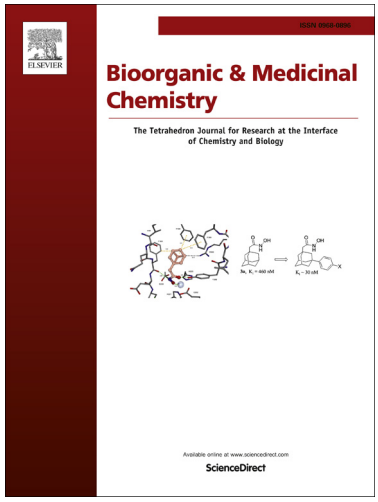

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Combination of 2-methoxy-3-phenylsulfonylaminobenzamide and 2-aminobenzothiazole to discover novel anticancer agents

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Abstract

The fragment of 2-substituted-3-sulfonylaminobenzamide has been proposed to replace the fragment of 2-substituted-3-sulfonylaminopyridine in PI3K and mTOR dual inhibitors to design novel anticancer agents based on bioisostere. The combination of the fragment of 2-substituted-3-sulfonylaminobenzamide with the fragment of 2-aminobenzothiazole or 2-aminothiazolo[5,4-*b*]pyridine, or 2-amino[1,2,4]triazolo[1,5-*a*]pyridine produced the novel structures of anticancer agents. As a result, nineteen target compounds were synthesized and characterized. Their antiproliferative activities *in vitro* were evaluated via MTT assay against four human cancer cell lines including HCT-116, A549, MCF-7 and U-87 MG. The SAR of target compounds was preliminarily discussed. Compound **1g** with potent antiproliferative activity was examined for its effect on the AKT and p-AKT^{473}. The

anticancer effect of **1g** was evaluated in established nude mice HCT-116 xenograft model. The results suggested that compound **1g** can block PI3K/AKT/mTOR pathway and significantly inhibit tumor growth. These findings strongly support our assumption that the fragment of benzamide can replace the pyridine ring in some PI3K and mTOR dual inhibitor to design novel anticancer agents.

Key Words benzamide ∗ benzothiazole ∗ synthesis ∗ anticancer effect∗ bioisostere

1. Introduction

The chemical and biological study of heterocyclic compounds has been an interesting field for a long time in medicinal chemistry. Benzothiazole consists of thiazole ring fused with benzene ring and its derivatives exhibit a broad spectrum of biological activities such as antitumor, antimicrobial, anti-inflammatory, anticonvulsant and antidiabetic activity. Khokra *et al*. reviewed the progress on the synthesis and biological activities of benzothiazole derivatives in 2011.[1] Recently, Shi *et al.* reported that pyridinyl-2-amine linked benzothiazole-2-thiol compounds exhibited potent and broad-spectrum inhibitory activities against human tumor cell lines.[2] Caputo *et al.* reported benzothiazole derivatives linked arylurea moiety at C-2 also showed significant growth inhibitory activities against several human tumor cell lines.[3] Hong *et al.* discovered benzothiazole derivatives linked methylurea moiety at C-2 were effective inhibitors against the wild-type and T315I mutant of Bcr-Abl kinase.[4] Noolvi *et al.* synthesized a series of 2-arylaminobenzothiazole and evaluated their antitumor effects in vitro.[5] Our group reported that 1-(2-acetylamidobenzothiazol -6-yl)-3-(4-(1-methylpiperazine-4-carbinyl)phenyl)urea displayed potent cytotoxic activity against A549.[6] There are two hydrogen-bond acceptors and one hydrogen-bond donor in the 2-acetylaminobenzothiazole. Thus it is a desired fragment in drug design.

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.[7] 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.[8] In many kinds of cancers, the PI3K/AKT/mTOR signal transduction pathway is dysregulated contributing to cellular transformation and tumor growth.[9] Therefore, PI3K and mTOR have become the targets of intense research for anticancer drug discovery.[10, 11] In recent years, a significant progress has been made on the design, synthesis and evaluation of PI3K and mTOR dual inhibitors, and thereupon the pharmacophore of the dual inhibitors has been proposed.[12] 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 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.[13] Amgen Inc. designed, synthesized and evaluated several classes of *N*-(2, 5-disubstitutedpyridin-3-yl)phenylsulfonamides. Thus they discovered that *N*-(2-chloro-5-(4-morpholinoquinilin-6-yl)pyridin-3-yl)-4-fluorophenylsulfonamide,[14] *N*-(2-chloro-5-(2-acetylaminobenzo[*d*]thiazol-6-yl)pyridin-3-yl)-4-fluorophenyl sulfonamide (compound **A**, Figure 1),[15] *N*-(2-chloro-5-(2-acetylaminoimidazo [1,2-b]pyridazin-6-yl)pyridin-3-yl)-4-fluorophenyl sulfonamide[16] and AMG 511[17] are excellent PI3Kα/mTOR dual inhibitors or PI3Kα selective inhibitor, and orally bioavailable anticancer agents as well. The analogue of compound **A**, N-(6-(2-methoxy-3-(4-fluorophenylsulfonlyamino)pyridin-5-yl)[1,2,4]triazolo[1,5-a] pyridin-2-yl)acetamide, also possesses the same pharmacophore as compound A and displayed anticancer effect.[18] Recently, QSAR and pharmacophore of analogues of compound **A** were studied.[19] BEZ235[20] and PF-04979064[21] possess the similar pharmacophore with GSK2126458. As PI3Kα and mTOR dual inhibitors, they are in

phase I/II clinical trials for treating solid tumors.

[Figure 1.]

Compound **A** exhibited very high antitumor effect *in vivo*. Its EC_{50} ranges from 0.26 mg/kg to 0.53 mg/kg against three established nude mice human cancer cell xenograft models. The interaction of compound **A** with PI3K was clarified by the co-crystal of compound **A** with PI3Kγ protein.[15] According to the co-crystal structure, the N-acetylaminobenzothiazole moiety can form two hydrogen bonds with the NH and the carbonyl group of backbone Val882. A water molecule bridge is formed among the pyridine nitrogen, the carboxyl in Asp841 and the hydroxyl in Tyr867. The interaction is outlined in Figure 2. Based on bioisostere, we propose that the water molecule located among the pyridine ring, the Tyr867 and Asp841 residues can be replaced with a small group linked to the pyridine ring. This reasoning was based on the realization that (a) displacing a 'trapped' water molecule may sometimes have an entropic advantage;[22] (b) replacing a water molecule may enable additional favorable direct interactions between ligand and protein. The amide group is the most favorable because the amide would project its oxygen and hydrogen to the positions similar to that of water. Thus, the O-H···O hydrogen bond between water and Asp841 and the O···H-O hydrogen bond between water and Tyr867 can be replaced by the N-H···O and O···H-O hydrogen bonds formed between the benzamide and the Asp841 and the Tyr867 residues directly. In other words, 2-methoxy-3-phenylsulfonylamino-5-(acetylaminobenzo[*d*]thiazol-6-yl) benzamide might interact with PI3K without the aid of water molecule. In this novel scaffold, 2-methoxy-3-phenylsulfonylamino-5-(acetylaminobenzo[*d*]thiazol-6-yl) benzamide shares the same pharmacophore (labeled as red in Figure 2) with compound **A**. In this way, benzamide fragment can replace pyridine ring fragment in compound A to design novel structures of PI3K inhibitors and anticancer agents.

[Figure 2.]

In our previous work, we reported the design, synthesis and evaluation of

2-methoxy-3-phenylsulfonylamino-5-(quinazolin-6-yl or quinolin-6-yl)benzamide as novel PI3K inhibitors and anticancer agents.[23] In this work, we combined the fragment of benzamide with benzothiazole or thiazolo[5,4-*b*]pyridine, or [1,2,4]triazolo[1,5-*a*]pyridine into one molecule to design novel anticancer agents (Figure 3, compounds **1**, **2** and **3**). Herein, we report our studies on the design and synthesis of compounds **1**, **2** and **3** and demonstrate the biological activities of these compounds.

[Figure 3.]

2. Results and discussion

2.1. Chemistry

The synthetic route of compounds **1** is outlined in Scheme 1.

[Scheme 1.]

In the presence of TEA or DMAP, commercially available compound **4a** or **4b** was acylated with formic acetic anhydride, acetic anhydride, or cyclopropylcarbonyl chloride respectively, to produce intermediates **5**. The reaction of 2-formylamino-6-bromobenzothiazole (**5a**) with ethyl bromoacetate afforded compound **6** in the presence of sodium hydride. Formyl group in intermediate **5a** was removed in work-up. Conversion of **4** to **7** was completed via a two-step process of diazo-reaction and bromo-substitution. Without further purification, compound **7** was converted to **8** via nucleophilic substitution of **7a** or **7b** with cyclopropylamine. The intermediates **10**, sulfonamide, were prepared from 5-bromobenzoic acid derivatives according to the synthetic route reported in our previous work.[23] Catalyzed by $PdCl_2$(dppf), intermediate **10** was reacted with bis(pinacolato)diboron to produce corresponding arylboronic esters. Without isolation of arylboronic esters, intermediate **5**, or **6,** or **8**, $PdCl_2$(dppf), water and potassium carbonate as well were added to the above reaction mixture. The resulted mixture was refluxed to produce the title compounds **1a**-**1j**. The preparation of arylboronic esters and Suzuki coupling were completed in one pot.

To expand the structural diversity of title compounds, 6-position or 7-position of

2-substitutedamino-[1,2,4]triazolo[1,5-*a*]pyridine was combined with intermediate **10** to produce compounds **2** or **3**. The synthetic routes are shown in Scheme 2. The acylation of commercially available 6-bromo-[1,2,4]triazolo[1,5-a]pyridin-2-amine (**11**) or 7-bromo-[1,2,4]triazolo[1,5-a]pyridin-2-amine (**13**) gave intermediates **12** or **14**. Compounds **2** and **3** were synthesized in a similar approach as compounds **1**.

[Scheme 2]

2.2. Antiproliferative assays *in vitro*

We first evaluated the antiproliferative activities of synthesized compounds against human colon carcinoma cell line (HCT-116) by applying the MTT colorimetric assay. The PI3K and mTOR dual inhibitors BEZ235 and compound **A** were used as the positive controls. For the active compounds, we next evaluated their antiproliferative activities against human breast adenocarcinoma carcinoma cell line (MCF-7), lung adenocarcinoma epithelial cell line (A549) and glioma cell line (U-87 MG). The results are summarized in Table 1.

[Table 1]

As expected, some compounds significantly exhibited antiproliferative activities against HCT-116. Notably, compounds **1g** (IC_{50} = 1.95 μM against HCT-116) and **1h** (IC_{50} = 4.25 μM against HCT-116) showed approximate 10 times more potencies than compounds **2b** (IC_{50} = 27.33 μM against HCT-116) and **3b** (IC_{50} = 32.63 μM against HCT-116). Similarly, compounds **1c** (IC_{50} = 2.01 μM against HCT-116) and **1i** (IC_{50} = 2.50 μM against HCT-116) were more active than compounds **2f** (IC_{50} = 43.5 μM against HCT-116) and **3c** (IC_{50} > 50 μM against HCT-116). These data indicated that the benzothiazole fragment was a little better than thiazolo[5,4-*b*]pyridine fragment and much better than [1,2,4]triazolo[1,5-*a*]pyridine fragment in the structures of target compounds on antiproliferative activity. To further determine whether benzothiazole ring was a better selection than thiazolo[5,4-*b*]pyridine ring, we examined the antiproliferative activities of compounds **1c**, **1g, 1i** and **1h** against the other three cancer lines MCF-7, A549 and U-87 MG. The results (shown in Table 1) revealed that the benzothiazole was optimal fragment in these series. The results were consistent

with the activity of similar kinase inhibitors.[4] As for R^1 substituted group, the 4-fluorophenyl was an optimal fragment for this series (compared **1c** with **1d**) and our reported compounds.[22] The fact that compound **1c** (IC_{50} =2.87 μM against U-87 MG) was more potent than compounds **1b** (IC_{50} = 17.93 μM against U-87 MG) and **1a** (IC_{50}> 50 μM against U-87 MG) indicated that the compound with a methoxy group at 2-position (R^2) of benzene ring was important to improve the antiproliferative activity. From the docking study we found that the oxygen atom in the methoxy group appeared to establish an additional hydrogen bond with the Lys833 backbone. Thus an additional hydrogen bond would contribute to strengthening inhibitory activity. We further attempted to explore the SAR of the side chain linked to the 2-position (R^3) of benzothiazole by replacing the acetyl group in **1g** at position of R^3 with different groups. Replacement of the acetyl group with cyclopropylcarbonyl group (**1g** and **1c**) displayed a negative effect on potency. Replacement of the acetyl with the cyclopropyl or ethoxycarbonylmethyl (**1e** and **1f**) resulted in a complete loss of activity, which reveals the indispensable nature of the acetyl group at this position for maintaining activity in this series. From above mentioned results we could reveal a SAR that the benzamide fragment combined with benzothiazole fragment with aryl at R^1, methoxy at R^2 and acetyl at R^3 were favorable for antiproliferative activity. In addition, the IC_{50} of compound **1g** against MCF-7 was comparable to that of positive drugs compound **A** and BEZ235. Therefore, compound **1g** was further investigated.

2.3. Western blot assay

To determine whether these compounds inhibit the PI3K, we evaluated the suppressive effects of compound **1g** at 0.1, 1.0 and 10.0 μM and positive BEZ235 at 1.0 μM on AKT and p-AKT^{473} in HCT-116 cells through Western blot. As shown in Figure 4, the suppressive effects of compound **1g** on p-AKT^{473} were almost equal to BEZ235 at 1.0 μM. The results suggest compound **1g** can block the PI3K/AKT/mTOR pathway and thus **1g** is a potential PI3K inhibitor.

[Figure 4.]

2.4. Anticancer effects in established nude mouse HCT-116 xenograft model *in vivo*

Compound **1g**, displayed evident activities against the four carcinoma cell lines, was chosen to evaluate its anticancer effect in the nude mouse HCT-116 colon adenocarcinoma xenograft model. BEZ235 (30 mg/kg) was used as positive drug. **1g** was dosed orally at 10 mg/kg or 30 mg/kg once a day for 14 days. The control group was administrated orally the solvent only.

The change in the tumor volumes was observed in Figure 5 (A). In this model, treatment groups with compound **1g** at 10 mg/kg or 30 mg/kg significantly inhibit tumor growth by 40.06% and 46.87%, compared to the control group, respectively. BEZ235 group displayed a delay in tumor growth by 24.22% with 30 mg/kg. The low suppression ratio in positive group may be related to the poor solubility of BEZ235. These results suggest that **1g** display significant inhibitory effect on the tumor growth.

[Figure 5.]

During the experimental period, the weight loss of tested animals was observed in BEZ235 group, indicating that BEZ235 displays some toxicity at the dosage administration. But compound **1g** was well tolerated at the dose of 10 mg/kg and 30 mg/kg in terms of its effect on body weight (Figure 5, B). This is due to the low toxicity of compound **1g**.

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 compound **1g** with PI3K protein. Docking simulations were performed on human PI3Kγ (PDB code 3QKO). [15]

[Figure 6.]

The docking result of compound **1g** with PI3Kγ (Figure 6) has the following three indications: (a) 2-acetylaminobenzothiazole fragment can form two hydrogen bonds with Val882. (b) The oxygen atom of benzamide can form two hydrogen bonds, one with Tyr867 and the other with Asp964. The hydrogen atom of benzamide can form a

hydrogen bond with Asp841. These interactions revealed that benzamide can replace the pyridine ring in the structure of compound **A**. (c) The oxygen atoms of sulfamide and the methoxy group at 2-position of benzamide can form two hydrogen bonds with Lys833. These interactions suggested that compound **1g** displays rational interaction with PI3Kγ, and the interaction mode of compound **1g** is similar with that of compound **A**. As for compound **1b** or **1a**, the chloride or hydrogen atom at 2-position of benzamide cannot form hydrogen bond with Lys833, which suggests that the methoxy group is a more suitable substitute group than chloride and hydrogen in the target compound **1**.

3. Conclusion

In the present study, we proposed that the combination of 2-substituted-3-sulfonylaminobenzamide with benzothiazole, or thiazolo[5,4-*b*]pyridine, or [1,2,4]triazolo[1,5-*a*]pyridine could produce the novel structures of anticancer agents. Nineteen compounds were synthesized and characterized. The results of antiproliferative activities indicated that compound **1g** significantly exhibits inhibitory activity against the four cancer cell lines. The SAR of these compounds indicated that 2-methoxy-3-(4-fluorophenylsulfonylamino) benzamide and 2-acetylamino-5-benzothiazol-6-yl are optimal fragments. The Western blot assay results of compound **1g** suggested that these compounds can block the PI3K/AKT/mTOR pathway. Compound **1g** significantly displayed inhibitory effect on tumor growth in established nude mice HCT-116 xenograft model. These findings strongly support our assumption that the fragment of benzamide can replace the pyridine ring in compound **A** to design novel anticancer agents.

4. Experimental

4.1. Chemistry

Unless specified otherwise, all starting materials, reagents and solvents were commercially available. All reactions were monitored by thin-layer chromatography on silica gel plates (GF-254) and visualized with UV light. All the melting points

were determined on a Beijing micro melting-point apparatus and thermometer was uncorrected. ^{1}H-NMR spectra were recorded on a 400 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. *N*-(6-bromobenzo[d]thiazol-2-yl)formamide (5a)

The mixture of acetic anhydride (7.0 g, 69 mmol) and formic acid (85%, 2.5 g, 54 mmol) was heated at 60 °C for 2 h, cooled. 6-Bromobenzothiazole-2-amine (2.0 g, 8.7 mmol) was added portion wise to the resulting anhydride while keeping the temperature below 40 °C. Then ether (15 ml) was added and the mixture was stirred at room temperature overnight. The precipitated crystals was collected by filtration and washed with ether to give **5a** (1.7 g, 77%) as white solid. Mp: 235.0-236.0 °C; ^{1}H NMR (DMSO-d_6): δ 12.60 (s, 1H, NH), 8.61 (s, 1H, COH), 8.28 (s, 1H, Ar-H), 7.71 (d, J = 8.4 Hz, 1H, Ar-H), 7.59 (d, J = 8.4 Hz, 1H, Ar-H). ESI-HRMS m/z: calcd for $C_8H_5BrN_2NaOS$ $[M+Na]^+$: 278.9204; found 278.9203.

4.1.2. *N*-(6-bromobenzo[d]thiazol-2-yl)acetamide (5b)

6-Bromobenzo[d]thiazol-2-amine (2.3 g, 10.0 mmol) was suspended in dichloromethane (30 ml), and then 4-dimethylaminopyridine (1.3 g, 11.5 mmol) was added. The mixture was cooled in an ice-water bath under nitrogen, and acetic anhydride (1.0 ml, 11.5 mmol) was added. The resulting mixture was allowed to slowly warm to room temperature while being stirred overnight under nitrogen. Then hydrochloric acid (10%, 10 ml) was added and **5b** (2.3 g, 85%) was obtained by filtration as white solid. Mp: 217.0-218.0 °C; ^{1}H NMR (DMSO-d_6): δ 12.46 (s, 1H, NH), 8.24 (s, 1H, Ar-H), 7.67 (d, J = 7.2 Hz, 1H, Ar-H), 7.57 (d, J = 6.8 Hz, 1H, Ar-H), 2.21 (s, 3H, CH_3). ESI-HRMS m/z: calcd for $C_9H_7BrN_2NaOS$ $[M+Na]^+$: 292.9360; found 292.9360.

4.1.3. *N*-(6-bromobenzo[d]thiazol-2-yl)cyclopropanecarboxamide (5c)

6-Bromobenzothiazol-2-amine (0.6 g, 2.6 mmol) was suspended in dichloromethane (15 ml), and then triethylamine (6 ml) was added. The mixture was cooled in an ice-water bath, and cyclopropanecarbonyl chloride (0.29 ml, 3.2 mmol) was added dropwise. The mixture was allowed to slowly warm to room temperature, and stirred at room temperature for 1 h. Then the mixture was heated to reflux for 5 h under nitrogen, cooled to room temperature and the solvent was evaporated off in vacuo. The residue was extracted with dichloromethane (100 ml × 1, 50 ml × 2), and then the organic layer was collected, washed with 2N HCl and saturated salt water, then dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated off to give **5c** (0.5 g, 64%) as white solid. Mp: 235.0-236.0 °C; ^{1}H NMR (DMSO-d_6): δ 12.75 (s, 1H, NH), 8.24 (d, J = 2.0 Hz, 1H, Ar-H), 7.67(d, J = 8.4 Hz, 1H, Ar-H), 7.57 (dd, J = 2.0, 8.4 Hz, 1H, Ar-H), 2.00 (m, 1H, CH), 0.96 (m, 4H, 2CH_2). ESI-HRMS m/z: calcd for $C_{11}H_9BrN_2NaOS$ $[M+Na]^+$: 320.1607; found 320.1608.

4.1.4. *N*-(6-bromothiazolo[5,4-b]pyridin-2-yl)acetamide (5d)

In a similar manner as for **5b**, started with **4b** (0.25 g, 1.09 mmol) to produce **5d** (0.23 g, 80%). Mp: 240.0-242.0 °C; ^{1}H NMR (DMSO-d_6): δ 12.62 (s, 1H, NH), 8.16 (d, J = 8.8 Hz, 1H, Ar-H), 7.57 (d, J = 8.8 Hz, 1H, Ar-H), 2.24 (s, 3H, CH_3). ESI-HRMS m/z: calcd for $C_8H_6BrN_3NaOS$ $[M+Na]^+$: 293.9313; found 293.9315.

4.1.5. *N*-(5-bromothiazolo[5,4-b]pyridin-2-yl)cyclopropanecarboxamide (5e)

In a similar manner as for **5c**, started with **4b** (0.30 g, 1.62 mmol) to produce **5e** (0.35 g, 72%). Mp: >270 °C; ^{1}H NMR (DMSO-d_6): δ 12.94 (s, 1H, NH), 8.16 (d, J = 8.8 Hz, 1H, Ar-H), 7.57 (d, J = 8.8 Hz, 1H, Ar-H), 2.03 (m, 1H, CH), 0.99 (m, 4H, CH_2). ESI-HRMS m/z: calcd for $C_{10}H_8BrN_3OS$ $[M+H]^+$: 297.9650; found 297.9659.

4.1.6. Ethyl 2-(6-bromobenzo[d]thiazol-2-ylamino)acetate (6)

To the suspension of NaH (60%, 0.16 g) in DMF (20 ml) was added **5a** (1.7 g，6.6 mmol) in small portions. After stirring for 30 min, the solution of ethyl bromoacetate

(0.81 ml, 7.3 mmol) in DMF (4 ml) was added and the resulting mixture was stirred at room temperature for 4 h, poured into 50 ml of water. Compound **6** (1.5 g, 74%) was obtained by filtration as white solid. Mp: 210.0-211.0 °C; ^{1}H NMR (DMSO-d_6): δ 8.86 (s, 1H, NH), 8.33 (s, 1H, Ar-H), 7.73 (d, J = 8.8 Hz, 1H, Ar-H), 7.60 (d, J = 8.8 Hz, 1H, Ar-H), 4.98 (s, 2H, CH_2), 4.18 (q, J = 7.2 Hz, 2H, OCH_2), 1.20 (t, J = 7.2 Hz, 3H, CH_3). ESI-HRMS m/z: calcd for $C_{11}H_{12}BrN_2O_2S$ $[M+H]^+$: 314.9803; found 314.9804.

4.1.7. 6-Bromo-*N*-cyclopropylbenzo[d]thiazol-2-amine (8a)

Compound **4a** (0.8 g, 3.5 mmol) was mixed under intensive stirring with 85% phosphoric acid (8 ml) at 50 °C. The solution was cooled to -20 °C and a solution of sodium nitrite (0.29 g, 4.2 mmol) in water (2 ml) was added slowly to keep the temperature below -10 °C. After stirring for 1 h, the resulting diazonium salt was poured into a solution of active copper (I) bromide (0.65 g, 4.5 mmol) in 48% HBr (5 ml). The mixture was intensively stirred for 1 h at room temperature, at 40 °C for 2 h and then at room temperature overnight, poured into ice-water (100 ml) then extracted with ethyl acetate (100 ml × 1, 50 ml × 2). The organic layer was collected and washed with saturated Na_2CO_3, saturated salt water, then dried over anhydrous Na_2SO_4 and filtered. Then ethyl acetate was evaporated off to give **7a** as brown crude product which was used for the next step directly.

Compound **7a** (1.0 g, 3.4 mmol) was suspended in 1,4-dioxane (10 ml), and cyclopropylamine (3.6 ml, 51.0 mmol) was added. The resulting mixture was heated to reflux under nitrogen for 6 h, cooled to room temperature. The solvent was evaporated off to give crude product which was purified through column chromatograph to give **8a** (0.44 g, 48%) as off-white solid. Mp: 183.0-184.0 °C; ^{1}H NMR (DMSO-d_6): δ 8.49 (s, 1H, NH), 7.97 (d, J = 2.0 Hz, 1H, Ar-H), 7.37 (d, J = 8.8 Hz, 1H, Ar-H), 7.33 (m, 1H, Ar-H), 2.68 (m, 1H, CH), 0.77 (m, 2H, CH_2), 0.57 (m, 2H, CH_2). ESI-HRMS m/z: calcd for $C_{10}H_{10}BrN_2S$ $[M+H]^+$: 268.9748; found 268.9749.

4.1.8. 5-Bromo-*N*-cyclopropylthiazolo[5,4-b]pyridin-2-amine (8b)

In a similar manner as for compound **8a**, started with compound **4b** to produce compound **7b** as crude product which was used for the next step directly, then following the procedure to prepare **8a** and using compound **7b** (0.5 g, 2.0 mmol) to produce **8b** (0.35 g, 77%). Mp: 163.0-165.0 °C; ^{1}H NMR (DMSO-d_6): δ 8.78 (s, 1H, NH), 8.16 (d, J = 8.8 Hz, 1H, Ar-H), 7.57 (d, J = 8.8 Hz, 1H, Ar-H), 2.78 (m, 1H, CH), 0.82 (m, 2H, CH_2), 0.62 (m, 2H, CH_2). ESI-HRMS m/z: calcd for $C_9H_9BrN_3S$ $[M+H]^+$: 269.9701; found 269.9702.

4.1.9. General procedure for the synthesis of 10a-10f

The compounds were prepared according to previously described methods. [23]

4.1.9.1. 3-Bromo-5-(4-fluorophenylsulfonylamino)benzamide (10a)

White solid; yield: 83%; Mp: 238.0-240.0 °C; ^{1}H NMR (DMSO-d_6): δ 10.77 (1H, s, SO_2NH), 8.06 (1H, s, Ar-H), 7.84 (2H, dd, J = 8.8, 5.2 Hz, Ar-H), 7.74 (1H, s, $CONH_2$), 7.56 (1H, s, $CONH_2$), 7.60 (1H, s, Ar-H), 7.44 (2H, t, J = 8.8 Hz, Ar-H), 7.38 (1H, s, Ar-H). ESI-HRMS *m/z*: calc'd for $C_{13}H_{11}BrFN_2O_3S$ $[M+H]^+$: 372.9658; found 372.9655.

4.1.9.2. 5-Bromo-2-chloro-3-(4-fluorophenylsulfonylamino)benzamide (10b)

White solid; yield: 75%; Mp: 228.0-229.0 °C; ^{1}H NMR (DMSO-d_6): δ 10.47 (1H, s, SO_2NH), 7.96 (1H, s, $CONH_2$), 7.82 (2H, dd, J = 5.2, 8.8 Hz, Ar-H), 7.45 (2H, t, J = 8.8 Hz, Ar-H), 7.70 (1H, s, $CONH_2$), 7.47 (1H, s, Ar-H), 7.41 (1H, d, J = 2.0 Hz, Ar-H). ESI-HRMS *m/z*: calc'd for $C_{13}H_{10}BrClFN_2O_3S$ $[M+H]^+$: 406.9268; found 406.9264.

4.1.9.3. 5-Bromo-3-(cyclopropanesulfonylamino)-2-methoxybenzamide (10c)

White solid; yield: 57%; Mp: 175.5-177.0 °C; ^{1}H NMR (DMSO-d_6): δ 9.55 (1H, s, SO_2NH), 7.85 (1H, s, $CONH_2$), 7.69 (1H, s, $CONH_2$), 7.64 (1H, d, J = 2.4 Hz, Ar-H), 7.39 (1H, d, J = 2.4 Hz, Ar-H), 3.77 (3H, s, OCH_3), 2.82 (1H, m, CH), 0.99

(4H, m, $2CH_2$). ESI-HRMS *m/z*: calc'd for $C_{11}H_{14}BrN_2O_4S$ $[M+H]^+$: 348.9858; found 348.9860.

4.1.9.4. 5-Bromo-3-(4-fluorophenylsulfonamido)-2-methoxybenzamide (10d)

White solid; yield: 85%; Mp: 186.0-188.0 °C; ^{1}H NMR (DMSO-d_6): δ 7.88 (2H, dd, *J* = 5.2, 8.8 Hz, Ar-H), 7.82 (1H, s, Ar-H), 7.75 (1H, s, SO_2NH), 7.19 (3H, t, *J* = 8.4 Hz, Ar-H), 6.94 (1H, s, NH), 3.51 (3H, s, OCH_3), 3.07 (3H, s, NCH_3). ESI-HRMS *m/z*: calc'd for $C_{14}H_{13}BrFN_2O_4S$ $[M+H]^+$: 402.9763; found 402.9760.

4.1.9.5. 5-Bromo-3-(4-chlorophenylsulfonylamido)-2-methoxybenzamide (10e)

White solid; yield: 84%; Mp: 177.5-178.5 °C; ^{1}H NMR (DMSO-d_6): δ 10.26 (1H, s, SO_2NH), 7.82 (2H, d, *J* = 8.8 Hz, Ar-H), 3.45 (3H, s, OCH_3), 7.81 (1H, s, $CONH_2$), 7.70 (2H, d, *J* = 8.8 Hz, Ar-H), 7.64 (1H, s, $CONH_2$), 7.48 (1H, d, *J* = 2.4 Hz, Ar-H), 7.34 (1H, d, *J* = 2.4 Hz, Ar-H). ESI-HRMS *m/z*: calc'd for $C_{14}H_{13}BrClN_2O_4S$ $[M+H]^+$: 418.9468; found 418.9466.

4.1.9.6. 5-Bromo-2-methoxy-3-(4-methylphenylsulfonylamino)benzamide (10f)

White solid; yield: 87%; Mp: 169.0-170.5 °C; ^{1}H NMR (DMSO-d_6): δ 10.11 (1H, s, SO_2NH), 7.81 (1H, s, $CONH_2$), 7.72 (2H, d, *J* = 8.0 Hz, Ar-H), 7.64 (1H, s, $CONH_2$), 7.50 (1H, d, *J* = 2.4 Hz, Ar-H), 7.41 (2H, d, *J* = 8.4 Hz, Ar-H), 7.29 (1H, d, *J* = 2.4 Hz, Ar-H), 3.44 (3H, s, OCH_3), 2.36 (3H, s, Ar-CH_3). ESI-HRMS *m/z*: calc'd for $C_{15}H_{16}BrN_2O_4S$ $[M+H]^+$: 399.0014; found 399.0010.

4.1.10. General procedure for the preparation of 12a-12b and 14a-14b

The compounds were prepared according to previously described methods.[18]

4.1.10.1. *N*-(6-bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)acetamide (12a)

White solid; yield: 85%. Mp: 222.0-225.0 °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.

4.1.10.2. *N*-(6-bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)cyclopropanecarboxamide (12b)

White solid; yield: 77%; Mp: 216.0-219.0 °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* = 9.2Hz, 1H, Ar-H), 2.03 (m, 1H, CH), 0.83 (d, *J* = 5.6 Hz, 4H, 2CH_2). ESI-HRMS *m/z*: calc'd for $C_{10}H_9BrN_4NaO$ $[M + Na]^+$: 302.9857; found 302.9857.

4.1.10.3. *N*-(7-bromo-[1,2,4]triazolo[1,5-a]pyridin-2-yl)acetamide (14a)

White solid; yield: 62%; Mp: 228.0-230.0 °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.

4.1.10.4. *N*-(7-bromo-[1,2,4]triazolo[1,5-a]pyridin-2-yl)cyclopropanecarboxamide (14b)

White solid; yield: 70%; Mp: 212.0-214.0 °C; ^{1}H NMR (DMSO-d6): δ 11.12 (s, 1H, NH), 8.85 (d, *J* = 5.6 Hz, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 7.35 (d, *J* = 5.6 Hz, 1H, Ar-H), 2.03 (m, 1H, CH), 0.83 (d, *J*= 5.6 Hz, 4H, CH_2). ESI-HRMS *m/z*: calc'd for $C_{10}H_9BrN_4NaO$ $[M + Na]^+$: 302.9857; found 302.9857.

4.1.11. General procedure for the preparation of 1a-1j, 2a-2e and 3a-3c.

To a round bottomed flask were added compound **10** (0.45 mmol), bis(pinacolato)diboron (0.54 mmol), potassium acetate (1.35 mmol), $PdCl_2$(dppf) (0.03 mmol) and 1,2-dimethoxyethane (6 ml), then the mixture was heated to reflux for 4 h under nitrogen atmosphere, cooled to room temperature. Then compound **5a-5e, or 6, or 8a-8b, or 12a-12b, or 14a-14b** (0.45 mmol), potassium carbonate (1.35 mmol), $PdCl_2$(dppf) (0.03 mmol) and water (1 ml) were added. The resulting mixture was refluxed for another 1-3 h under nitrogen atmosphere. The solvent was

evaporated off under vacuum and the residue was purified through column chromatography ($CHCl_3$: MeOH = 30:1, v/v) to produce **1a-1j, 2a-2f** and **3a-3c** as white solid.

4.1.11.1. 3-(2-(Cyclopropanecarboxamido)benzo[d]thiazol-6-yl)-5-(4-fluorophenylsulfonylamino)benzamide (1a)

Yield: 78%; Mp: >270 °C; ^{1}H NMR (DMSO-d_6): δ 12.77 (1H, s, CONH), 10.61 (1H, s, SO_2NH), 8.23 (1H, s, Ar-H), 8.12 (1H, s, Ar-H), 7.92 (1H, s, $CONH_2$), 7.87 (2H, dd, *J* = 8.4, 4.8 Hz, Ar-H), 7.84 (1H, d , *J* = 8.4 Hz, Ar-H), 7.64 (1H, d, *J* = 8.4 Hz, Ar-H), 7.57 (1H, s, $CONH_2$), 7.56 (1H, s, Ar-H), 7.49 (1H, s, Ar-H), 7.42 (2H, t, *J* = 8.6 Hz, Ar-H), 2.02 (1H, m, CH), 0.98 (4H, m, $2CH_2$). ESI-HRMS m/z: calcd for $C_{24}H_{19}FN_4NaO_4S_2$ $[M+Na]^+$: 533.0729; found 533.0737.

4.1.11.2. 2-Chloro-5-(2-(cyclopropanecarboxamido)benzo[d]thiazol-6-yl)-3-(4-fluorophenylsulfonylamino)benzamide (1b)

Yield: 73%; Mp: >270 °C; ^{1}H NMR (DMSO-d_6): δ 12.79 (1H, s, CONH), 10.35 (1H, s, SO_2NH), 8.24 (1H, s, Ar-H), 7.97 (1H, s, $CONH_2$), 7.85 (2H, dd, *J* = 8.4, 5.6 Hz, Ar-H), 7.82 (1H, d , *J* = 9.2 Hz, Ar-H), 7.67 (1H, s, $CONH_2$), 7.63 (1H, d, *J* = 10.0 Hz, Ar-H), 7.61 (1H, s, Ar-H), 7.56 (1H, s, Ar-H), 7.45 (2H, t, *J* = 8.6 Hz), 2.02 (1H, m, CH), 0.98 (4H, m, $2CH_2$). ESI-HRMS m/z: calcd for $C_{24}H_{18}ClFN_4NaO_4S_2$ $[M+Na]^+$: 567.034; found 567.0343.

4.1.11.3. 5-(2-(Cyclopropanecarboxamido)benzo[d]thiazol-6-yl)-3-(4-fluoro phenylsulfonylamino)-2-methoxybenzamide (1c)

Yield: 68%; Mp: 168.0-170.0 °C; ^{1}H NMR (DMSO-d_6): δ 12.74 (1H, s, CONH), 10.04 (1H, s, SO_2NH), 8.18 (1H, s, Ar-H), 7.92 (2H, dd, *J* = 8.4, 5.6 Hz, Ar-H), 7.81 (1H, d, *J* = 8.4 Hz, Ar-H), 7.80 (1H, s, $CONH_2$), 7.65 (1H, s, $CONH_2$), 7.60 (1H, d , *J* = 3.2 Hz, Ar-H), 7.58 (1H, d, *J* = 9.6 Hz, Ar-H), 7.56 (1H, s, Ar-H), 7.45 (2H, t, *J* = 8.8 Hz, Ar-H), 3.49 (3H, s, OCH_3), 2.02 (1H, s, COCH), 0.98 (4H, m, CH_2). ESI-HRMS m/z: calc'd for $C_{25}H_{21}FN_4NaO_5S_2$ $[M+Na]^+$: 563.0835; found 563.0847.

4.1.11.4. 5-(2-(Cyclopropanecarboxamido)benzo[d]thiazol-6-yl)-3-(cyclo propanesulfonamido)-2-methoxybenzamide (1d)

Yield: 50%; Mp: 209.5-211.0 °C; ^{1}H NMR (DMSO-d_6): δ 12.74 (1H, s, CONH), 9.40 (1H, s, SO_2NH), 8.27 (1H, s, Ar-H), 7.87 (1H, s, $CONH_2$), 7.83 (1H, s, Ar-H), 7.80 (1H, d , J = 4.8 Hz, Ar-H), 7.69 (1H, s, Ar-H), 7.67 (1H, s, $CONH_2$), 7.62 (1H, s, Ar-H), 3.84 (3H, s, OCH_3), 2.85 (1H, m, SO_2CH), 2.02 (1H, s, COCH), 0.98 (8H, m, CH_2). ESI-HRMS m/z: calcd for $C_{22}H_{22}N_4NaO_5S_2$ $[M+Na]^+$: 509.0929; found 509.0939.

4.1.11.5. 5-(2-(Cyclopropylamino)benzo[d]thiazol-6-yl)-3-(4-fluorophenyl sulfonylamino)-2-methoxybenzamide (1e)

Yield: 41%; Mp: 173.0-174.0 °C; ^{1}H NMR (DMSO-d_6): δ 10.01 (s, 1H, NH), 8.48 (s, 1H, NH), 7.94 (s, 1H, Ar-H), 7.9.-7.92 (m, 2H, Ar-H), 7.79 (s, 1H, Ar-H), 7.59 (s, 2H, Ar-H), 7.49 (s, 1H, $CONH_2$), 7.47 (s, 1H, $CONH_2$), 7.45 (s, 1H, Ar-H), 7.44 (d, J = 8.8 Hz, 1H, Ar-H), 7.38 (d, J = 8.4 Hz, 1H, Ar-H), 3.47 (s, 3H, OCH_3), 2.72 (m, 1H, CH), 0.79 (m, 2H, CH_2), 0.59 (m, 2H, CH_2). ESI-HRMS m/z: calcd for $C_{24}H_{22}FN_4O_4S_2$ $[M+H]^+$: 513.1066; found 513.1082.

4.1.11.6. Ethyl-2-(6-(3-carbamoyl-5-(4-fluorophenylsulfonylamino)-4-methoxy phenyl)benzo[d]thiazol-2-ylamino)acetate (1f)

Yield: 22%; Mp: 203.0-204.0 °C; ^{1}H NMR (DMSO-d_6): δ 10.01 (s, 1H, NH), 8.55 (t, J = 5.6 Hz, 1H, NH), 7.90 - 7.92 (m, 2H, Ar-H), 7.89(s, 1H, Ar-H), 7.79 (s, 1H, Ar-H), 7.59 (m, 2H, Ar-H), 7.48 (s, 1H, $CONH_2$), 7.47 (s, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 7.43 (d, J = 8.8 Hz, 1H, Ar-H), 7.39 (d, J = 7.6 Hz, 1H, Ar-H), 4.21 (d, J = 5.6 Hz, 2H, CH_2), 4.15 (q, J = 7.2 Hz, 2H, OCH_2), 3.46 (s, 3H, OCH_3), 1.22 (t, J = 7.2 Hz, 3H, CH_3). ESI-HRMS m/z: calcd for $C_{25}H_{24}FN_4O_6S_2$ $[M+H]^+$: 559.1121; found 559.1133.

4.1.11.7. 5-(2-Acetamidobenzo[d]thiazol-6-yl)-3-(4-fluorophenylsulfonylamino)-

2-methoxybenzamide (1g)

Yield: 31%; Mp: 191.0-192.0 °C; ^{1}H NMR (DMSO-d_6): δ 12.44 (s, 1H, CONH), 10.05 (s, 1H, SO_2NH), 8.18 (s, 1H, Ar-H), 7.93 (dd, 2H, J = 8.4, 5.2 Hz, Ar-H), 7.81 (d, 1H, J=8.0Hz, Ar-H), 7.80 (s, 1H, Ar-H), 7.65 (s, 1H, $CONH_2$), 7.60 (d, 1H, J = 7.2 Hz, Ar-H), 7.58 (s, 1H, Ar-H), 7.55 (s, 1H, $CONH_2$), 7.45 (t, 2H, J = 8.8 Hz, Ar-H), 3.48 (s, 3H, OCH_3), 2.22 (s, 3H, $COCH_3$); HRMS m/z: calcd for $C_{23}H_{19}FN_4NaO_5S_2$ $[M+Na]^+$: 537.0679; found 537.0691.

4.1.11.8. 5-(2-Acetamidothiazolo[5,4-b]pyridin-5-yl)-3-(4-fluorophenylsulfonyl amino)-2-methoxybenzamide (1h)

Yield: 26%; Mp: >270 °C; ^{1}H NMR (DMSO-d_6): δ 12.56 (s, 1H, NH), 10.05 (s, 1H, NH), 8.16 (d, J = 8.4 Hz, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 7.94 (d, J = 8.4 Hz, 1H, Ar-H), 7.88-7.92 (m, 2H, Ar-H), 7.84 (s, 1H, $CONH_2$), 7.63 (s, 1H, $CONH_2$), 7.42-7.46 (m, 2H, Ar-H), 3.52 (s, 3H, OCH_3), 2.25 (s, 3H, CH_3). ESI-HRMS m/z: calcd for $C_{22}H_{18}FN_5NaO_5S_2$ $[M+Na]^+$: 538.0631; found 538.0638.

4.1.11.9. 5-(2-(Cyclopropanecarboxamido)thiazolo[5,4-b]pyridin-5-yl)-3-(4-fluorophenylsulfonylamino)-2-methoxybenzamide (1i)

Yield: 33%; Mp: 234.0-235.0 °C; ^{1}H NMR (DMSO-d_6): δ 12.87 (s, 1H, NH), 10.04 (s, 1H, NH), 8.16 (d, J = 8.4 Hz, 1H, Ar-H), 8.08 (s, 1H, Ar-H), 7.97 (s, 1H, Ar-H), 7.95 (d, J = 8.4 Hz, 1H, Ar-H), 7.88-7.92 (m, 2H, Ar-H), 7.84 (s, 1H, $CONH_2$), 7.63 (s, 1H, $CONH_2$), 7.42-7.46 (m, 2H, Ar-H), 3.52 (s, 3H, OCH_3), 2.04 (m, 1H, CH), 1.00 (m, 4H, CH_2). ESI-HRMS m/z: calcd for $C_{24}H_{21}FN_5O_5S_2$ $[M+H]^+$: 542.0968; found 542.0978.

4.1.11.10. 5-(2-(Cyclopropylamino)thiazolo[5,4-b]pyridin-5-yl)-3-(4-fluoro phenylsulfonylamino)-2-methoxybenzamide (1j)

Yield: 42%; Mp: 155.0-156.0 °C; ^{1}H NMR (DMSO-d_6): δ 9.72 (s, 1H, NH), 8.74 (s, 1H, NH), 8.00 (s, 1H, Ar-H), 7.88-7.91 (m, 2H, Ar-H), 7.87 (s, 1H, Ar-H), 7.80 (s, 1H, $CONH_2$), 7.77 (d, J = 8.4 Hz, 1H, Ar-H), 7.72 (d, J = 8.4 Hz, 1H, Ar-H), 7.59 (s,

1H, $CONH_2$), 7.41-7.45 (m, 2H, Ar-H), 3.51 (s, 3H, OCH_3), 2.78 (m, 1H, CH), 0.82 (m, 2H, CH_2), 0.62 (m, 2H, CH_2). ESI-HRMS m/z: calcd for $C_{23}H_{21}FN_5O_4S_2$ $[M+H]^+$: 514.1019; found 514.1027.

4.1.11.11. 5-(2-Acetamido-[1,2,4]triazolo[1,5-a]pyridin-6-yl)-3-(cyclopropane sulfonylamino)-2-methoxybenzamide (2a)

Yield: 50%; Mp: 257.0-259.0 °C; ^{1}H NMR (DMSO-d_6): δ 10.84 (s, 1H, NH), 9.41 (s, 1H, NH), 9.20 (s, 1H, Ar-H), 7.92 (d, J = 9.2 Hz, 1H, Ar-H), 7.86 (s, 1H, Ar-H), 7.78 (m, 3H, Ar-H), 7.66 (s, 2H, NH_2), 3.85 (s, 3H, OCH_3), 2.51 (m, 1H, CH), 2.16 (s, 3H, CH_3), 1.00 (d, 4H, J = 6.6 Hz, CH_2). ESI-HRMS *m/z*: calc'd for $C_{23}H_{22}N_6O_5S$ $[M+H]^+$: 495.5309; found 495.5312.

4.1.11.12. 5-(2-Acetamido-[1,2,4]triazolo[1,5-a]pyridin-6-yl)-2-methoxy-3-(4-methylphenylsulfonylamino)benzamide (2b)

Yield: 46%; Mp: 224.0-226.0 °C; ^{1}H NMR (DMSO-d_6): δ 10.85 (s, 1H, NH), 9.92 (s, 1H, NH), 9.05 (s, 1H, Ar-H), 7.76 (m, 5H, Ar-H), 7.68 (s, 1H, Ar-H), 7.58 (s, 2H, NH_2), 7.39 (d, J = 7.6 Hz, 2H, Ar-H), 3.47 (s, 3H, OCH_3), 2.36 (s, 3H, CH_3), 2.16 (s, 3H, CH_3). ESI-HRMS *m/z*: calc'd for $C_{23}H_{22}N_6O_5S$ $[M+H]^+$: 495.5309; found 495.5312.

4.1.11.13. 5-(2-Acetamido-[1,2,4]triazolo[1,5-a]pyridin-6-yl)-3-(4-chlorophenyl sulfonylamino)-2-methoxybenzamide (2c)

Yield: 35%; Mp: 248.0-251.0 °C; ^{1}H NMR (DMSO-d_6): δ 10.85 (s, 1H, NH), 10.12 (s, 1H, NH), 9.13 (s, 1H, Ar-H), 7.82 (m, 5H, Ar-H), 7.72 (s, 1H, Ar-H), 7.67 (d, J = 8.0 Hz, 2H, Ar-H), 7.62 (s, 2H, NH_2), 3.46 (s, 3H, OCH_3), 2.16 (s, 3H, CH_3). ESI-HRMS *m/z*: calc'd for $C_{22}H_{20}ClN_6O_5S$ $[M+H]^+$: 515.0904; found 515.0902.

4.1.11.14. 5-(2-Acetamido-[1,2,4]triazolo[1,5-a]pyridin-6-yl)-3-(4-fluorophenyl sulfonylamino)-2-methoxybenzamide (2d)

Yield: 49%; Mp: 252.0-254.0 °C; ^{1}H NMR (DMSO-d_6): δ 10.85 (s, 1H, NH),

10.06 (s, 1H, NH), 9.12 (s, 1H, Ar-H), 7.92 (m, 2H, Ar-H), 8.03 (s, 1H, Ar-H), 7.80 (m, 3H, Ar-H), 7.73 (s, 1H, Ar-H), 7.61 (s, 2H, NH_2), 7.33 (m, 2H, Ar-H), 3.46 (s, 3H, OCH_3), 2.16 (s, 3H, CH_3). ESI-HRMS *m/z*: calc'd for $C_{22}H_{20}FN_6O_5S$ $[M+H]^+$: 499.1200; found 499.1202.

4.1.11.15.

5-(2-Acetamido-[1,2,4]triazolo[1,5-a]pyridin-6-yl)-2-chloro-3-(4-fluoro phenylsulfonylamino)benzamide (2e)

Yield: 35%; Mp: 264.0-266.0 °C; ^{1}H NMR (DMSO-d_6): δ 10.88 (s, 1H, NH), 10.38 (s, 1H, NH), 9.24 (s, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 7.84 (m, 4H, Ar-H, NH_2), 7.72 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.67 (s, 1H, Ar-H), 7.43 (m, 2H, Ar-H), 2.17 (s, 3H, CH_3). ESI-HRMS *m/z*: calc'd for $C_{21}H_{17}ClFN_6O_4S$ $[M+H]^+$: 503.0705; found 503.0701.

4.1.11.16.

5-(2-(Cyclopropanecarboxamido)-[1,2,4]triazolo[1,5-a]pyridin-6-yl)-3-(4-fluorophenylsulfonylamino)-2-methoxybenzamide (2f)

Yield: 48%; Mp: 225.0-228.0 °C; ^{1}H-NMR (DMSO-d_6): δ 11.12 (s, 1H, NH), 10.06 (s, 1H, NH), 9.10 (s, 1H, Ar-H), 7.93 (m, 2H, Ar-H), 7.81 (m, 3H, 2Ar-H, CONH), 7.73 (s, 1H, Ar-H), 7.62 (m, 2H, CONH, Ar-H), 7.44 (t, *J* = 8.8 Hz, 2H, Ar-H), 3.47 (s, 3H, OCH_3), 2.07 (s, 1H, CH), 0.85 (d, *J* = 6.0 Hz, 4H, $2CH_2$). ESI-HRMS *m/z*: calc'd for $C_{24}H_{22}FN_6O_5S$ $[M+H]^+$: 525.1356; found 525.1353.

4.1.11.17. 5-(2-Amino-[1,2,4]triazolo[1,5-a]pyridin-7-yl)-3-(4-fluorophenyl sulfonylamino)-2-methoxybenzamide (3a)

Yield: 38%; Mp: 210.0-213.0 °C; ^{1}H NMR (DMSO-d_6): δ 10.08 (s, 1H, NH), 8.60 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.92 (m, 2H, Ar-H), 7.85 (s, 1H, CONH), 7.70 (s, 1H, Ar-H), 7.62 (s, 2H, $CONH_2$, Ar-H), 7.46 (m, 3H, Ar-H), 7.07 (d, *J* = 6.8 Hz, 1H, Ar-H), 6.10 (s, 2H, NH_2). ESI-HRMS *m/z*: calc'd for $C_{22}H_{20}FN_6O_5S$ $[M+H]^+$: 499.1200; found 499.1201.

4.1.11.18. 5-(2-Acetamido-[1,2,4]triazolo[1,5-a]pyridin-7-yl)-3-(4-fluorophenyl sulfonylamino)-2-methoxybenzamide (3b)

Yield: 42%; Mp: 225.0-227.0 °C; ^{1}H NMR (DMSO-d_6): δ 10.84 (s, 1H, NH) 10.10 (s, 1H, NH), 8.91 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.92 (m, 2H, Ar-H), 7.86 (s, 1H, CONH), 7.83 (s, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 7.62 (s, 1H, Ar-H), 7.63 (s, 1H, CONH), 7.44 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.34 (d, *J* = 6.8 Hz, 1H, Ar-H), 3.50 (s, 3H, OCH_3), 2.17 (s, 3H, CH_3). ESI-HRMS *m/z*: calc'd for $C_{22}H_{20}FN_6O_5S$ $[M+H]^+$: 499.1200; found 499.1205.

4.1.11.19. 5-(2-(Cyclopropanecarboxamido)-[1,2,4]triazolo[1,5-a]pyridin-7-yl)-3-(4-fluorophenylsulfonylamino)-2-methoxybenzamide (3c)

Yield: 42%; Mp: 233.0-235.0 °C; ^{1}H NMR (DMSO-d_6): δ 11.12 (s, 1H, NH) 10.10 (s, 1H, NH), 8.90 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.92 (m, 2H, Ar-H), 7.86 (s, 1H, CONH), 7.83 (s, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.68 (s, 1H, Ar-H), 7.63 (s, 1H, CONH), 7.44 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.34 (d, *J* = 7.2 Hz, 1H, Ar-H), 3.50 (s, 3H, OCH_3), 2.06 (s, 1H, CH), 0.84 (d, *J* = 6.0 Hz, 4H, CH_2). ESI-HRMS *m/z*: calc'd for $C_{24}H_{22}FN_6O_5S$ $[M+H]^+$: 525.1356; found 525.1354.

4.2. Biological assay methods

4.2.1. Cell culture

The human cell lines HCT-116, A549, 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

Compound **A** was synthesized in our laboratory (Purity: 96%, HPLC). BEZ235 was purchased from Shanghai Biochempartner Company (Purity: 99%, HPLC). 3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-*2H*- tetrazolium bromide (MTT) was purchased

from Sigma (St. Louis, MO, USA). Cellular chemosensitivity was determined by using a modified MTT method assay in vitro. In brief, HCT-116, A549, 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 72 h incubation period. Cells were treated with final concentrations of 100.0, 10.0, 1.0, 0.1 and 0.01 μM of tested compounds simultaneously and incubated for 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 compounds **1g**, **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 12000 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 complexs were detected by chemiluminescence with an GeneGnome5 system (Syngene, UK). Protein bands were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

4.2.4. Anticancer effects in established nude mouse HCT-116 xenograft model *in vivo*

Mice (BALB/C, SPF grade, male, 18-20 g) were purchased from Shanghai Slakey Laboratory Animal Co., LTD and fed at Shanghai Institute of Pharmaceutical Industry. The experimental protocol was approved by Ethic Committee of Shanghai Institute of Pharmaceutical Industry.

HCT-116 cells at 3×10^6 were injected subcutaneously into the flank. Once the tumor xenografts reached 100 mm^3, all tumor-bearing nude mice were randomly divided into four groups, with 8 mice in control group and 5 mice in each drug group. In the control group, the same volume of solvent was administered orally. BEZ235 and **1g** were dissolved in NMP/PEG300 (1: 9). BEZ235 was dosed orally at 20 mg/kg to positive group. **1g** was dosed orally at 10 mg/kg and 30 mg/kg for the low and high dosage groups once a day for 14 days, respectively. Tumor volumes and body weights were recorded at intervals of 3 or 4 days. Tumor volume was calculated as length × width × width/2 and is reported in mm^3. Results are expressed as the mean ± standard error. The mice were anesthetized and sacrificed on day 26. The weights of the body, the neoplasm, and the volumes of neoplasm, were measured and inhibitory ratios for tumor volume and weight was calculated.

Molecular modeling

The protein-ligand complex crystal structure of compound A bound to PI3Kγ was chosen as the template to compare the docking mode compound **A** bound to PI3Kγ and **1g** bound to PI3Kγ. The molecular docking procedure was performed by using C-DOCKER protocol within Discovery Studio 2.5. For enzyme preparation, the hydrogen atoms were added. The whole PI3Kγ enzyme was defined as a receptor and the site sphere was selected on the basis of the ligand binding location of compound **A**.

Compound **A** was removed and compound **1g** was placed. After end of molecular docking, ten docking poses was scored and selected based on calculated C-DOCKER energy.

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References

1. Khokra, S. L.; Arora, K.; Mehta1, H.; Aggarwal, A.; Yadav, M. *Int. J. Pharm. Sci. Res.* **2011**, *2*, 748.
2. Shi, X. H.; Wang, Z.; Xia, Y.; Ye, T. H.; Deng. M.; Xu, Y. Z.; Wei, Y. Q.; Yu, L. T. *Molecules* **2012**, *17*, 3933.
3. Caputo, R.; Calabro, ML.; Micale, N.; Schimmer, A. D.; Ali, M.; Zappala, M.; Grasso, S. *Med. Chem. Res*. **2011**, *21*, 2644.
4. Hong, S.; Kim, J.; Yun, S. M.; Lee, H.; Park, Y.; Hong, S. S.; Hong, S. *J. Med. Chem.* **2013**, *56*, 3531.
5. Noolvi, M. N.; Patel, H. M.; Kaur, M. *Eur. J. Med. Chem.* **2012**, *54*, 447.
6. Xuan, W.; Ding, W.; Hui, H. X.; Zhang, S.Q. *Med. Chem. Res.* **2013**, *22*, 3857.
7. Grant, S. K. *Cell. Mol. Life Sci.* **2009**, *66*, 1163.
8. Engelman, J. A.; Luo, J.; Cantley, L.C. *Nat. Rev. Genet.* **2006**, *7*, 606.
9. Salmena, L.; Carracedo, A.; Pandolfi, P. P. *Cell.* **2008**, *133*, 403.
10. Liu, P.; Cheng, H.; Roberts, T. M.; Zhao, J. *Nat. Rev. Drug. Discov.* **2009**, *8*, 627.
11. Liu, Q.; Thoreen, C.; Wang, J. H.; Sabatini, D.; Gray, N. S. *Drug Discov. Today Ther. Strateg.* **2009**, *6*, 47.
12. Wu, P.; Hu, Y. Z. *Med. Chem. Commun.* **2012**, *3*, 1337.
13. Knight, S. D.; Adams, N. D.; Burgess, J. L.; Chaudhari, A. M.; Darcy, M. G.; Donatelli, C. A.; Luengo, J. I.; Newlander, K. A.; Parrish, C. A.; Ridgers, L. H.; Sarpong, M. A.; Van Aller, G. S.; Carson, J. D.; Diamond, M. A.; Elkins, P. A.; Gardiner, C. M.; Garver, E.; Gilbert, S. A.; Gontarek, R. R.; Jackson, J. R.; Kershner, K. L.; Luo, L.; Raha, K.; Sherk, C. S.; Sung, C. M.; Sutton, D.;

Tummino, P. J.; Wegrzyn, R. J.; Auger, K. R.; Dhanak, D. *ACS Med. Chem. Lett.* **2010**, *1,* 39.

14. Nishimura, N.; Aaron, S.; Liu, L.; Yang, K.; Bryan, M. C.; Andrews, K. L.; Bo, Y. Y.; Booker, S. K.; Caenepeel, S.; Freeman, D.; Liao, H.; McCarter, J.; Mullady, E. L.; Miguel, T. S.; Subramanian, R.; Tamayo, N.; Wang, L.; Whittington, D. A.; Zalameda, L.; Zhang, N.; Hughes, P. E.; Norman, M. H. *J. Med. Chem.* **2011**, *54*, 4735.
15. D'Angelo, N. D.; Kim, T.-S.; Andrews, K.; Booker, S. K.; Caenepeel, S.; Chen, K.; D'Amico, D.; Freeman, D.; Jiang, J.; Liu, L.; McCarter, J. D.; SanMiguel, T.; Mullady, E. L.; Schrag, M.; Subramanian, R.; Tang, J.; Wahl, R.C.; Wang, L.; Whittington, D. A.; Wu, T.; Xi, N.; Xu, Y.; Yakowec, P.; Yang, K.; Zalameda, L. P.; Zhang, N.; Hughes, P.; Norman, M. H. *J. Med. Chem.* **2011**, *54*, 1789.
16. Stec, M. M.; Andrews, K. L.; Booker, S. K.; Caenepeel, S.; Freeman, D. J.; Jiang, J.; Liao, H. Y.; McCarter, J.; Mullady, E. L.; Miguel, T. S.; Subramanian, R.; Tamayo, N.; Wang, L.; Yang, K.; Zalameda, L. P.; Zhang, N.; Hughes, P. E.; Norman, M. H. *J. Med. Chem.* **2011**, *54*, 5174.
17. Norman, M. H.; Andrews, K. L.; Bo, Y. Y.; Booker, S. K.; Caenepeel, S.; Cee, V. J.; D'Angelo, N. D.; Freeman, D. J.; Herberich, B. J.; Hong, F. T.; Jackson, C. M.; Jiang, J.; Lanman, B. A.; Liu, L.; McCarter, J. D.; Mullady, E. L.; Nishimura, N.; Pettus, L. H.; Reed, A. B.; Miguel, T. S.; Smith, A. L.; Stec, M. M.; Tadesse, S.; Tasker, A.; Aidasani, D.; Zhu, X.; Subramanian, R.; Tamayo, N. A.; Wang, L.; Whittington, D. A.; Wu, B.; Wu, T.; Wurz, R. P.; Yang, K.; Zalameda, L.; Zhang, N.; Hughes, P. E. *J. Med. Chem.* **2012**, *55*, 7796.
18. Wang, X. M.; Xu, J.; Li, Y. P.; Li, H.; Jiang, C. S.; Yang, G. D.; Lu, S. M.; Zhang, S. Q. *Eur. J. Med. Chem.* **2013**, *67*, 243.
19. Bharate, S. B.; Singh, B.; Bharate, J. B.; Jain, S. K.; Meena, S.; Vishwakarma, R. A. *Med. Chem. Res.* **2013**, *22*, 890.
20. Maira, S. M.; Stauffer, F.; Brueggen, J.; Furet, P.; Schnell, C.; Fritsch, C.; Brachmann, S.; Chene, P.; Pover, A. D.; Schoemaker, K.; Fabbro, D.; Gabriel, D.; Simonen, M.; Murphy, L.; Finan, P.; Sellers, W.; Garcia-Echeverria, C. *Mol.*

Cancer Ther. **2008**, *7*, 1851.

21. Cheng, H.; Li, C.; Bailey, S.; Baxi, S. M.; Goulet, L.; Guo, L.; Hoffman, J.; Jiang, Y.; Johnson, T. O.; Johnson, T. W.; Knighton, D. R.; Li, J.; Liu, K. K.-C.; Liu, Z.; Matthew, A. M.; Walls, M.; Peter, A. W.; Yin, M.; Zhu, J.; Zientek, M. *ACS Med. Chem. Lett.* **2013**, *4*, 91.
22. Dunitz, J. D. Science **1994**, *264*, 670.
23. Shao, T.; Wang, J.; Chen, J. G.; Wang, X. M.; Li, Y. P.; Yang, G. D.; Mei, Q. B.; Zhang, S. Q. *Eur. J. Med. Chem.* **2014**, 75, 96.

Figure and scheme Legends

Figure 1. The structures of some PI3Kα and mTOR dual inhibitors

Figure 2. Discovery of novel PI3K inhibitor based on bioisostere

Figure 3. Proposed novel structures

Figure 4. Effect of **1g** and BEZ235 on AKT in HCT-116 cells. (**A**) Effect of compound **1g** and BEZ235 on the AKT and p-AKT^{473}. (**B**) The quantified effect of compounds **1g** and BEZ235 on the AKT and p-AKT^{473}. Results are $\overline{x} \pm s$ for three independent experiments each performed in duplicate. **P<0.01 *vs* DMSO group.

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Figure 6. Docking mode of compound **1g** with PI3Kγ. Selected residues Val882, Tyr867, Lys833, Asp964 and Asp841 are shown. Green dashed lines indicate hydrogen bond.

Scheme 1. Reagents and conditions: (a) Ac_2O, 85% HCO_2H, 60°C, 2 h; then, rt, **4a**, ether, overnight; or **4a** or **4b**, Ac_2O, DMAP, CH_2Cl_2, rt, overnight; or **4a** or **4b**, cPrCOCl, TEA, CH_2Cl_2, reflux, 3 h; (b) **5a**, $BrCH_2CO_2Et$, NaH, DMF, rt, 4 h; (c) **4a** or **4b**, H_3PO_4, $NaNO_2$, -10°C, 1 h, then CuBr, 40% HBr, rt, 1 h, 40°C, 2h; (d) **7a** or **7b**, cyclopropylamine, 1,4-dioxane, 45°C, 4 h; (e) **9a-9c**, $SnCl_2 \cdot 2H_2O$, EtOAc, reflux, 4 h; (f) RSO_2Cl, Py., THF, rt, overnight; (g) **10a** - **10f**, bis(pinacolato)diboron, AcOK, $PdCl_2$(dppf), 1,4-dioxane, reflux under N_2, 3-5 h; (h) **5b**-**5e** or **6** or **8a-8b**, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/water (5:1), reflux under N_2, 1-5 h.

Scheme 2. Reagents and conditions: (a) (i) **11** or **13**, Ac_2O, DMAP, CH_2Cl_2, rt,

overnight; or (ii) **11** or **13**, cPrCOCl, Et_3N, CH_2Cl_2, reflux, 3 h; (b) **10**, bis(pinacolato)diboron, AcOK, $PdCl_2$(dppf), 1,4-dioxane, reflux under N_2, 3-5 h; (h) **12a-12b** or **14a-14b**, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/water, reflux under N_2, 1-3 h.

Table 1 Antiproliferative activities of synthesized compounds against HCT-116, MCF-7, A549 and U-87 MG ($\overline{x} \pm s$, n = 3)

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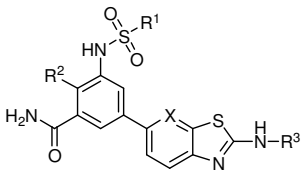

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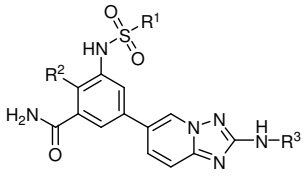

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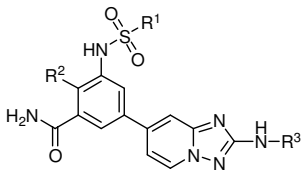
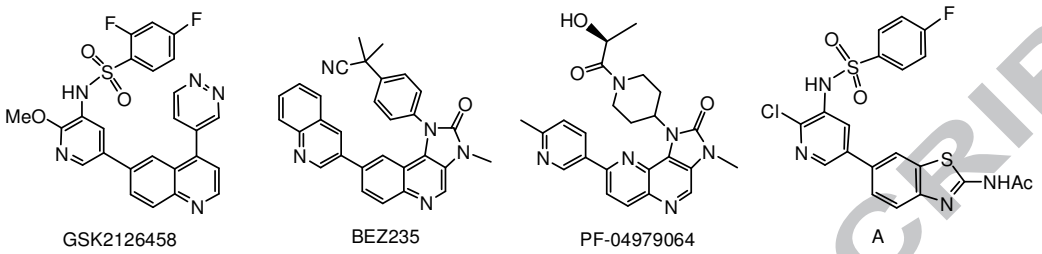


3

Compds	R^1	R^2	R^3	X	IC_{50}(μM)			
					HCT-116	MCF-7	A549	U-87 MG
1a	4-FPh	H	cPrCO	CH	>50	nt	nt	nt
1b	4-FPh	Cl	cPrCO	CH	11.40 ± 2.35	>50	>50	17.93 ± 2.86
1c	4-FPh	OMe	cPrCO	CH	2.01 ± 0.95	1.58 ± 0.24	6.34 ± 2.22	2.87 ± 0.40
1d	cPr	OMe	cPrCO	CH	14.50 ± 1.88	nt	46.30 ± 8.65	8.22 ± 0.08
1e	4-FPh	OMe	cPr	CH	>50	nt	nt	nt
1f	4-FPh	OMe	CH_2CO_2Et	CH	>50	nt	nt	nt
1g	4-FPh	OMe	Ac	CH	1.95 ± 0.66	0.50 ± 0.24	1.70 ± 1.50	4.75 ± 1.92
1h	4-FPh	OMe	Ac	N	4.25 ± 1.06	12.54 ± 3.60	4.31 ± 1.90	21.70 ± 5.35
1i	4-FPh	OMe	cPrCO	N	2.50 ± 0.89	22.10 ± 3.60	8.05 ± 1.91	20.47 ± 3.82
1j	4-FPh	OMe	cPr	N	35.72 ± 8.26	33.13 ± 9.20	30.25±7.38	41.68 ± 9.15
2a	cPr	OMe	Ac		36.33 ± 4.16	nt	nt	nt
2b	4-MePh	OMe	Ac		27.33 ± 2.08	nt	nt	nt
2c	4-ClPh	OMe	Ac		31.67 ± 5.69	nt	nt	nt
2d	4-FPh	OMe	Ac		25.40 ± 3.61	nt	nt	nt
2e	4-FPh	Cl	Ac		>50	nt	nt	nt
2f	4-FPh	OMe	cPrCO		43.50 ± 5.07	nt	nt	nt
3a	4-FPh	OMe	H		>50	nt	nt	nt
3b	4-FPh	OMe	Ac		32.63 ± 8.30	nt	nt	nt
3c	4-FPh	OMe	cPrCO		>50	nt	nt	nt
A					0.51 ± 0.10	0.52 ± 0.41	0.20 ± 0.08	2.79 ± 1.06
BEZ235					0.32 ± 0.39	0.55 ± 0.23	0.10 ± 0.06	2.34 ± 0.36

nt: not tested



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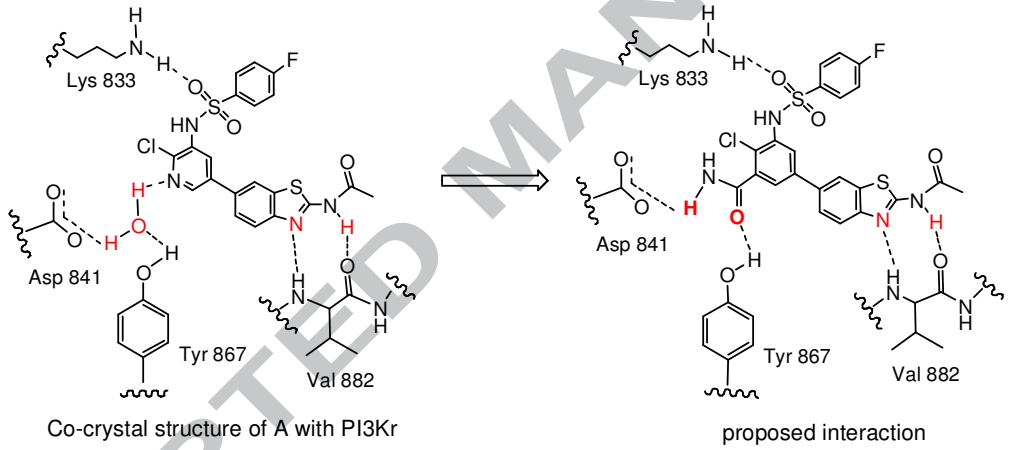


Figure 2. Discovery of novel PI3K inhibitor based on bioisostere

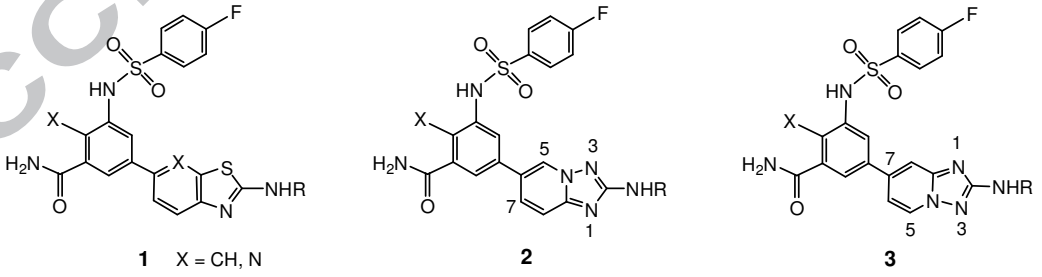
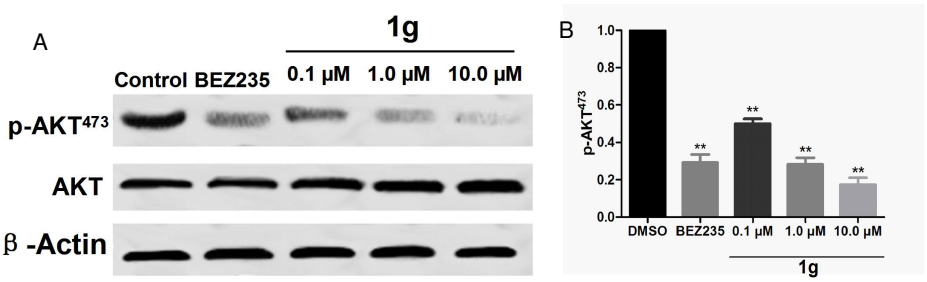


Figure 3. Proposed novel structures



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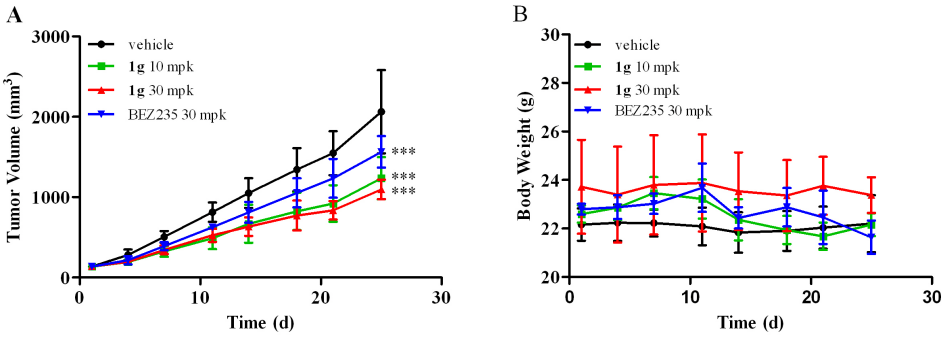

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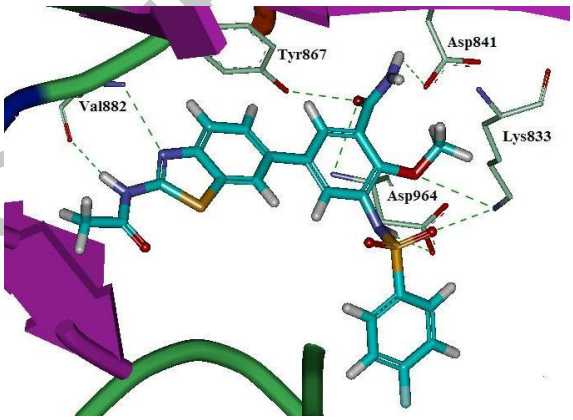

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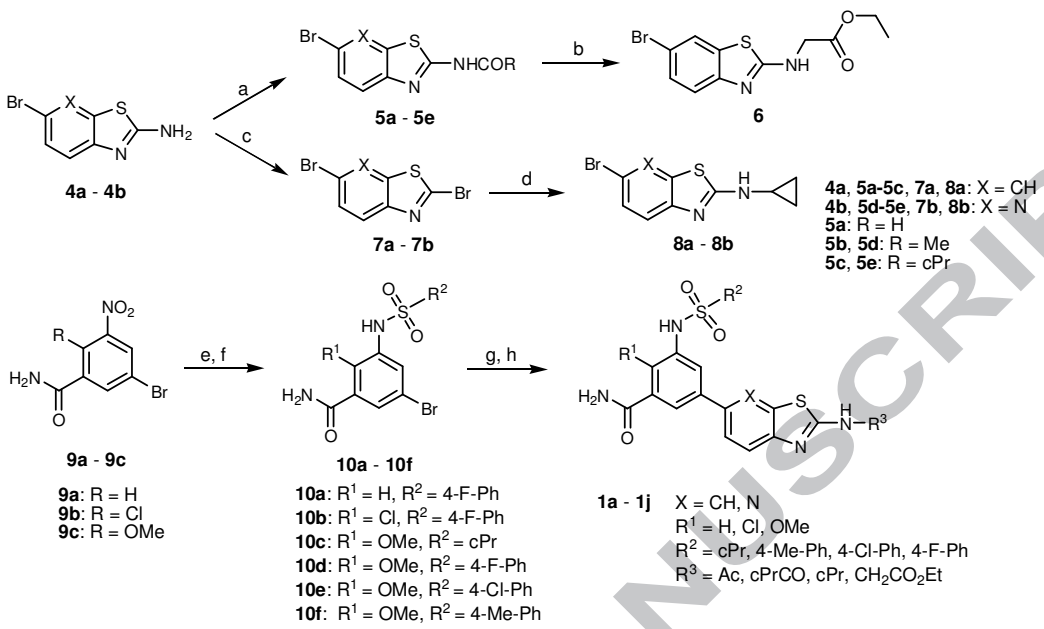
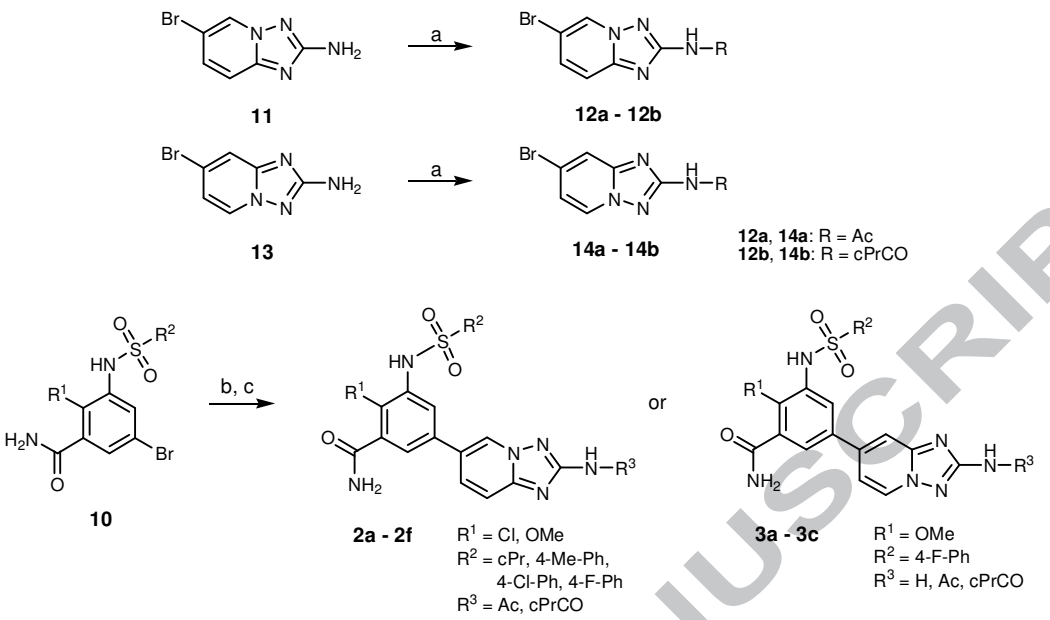


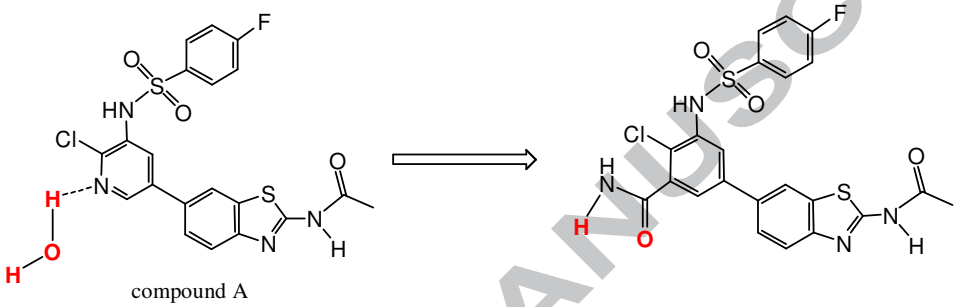
Scheme 1. Reagents and conditions: (a) Ac_2O, 85% HCO_2H, 60°C, 2 h; then, rt, **4a**, ether, overnight; or **4a** or **4b**, Ac_2O, DMAP, CH_2Cl_2, rt, overnight; or **4a** or **4b**, cPrCOCl, TEA, CH_2Cl_2, reflux, 3 h; (b) **5a**, $BrCH_2CO_2Et$, NaH, DMF, rt, 4 h; (c) **4a** or **4b**, H_3PO_4, $NaNO_2$, -10°C, 1 h, then CuBr, 40% HBr, rt, 1 h, 40°C, 2h; (d) **7a** or **7b**, cyclopropylamine, 1,4-dioxane, 45°C, 4 h; (e) **9a-9c**, $SnCl_2 \cdot 2H_2O$, EtOAc, reflux, 4 h; (f) RSO_2Cl, Py., THF, rt, overnight; (g) **10a** - **10f**, bis(pinacolato)diboron, AcOK, $PdCl_2$(dppf), 1,4-dioxane, reflux under N_2, 3-5 h; (h) **5b**-**5e** or **6** or **8a-8b**, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/water (5:1), reflux under N_2, 1-5 h.



Scheme 2. Reagents and conditions: (a) (i) **11** or **13**, Ac_2O, DMAP, CH_2Cl_2, rt, overnight; or (ii) **11** or **13**, cPrCOCl, Et_3N, CH_2Cl_2, reflux, 3 h; (b) **10**, bis(pinacolato)diboron, AcOK, $PdCl_2$(dppf), 1,4-dioxane, reflux under N_2, 3-5 h; (h) **12a-12b** or **14a-14b**, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/water, reflux under N_2, 1-3 h.

Graphical abstract

The structure of benzamide can replace the complex of the pyridine in compound A with water molecule to design novel anticancer agents based on bioisostere.



compound A