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Original article

Synthesis and anticancer activity evaluation of a series of [1,2,4]triazolo[1,5-a]pyridinylpyridines *in vitro* and *in vivo*

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ABSTRACT

A series of [1,2,4]triazolo[1,5-a]pyridinylpyridines were synthesized and characterized. Their antiproliferative activities *in vitro* were evaluated by MTT against three human cancer cell lines including HCT-116, U-87 MG and MCF-7 cell lines. The SAR of target compounds was preliminarily discussed. The compounds **1c** and **2d** with potent antiproliferative activities were tested for their effects on the AKT and p-AKT^{473}. The anticancer effect of **1c** was evaluated in mice bearing sarcoma S-180 model. The results suggest that the title compounds are potent anticancer agents.

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. 2-Amino-[1,2,4]triazolo[1,5-a]pyridine, with two hydrogen bond acceptors and a hydrogen bond donor, is considered as an ideal fragment or a scaffold in drug design. Its derivatives exhibit a broad spectrum of biological activities such as anticancer, anti-inflammatory, antimicrobial and antidiabetic activity. Dugan et al. reported the structure–activity relationships and biological evaluation of a novel series of compounds based on 1,2,4-triazolo[1,5-a]pyridine scaffold and discovered that CEP-33779 is a novel, selective, and orally bioavailable inhibitor of JAK2 [1]. Thus CEP-33779 can be used in anticancer therapy [2] and rheumatoid arthritis treatment [3]. Wyatt et al. identified 2-acylamino-6-aryl[1,2,4]triazolo[1,5-a]pyridines as inhibitors of the leishmania cdc 2-related protein kinase CRK3 and the inhibitors may be used to cure 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]. Bell et al. described the identification and SAR of 6-aryl-[1,2,4]triazolo[1,5-a]pyridines and discovered that CZC19945 and CZC24832 display potent inhibitory activities against PI3Kγ with good *in vivo* PK profile [7]. Bergamini et al. further proved that CZC24832 is efficacious in *in vitro* and *in vivo* model of inflammatory diseases in rodents and humans and it will be of use for the treatment of autoimmune and inflammatory disorders [8]. Ellard et al. synthesized a series of 2-ureido-[1,2,4]triazolo[1,5-a]pyridine and discovered two compounds exhibit excellent PI3Kγ/δ potency with high selectivity over the other isoforms and the general kinome [9]. In addition, Kuroyanagi et al. found that polysubstituted [1,2,4]triazolo[1,5-a]pyridines is the specific inhibitor of β-1,6-glucan synthesis with potent antifungal growth inhibition [10]. East et al. reported that 2-ureido-5,7-disubstituted-[1,2,4]triazolo[1,5-*a*]pyridine displays good antibacterial activity, particularly against gram-positive organisms [11]. Edmondson et al. reported that 6-substituted [1,2,4]triazolo[1,5-a]pyridine is a potent DPP-4 inhibitor and may be used to treat type 2 diabetes mellitus [12].

Cancer, as a major cause of death in the world, has posed a great challenge to the fields of medicine and immunology. Chemotherapy has been widely employed for various cancer treatments. The toxicity and resistance of traditional chemotherapeutic drugs makes it urgent to develop new targets and novel drugs for the cancer therapy. Kinases are well-known targets for a variety of diseases and disorders [13].

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PI3K (a family of lipid kinase) and mTOR (mammalian target of rapamycin) have been found to play a regulatory role in many cellular processes, including cell growth, proliferation, differentiation, motility and survival [14]. In many cancers the PI3K/AKT/mTOR signal transduction pathway is dysregulated contributing to cellular transformation and tumor growth [15]. Therefore, PI3Kα and mTOR have become the targets of intense research for anticancer drug discovery [16,17]. In recent years, a significant progress has been made on the design, synthesis and evaluation of PI3Kα and mTOR dual inhibitors, and thereupon the pharmacophore of the dual inhibitors has been proposed [18]. Among the reported PI3K/mTOR dual inhibitors, *N*-(5-quinilin-6-yl)pyridine-3-yl) phenylsulfonamide is a class of important active compounds. Two ring nitrogen atoms in pyridine and quinoline are the main components of pharmacophore. GlaxoSmithKline company identified GSK2126458, *N*-(2-methoxy-5-(4-pyridazin-4-yl) quinilin-6-yl)pyridine-3-yl)-2,4-difluorobenzenesulfonamide, as a potent, orally bioavailable inhibitor of PI3Kα and mTOR [19]. Amgen Inc. designed, synthesized and evaluated several classes of *N*-(2, 5-disubstituted-pyridine-3-yl)phenylsulfonamides. Thus they discovered that *N*-(2-chloro-5-(4-morpholinoquinilin-6-yl)pyridine-3-yl)-4-fluorophenylsulfonamide [20], *N*-(2-chloro-5-(2-acetylamonobenzo[*d*] thiazol-6-yl)pyridine-3-yl)-4-fluorophenyl sulfonamide (compound **A**, Fig. 1) [21], *N*-(2-chloro-5-(2-acetylamonoimidazo[1,2-b]pyridazin-6-yl)pyridine-3-yl)-4-fluorophenylsulfonamide [22] and AMG 511 [23] are excellent PI3Kα/mTOR dual inhibitors or PI3Kα selective inhibitor, and orally bioavailable anticancer agents as well.

In an attempt to develop novel anticancer agents, we try to combine 2-amino-[1,2,4]triazolo[1,5-*a*]pyridine with *N*-pyridin-3-ylphenylsulfonamide to design and synthesize the analogues of compound A. Herein, the benzo[*d*]thiazole moiety was replaced by [1,2,4]triazolo[1,5-*a*]pyridine (Fig. 1). Compared with compound **A**, the designed compounds **1** and **2** have the same pharmacophore with compound **A**. Here, we report the synthesis and anticancer activity evaluation of a series of [1,2,4]triazolo[1,5-a]pyridinylpyridines *in vitro* and *in vivo*.

2. Results and discussion

2.1. Chemistry

The synthetic route to obtain the compounds **1a**–**1p** is outlined in Scheme 1.

6-Bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-ylamine **3** was acylated to give acetyl amide **4a**, cyclopropylcarbonyl amide **4b** and formamide **4c**, respectively [24]. Reaction of **4c** with alkyl halides afforded **5a**–**5c** in the presence of sodium hydride [25]. Reduction of 5-bromo-2-chloro-3-nitropyridine **6a** or 5-bromo-2-methoxy-3-nitropyridine **6b** afforded corresponding amines. Without further purification, the amines were converted to sulfamides **7a**–**7g** [26]. Catalyzed by $PdCl_2$(dppf), sulfamides **7a**–**7g** were reacted with bis(-pinacolato)diboron to produce arylboronic esters. Without isolation of arylboronic esters, intermediate **3**, or **4** or **5**, $PdCl_2$(dppf), water and sodium carbonate as well were added to the above reaction mixture. The resulted mixture was refluxed for 1 h to produce the title compounds **1a**–**1p**. In the case of preparation of **1m**, the refluxing was lasted for 5 h to make hydrolysis of ester group. The preparation of arylboronic esters and Suzuki coupling were completed in one pot. Formyl group in intermediate **5** was removed in last step.

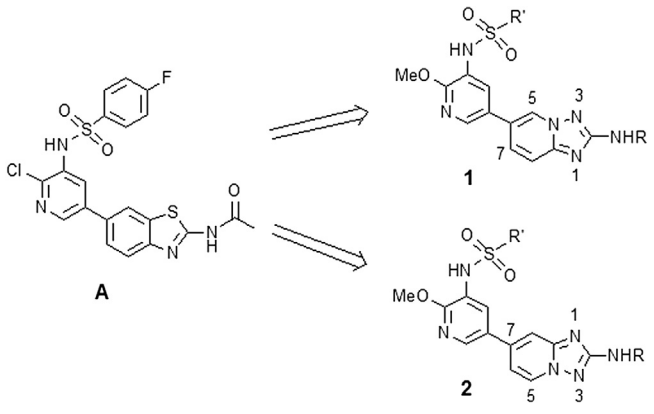


Fig. 1. The structures of compound **A** and target compounds **1** and **2**.

The synthetic route to obtain the compounds **1q** and **1r** is shown in Scheme 2. Ammonolysis of ester group in compound **1p** produced the compounds **1q** and **1r**.

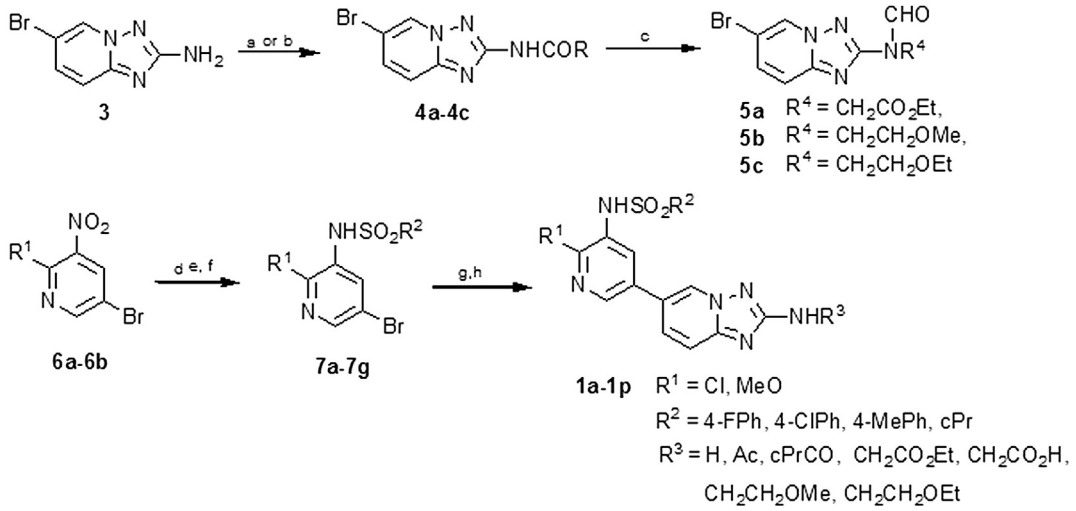
To expand the structural diversity of the title compounds, 7-position of 2-acetylamino-[1,2,4]triazolo[1,5-*a*]pyridine was combined with 3-sulfonamidopyridinyl to yield compound **2**. The acetylation of 7-bromo-[1,2,4]triazolo[1,5-*a*]pyridine-2-ylamine **8** produced compound **9**. Compounds **2a**–**2d** were synthesized by Suzuki coupling in a same manner as preparation of compounds **1a**–**1p**. The synthetic route for the compounds **2a**–**2d** is shown in Scheme 3.

2.2. In vitro antiproliferative assays

We evaluated antiproliferative activities of compounds **1a**–**1r** and **2a**–**2d** against three human cancer cell lines including human colon carcinoma cell line (HCT-116), glioma cell line (U-87 MG) and human breast adenocarcinoma cell line (MCF-7) by applying the MTT colorimetric assay. The PI3K and mTOR dual inhibitors **A** and BEZ235 were used as the positive controls. The results of the cell viability assay of compounds are summarized in Table 1.

First we synthesized a series of compound **1** by changing substituent R^1, R^2 and R^3. As expected, all compounds exhibited significant effects on antiproliferative activities *in vitro*, especially against HCT-116. This may be related to PI3Kα mutant in HCT-116. The fact that compounds **1c** (IC_{50} = 0.85 μM against HCT-116) and **1e** (IC_{50} = 0.60 μM against HCT-116) were more potent than compounds **1a** (IC_{50} = 9.23 μM against HCT-116) and **1b** (IC_{50} = 2.65 μM against HCT-116) indicated that the compound with a methoxy at 2-position (R^1) of pyridine ring may improve the antiproliferative activity. Compounds **1c**, **1d**, **1e** and **1f** exhibited similar activities against HCT-116, U-87 MG and MCF-7, which suggests that the substituent at the phenyl ring of R^2 has less effect on the antiproliferative activity. However, **1g** (IC_{50} = 2.22 μM against HCT-116) was less potent than **1c**, **1e**, **1d** and **1f** (IC_{50} = 0.60–0.85 μM against HCT-116), indicating that aromatic group at position of R^2 is more beneficial to the activity than cyclopropyl group. To further study structure–activity relationship (SAR) of compound **1**, we replaced acetyl at position of R^3 with different groups. When acetyl at position of R^3 was removed, the activity of compounds **1j**–**1l** decreased against all three human cell lines. Compared with compounds **1a** and **1c** with an acetyl group at R^3, compounds **1h** and **1i** with cyclopropylcarbonyl group at R^3 were less effect against three human cells. In addition, we replaced acetyl at R^3 with substituted methyl to produce **1m**, **1p**, **1q** and **1r** or with substituted ethyl to produce **1n** and **1o**. The antiproliferative activities of compounds **1m**–**1r** were not improved. Therefore, the acetyl at R^3 position is effective on antiproliferative activity *in vitro*.

In our second approach, we changed the pyridinyl from 6-position of 2-amino-[1,2,4]triazolo[1,5-a]pyridine to 7-position to produce compound **2**. Compared with compounds **1c**, **1d** and **1e**, compounds **2a**, **2b** and **2c** were less potent. Interestingly, compound **2d** (IC_{50} = 0.82 μM against HCT-116) with H at R^3 is more potent than **2c** (IC_{50} = 1.32 μM) with acetyl at position R^3, which is



Scheme 1. Reagents and conditions: (a) (i) AcCl or cPrCOCl, THF, rt; (ii) 7 N NH_3 in MeOH, rt, overnight; (b) MeCOOCHO, Et_2O, rt; (c) NaH, RX, DMF; (d) $SnCl_2 \cdot 2H_2O$, conc. HCl, rt; (e) RSO_2Cl, pyridine, rt or reflux; (f) K_2CO_3, MeOH, rt; (g) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, 1,2-dimethoxyethane, reflux; (h) **3** or **4a**–**4c** or **5a**–**5c**, $PdCl_2$(dppf), Na_2CO_3, EtOH/H_2O 3:2, reflux.

different from compound **1**. From above mentioned results we could reveal a SAR in which methoxy at R^1, aryl at R^2 and acetyl at R^3 are favorable for antiproliferative activity. The IC_{50}s of compounds **1c** and **2d** are comparable to that of PI3K and mTOR dual inhibitor **BEZ235**. Therefore, compounds **1c** and **2d** were further investigated.

2.3. Western blot assay

To determine whether these compounds inhibit the PI3K, we evaluated the suppressive effects of compounds **1c** and **2d** at 10 μM and positive compound **A** on AKT and p-AKT^{473} in HCT-116 cells through Western blot. As shown in Fig. 2, the suppressive effects of compounds **1c** and **2d** on p-AKT^{473} were slightly weaker than that of positive compound **A**.

2.4. Anticancer effects in established mouse S180 homograft models in vivo

We next evaluated whether compounds **1c** and **2d** could inhibit tumor growth in established mouse homograft models. Though favorable activity of compound **2d** against human cancer cells, it led to death of mice at dose of 20 mg/kg. Compound **1c** can be tolerated by mice at dose of 20 mg/kg and then we evaluated the activity of compound **1c** inhibiting tumor growth. A study using mice bearing sarcoma S-180 was performed. Compound **1c** was dosed orally at 1 mg/kg or 5 mg/kg once a day. In this model the volumes of S-180 tumor is difficult to measure, so tumor weights were used as evaluating indicators which are illustrated in Table 2.

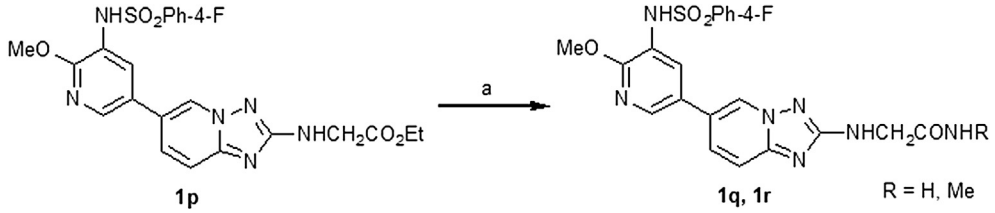
The inhibitory ratios at 1 mg/kg and 5 mg/kg were 20.0% and 75.8%, respectively. The results suggested that compound **1c** distinctly exhibited inhibiting effect on tumor growth. Mouse body weights loss of tested animals was not observed at 1 mg/kg group. This is likely due to the low dosage. However, the body weight dropped below 90% of the starting body weight over the course of the study at 5 mg/kg group, which indicated that compound **1c** still has some toxicity at the dosage.

2.5. Docking studies

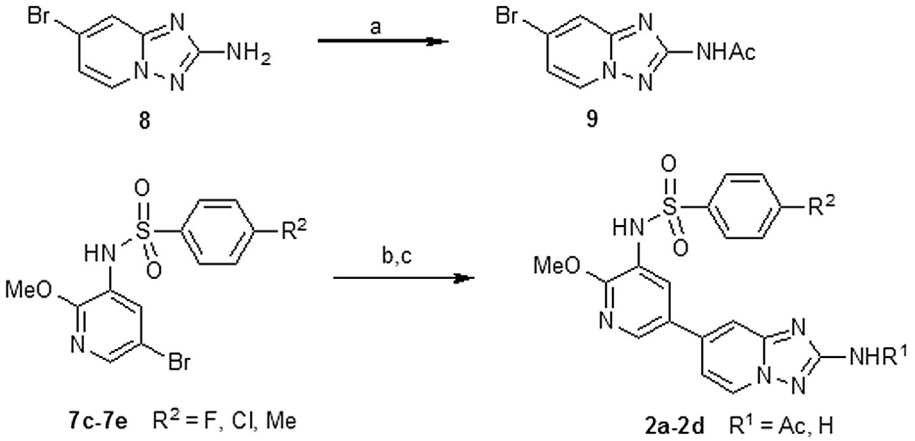
Docking analysis utilizing the C-DOCKER program within Discovery Studio 2.5 software package was performed to further explore the binding mode of the target compounds **1a** and **1c**. Docking simulations were performed on human PI3Kγ (PDB code 3QKO) [21] (Fig. 3). The docking result of compound **1a** with PI3Kγ (Fig. 3A) indicated that: (a) the 2-acetyl[1,2,4]triazolo[1,5-*a*]pyridine forms two hydrogen bonds with Val882; (b) the nitrogen of 2-chloropyridine forms a hydrogen bond with Asp964 and two hydrogen bonds are formed via an ordered water molecule located between the 2-chloropyridine ring nitrogen and the Tyr867 and Asp841 residues; (c) the two oxygens of sulfonylamide form two hydrogen bonds with Lys833. By contrast, compound **1c** formed an extra hydrogen bond via the oxygen of methoxypyridine (Fig. 3B), which explains the methoxy is more suitable than chloro in compound **1**.

3. Conclusion

A series of 5-(6-[1,2,4]triazolo[1,5-a]pyridinyl)pyridines and 5-(7-[1,2,4]triazolo[1,5-a]pyridinyl)pyridines were synthesized. Their antiproliferative activities were evaluated *in vitro* against human cancer cell lines including HCT-116, U-87 MG and MCF-7.



Scheme 2. Reagents and conditions: (a) NH_3 or $MeNH_2$ in MeOH, rt.



Scheme 3. Reagents and conditions: (a) (i) AcCl, THF, rt; (ii) 7 N NH_3 in MeOH, rt, overnight; (b) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, 1,2-dimethoxyethane E, reflux; (c) **8** or **9**, $PdCl_2$(dppf), Na_2CO_3, EtOH/H_2O, 3:2, reflux.

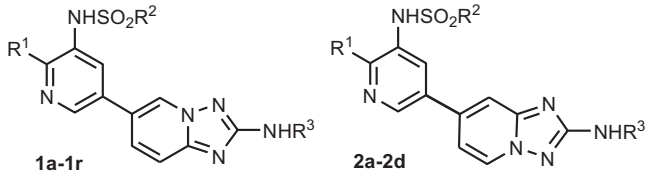
Compounds displayed IC_{50} around 1 μM. The SAR of these compounds indicated that methoxy at 2-position, arylsulfonamino at 3-positon and 2-acetylamino-[1,2,4]triazolo[1,5-a]pyridinyl at 5-position of pyridine ring can improve the inhibitory activity. The Western blot assay results of compounds **1c** and **2d** suggested that these compounds can inhibit the PI3K/AKT/mTOR pathway. Compound **1c** distinctly exhibited inhibiting effect on tumor growth in mice bearing sarcoma S-180 model. These results suggest the title compounds are potent anticancer agents.

4. Experimental protocols

4.1. Chemistry and chemical methods

3-[4, 5-Dimethyl-2-thiazolyl]-2, 5-diphenyl-*2H*-tetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO, USA). 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

Table 1
Antiproliferative activities against HCT-116, U-87 MG and MCF-7 ($\bar{x} \pm s$, $n = 3$).



Compds	R^1	R^2	R^3	IC_{50} (μM)		
				HCT-116	U-87 MG	MCF-7
1a	Cl	4-FPh	Ac	9.23 ± 1.00	6.95 ± 0.95	19.79 ± 2.84
1b	Cl	4-MePh	Ac	2.65 ± 0.48	3.76 ± 0.37	3.25 ± 0.65
1c	OMe	4-FPh	Ac	0.85 ± 0.40	1.82 ± 0.48	0.84 ± 0.30
1d	OMe	4-ClPh	Ac	0.74 ± 0.43	2.09 ± 0.89	2.01 ± 0.78
1e	OMe	4-MePh	Ac	0.60 ± 0.09	1.60 ± 0.27	0.81 ± 0.25
1f	OMe	2,4-diFPh	Ac	0.74 ± 0.34	2.00 ± 0.46	0.87 ± 0.21
1g	OMe	cPr	Ac	2.22 ± 1.23	3.73 ± 0.91	1.64 ± 0.69
1h	OMe	4-FPh	cPrCO	1.74 ± 0.30	4.85 ± 1.33	2.59 ± 1.27
1i	Cl	4-FPh	cPrCO	9.98 ± 2.89	10.96 ± 4.00	>60
1j	OMe	4-FPh	H	2.97 ± 2.19	3.95 ± 0.90	1.93 ± 1.13
1k	OMe	4-ClPh	H	2.50 ± 1.99	3.11 ± 0.72	1.82 ± 1.03
1l	OMe	4-MePh	H	3.40 ± 0.51	2.51 ± 0.99	1.55 ± 0.41
1m	OMe	4-FPh	CH_2CO_2H	8.64 ± 2.88	9.81 ± 2.91	12.64 ± 2.94
1n	OMe	4-FPh	CH_2CH_2OMe	4.61 ± 2.90	6.39 ± 1.02	2.41 ± 0.64
1o	OMe	4-FPh	CH_2CH_2OEt	9.35 ± 3.77	8.15 ± 1.52	1.35 ± 0.32
1p	OMe	4-FPh	CH_2CO_2Et	4.37 ± 1.93	7.94 ± 2.17	4.41 ± 2.23
1q	OMe	4-FPh	CH_2CONH_2	6.10 ± 1.61	5.80 ± 1.89	8.40 ± 3.57
1r	OMe	4-FPh	$CH_2CONHMe$	4.91 ± 2.53	7.29 ± 2.24	2.54 ± 0.69
2a	OMe	4-ClPh	Ac	1.33 ± 0.49	2.12 ± 0.13	1.24 ± 0.30
2b	OMe	4-MePh	Ac	1.38 ± 0.48	2.03 ± 0.47	1.36 ± 0.54
2c	OMe	4-FPh	Ac	1.32 ± 0.33	2.41 ± 0.51	1.73 ± 0.31
2d	OMe	4-FPh	H	0.82 ± 0.06	1.77 ± 0.34	0.92 ± 0.22
BEZ235				1.16 ± 0.15	1.32 ± 0.41	0.73 ± 0.21
A				nt	nt	0.23 ± 0.09

nt: not tested.

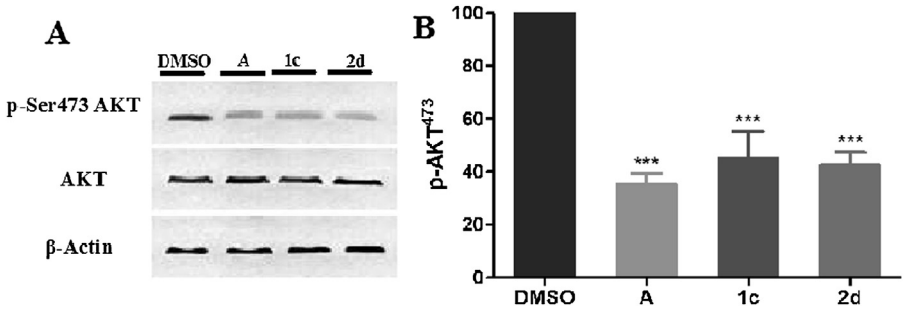


Fig. 2. Effect of compounds **A**, **1c** and **2d** on AKT in HCT116 cells. (A) Effect of compounds **1c**, **2d** and **A** on the AKT and p-AKT^{473}. (B) The quantified effect of compounds **1c**, **2d** and **A** on the AKT and p-AKT^{473}. Results are $\bar{x} \pm s$ for three independent experiments each performed in duplicate. ***$P < 0.001$.

UV light. All the melting points were determined on a Beijing micromelting-point apparatus and thermometer was uncorrected. ^{1}H NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer with tetramethylsilane (TMS) as an internal reference. ^{13}C NMR spectra were recorded on a 100 MHz 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 procedures for the synthesis of compounds **4a**, **4b** and **9***

To a stirred solution of 6-bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-ylamine **3** (2.00 g, 9.3 mmol) or 7-bromo-[1,2,4]triazolo[1,5-*a*]pyridine-2-ylamine **8** (2.00 g, 9.3 mmol) in THF (40 ml) was added *N*, *N*-diisopropylethylamine (4.87 ml, 27.9 mmol), and then acyl chloride (27.9 mmol) was added. The reaction mixture was stirred at room temperature until all starting material was consumed. The solvent was evaporated under vacuum. The solution of ammonia in methanol (7 N, 20 ml) was added to the residue, and the resulted mixture was stirred at room temperature overnight. The solvent was evaporated under vacuum. Ether (20 ml) was added to the residue, and the solid was collected by filtered, washed with water (20 ml), acetone (20 ml) and then dried to afford a off-white solid.

*4.1.1.1. N-(6-bromo-[1,2,4]triazolo[1,5-a]pyridin-2-yl)acetamide (**4a**).* Yield 84.6%, mp 222–225 °C. ^{1}H NMR (DMSO-d_6): δ 10.87 (s, 1H, NH), 9.29 (s, 1H, Ar–H), 7.78 (d, $J = 9.2$ Hz, 1H, Ar–H), 7.78 (d, $J = 9.2$ Hz, 1H, Ar–H), 2.13 (s, 3H, CH_3). ESI–HRMS *m*/*z*: calc'd for $C_8H_7BrN_4NaO$ $[M + Na]^+$: 276.9701; found 276.9699.

*4.1.1.2. N-(6-bromo-[1,2,4]triazolo[1,5-a]pyridin-2-yl)cyclopropanecarboxamide (**4b**).* Yield 77.1%, mp 216–219 °C. ^{1}H NMR (DMSO-d_6): δ 11.14 (s, 1H, NH), 9.28 (s, 1H, Ar–H), 7.78 (d, $J = 9.6$ Hz, 1H, Ar–H), 7.66 (d, $J = 9.2$ Hz, 1H, Ar–H), 2.03 (m, 1H, CH), 0.83 (d, $J = 5.6$ Hz, 4H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.65, 159.25, 148.68, 133.81, 129.66, 115.90, 106.91, 14.41, 8.34, 8.34. ESI–HRMS *m*/*z*: calc'd for $C_{10}H_9BrN_4NaO$ $[M + Na]^+$: 302.9857; found 302.9857.

*4.1.1.3. N-(7-bromo-[1,2,4]triazolo[1,5-a]pyridin-2-yl)acetamide (**9**).* Yield 62.1%, mp 228–230 °C. ^{1}H NMR (DMSO-d_6): δ 10.87 (s, 1H, NH), 8.82 (d, $J = 5.6$ Hz, 1H, Ar–H), 8.05 (s, 1H, Ar–H), 7.30 (d, $J = 5.6$ Hz, 1H, Ar–H), 2.14 (s, 3H, CH_3). ESI–HRMS *m*/*z*: calc'd for $C_8H_7BrN_4NaO$ $[M + Na]^+$: 276.9701; found 276.9699.

*4.1.2. N-(6-bromo-[1,2,4]triazolo[1,5-a]pyridin-2-yl)formamide (**4c**)*

The mixture of acetic anhydride (0.76 ml, 8 mmol) and 88% formic acid (0.34 ml, 8 mmol) was stirred at 60 °C for 2 h, cooled to room temperature. To the resulting anhydride 6-bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-ylamine **3** (0.43 mg, 2 mmol) and ether (10 ml) were added. The mixture was stirred at room temperature overnight, filtered. The solid was washed by ether and dried to afford a white solid. Yield: 97.5%, mp 217–220 °C. ^{1}H NMR (DMSO-

Table 2
Inhibitory ratios on cancer weights ($\bar{x} \pm s$, $n = 6$).

Groups	Dosage (mg/kg)	Average weights (g)	Inhibition rate in weight (%)
Solvent	10 ml/kg	0.95 ± 0.35	
1c	1.0	0.76 ± 0.25	20.0*
1c	5.0	0.23 ± 0.16	75.8***

*$P < 0.05$ *vs* solvent; ***$P < 0.001$ *vs* solvent.

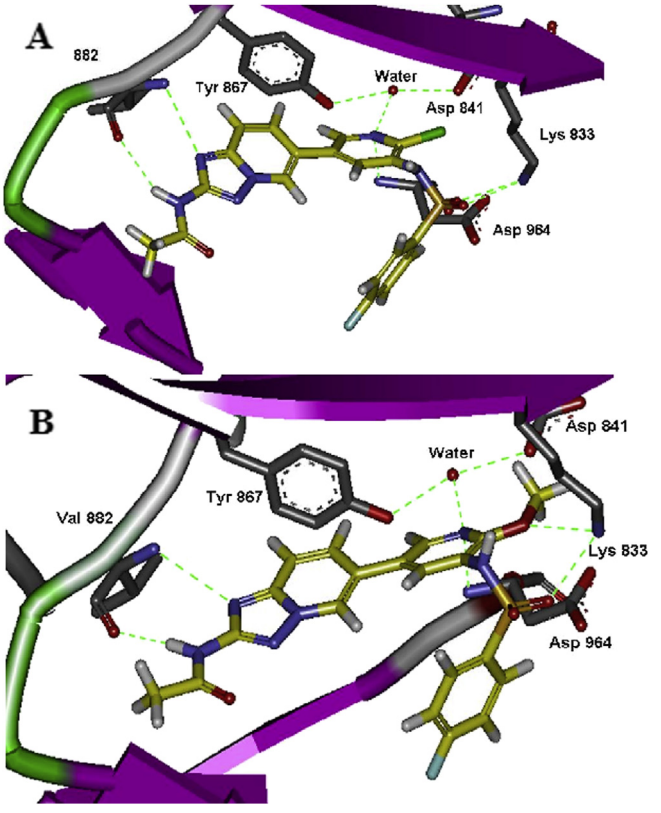


Fig. 3. Docking mode comparison between **1a** and **1c**. (A) Ribbon show of compound **1a** bound to PI3Kγ; (B) ribbon show of compound **1c** bound to PI3Kγ. Compounds **1a** and **1c** are shown in yellow backbones. Selected residues Val882, Tyr867, Lys833, Asp841 and Asp964 are shown based on elements and green dashed lines indicate hydrogen bonds. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

d_6): δ 11.32 (d, J = 10.0 Hz, NH), 9.28 (s, 1H, Ar–H), 9.13 (d, J = 10.0 Hz, CHO), 7.82 (d, J = 9.2 Hz, 1H, Ar–H), 7.67 (d, J = 9.2 Hz, 1H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.06, 160.05, 149.33, 134.26, 129.70, 115.77, 107.14. ESI–HRMS m/z: calc'd for $C_7H_5BrN_4NaO$ $[M + Na]^+$: 262.9544; found 262.9543.

4.1.3. General procedures for the synthesis of compounds ***5a–5c***

Compound **4c** (0.41 g, 1.7 mmol) was added to the suspension of sodium hydride (60%, 0.07 g, 1.7 mmol) in DMF (6 ml) and the mixture was stirred at room temperature for 0.5 h. The alkyl halide (1.83 mmol) was added dropwise to the reaction mixture and then the mixture was stirred at room temperature until all starting material was consumed. Ice-water (20 ml) was poured into the mixture and the mixture was filtered, washed by water and dried to afford the white solid [25].

*4.1.3.1. Ethyl 2-(N-(6-bromo-[1,2,4]triazolo[1,5-a]pyridin-2-yl)formamido)acetate (**5a**).* Yield 97.3%, mp 140–142 °C. ^{1}H NMR (DMSO-d_6): δ 9.58 (s, 1H, CHO), 8.62 (s, 1H, Ar–H), 7.63 (d, J = 8.4 Hz, 1H, Ar–H), 7.52 (d, J = 9.2 Hz, 1H, Ar–H), 4.75 (s, 2H, CH_2), 4.25 (dd, J_1 = 14.4 Hz, J_2 = 14.4 Hz, 2H, OCH_2), 1.31 (t, J = 6.8 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.13, 161.47, 161.09, 149.39, 134.89, 129.95, 116.08, 107.75, 61.67, 43.26, 14.45. ESI–HRMS m/z: calc'd for $C_{11}H_{11}BrN_4NaO_3$ $[M + Na]^+$: 348.9912; found 348.9907.

*4.1.3.2. N-(6-bromo-[1,2,4]triazolo[1,5-a]pyridin-2-yl)-N-(2-ethoxyethyl)formamide (**5b**).* Yield 66.9%, mp 145–147 °C. ^{1}H NMR (DMSO-d_6): δ 9.52 (s, 1H CHO), 8.63 (s, 1H, Ar–H), 7.63 (d, J = 9.6 Hz, 1H, Ar–H), 7.51 (d, J = 9.2 Hz, 1H, Ar–H), 4.23 (t, J = 6.2 Hz, 2H, OCH_2), 3.74 (t, J = 6.2 Hz, 2H, CH_2), 3.54 (dd, J_1 = 14.0 Hz, J_2 = 14.0 Hz, 2H, OCH_2), 1.56 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.75, 161.57, 149.36, 134.67, 129.89, 116.02, 107.51, 66.47, 65.77, 41.17, 15.47. ESI–HRMS m/z: calc'd for $C_{11}H_{13}BrN_4NaO_2$ $[M + Na]^+$: 335.0120; found 335.0118.

*4.1.3.3. N-(6-bromo-[1,2,4]triazolo[1,5-a]pyridin-2-yl)-N-(2-methoxyethyl)formamide (**5c**).* Yield 53.5%, mp 121–123 °C. ^{1}H NMR (DMSO-d_6): δ 9.52 (s, 1H, CHO), 8.64 (s, 1H, Ar–H), 7.63 (d, J = 9.2 Hz, 1H, Ar–H), 7.52 (d, J = 9.2 Hz, 1H, Ar–H), 4.24 (t, J = 5.8 Hz, 2H, CH_2), 3.71 (t, J = 5.8 Hz, 2H, CH_2), 3.37 (s, 3H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.78, 161.56, 149.35, 134.66, 129.88, 116.01, 107.51, 68.73, 58.40, 41.02. ESI–HRMS m/z: calc'd for $C_{10}H_{11}BrN_4NaO_2$ $[M + Na]^+$: 320.9963; found 320.9964.

*4.1.4. General procedure for the synthesis of N-(5-bromo-3-pyridinyl)-4-arylsulfonamide (**7a–7g**)*

Compounds **7a–7g** were synthesized according to the procedure described in Ref. [26].

*4.1.4.1. N-(5-bromo-2-chloro-3-pyridinyl)-4-fluorophenylsulfonamide (**7a**).* Yield 82.1%, mp 175–178 °C. ^{1}H NMR ($CDCl_3$): δ 8.22 (s, 1H, NH), 8.17 (s, 1H, Ar–H), 7.85 (m, 2H, Ar–H), 7.21 (t, J = 8.4 Hz, 2H, Ar–H), 7.01 (s, 1H, Ar–H). ESI–HRMS m/z: calc'd for $C_{11}H_7BrClFN_2NaO_2S$ $[M + Na]^+$: 363.9084; found 363.9087.

*4.1.4.2. N-(5-bromo-2-chloro-3-pyridinyl)-4-methylphenylsulfonamide (**7b**).* Yield 74.7%, mp 162–165 °C. ^{1}H NMR ($CDCl_3$): δ 8.17 (d, J = 5.6 Hz, 2H, NH, Ar–H), 7.72 (d, J = 7.6 Hz, 2H, Ar–H), 7.32 (d, J = 6.8 Hz, 2H, Ar–H), 7.03 (s, 1H, Ar–H), 2.44 (s, 3H, CH_3). HRMS m/z: calc'd for $C_{12}H_{10}BrClN_2NaO_2S$ $[M + Na]^+$: 382.9233; found 382.9238.

*4.1.4.3. N-[5-bromo-2-methoxy-3-pyridinyl]-4-fluorophenylsulfonamide (**7c**).* Yield 85.0%, mp 154–157 °C. ^{1}H NMR ($CDCl_3$): δ 7.92 (s, 1H, Ar–H), 7.85 (m, 2H, Ar–H), 7.18 (m, 2H, Ar–H), 6.92 (s, 1H, Ar–H), 3.85 (s, 3H, OCH_3). HRMS m/z: calc'd for $C_{12}H_{10}BrFN_2NaO_3S$ $[M + Na]^+$: 382.9477; found 382.9482.

*4.1.4.4. N-[5-bromo-2-methyloxy-3-pyridinyl]-4-methylphenylsulfonamide (**7d**).* Yield 86.0%, mp 126–129 °C. ^{1}H NMR ($CDCl_3$): δ 7.89 (d, J = 7.2 Hz, 2H, NH, Ar–H), 7.72 (d, J = 7.6 Hz, 2H, Ar–H), 7.29 (d, J = 6.8 Hz, 2H, Ar–H), 6.95 (s, 1H, Ar–H), 3.85 (s, 3H, OCH_3), 2.42 (s, 3H, CH_3). ESI–HRMS m/z: calc'd for $C_{13}H_{13}BrN_2NaO_3S$ $[M + Na]^+$: 378.9728; found 378.9736.

*4.1.4.5. N-[5-bromo-2-methyloxy-3-pyridinyl]-4-chlorophenylsulfonamide (**7e**).* Yield 86.3%, mp 154–157 °C. ^{1}H NMR ($CDCl_3$): δ 7.92 (d, J = 6.8 Hz, 2H, NH, Ar–H), 7.77 (d, J = 8.0 Hz, 2H, Ar–H), 7.48 (d, J = 8.0 Hz, 2H, Ar–H), 6.95 (s, 1H, Ar–H), 3.84 (s, 3H, OCH_3). ESI–HRMS m/z: calc'd for $C_{12}H_{10}BrClN_2NaO_3S$ $[M + Na]^+$: 398.9182; found 398.9187.

*4.1.4.6. N-[5-bromo-2-methyloxy-3-pyridinyl]-2,4-difluorophenylsulfonamide (**7f**).* Yield 88.2%, mp 158–160 °C. ^{1}H NMR ($CDCl_3$): δ 10.48 (s, 1H, NH), 8.13 (s, 1H, Ar–H), 7.76 (t, J = 11.6 Hz, 2H, Ar–H), 7.56 (t, J = 9.8 Hz, 1H, Ar–H), 7.23 (t, J = 8.6 Hz, 1H, Ar–H). ESI–HRMS m/z: calc'd for $C_{12}H_9BrF_2N_2NaO_2S$ $[M + Na]^+$: 400.9383; found 400.9386.

*4.1.4.7. N-[5-bromo-2-methyloxy-3-pyridinyl]-cyclopropylcarbonylsulfonamide (**7g**).* Yield 95.8%, mp 127–129 °C. ^{1}H NMR ($CDCl_3$): δ 7.95 (d, J = 12.8 Hz, 2H, NH, Ar–H), 6.73 (s, 1H, Ar–H), 4.02 (s, 3H, OCH_3), 2.54 (m, 1H, CH),1.25 (m, 2H, CH_2), 1.03 (m, 2H, CH_2). ESI–HRMS m/z: calc'd for $C_9H_{11}BrN_2NaO_3S$ $[M + Na]^+$: 328.9571; found 328.9573.

4.1.5. General procedures for the synthesis of compounds ***1a–1p*** *and* ***2a–2d***

To a round bottomed flask were added compound **7** (0.4 mmol), bis(pinacolato)diboron (0.13 g, 0.5 mmol), potassium acetate (0.10 g, 1.0 mmol), $PdCl_2$(dppf) (0.22 g, 0.03 mmol) and 1,2-dimethoxyethane (10.5 ml) and the mixture was refluxed for 4 h under N_2 atmosphere, cooled to room temperature. Then, compound **3** or **4** or **5** or **8** or **9** (0.3 mmol), sodium carbonate (0.74 g, 0.7 mmol) and EtOH/H_2O (3:2, v/v, 7.5 ml) as well were added to the mixture. The mixture was refluxed under stirring for 1 h under N_2 atmosphere. The solvent was evaporated under vacuum and the residue was purified by chromatography on silica gel ($CHCl_3$:MeOH = 30:1, v/v) to produce a white or off-white solid.

*4.1.5.1. N-(6-(6-chloro-5-(4-fluorophenylsulfonamido)pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl)acetamide (**1a**).* Yield 65.2%, mp 234–235 °C. ^{1}H NMR (DMSO-d_6): δ 10.90 (s, 1H, NH), 10.56 (s, 1H, NH), 9.39 (s, 1H, Ar–H), 8.71 (s, 1H, Ar–H), 8.18 (s, 1H, Ar–H), 7.97 (d, J = 9.2 Hz, 1H, Ar–H), 7.85 (m, 3H, Ar–H), 7.43 (m, 2H, Ar–H), 2.17 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.31, 163.80, 159.73, 149.34, 145.89, 145.66, 136.94, 135.17, 132.35, 131.06, 130.35, 130.25, 129.99, 127.38, 121.98, 117.16, 116.94, 115.33, 24.18. ESI–HRMS m/z: calc'd for $C_{19}H_{14}ClFN_6NaO_3S$ $[M + Na]^+$: 483.0418; found 483.0409.

*4.1.5.2. N-(6-(6-chloro-5-(4-methylphenylsulfonamido)pyridin-3-yl)-[1,2,4]triazolo[1,5-a pyridin-2-yl)acetamide (**1b**).* Yield 57.4%, mp 246–248 °C. ^{1}H NMR (DMSO-d_6): δ 10.89 (s, 1H, NH), 10.39 (s, 1H, NH), 9.34 (s, 1H, Ar–H), 8.68 (s, 1H, Ar–H), 8.10 (s, 1H, Ar–H), 7.91 (d, J = 9.2 Hz, 1H, Ar–H), 7.82 (d, J = 9.2 Hz, 1H, Ar–H), 7.65 (d, J = 8.0 Hz, 2H, Ar–H), 7.38 (d, J = 7.6 Hz, 2H, Ar–H), 2.39 (s, 3H, CH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.40, 159.73, 149.32, 145.61, 145.26, 144.15, 137.59, 134.34, 132.20, 131.14, 130.26, 130.26, 129.63, 127.31, 127.25, 127.25, 122.04, 115.37, 24.18,

21.49. ESI–HRMS *m/z*: calc'd for $C_{20}H_{17}ClN_6NaO_3S$ $[M + Na]^+$: 479.0669; found 479.0668.

*4.1.5.3. N-(6-(6-methoxy-5-(4-fluorophenylsulfonamido)pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl)acetamide (**1c**).* Yield 70.4%, mp 239–241 °C. ^{1}H NMR (DMSO-d_6): δ 10.84 (s, 1H, NH), 10.08 (s, 1H, NH), 9.23 (s, 1H, Ar–H), 8.40 (s, 1H, Ar–H), 8.00 (s, 1H, Ar–H), 7.90 (d, J = 9.2 Hz, 1H, Ar–H), 7.81 (m, 3H, Ar–H), 7.41 (m, 2H, Ar–H), 3.64 (s, 3H, OCH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.04, 163.54, 159.45, 157.36, 148.96, 142.33, 137.12, 132.95, 130.30, 130.21, 130.01, 126.21, 126.02, 123.39, 120.80, 116.73, 116.50, 115.18, 53.92, 24.15. ESI–HRMS *m/z*: calc'd for $C_{20}H_{17}FN_6NaO_4S$ $[M + Na]^+$: 479.0914; found 479.0909.

*4.1.5.4. N-(6-(6-methoxy-5-(4-chlorophenylsulfonamido)pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl)acetamide (**1d**).* Yield 55.6%, mp 233–235 °C. ^{1}H NMR (DMSO-d_6): δ 10.84 (s, 1H, NH), 10.15 (s, 1H, NH), 9.23 (s, 1H, Ar–H), 8.41 (s, 1H, Ar–H), 8.00 (s, 1H, Ar–H), 7.90 (d, J = 9.2 Hz, 1H, Ar–H), 7.76 (m, 3H, Ar–H), 7.65 (d, J = 8.0 Hz, 2H, Ar–H), 3.64 (s, 3H, OCH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.01, 159.46, 157.40, 148.97, 142.39, 139.63, 138.17, 133.05, 130.02, 129.59, 129.59, 129.10, 129.10, 126.22, 126.04, 123.38, 120.75, 115.18, 53.89, 24.16. ESI–HRMS *m/z*: calc'd for $C_{20}H_{17}ClN_6NaO_4S$ $[M + Na]^+$: 495.0618; found 495.0614.

*4.1.5.5. N-(6-(6-methoxy-5-(4-methylphenylsulfonamido)pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl)acetamide (**1e**).* Yield 66.8%, mp 249–252 °C. ^{1}H NMR (DMSO-d_6): δ 10.84 (s, 1H, NH), 9.94 (s, 1H, NH), 9.18 (s, 1H, Ar–H), 8.35 (s, 1H, Ar–H), 7.93 (s, 1H, Ar–H), 7.85 (d, J = 9.2 Hz, 1H, Ar–H), 7.77 (d, J = 9.2 Hz, 1H, Ar–H), 7.66 (d, J = 7.6 Hz, 2H, Ar–H), 7.36 (d, J = 8.0 Hz, 2H, Ar–H), 3.67 (s, 3H, OCH_3), 2.36 (s, 3H, CH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.88, 159.45, 157.02, 148.95, 143.73, 141.66, 137.77, 131.57, 129.97, 129.89, 129.89, 127.23, 127.23, 126.12, 125.88, 123.50, 121.35, 115.22, 53.04, 24.16, 21.43. ESI–HRMS *m/z*: calc'd for $C_{21}H_{20}N_6NaO_4S$ $[M + Na]^+$: 475.1164; found 475.1162.

*4.1.5.6. N-(6-(6-methoxy-5-(2,4-difluorophenylsulfonamido)pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl)acetamide (**1f**).* Yield 50.0%, mp 216–218 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.85 (s, 1H, NH), 10.35 (s, 1H, NH), 9.26 (s, 1H, Ar–H), 8.45 (s, 1H, Ar–H), 8.03 (s, 1H, Ar–H), 7.93 (d, J = 9.2 Hz, 1H, Ar–H), 7.76 (m, 2H, Ar–H), 7.59 (m, 1H, Ar–H), 7.20 (m, 1H, Ar–H), 3.64 (s, 3H, OCH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.84, 164.32, 159.46, 158.64, 158.29, 148.96, 143.12, 135.03, 132.30, 129.99, 126.22, 126.06, 123.25, 120.12, 115.17, 112.38, 112.14, 106.24, 53.89, 24.15. ESI–HRMS *m/z*: calc'd for $C_{20}H_{16}F_2N_6NaO_4S$ $[M + Na]^+$: 497.0819; found 497.0821.

*4.1.5.7. N-(6-(6-methoxy-5-(4-cyclopropylcarbonylsulfonamido)pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl)acetamide (**1g**).* Yield 4 7.0%, mp 240–242 °C. ^{1}H NMR (DMSO-d_6): δ 10.83 (s, 1H, NH), 9.43 (s, 1H, NH), 9.28 (s, 1H, Ar–H), 8.42 (s, 1H, Ar–H), 8.00 (s, 1H, Ar–H), 7.94 (d, J = 9.2 Hz, 1H, Ar–H), 7.70 (d, J = 9.2 Hz, 1H, Ar–H), 3.99 (s, 3H, OCH_3), 2.87 (m, 1H, CH), 2.16 (s, 3H, CH_3), 0.95 (m, 4H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.82, 159.41, 157.24, 148.94, 141.31, 131.36, 130.14, 126.22, 125.99, 126.64, 122.04, 115.13, 54.32, 30.97, 34.15, 5.62, 5.62. ESI–HRMS *m/z*: calc'd for $C_{17}H_{18}N_6NaO_4S$ $[M + Na]^+$: 425.1008; found: 425.1009.

*4.1.5.8. N-(6-(6-methoxy-5-(4-fluorophenylsulfonamido)pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl)cyclopropanecarboxamide (**1h**).* Yield 56.7%, mp 257–260 °C. ^{1}H NMR (DMSO-d_6): δ 11.12 (s, 1H, NH), 10.09 (s, 1H, NH), 9.21 (s, 1H, Ar–H), 8.40 (s, 1H, Ar–H), 8.00 (s, 1H, Ar–H), 7.86 (m, 4H, Ar–H), 7.41 (m, 2H, Ar–H), 3.64 (s, 3H, OCH_3), 2.07 (m, 1H, CH), 0.85 (s, 4H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.66, 163.59, 159.31, 157.37, 148.96, 142.34, 137.11, 132.95, 130.30, 130.20, 130.03, 126.20, 126.03, 123.40, 120.80, 116.73, 116.51, 115.18, 53.92, 14.41, 8.30, 8.30. ESI–HRMS *m/z*: calc'd for $C_{22}H_{19}FN_6NaO_4S$ $[M + Na]^+$: 505.1070; found 505.1071.

*4.1.5.9. N-(6-(6-chloro-5-(4-fluorophenylsulfonamido)pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl)cyclopropanecarboxamide (**1i**).* Yield 48.9%, mp 236–238 °C. ^{1}H NMR (DMSO-d_6): δ 11.12 (s, 1H, NH), 10.56 (s, 1H, NH), 9.37 (s, 1H, Ar–H), 8.71 (s, 1H, Ar–H), 8.18 (s, 1H, Ar–H), 7.97 (d, J = 9.2 Hz, 1H, Ar–H), 7.82 (d, J = 8.4 Hz, 3H, Ar–H), 7.43 (m, 2H, Ar–H), 2.06 (m, 1H, CH), 0.85 (d, J = 5.6 Hz, 4H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.69, 163.79, 159.59, 149.32, 145.88, 145.64, 136.25, 135.16, 132.35, 131.07, 130.34, 130.25, 129.99, 127.36, 121.99, 117.16, 116.93, 115.32, 14.44, 8.35, 8.35. ESI–HRMS *m/z*: calc'd for $C_{21}H_{16}ClFN_6NaO_3S$ $[M + Na]^+$: 509.0575; found 509.0574.

*4.1.5.10. N-(5-(2-amino-[1,2,4]triazolo[1,5-a]pyridin-6-yl)-2-methoxypyridin-3-yl)-4-fluorophenylsulfonamide (**1j**).* Yield 49.1%, mp 247–250 °C. ^{1}H NMR (DMSO-d_6): δ 10.06 (s, 1H, NH), 8.89 (s, 1H, Ar–H), 8.34 (s, 1H, Ar–H), 7.92 (s, 1H, Ar–H), 7.81 (s, 2H, Ar–H), 7.68 (d, J = 8.4 Hz, 1H, Ar–H), 7.42 (m, 3H, Ar–H), 6.11 (s, 2H, NH_2), 3.63 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.00, 163.45, 157.09, 150.17, 141.34, 137.44, 132.13, 130.25, 130.16, 128.42, 126.50, 125.01, 121.46, 121.35, 116.66, 116.43, 112.96, 53.82. ESI–HRMS *m/z*: calc'd for $C_{18}H_{15}FN_6NaO_3S$ $[M + Na]^+$: 437.0808; found 437.0805.

*4.1.5.11. N-(5-(2-amino-[1,2,4]triazolo[1,5-a]pyridin-6-yl)-2-methoxypyridin-3-yl)-4-chlorophenylsulfonamide (**1k**).* Yield 57.1%, mp 210–213 °C. ^{1}H NMR (DMSO-d_6): δ 10.13 (s, 1H, NH), 8.84 (s, 1H, Ar–H), 8.89 (s, 1H, Ar–H), 8.35 (s, 1H, Ar–H), 7.75 (d, J = 7.6 Hz, 2H, Ar–H), 7.66 (t, J_1 = 8.4 Hz, J_2 = 8.0 Hz, 3H, Ar–H), 7.45 (d, J = 8.8 Hz, 1H, Ar–H), 6.11 (s, 2H, NH_2), 3.62 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.02, 157.11, 150.20, 142.02, 139.62, 138.17, 132.82, 129.58, 129.58, 129.11, 129.11, 128.42, 126.57, 125.09, 121.19, 120.65, 112.96, 53.83. ESI–HRMS *m/z*: calc'd for $C_{18}H_{15}ClN_6NaO_3S$ $[M + Na]^+$: 453.0513; found 453.0513.

*4.1.5.12. N-(5-(2-amino-[1,2,4]triazolo[1,5-a]pyridin-6-yl)-2-methoxypyridin-3-yl)-4-methylphenylsulfonamide (**1l**).* Yield 49.8%, mp 235–237 °C. ^{1}H NMR (DMSO-d_6): δ 9.92 (s, 1H, NH), 8.84 (s, 1H, Ar–H), 8.29 (s, 1H, Ar–H), 7.86 (s, 1H, Ar–H), 7.64 (s, 3H, Ar–H), 7.44 (d, J = 7.6 Hz, 1H, Ar–H), 7.36 (d, J = 6.0 Hz, 2H, Ar–H), 6.10 (s, 2H, NH_2), 3.65 (s, 3H, OCH_3), 2.36 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.06, 156.72, 150.23, 143.74, 141.32, 137.73, 131.33, 129.90, 129.90, 128.37, 127.24, 127.24, 126.39, 125.50, 121.32, 121.21, 113.00, 53.89, 21.43. ESI–HRMS *m/z*: calc'd for $C_{19}H_{18}N_6NaO_3S$ $[M + Na]^+$: 433.1059; found 433.1058.

*4.1.5.13. 2-(6-5-(4-Fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-[1,2,4]triazol [1,5-a]pyridin-2-ylamino)acetic acid (**1m**).* Yield 89.0%, mp 225–228 °C. ^{1}H NMR (DMSO-d_6): δ 8.99 (s. 1H, Ar–H), 8.33 (d, J = 2.0 Hz, 1H, Ar–H), 7.93 (d, J = 2.4 Hz, 1H, Ar–H), 7.81 (m, 2H, Ar–H), 7.72 (d, J = 9.2 Hz, 1H, Ar–H), 7.51 (d, J = 9.2 Hz, 2H, Ar–H), 7.41 (t, J = 8.8 Hz, 2H, Ar–H), 6.98 (t, J = 6.4 Hz, 1H, NH), 3.93 (d, J = 6.8 Hz, 2H, CH_2), 3.63 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.94, 166.89, 163.53, 157.09, 150.16, 141.80, 137.15, 132.54, 130.30, 130.20, 128.77, 126.44, 125.35, 121.50, 120.87, 116.72, 116.49, 113.26, 53.88, 44.63. ESI–HRMS *m/z*: calc'd for $C_{20}H_{18}FN_6O_5S$ $[M + H]^+$: 473.1043; found 473.1046.

*4.1.5.14. N-(5-(2-(2-methoxyethylamino)-[1,2,4]triazolo[1,5-a]pyridin-6-yl)-2-methoxypyridin-3-yl)-4-fluorophenylsulfonamide (**1n**).* Yield 57.5%, mp 205–207 °C. ^{1}H NMR (DMSO-d_6): δ 10.07 (s, 1H, NH), 8.97 (s. 1H, Ar–H), 8.35 (s, 1H, Ar–H), 7.93 (s, 1H, Ar–H), 7.81

(t, J = 6.2 Hz, 2H, Ar–H), 7.70 (d, J = 9.2 Hz, 1H, Ar–H), 7.48 (d, J = 8.8 Hz, 1H, Ar–H), 7.41 (t, J = 8.4 Hz, 2H, Ar–H), 6.70 (t, J = 5.4 Hz, 1H, NH), 3.63 (s, 3H, OCH_3), 3.50 (t, J = 5.6 Hz, 2H, OCH_2), 3.41 (d, J = 6.0 Hz, CH_2), 3.27 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.03, 163.53, 157.06, 141.86, 137.14, 137.11, 132.58, 130.30, 130.20, 128.62, 126.49, 125.27, 121.24, 120.79, 116.71, 116.49, 113.03, 71.23, 58.42, 53.86, 42.52. ESI–HRMS m/z: calc'd for $C_{21}H_{21}FN_6NaO_4S$ $[M + Na]^+$: 495.1227; found 495.1231.

*4.1.5.15. N-(5-(2-(2-ethoxyethylamino)-[1,2,4]triazolo[1,5-a]pyridin-6-yl)-2-methoxypyridin-3-yl)-4-fluorophenylsulfonamide (**1o**).* Yield 58.6%, mp 209–212 °C. ^{1}H NMR (DMSO-d_6): δ 10.08 (s, 1H, NH), 8.99 (s. 1H, Ar–H), 8.35 (s, 1H, Ar–H), 7.93 (s, 1H, Ar–H), 7.81 (m, 2H, Ar–H), 7.70 (d, J = 9.2 Hz, 1H, Ar–H), 7.48 (d, J = 8.8 Hz, 1H, Ar–H), 7.41 (m, 2H, Ar–H), 6.68 (t, J = 5.8 Hz, 1H, NH), 3.63 (s, 3H, OCH_3), 3.54 (t, J = 6.0 Hz, 2H, OCH_2), 3.47 (dd, J_1 = 14.0 Hz, J_2 = 14.4 Hz, 2H, OCH_2), 3.40 (d, J = 6.0 Hz, CH_2), 1,20 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.03, 163.54, 157.06, 150.23, 141.89, 137.12, 132.61, 130.25, 130.20, 128.63, 126.50, 125.26, 121.22, 120.76, 116.72, 116.49, 113.02, 69.10, 65.92, 53.86, 42.78, 15.62. ESI–HRMS m/z: calc'd for $C_{22}H_{23}FN_6NaO_4S$ $[M + Na]^+$: 509.1383; found 509.1390.

*4.1.5.16. Ethyl 2-(6-(6-methoxy-5-(4-fluorophenylsulfonamido)pyridin-3-yl)-[1,2,4] triazolo[1,5-a]pyridin-2-ylamino)acetate (**1p**).* Yield 66.0%, mp 202–204 °C. ^{1}H NMR (DMSO-d_6): δ 10.07 (s, 1H, NH), 8.99 (s. 1H, Ar–H), 8.35 (d, J = 1.2 Hz, 1H, Ar–H), 7.94 (s, 1H, Ar–H), 7.81 (m, 2H, Ar–H), 7.73 (d, J = 9.2 Hz, 1H, Ar–H), 7.52 (d, J = 8.8 Hz, 2H, Ar–H), 7.41 (m, 2H, Ar–H), 7.13 (t, J = 6.4 Hz, 1H, NH), 4.11 (dd, J_1 = 14.0 Hz, J_2 = 14.0 Hz, 2H, OCH_2), 4.02 (d, J = 6.4 Hz, 2H, CH_2), 3.63 (s, 3H, OCH_3), 1,20 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.21, 163.54, 161.52, 157.44, 149.63, 142.36, 137.12, 132.88, 131.04, 130.29, 130.20, 126.45, 125.69, 124.19, 120.91, 116.71, 116.49, 115.35, 61.66, 53.94, 43.27, 14.47. ESI–HRMS m/z: calc'd for $C_{22}H_{21}FN_6NaO_5S$ $[M + Na]^+$: 523.1176; found 523.1178.

*4.1.5.17. N-(7-(6-methoxy-5-(4-chlorophenylsulfonamido)pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl)acetamide (**2a**).* Yield 72.5%, mp 226–229 °C. ^{1}H NMR (DMSO-d_6): δ 10.85 (s, 1H, NH), 10.21 (s, 1H, NH), 8.91 (d, J = 6.8 Hz, 1H, Ar–H), 8.52 (s, 1H, Ar–H), 8.08 (s, 1H, Ar–H), 7.96 (s, 1H, Ar–H), 7.75 (d, J = 8.0 Hz, 2H, Ar–H), 7.65 (d, J = 8.0 Hz, 2H, Ar–H), 7.42 (d, J = 6.8 Hz, 1H, Ar–H), 3.65 (s, 3H, OCH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.16, 159.62, 157.95, 150.09, 142.79, 139.64, 138.65, 138.17, 132.863, 129.60, 129.60, 129.39, 129.10, 129.10, 127.27, 120.95, 112.37, 111.09, 54.00, 24.16. ESI–HRMS m/z: calc'd for $C_{20}H_{17}ClN_6NaO_4S$ $[M + Na]^+$: 495.0618; found 495.0621.

*4.1.5.18. N-(7-(6-methoxy-5-(4-methylphenylsulfonamido)pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl)acetamide (**2b**).* Yield 74.3%, mp 216–218 °C. ^{1}H NMR (DMSO-d_6): δ 10.85 (s, 1H, NH), 10.01 (s, 1H, NH), 8.91 (d, J = 6.4 Hz, 1H, Ar–H), 8.46 (s, 1H, Ar–H), 7.99 (s, 1H, Ar–H), 7.90 (s, 1H, Ar–H), 7.65 (d, J = 7.6 Hz, 3H, Ar–H), 7.36 (d, J = 6.8 Hz, 3H, Ar–H), 3.68 (s, 3H, OCH_3), 2.36 (s, 3H, CH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.15, 159.62, 157.56, 150.09, 143.78, 142.15, 138.77, 137.71, 131.36, 129.93, 129.93, 129.43, 127.23, 127.23, 127.10, 121.043, 112.34, 110.99, 54.07, 24.16, 21.44. ESI–HRMS m/z: calc'd for $C_{21}H_{20}N_6NaO_4S$ $[M + Na]^+$: 475.1164; found 475.1168.

*4.1.5.19. N-(7-(6-methoxy-5-(4-fluorophenylsulfonamido)pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl) acetamide (**2c**).* Yield 85.0%, mp 233–235 °C. ^{1}H NMR (DMSO-d_6): δ 10.85 (s, 1H, NH), 10.14 (s, 1H, NH), 8.91 (d, J = 4.4 Hz, 1H, Ar–H), 8.51 (s, 1H, Ar–H), 8.05 (s, 1H, Ar–H), 7.95 (s, 1H, Ar–H), 7.81 (s, 2H, Ar–H), 7.41 (s, 3H, Ar–H), 3.65 (s, 3H, OCH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.05, 163.55, 159.60, 157.92, 150.08, 142.81, 138.65, 137.08, 132.81, 130.30, 130.21, 129.39, 127.25, 120.90, 116.76, 116.53, 112.36, 111.08, 54.04, 24.16. ESI–HRMS m/z: calc'd for $C_{20}H_{17}FN_6NaO_4S$ $[M + Na]^+$: 479.0914; found 479.0912.

*4.1.5.20. N-(5-(2-amino-[1,2,4]triazolo[1,5-a]pyridin-7-yl)-2-methoxypyridin-3-yl)-4-fluorophenylsulfonamide (**2d**).* Yield 65.5%, mp 232–235 °C. ^{1}H NMR (DMSO-d_6): δ 10.12 (s, 1H, NH), 8.61 (d, J = 6.8 Hz, 1H, Ar–H), 8.45 (d, J = 2.4 Hz, 1H, Ar–H), 7.97 (d, J = 2.4 Hz, 1H, Ar–H), 7.81 (m, 2H, Ar–H), 7.60 (d, J = 1.2 Hz, 1H, Ar–H), 7.42 (m, 2H, Ar–H), 7.14 (d, J = 6.8 Hz, 1H, Ar–H), 3.65 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.16, 163.56, 157.64, 151.25, 142.42, 137.22, 137.06, 132.46, 130.30, 130.20, 128.15, 127.72, 120.84, 116.77, 116.55, 110.04, 108.99, 55.40. ESI–HRMS m/z: calc'd for $C_{18}H_{15}FN_6NaO_3S$ $[M + Na]^+$: 437.0808; found 437.0810 $[M + Na]^+$.

*4.1.6. General procedures for the synthesis of compounds **1q–1r***

The solution of methylamine or ammonia in methanol (2 mmol) was added to the solution of compound **1p** (0.05 g, 0.1 mmol) in methanol (10 ml). The mixture was stirred at room temperature for 48 h. Then the solvent was evaporated under vacuum. Water (10 ml) was added to the residue, and the suspension was filtered, washed by water and dried to get a white solid.

*4.1.6.1. 2-(6-5-(4-Fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-ylamino)acetamide (**1q**).* Yield 78.5%, mp 249–251 °C. ^{1}H NMR (DMSO-d_6): δ 9.92 (s, 1H, NH), 8.84 (s, 1H, Ar–H), 8.29 (s, 1H, Ar–H), 7.86 (s, 1H, Ar–H), 7.64 (s, 3H, Ar–H), 7.44 (d, J = 7.6 Hz, 1H, Ar–H), 7.36 (d, J = 6.0 Hz, 2H, Ar–H), 6.10 (s, 2H, NH_2), 3.65 (s, 3H, OCH_3), 2.36 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.13, 166.05, 163.56, 157.64, 151.23, 142.43, 137.24, 137.03, 132.46, 130.30, 130.20, 128.16, 127.71, 120.83, 116.78, 116.55, 110.07, 108.98, 54.00, 53.94. ESI–HRMS m/z: calc'd for $C_{20}H_{18}FN_7NaO_4S$ $[M + Na]^+$: 494.1023; found 494.1030.

*4.1.6.2. 2-(6-5-(4-Fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-ylamino)-N-methylacetamide (**1r**).* Yield 68.4%, mp 229–231 °C. ^{1}H NMR (DMSO-d_6): δ 8.99 (s. 1H, Ar–H), 8.33 (s, 1H, Ar–H), 7.92 (s, 1H, Ar–H), 7.81 (s, 2H, Ar–H), 7.72 (d, J = 8.4 Hz, 1H, Ar–H), 7.51 (d, J = 8.8 Hz, 1H, Ar–H), 7.41 (m, 2H, Ar–H), 6.92 (s, 1H, NH), 3.82 (d, J = 3.6 Hz, 2H, CH_2), 3.63 (s, 3H, OCH_3), 3.63 (s, 3H, OCH_3), 2.59 (d, J = 2.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.80, 166.88, 163.52, 157.18, 150.18, 141.87, 137.14, 132.59, 130.30, 130.20, 128.78, 126.42, 125.35, 121.47, 120.85, 116.72, 116.49, 113.27, 53.87, 46.44, 26.01. ESI–HRMS m/z: calc'd for $C_{21}H_{20}FN_7NaO_4S$ $[M + Na]^+$: 508.1179; found 508.1184.

4.2. Biological assay methods

4.2.1. Cell culture

The human cell lines HCT-116, U-87 MG or MCF-7 were maintained as a monolayer culture in DMEM, supplemented with 10% FBS in a humidified atmosphere (5% CO_2) at 37 °C.

4.2.2. Antiproliferative assays

Cellular chemosensitivity was determined by using a modified MTT method assay *in vitro*. In brief, HCT-116, U-87 MG or MCF-7 cells in 200 μl culture medium were seeded into 96-well microplates at 3000–5000 cells per well respectively and cultured in DMEM 10% FBS, 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 48 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 48 h and then 20 μl of MTT solution (5 mg/ml in medium) was

added to each well and incubated for 4 h. The formed blue formazan crystals were pelleted to the bottom of the well by centrifugation, separated from the supernatant, and dissolved in 200 μl of DMSO. The optical density at 490 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.

4.2.3. Western blot assay

The suppressive activity of AKT and p-AKT^{473} in HCT-116 cells was determined by western blot. HCT-116 cells were seeded into six-well plates at 1×10^6 cells per well respectively and cultured in DMEM 10% FBS, incubated at 37 °C for 16 h prior to drug exposure. Cells were treated with final concentrations of 10 μM of compounds **1c**, **2d**, **A** and DMSO and incubated at 37 °C for 1 h. Cells were washed twice with ice-cold PBS and scraped into ice-cold cell lysis buffer. Then the cell lysates were clarified by centrifugation at 12,000 rpm for 20 min at 4 °C and the supernatant was collected. Protein content was measured by BCA protein Assay Kit and the proteins were separated on SDS–PAGE and then transferred onto nitrocellulose membranes. The membranes were incubated with antibodies against AKT (Cell Signaling Technology), p-Ser473 AKT (Cell Signaling Technology) and β-Acin (Santa Cruz), washed by TBST and then incubated with Mouse and rabbit horseradish peroxidase-conjugated secondary antibodies. The protein–antibody complexes were detected by chemiluminescence with a GeneGnome5 system (Syngene, UK). Protein bands were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

4.2.4. Anticancer effects in sarcoma S-180 model in vivo

Mice (20.1 ± 2.0 g) were purchased from Experiment Animal Center of Xi'an Jiaotong University College of Medicine and fed in the same place. The experimental protocol was approved by Ethic Committee of Xi'an Jiaotong University.

S-180 cells at 3×10^6 were injected subcutaneously into the flank of mice. All tumor-bearing mice were randomly divided into three groups, with 6 mice in each group. In the solvent group, the same volume of solvent was administered orally. Compound **1c** was dissolved in DMSO/PEG400/5% glucose (volume ratio, 10:60:30) and dosed orally at 1 mg/kg and 5 mg/kg for the low and high dosage groups once a day for 10 days, respectively. Body weights were recorded per day. The mice were anesthetized and sacrificed on Day 11. The weights of the body and the neoplasm were measured and inhibitory ratios for tumor weight was calculated.

4.3. 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 **1a** bound to PI3Kγ and **1c** 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 compounds **1a** and **1c** were placed. After end of molecular docking, ten docking poses was scored and selected based on calculated C-DOCKER energy.

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