 (C), 128.4 (CH), 126.5 (CH), 119.1 (CH), 119.0 (CH), 117.1 (CH), 57.9 (CH_2), 56.7 ($CH_2 \times 2$), 56.4 (CH_3), 41.2 (CH_2), 26.2 ($CH_2 \times 2$). ESI-HRMS m/z: calcd for $C_{25}H_{28}FN_8O_4S$ $[M+H]^+$: 555.1938; found: 555.1933.

5.1.4.9. 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonylamino) pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-(piperidin-1-yl)ethyl)urea (1i). White solid; yield: 63.2%; mp: 219–221 °C; ^{1}H NMR (DMSO-d_6): δ 10.05 (s, 1H, NH), 9.95 (m, 1H, NH), 9.09 (s, 1H, Ar-H), 8.49 (s, 1H, NH), 8.32 (d, J = 2.0 Hz, 1H, Ar-H), 7.95 (d, J = 2.0 Hz, 1H, Ar-H), 7.90 (dd, J_1 = 9.2 Hz, J_2 = 1.6 Hz, 1H, Ar-H), 7.81 (m, 2H, Ar-H), 7.79 (d, J = 9.2 Hz, 1H, Ar-H), 7.39 (m, 2H, Ar-H), 3.65 (s, 3H, OCH_3), 3.36 (d, J = 5.6 Hz, 2H, CH_2), 2.55 (m, 6H, $CH_2 \times 3$), 1.58 (m, 4H, $CH_2 \times 2$), 1.44 (d, J = 3.6 Hz, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 168.1 (C), 166.4 (C), 163.3 (C), 160.0 (CH), 156.9 (C), 151.4 (C), 143.9 (C), 140.3 (C), 134.5 (C), 132.9 (CH), 132.8 (CH), 132.7 (CH), 128.5 (CH), 126.4 (CH), 124.7 (C), 119.2 (CH), 119.1 (CH), 117.0 (CH), 60.3 (CH_2), 56.8 ($CH_2 \times 2$), 56.5 (CH_3), 39.6 (CH_2), 28.4 ($CH_2 \times 2$), 26.8 (CH_2).

ESI-HRMS *m*/*z*: calcd for $C_{26}H_{30}FN_8O_4S$ $[M+H]^+$: 569.2095; found: 569.2089.

5.1.4.10. 1-(2-(Diethylamino)ethyl)-3-(6-(2-Methoxy-3-(4-chlorophenylsulfonyl amino)pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)urea (1j). White solid; yield: 59.5%; mp: 200–202 °C; ^{1}H NMR (DMSO-d_6): δ 10.05 (s, 2H, NH), 9.05 (s, 1H, Ar-H), 8.44 (m, 1H, NH), 8.25 (d, J = 2.0 Hz, 1H, Ar-H), 7.90 (d, J = 2.4 Hz, 1H, Ar-H), 7.87 (dd, J_1 = 9.2 Hz, J_2 = 2.0 Hz, 1H, Ar-H), 7.74 (d, J = 8.4 Hz, 2H, Ar-H), 7.70 (d, J = 6.8 Hz, 1H, Ar-H), 7.61 (d, J = 8.4 Hz, 2H, Ar-H) 3.66 (s, 3H, OCH_3), 3.34 (s, 2H, CH_2), 2.66 (m, 6H, $CH_2 \times 3$), 1.05 (s, 6H, $CH_3 \times 2$). ^{13}C NMR (DMSO-d_6): δ 163.3 (C), 160.0 (CH), 157.0 (C), 156.9 (C), 151.4 (C), 143.5 (C), 142.8 (C), 140.1 (C), 133.5 (C), 132.9 (CH), 132.0 (CH × 2), 131.6 (CH × 2), 128.4 (CH), 128.3 (C), 126.6 (CH), 117.0 (CH), 56.4 (CH_3), 54.7 (CH_2), 49.7 ($CH_2 \times 2$), 40.4 (CH_2), 14.4 ($CH_3 \times 2$). ESI-HRMS *m*/*z*: calcd for $C_{25}H_{30}ClN_8O_4S$ $[M+H]^+$: 573.1799; found: 573.1794.

5.1.4.11. 1-(2-(Diethylamino)ethyl)-3-(6-(2-methoxy-3-(4-methylphenylsulfonyl amino)pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)urea (1k). White solid; yield: 68.0%; mp: 188–190 °C; ^{1}H NMR (DMSO-d_6): δ 10.02 (s, 2H, NH), 9.05 (s, 1H, Ar-H), 8.43 (m, 1H, NH), 8.29 (d, J = 2.0 Hz, 1H, Ar-H), 7.90 (d, J = 2.4 Hz, 1H, Ar-H), 7,86 (dd, J_1 = 9.2 Hz, J_2 = 1.2 Hz, 1H, Ar-H), 7.70 (d, J = 9.2 Hz, 1H, Ar-H), 7.64 (d, J = 8.0 Hz, 2H, Ar-H), 7.34 (d, J = 8.0 Hz, 2H, Ar-H) 3.66 (s, 3H, OCH_3), 3.31 (d, J = 5.6 Hz, 2H, CH_2), 2.60 (m, 6H, $CH_2 \times 3$), 1.02 (s, 6H, $CH_3 \times 2$). ^{13}C NMR (DMSO-d_6): δ 163.3 (C), 160.0 (CH), 156.9 (C), 151.4 (C), 146.0 (C), 143.4 (C), 140.8 (C), 133.4 (C), 132.8 (CH), 132.4 (CH × 2), 129.8 (CH × 2), 128.4 (CH), 128.4 (C), 126.4 (CH), 125.0 (C), 117.0 (CH), 56.5 (CH_3), 54.8 (CH_2), 49.7 ($CH_2 \times 2$), 40.7 (CH_2), 24.1 (CH_3), 14.8 ($CH_3 \times 2$). ESI-HRMS *m*/*z*: calcd for $C_{26}H_{33}N_8O_4S$ $[M+H]^+$: 553.2345; found: 553.2341.

5.1.4.12. 1-(2-(Diethylamino)ethyl)-3-(6-(2-methoxy-3-(2,4-difluorophenyl sulfonylamino)pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-[1,2,4]triazolo [1,5-*a*]pyridin-3-yl)urea (1l). White solid; yield: 41.5%; mp: 211–213 °C; ^{1}H NMR (DMSO-d_6): δ 10.08 (s, 2H, NH), 9.02 (s, 1H, Ar-H), 8.44 (s, 1H, NH), 8.20 (s, 1H, Ar-H), 7.85 (d, J = 7.6 Hz, 2H, Ar-H), 7.77 (m, 1H, Ar-H), 7.69 (d, J = 9.2 Hz, 1H, Ar-H), 7.47 (m, 1H, Ar-H), 7.16 (m, 1H, Ar-H), 3.68 (s, 3H, OCH_3), 3.38 (d, J = 5.6 Hz, 2H, CH_2), 2.75 (m, 6H, $CH_2 \times 3$), 1.07 (s, 6H, $CH_3 \times 2$). ^{13}C NMR (DMSO-d_6): δ 167.4 (C, J_{C-F} = 251 Hz), 163.2 (C), 162.2 (C, J_{C-F} = 246 Hz), 160.7 (CH), 157.0 (C), 151.3 (C), 141.9 (C), 134.7 (CH), 133.7 (C), 132.8 (CH), 130.1 (C), 128.3 (CH), 128.1 (CH), 126.8 (CH), 125.0 (C), 117.0 (CH), 114.4 (CH), 108.6 (CH), 56.3 (CH_3), 54.5 (CH_2), 49.8 ($CH_2 \times 2$), 40.1 (CH_2), 14.1 ($CH_3 \times 2$). ESI-HRMS *m*/*z*: calcd for $C_{25}H_{39}F_2N_8O_4S$ $[M+H]^+$: 575.2001; found: 575.1995.

5.1.4.13. 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonylamino)pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-oxo-2-(pyrrolidin-1-yl)ethyl)urea (1m). White solid; yield: 61.4%; mp: >250 °C; ^{1}H NMR (DMSO-d_6): δ 10.14 (s, 1H, NH), 10.10 (s, 1H, NH), 9.14 (s, 1H, Ar-H), 8.64 (s, 1H, NH), 8.40 (d, J = 2.4 Hz, 1H, Ar-H), 8.00 (d, J = 2.4 Hz, 1H, Ar-H), 7.92 (dd, J_1 = 9.2 Hz, J_2 = 1.6 Hz, 1H, Ar-H), 7.82 (m, 2H, Ar-H), 7.74 (d, J = 9.2 Hz, 1H, Ar-H), 7.42 (m, 2H, Ar-H), 4.06 (d, J = 4.4 Hz, 2H, CH_2), 3.64 (s, 3H, OCH_3), 3.44 (m, 2H, CH_2), 3.34 (d, J = 6.8 Hz, 2H, CH_2), 1.91 (m, 4H, $CH_2 \times 2$), 1.79 (m, 4H, $CH_2 \times 2$). ^{13}C NMR (DMSO-d_6): δ 169.7 (C), 168.2 (C), 166.6 (C), 163.2 (C), 160.0 (CH), 156.8 (C), 151.5 (C), 144.8 (C), 139.7 (C), 135.2 (C), 132.9 (CH), 132.9 (CH), 132.9 (CH), 128.5 (C), 126.2 (CH), 123.5 (CH), 119.3 (CH), 119.2 (CH), 117.2 (CH), 56.6 (CH_3), 47.9 ($CH_2 \times 2$), 45.6 (CH_2), 26.8 ($CH_2 \times 2$). ESI-HRMS *m*/*z*: calcd for $C_{25}H_{25}FN_8NaO_5S$ $[M+Na]^+$: 591.1550; found: 591.1545.

5.1.4.14. 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonylamino)-pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-oxo-2-(piperidin-1-yl)urea (1n). White solid; yield: 67.8%; mp: >250 °C; ^{1}H NMR (DMSO-d_6): δ 10.14 (s, 1H, NH), 10.10 (s, 1H, NH), 9.15 (s, 1H, Ar-H), 8.65 (s, 1H, NH), 8.40 (d, J = 2.0 Hz, 1H, Ar-H), 8.00 (d, J = 2.4 Hz, 1H, Ar-H), 7.92 (dd, J_1 = 9.2 Hz, J_2 = 1.2 Hz, 1H, Ar-H), 7.82 (m, 2H, Ar-H), 7.74 (d, J = 9.2 Hz, 1H, Ar-H), 7.42 (m, 2H, Ar-H), 4.13 (d, J = 5.6 Hz, 2H, CH_2), 3.63 (s, 3H, OCH_3), 3.46 (m, 2H, CH_2),3.36 (s, 2H, CH_2), 1.57 (m, 4H, $CH_2 \times 2$), 1.46 (d, J = 3.6 Hz, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 169.6 (C), 168.2 (C), 166.6 (C), 163.3 (C), 160.0 (CH), 156.7 (C), 151.5 (C), 144.9 (C), 139.7 (C), 135.4 (C), 132.9 (CH), 132.9 (CH), 132.9 (CH), 128.6 (C), 126.1 (CH), 123.5 (CH), 119.3 (CH), 119.2 (CH), 117.2 (CH), 56.5 (CH_3), 48.0 (CH_2), 45.4 (CH_2), 44.8 (CH_2), 28.9 (CH_2), 28.3 (CH_2), 27.1 (CH_2). ESI-HRMS *m*/*z*: calcd for $C_{26}H_{27}FN_8NaO_5S$ $[M+Na]^+$: 605.1707; found: 605.1701.

5.1.4.15. 1-(6-(2-Methoxy-3-(4-fluorophenylsulfonylamino)-pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)-3-(2-(morpholino-2-oxoethyl)urea (1o). White solid; yield: 64.2%; mp: >250 °C; ^{1}H NMR (DMSO-d_6): δ 10.16 (s, 1H, NH), 10.10 (s, 1H, NH), 9.15 (s, 1H, Ar-H), 8.65 (s, 1H, NH), 8.40 (d, J = 2.0 Hz, 1H, Ar-H), 8.00 (d, J = 2.4 Hz, 1H, Ar-H), 7.92 (dd, J_1 = 9.2 Hz, J_2 = 1.2 Hz, 1H, Ar-H), 7.82 (m, 2H, Ar-H), 7.75 (d, J = 9.2 Hz, 1H, Ar-H), 7.41 (m, 2H, Ar-H), 4.16 (d, J = 2.0 Hz, 2H, CH_2), 3.64 (s, 3H, OCH_3),3.60 (m, 4H, $CH_2 \times 2$), 3.47 (m, 4H, $CH_2 \times 2$). ^{13}C NMR (DMSO-d_6): δ 170.4 (C), 168.2 (C), 166.5 (C), 163.2 (C), 160.0 (CH), 156.8 (C), 151.5 (C), 144.8 (C), 139.7 (C), 135.2 (C), 132.9 (CH), 132.9 (CH), 132.8 (CH), 128.5 (CH), 126.2 (C), 123.5 (CH), 119.3 (CH), 119.1 (CH), 117.2 (CH), 69.1 (CH_2), 69.0 (CH_2), 56.5 (CH_3), 47.5 (CH_2), 44.9 (CH_2), 44.7 (CH_2). ESI-HRMS *m*/*z*: calcd for $C_{25}H_{25}FN_8NaO_6S$ $[M+Na]^+$: 607.1499; found: 607.1494.

5.1.4.16. 1,1-Dimethyl-3-(6-(2-methoxy-3-(4-fluorophenylsulfonylamino)pyridine-5-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)urea (1p). White solid; yield: 53.7%; mp: 153–155 °C; ^{1}H NMR (DMSO-d_6): δ 10.10 (s, 1H, NH), 9.40 (s, 1H, Ar-H), 9.16 (s, 1H, Ar-H), 8.40 (s, 1H, NH), 7.99 (s, 1H, Ar-H), 7.84 (m, 3H, Ar-H), 7.72 (d, J = 8.8 Hz, 1H, Ar-H), 7.42 (d, J = 8.4 Hz, 2H, Ar-H), 3.63 (s, 3H, OCH_3), 2.94 (s, 6H, $CH_3 \times 2$). ^{13}C NMR (DMSO-d_6): δ 168.2 (C), 166.5 (C), 163.8 (C), 160.0 (CH), 157.6 (C), 151.8 (C), 144.7 (C), 139.8 (C), 135.3 (CH), 132.9 (CH), 132.8 (CH), 132.2 (CH), 128.6 (CH), 125.7 (CH), 123.7 (C), 119.3 (CH), 119.1 (CH), 117.5 (CH), 56.5 (CH_3), 39.5 ($CH_3 \times 2$). ESI-HRMS *m*/*z*: calcd for $C_{21}H_{20}FN_7NaO_4S$ $[M+Na]^+$: 508.1179; found: 508.1174.

5.1.4.17. 1,1-Dimethyl-N-(6-(5-(4-fluorophenylsulfonamido)-6-methoxypyridin -3-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)pyrrolidine-1-carboxamide (1q). White solid; yield: 48.3%; mp: 155–156 °C; ^{1}H NMR (DMSO-d_6): δ 10.10 (s, 1H, NH), 9.26 (s, 1H, Ar-H), 9.16 (s, 1H, Ar-H), 8.40 (m, 1H, NH), 7.99 (s, 1H, Ar-H), 7.80 (m, 3H, Ar-H), 7.72 (d, J = 8.8 Hz, 1H, Ar-H), 7.41 (m, 2H, Ar-H), 3.63 (s, 3H, OCH_3), 3.40 (s, 4H, $CH_2 \times 2$), 1.85 (s, 4H, $CH_2 \times 2$). ^{13}C NMR (DMSO-d_6): δ 168.2 (C), 166.6 (C), 163.8 (C), 160.0 (CH), 155.6 (C), 151.7 (C), 144.8 (C), 139.8 (C), 135.4 (CH), 132.9 (CH), 132.8 (CH), 132.2 (CH), 128.6 (CH), 125.7 (CH), 123.6 (C), 119.3 (CH), 119.1 (CH), 117.5 (CH), 56.5 (CH_3), 49.0 ($CH_2 \times 2$), 28.1 ($CH_2 \times 2$). ESI-HRMS *m*/*z*: calcd for $C_{23}H_{22}FN_7NaO_4S$ $[M+Na]^+$: 534.1336; found: 534.1330.

5.2. Biology

5.2.1. Cell culture

The four human tumor cell lines including HCT-116, MCF-7, U87 MG and A549 were grown as a monolayer respectively. HCT-116, MCF-7 and U87 MG were maintained in DMEM medium supplemented with 10% heat inactivated fetal bovine serum (FBS). A549 was maintained in RPMI-1640 medium, with 10% heat inactivated calf serum. All cancer cells were incubated in a humidified atmosphere containing 5% CO_2 at 37 °C.

5.2.2. MTT assay

The in vitro antiproliferative activities of compounds were determined by MTT assay. 1500–4000 cells per well were seeded into 96-well plates in 200 μL medium and incubated for 24 h. A series of concentrations of synthesized compounds and **BEZ235** were added to the wells with DMSO as vehicle control. The mixture was incubated at 37 °C, with a final concentration of 1% DMSO. After 72 h of incubation, 20 μL of MTT solution (5 mg/mL in PBS) was added to each well and incubated at 37 °C for 4 h. The supernatant of each well was removed and the formed blue formazan crystals were dissolved in 200 μL of DMSO. The optical density at 490 nm wavelength 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, were calculated by means of GraphPad Prism 5 Software.

5.2.3. PI3Ks and mTOR kinase assay

Inhibitory PI3Ks enzymatic activities of compounds **T1c**, **T1e** and **BEZ235** were evaluated according to the reported method.[26,27] Compounds **T1c**, **T1e** 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) or mTOR 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 PI3Ks or mTOR activity.

The percentage of inhibition was calculated based on the following equation

$$\%\,\text{inhibition} = \left[1 - (\text{Lu}_{\text{compound}} - \text{Lu}_{\text{min}})/(\text{Lu}_{\text{max}} - \text{Lu}_{\text{min}})\right] \times 100\%,$$

where $\text{Lu}_{\text{compound}}$ is the signal at a given compound concentration, Lu_{max} is the signal of PI3Ks or mTOR 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 Prism 5 Software.

5.2.4. Acute oral toxicities assay

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 1:8:1 in volume ratio). The UDP study was according to the principles of OECD guideline 425.[29] The starting dose of compounds **B**, **1b** and **1c** was 50 mg/kg and the starting dose of compounds **1e** was 100 mg/kg. The slope factor sigma of all these compounds was 0.12. Dose progression was stopped when one of the three stopping rules of the AOT425StatPgm program was satisfied.[30] The LD_{50} values and 95% confidence intervals were calculated using the AOT425StatPgm program.

5.2.5. In vivo antitumor effect in established mice S180 homograft models

Mice (20.3 ± 1.6 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 Xian 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 **1e** was dissolved in NMP/PEG400/H_2O (1:6:3) and dosed orally at 5 mg/kg, 10 mg/kg or 20 mg/kg 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.

5.3. Molecule docking

The protein–ligand complex crystal structure[21] of compound **A** with PI3Kγ was chosen as the template to elucidate the docking modes of **1c** and **1e** with PI3Kγ. Protein structure was downloaded from Protein Data Bank (PDB code 3QK0) and the C-DOCKER protocol within Discovery Studio 2.5 was used for molecular docking. The PI3Kγ enzyme was prepared by added hydrogen atoms and then defined as a receptor. The site sphere was selected on the basis of the ligand binding location of compound **A**, which was replaced by compound **1c** or **1e**. After end of molecular docking, 10 docking poses were scored and selected based on calculated C-DOCKER energy.

5.4. Statistical analysis

The data in Table 1 are reported as mean ± standard deviation (SD) for at least three experiments. Statistical differences were analyzed according to one way ANOVA test wherein the differences were considered to be significant at $P<0.05$. All statistics were calculated using a statistical program GraphPad Prism 5.

Acknowledgment

Financial support from National Natural Science Foundation of China (Grant Nos. 21072156 and 81272448) is gratefully acknowledged.

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Please cite this article in press as: Wang, X.-M.; et al. *Bioorg. Med. Chem.* (2015), http://dx.doi.org/10.1016/j.bmc.2015.07.017