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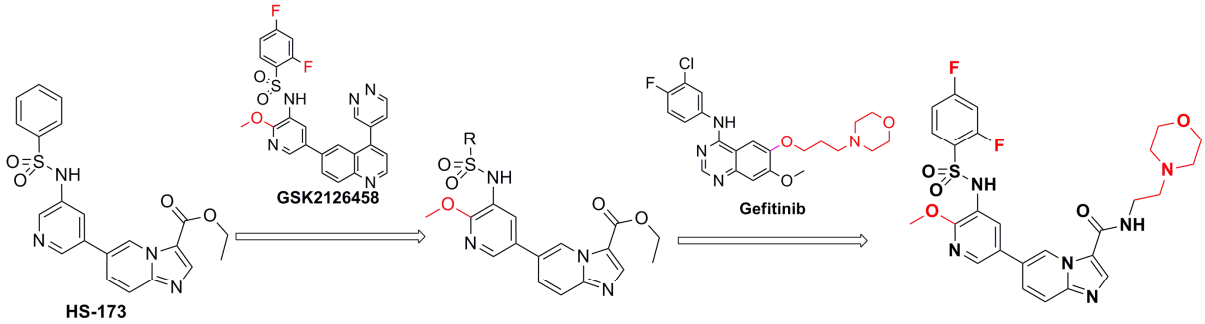

1i

PI3Kα： IC_{50} = 0.5 nM
HCT-116： IC_{50} = 10 nM
SNU638： IC_{50} = 10 nM

Design, synthesis, and biological evaluation of novel 3-substituted imidazo[1,2-a]pyridine and quinazolin-4(3H)-one derivatives as PI3Kα inhibitors

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Abstract: Phosphatidylinositol 3-kinase (PI3K) is a pivotal regulator of intracellular signaling pathways and considered as a promising target in the development of a therapeutic treatment of cancer. Among the different PI3K subtypes, the PIK3CA gene encoding PI3K p110α is frequently mutated and overexpressed in majority of human cancers. Therefore, the inhibition of PI3Kα has been considered to be an efficient approach for the treatment of cancer. In this study, two series compounds containing hydrophilic group in imidazo[1,2-a]pyridine and quinazolin-4(3H)-one were synthesized and their antiproliferative activities against five cancer cell lines, including HCT-116, SK-HEP-1, MDA-MB-231, SNU638 and A549, were evaluated. Compound **1i** with most potent antiproliferative activity was selected for further biological evaluation. PI3K kinase assay showed that **1i** has selectivity for PI3Kα distinguished from other isoforms. The western blot assay indicated that **1i** is more effective than HS-173, an imidazopyridine-based PI3Ka inhibitor, in reducing the levels of phospho-Akt. All these results suggested that **1i** is a potent PI3Kα inhibitor and could be considered as a potential candidate for the development of anticancer agents.

Keywords: Phosphatidylinositol 3-kinase, imidazo [1, 2-a] pyridine, quinazolin-4(3H)-one, PIK3CA gene, anticancer agents

Abbreviations: PI3K, Phosphatidylinositol 3-kinase; RTK, Receptor tyrosine kinases;

1. Introduction

The phosphatidylinositol-3-kinase (PI3K) signaling pathway is crucial to multiple cellular functions including cell growth, cell cycle, cell survival, differentiation and invasion. The PI3K pathway is frequently activated, amplified and mutated in human cancers [1-5]. Aberrant PI3K activity is often involved in malignant transformation, suggesting that the inhibition of this pathway is a promising approach for novel chemotherapeutic agents [5-8]. PI3K-AKT signaling is activated in cancers by several different mechanisms (Fig. 1). Receptor tyrosine kinase (RTKs) activation or mutation, Ras mutation, PTEN loss and PIK3CA mutation can lead to PI3K signaling activation. PIK3CA, which encodes the p110 catalytic subunit, is the only gene with somatic mutations and is the most frequently mutated gene in human cancers [9]. In recent years, it is evident that many human tumors carry somatic missense mutations in PIK3CA at high frequency [10-12].

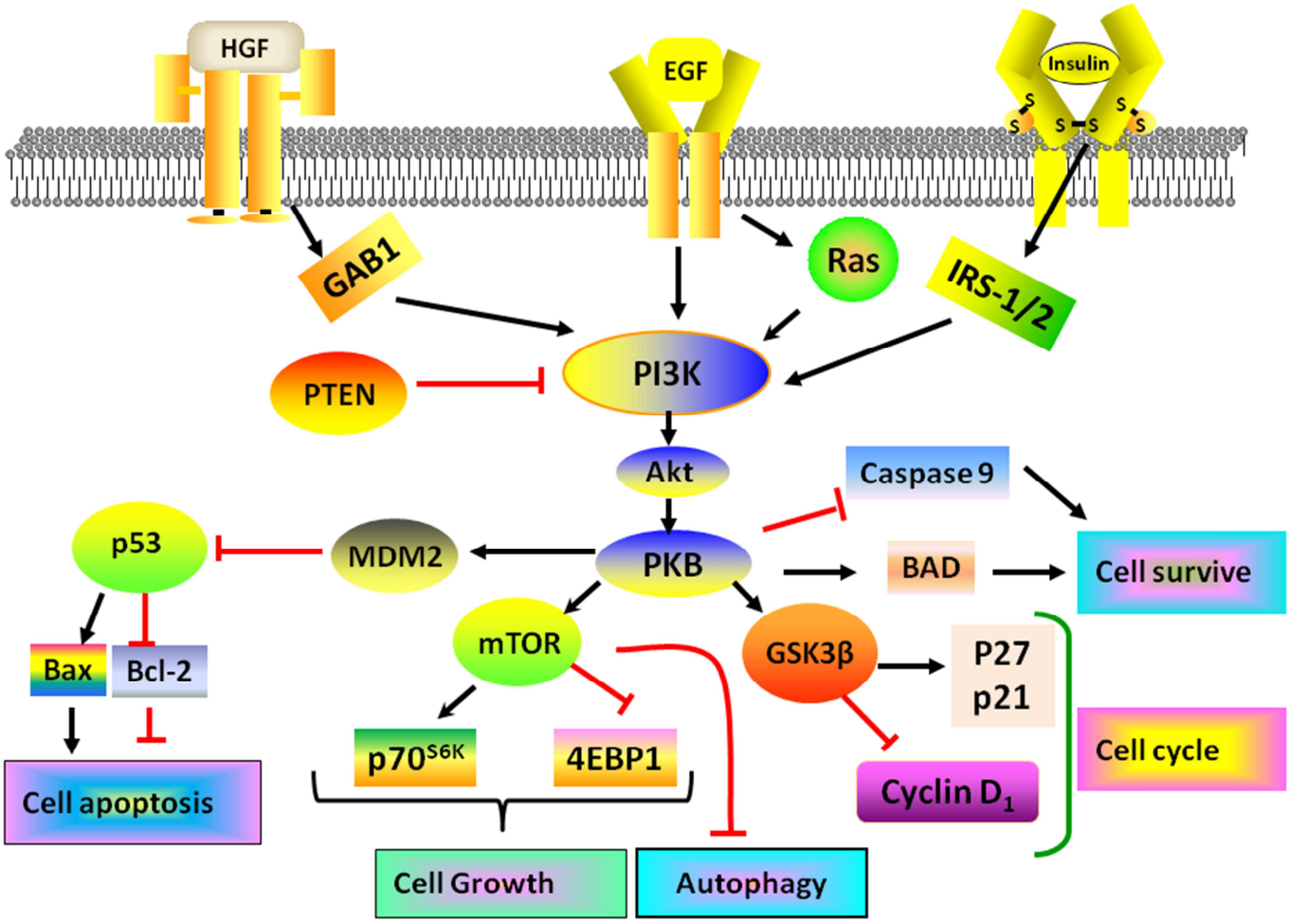


Fig.1. RTK/PI3K/Akt signaling cascade in cancer.

Several inhibitors targeting PI3K have been developed, and are being evaluated in preclinical studies and early clinical trials [13-15]. These include pan-PI3K and isoform-specific PI3K inhibitors that block the catalytic site of p110 isoforms. Among them, GSK2126458 has been identified as highly potent, orally bioavailable inhibitor of PI3K with picomolar activity and is currently under evaluation in clinical trials for

oncology applications [16-17] (Fig.2). In addition, there are a number of potential PI3K inhibitors have been developed and under preclinical tests [18-21]. HS-173, exhibiting a potent and highly selective PI3Kα inhibitory activity with an IC_{50} of 0.8 nM, showed remarkable antitumor activity *in vitro* and *in vivo* [18, 22-23] (Fig.2).

Previous study indicates that difluorophenyl and 2-methyloxy group of GSK2126458 are vital to its PI3K inhibitory activity [16]. Hence we introduce these two groups into HS-173. However, HS-173 is somewhat unstable in an aqueous phosphate buffer with pH 6.7 and this instability is probably due to the hydrolysis of the ester linkage [19]. It is known that amide is more stable than ester in the buffer solution having a pH ranging from 4.5 to 7.4. Therefore, the stability of HS-173 might be increased if the ester linkage is replaced with amide group. In addition, we also tried to improve its physical and chemical properties via introducing a substituent on amide nitrogen. Many drugs approved for marketing, such as Gefitinib [24], Lapatinib [25] and Bosutinib [26], contain a hydrophilic group extending into the solvent region, , to increase their water solubility and bioavailability. Therefore, we proposed replacing the ester linkage with amide group containing hydrophilic substituent, to eliminate the inherent hydrolysis of ester as well as providing a potential means to enhance the solubility of the molecules. In addition, we also tried to replace the imidazopyridine moiety with indole ring, however, this decreased the PI3Kα inhibitory activity. Moreover, the removal of the ester linkage from C3 position to C2 position led to a decrease in PI3Kα inhibitory activity. Thus it suggested that the 3-formyl imidazopyridine moiety is essential for its PI3Kα inhibitory activity.

Separately, the design strategy of quinazolin-4(3H)-one derivative was derived from Gefitinib and several other clinical EGFR-TKIs owing to their quinazoline scaffold, whose nitrogen atom in 1 position is very similar to the quinoline's nitrogen atom of GSK2126458. As the quinoline nitrogen of GSK2126458 forms a hydrogen bond with the hinge of PI3K, we hypothesize that nitrogen atom in position 1 of quinazoline ring may also form a hydrogen bond in the binding site of PI3K. In addition, it just so happened that position3 of quinazolin-4(3H)-one moiety can introduce an ethyl acetate group similar to that of HS-173to afford **10a**. **10a** showed modest specificity profiles against five cancer cell lines. According to the above-mentioned findings, we further introduced different hydrophilic group to quinazoline scaffold and compounds **10b-10g** were obtained. As expected, the activity was improved to some extent compared to that of **10a**.

In this study, we synthesized two series of compounds containing hydrophilic group at position 3 in imidazo[1,2-a]pyridine and quinazolin-4(3H)-one (Fig.3), with the goal of developing some new PI3K inhibitors. These compounds were prepared and evaluated for their PI3K inhibitory activities and anticancer effects *in vitro*.

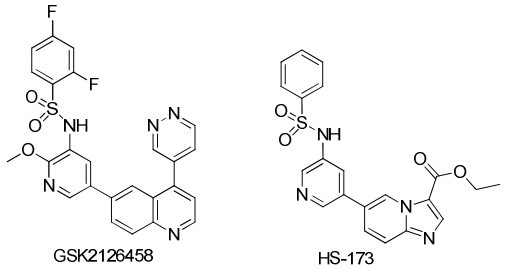


Fig.2. The structures of PI3K inhibitors.

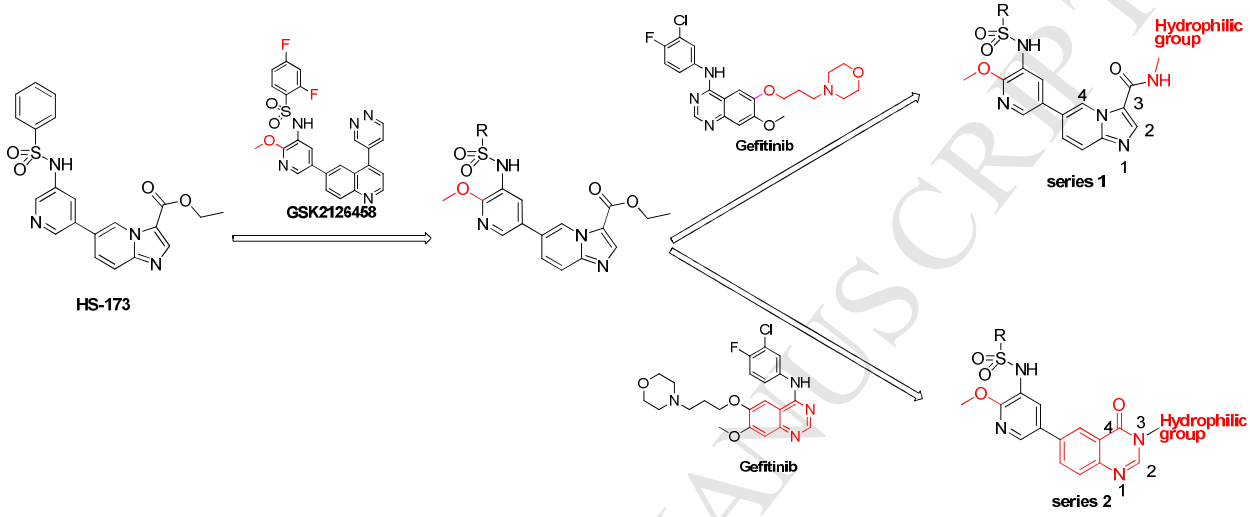


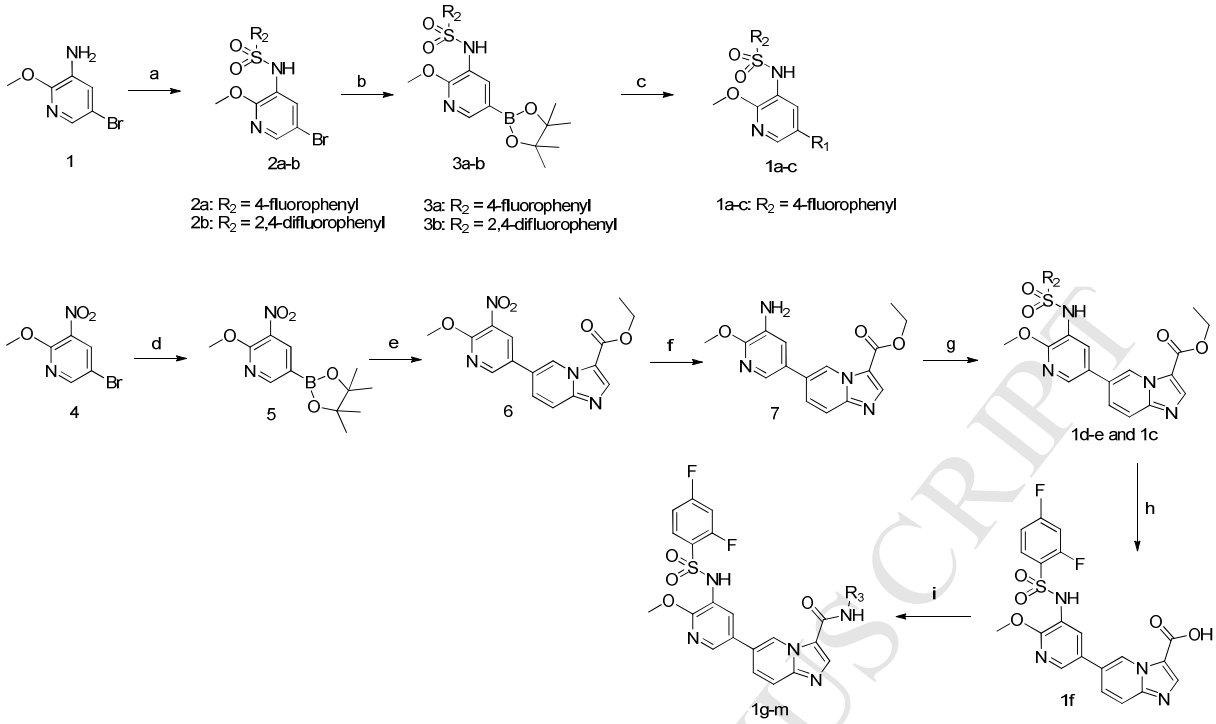
Fig.3. The design strategy based on HS-173 and GSK2126458

2. Results and discussion

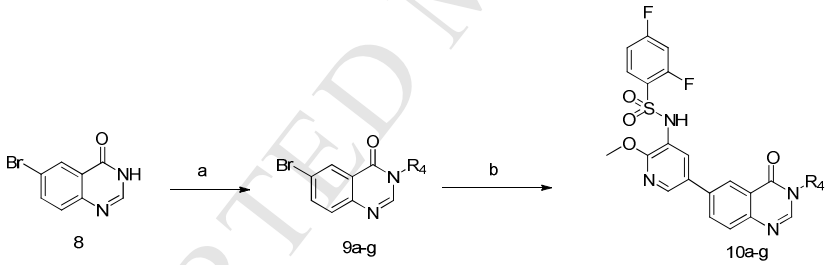
2.1. Chemistry

The synthetic routes for the target compounds are outlined in Schemes 1-2. Sulfonylation of **1** reacted with two benzenesulfonyl chlorides to yield **2a** and **2b**, which were then subjected to Suzuki coupling with bis(pinacolato)diborane to afford arylboronic ester **3a** and **3b**. Coupling **3a** with three intermediates containing five-membered rings of benzo derivatives via Suzuki reaction to yield corresponding target compounds **1a-c**. Using the same synthetic method as **3a**, intermediate **5** was obtained by the reaction of **4**. Intermediate **5** was coupled with ethyl 6-bromoimidazo[1,2-a]pyridine-3-carboxylate via Suzuki reaction to yield **6**. Subsequently, the nitro group was reduced to produce amide **7**. Sulfonylation of **7** with different sulfonyl chlorides gave the title compounds **1d-e** and the compound **1c** could also be obtained by this method. The compound **1e** was refluxed in EtOH-H_2O-NaOH (1.5 mol/L), acidified by HCl (2 mol/L) to afford **1f**, which was condensed with different amines to afford the target compounds **1g-m**.

The quinazolin-4(3H)-one derivatives were synthesized according to the Scheme 2. Commercial agent **8** reacted with different types of chlorinated compounds to afford **9a-g**, which were coupled with **3b** via Suzuki reaction to get **10a-g**.



Scheme 1. (a) Benzenesulfonyl chloride, pyridine, rt, 24 h; (b) Bis(pinacolato)diborane, $Pd(dppf)_2Cl_2$, AcOK, DMF, 100 ºC, 8 h; (c) $Pd(dppf)_2Cl_2$, Cs_2CO_3, DMF/ H_2O, 90 ºC, 8 h; (d) Bis(pinacolato)diborane, $Pd(dppf)_2Cl_2$, AcOK, DMF, 100 ºC, 8 h; (e) $Pd(dppf)_2Cl_2$, Cs_2CO_3, DMF, 90ºC, 8h; (f) Fe, HCl/H_2O, 80 ºC , 4 h; (g) Sulfonyl chloride, pyridine, rt, 24 h; (h) NaOH/Ethanol/ H_2O, 80 ºC, 0.5 h, then HCl (2 mol/L); (i) DIPEA, HOBt, EDCI, THF,12 h, rt.



Scheme 2. (a) Chlorinated compounds, K_2CO_3, DMF, 60 ºC, 12 h; (b) **3b**, $Pd(dppf)_2Cl_2$, Cs_2CO_3, DMF/ H_2O, 90ºC, 8h.

2.2. Antiproliferative assays in vitro

All synthesized compounds were evaluated for their cytotoxicities *in vitro* against five human cancer cell lines including HCT-116, A549, SK-HEP-1, SNU638 and MDA-MB-231. PI3K/AKT signaling is activated in all these five cancer cell lines [27-31]. In addition, we also examined the cytotoxicity of three selected compounds against HUVEC, which is a human normal cell.

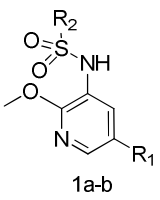
The antiproliferative results of all the compounds are summarized in Table 1 and Table 2. The results of antiproliferative effect assay showed most of the derivatives exhibited stronger antiproliferative effects, especially against HCT-116 and SNU638. Compound **1c** (IC_{50} = 0.64 μM against HCT-116) was more potent than compound **1a** (IC_{50} = 1.3 μM against HCT-116) and **1b** (IC_{50} >20 μM against HCT-116), suggesting that imidazo[1,2-a]pyridine group, especially substitution at its position 3, can improve the antiproliferative activity. Compound **1f** (IC_{50} = 0.09 μM against HCT-116) was more potent than **1c**, **1d** (0.32 μM against HCT-116) and

1e (4.94 μM against HCT-116), indicating that aromatic group at position of R_2 is more beneficial to activity than alkyl group. Moreover, compared to 4-fluorophenyl, 2,4-difluorophenyl at R_2 position can improve antiproliferative activity. To confirm our conjecture that hydrophilic group at R_3 position can enhance activity, we synthesized compounds **1h** to **1m** by changing substituent R_3. As we had expected, almost all the activities of compounds **1h** to **1m** were improved and basically reached the nanomolar level. Among them, compound **1i** showed the best anticancer activity with an IC_{50} value of 0.01 μM against HCT-116 and SNU638. Besides, compound **1j** also showed significant antiproliferative effects against HCT-116. Previous studies have reported that HCT116 cells contain a PIK3CA kinase domain mutation and Met amplification has been reported in SNU638 cells [31-33]. Considering that both of PIK3CA mutation and Met amplification could activate PI3K/Akt pathway, it is speculated this contribute to the stronger antiproliferative effects of our compounds against HCT116 and SNU638. Moreover, compound **1i** also showed better antiproliferative effects in A549 and MDA-MB-231 cells with Ras mutation [34-35] than those in SK-Hep-1 cells, which is a wild type for PIK3CA, PTEN and Ras. All the results indicated that our compounds inhibit the cell growth more effective in PI3K signal activated cells.

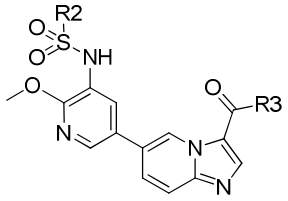
To develop more core structure, we synthesized another series of compounds **10a** to **10g** with quinazolin-4(3H)-one scaffold. However, the activities of this series were not as good as those of the imidazo[1,2-a]pyridine derivatives. Thus we confirmed the imidazopyridine moiety is essential to its PI3Kα inhibitory activity. However, very similar to **1a**-**1m**, the introduction of hydrophilic groups at position 3 of quinazolin could also improve their biological activity. Nevertheless, the antiproliferation activity could be decreased by the increased length of alkyl group. Among **10a** to **10g**, compound **10b** showed significant antiproliferative effects against all these five human cancer cell lines.

Not only that, we selected three representative compounds **1i**, **1j**, **10b** with better inhibitory activity to investigate their cytotoxicity to normal cells. All of the three compounds displayed much lower cytotoxicity in HUVEC. **1i** showed an IC_{50} value of 2.93 μM against HUVEC, which is at least 290 times higher than that of HCT-116 and MDA-MB-231 cells (Table 2). These indicated that our compounds could inhibit the growth of cancer cell in effective concentration *in vitro* without damaging the normal cells.

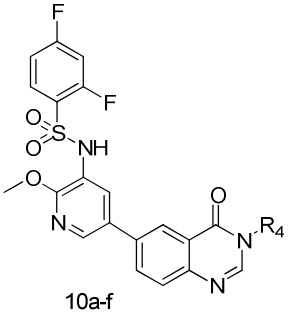
Table 1 Cytotoxicity *in vitro* of target compounds against five cancer cell lines (IC_{50} Values[a] in μM).



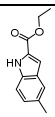
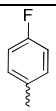
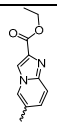
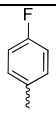
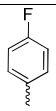
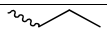
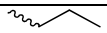
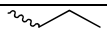
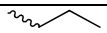
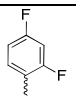
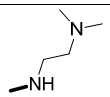
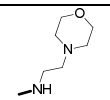
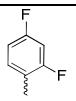
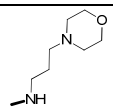
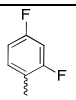
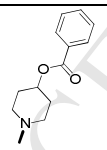
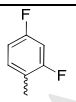
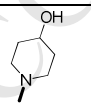
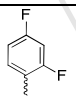
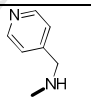
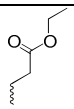
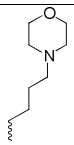
1a-b

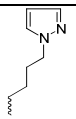
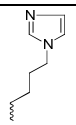
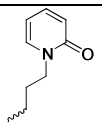
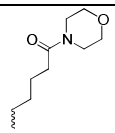
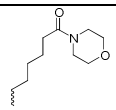


1c-m



10a-f

Cells					HCT-116	SK-HEP-1	MDA-MB-231	SNU638	A549
Comp.	R_1	R_2	R_3	R_4					
1a					1.3±0.25	0.3±0.02	0.15±0.05	15.9±0.94	>20
1b					>20	>20	>20	>20	>20
1c					0.64±0.29	>20	0.53±0.06	>20	0.51±0.05
1d					0.32±0.03	0.17±0.02	0.19±0.02	0.09±0.01	0.23±0.04
1e					4.94±0.65	0.83±0.07	2.32±0.12	0.51±0.05	3.56±0.15
1f					0.09±0.01	0.3±0.05	0.12±0.02	0.36±0.04	0.3±0.02
1g			H		1.31±0.19	0.75±0.11	0.37±0.07	1.46±0.25	1.15±0.09
1h					0.14±0.01	>20	1.8±0.58	0.51±0.04	0.4±0.3
1i					0.01±0.003	0.11±0.04	0.04±0.002	0.01±0.005	0.04±0.004
1j					0.1±0.04	0.4±0.08	0.3±0.07	0.3±0.03	0.5±0.08
1k					1.23±0.29	2.05±0.31	1.45±0.29	1.39±0.26	1.62±0.31
1l					0.49±0.03	0.79±0.04	0.63±0.03	0.27±0.02	0.48±0.05
1m					0.31±0.07	0.98±0.11	0.42±0.03	0.49±0.06	0.5±0.04
10a					0.82±0.12	1.4±0.14	0.12±0.01	0.3±0.03	0.1±0.02
10b					0.2±0.01	0.6±0.09	0.5±0.11	0.28±0.06	0.1±0.02

10c		4.0±0.78	>20	>20	0.36±0.04	0.2±0.05
10d		0.8±0.07	>20	11.7±0.87	0.25±0.03	0.2±0.03
10e		1.36±0.18	0.81±0.11	0.15±0.04	2.26±0.23	0.57±0.06
10f		1.11±0.09	2.25±0.18	1.83±0.11	>20	2.54±0.13
10g		2.23±0.09	11.49±1.27	2.15±0.89	19.72±1.72	7.6±1.18
HS-173		0.54±0.13	1.09±0.16	0.76±0.16	0.68±0.11	0.26±0.04

[a]IC_{50} values are the mean of triplicate measurements.

Table 2 Cytotoxicity assay against HUVE cells (HUVEC) (IC_{50} Values[a] in μM).

Compound	HUVE cells
1i	2.94±0.77
1j	3.56±0.95
10b	8.97±1.02

[a]IC_{50} values are the mean of triplicate measurements.

2.3. PI3K enzymatic activity assays

To elucidate the mechanism of antiproliferative activities of these active compounds, we selected the most potent compounds **1i**, **1j** and **10b** according to the antiproliferative results for the further PI3K enzymatic activity assays. HS-173 was selected as the positive drug. As shown in Table 3, kinase inhibition activity assay indicated that these three compounds above showed a highly potent inhibitory activity against PI3Kα when compared to that of other class I PI3Ks (PI3Kβ, PI3Kγ and PI3Kδ). Most important, the kinase inhibitory activity of **1i** is better than that of HS-173.

Table 3 Activities of **1i**, **1j**, **10b** and HS-173 against Class I PI3K (IC_{50} Values in nM)

	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
1i	0.5	89	45	93
1j	3.8	106	37	101
10b	6.5	108	116	69
HS-173	1.1	59	104	87

2.4. Molecular docking

Molecular docking study was performed to elucidate the binding model of **1i** in the binding site of PI3Kα. As showed in Fig.4, the amidogen of sulfonamide group forms three hydrogen bond interaction with Val859 in the hinge binder region of PI3Kα. In addition, the morpholinyl formed an additional hydrogen bond with Lys802 and the nitrogen of pyridyl group formed another hydrogen bond with the side chain of Ser854. All these binding interaction were further stabilized in the binding site via the hydrophobic interactions with residues including Lys802, Tyr836, Ile848, Val851 and Ile932 in the back pocket of the enzyme.

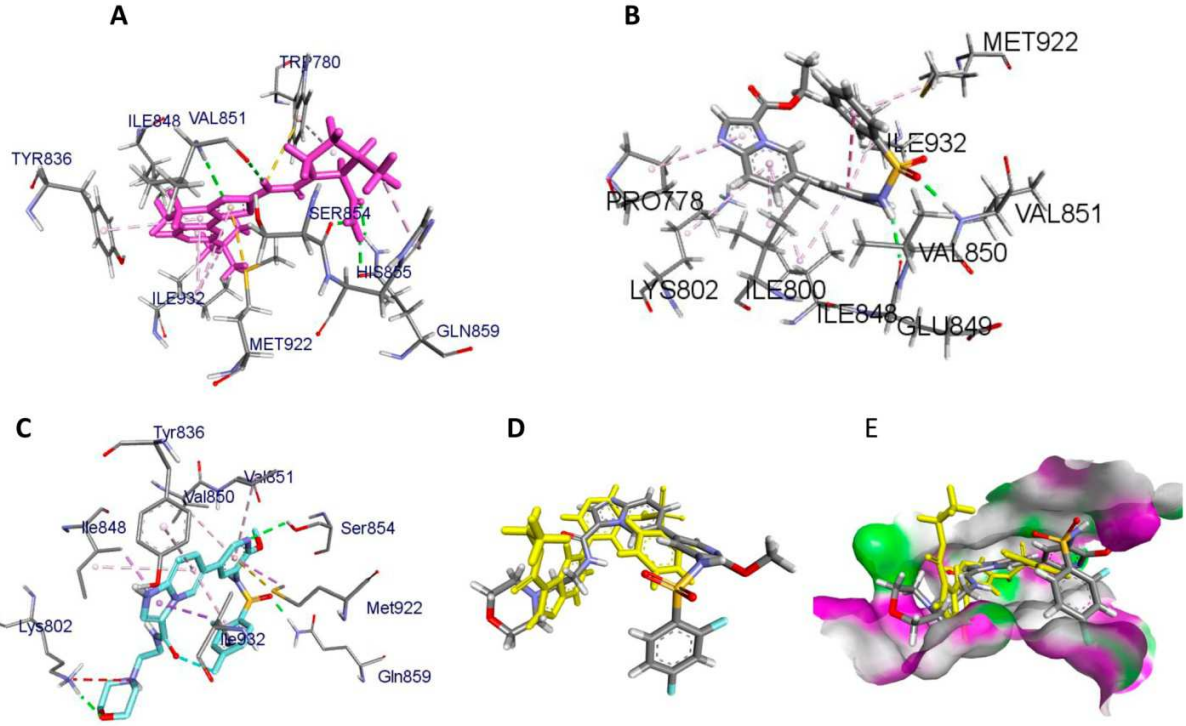


Fig.4. Docking mode of **1i** and HS-173 into protein crystal structure of PI3Kα. (A) Key interactions of the crystal ligand in the active site of PI3Kα (PDB: 4ZOP). (B) Key interactions of HS-173 in the active site of PI3Kα. (C) Key interactions of compound **1i** in the active site of PI3Kα. (D) The binding pose of **1i** and HS-173 in the active site of PI3Kα. **1i** was highlighted with yellow. (E) Binding modes of **1i** and HS-173 in the binding pocket of PI3Kα. The ligands are shown in stick model, while the proteins are shown in surface model for better visualization in the three dimensional combination models. **1i** was highlighted with yellow.

2.5. Western blot assay

Activation of the PI3K pathway leads to phosphorylation of Akt and subsequently regulated the downstream targets. PI3K inhibitors show significant tumor growth inhibition via suppression of Akt phosphorylation and then inhibit phosphorylation of other proteins downstream of PI3K [24-26]. To further determine whether these compounds affect the PI3K/Akt signaling pathway, we evaluated the effects of **1i** on the related protein levels including Akt and phospho-Akt (p-Akt, S473) in HCT-116 cells by Western blot. As shown in Fig.5, compound **1i** obviously decreased the phospho-Akt (S473) in a dose-dependent manner,

indicating that **1i** might act as a potential PI3K inhibitor (Fig. 5A). Moreover, compound **1i** is more effective than HS-173 in reducing the phospho-Akt (S473) levels at the concentration of 0.5 μM (Fig.5B).

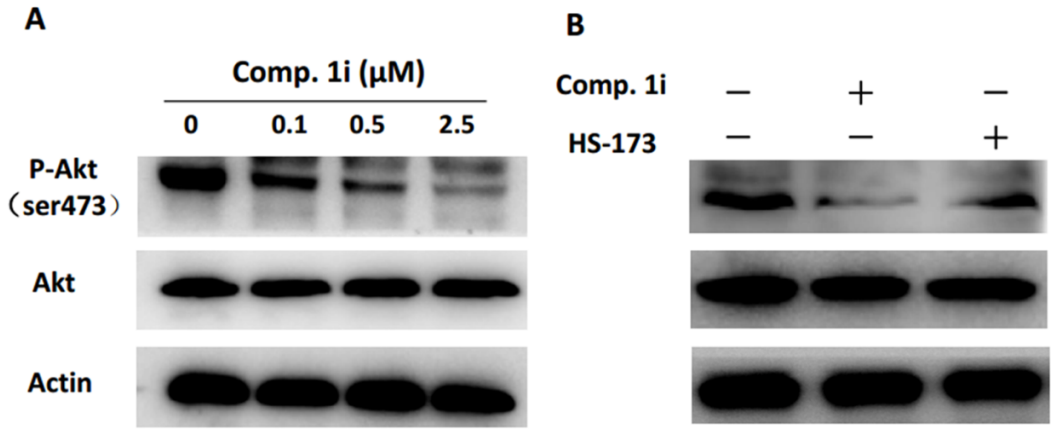


Fig. 5. Effect of compound **1i** on PI3K pathway in HCT-116 cells. (A) Compound **1i** decreased phospho-Akt (S473) in a dose-dependent manner. (B) Compound **1i** is more effective than HS-173 in reducing the levels of phospho-Akt (S473). Cells were treated with 0.1% DMSO, 0.5μM compound **1i** or HS-173 for 24 h.

3. Conclusion

In summary, two series of compounds containing hydrophilic group in imidazo[1,2-a]pyridine and quinazolin-4(3H)-one were designed and synthesized. Their PI3Kα inhibitory activities and antiproliferative activities against various cancer cell lines were evaluated *in vitro*. Compound **1i** as a potential PI3Kα inhibitor significantly inhibited the PI3K/Akt/mTOR pathway and could be considered as a potential candidate for the development of anticancer agents. According to these results, it can be concluded that derivatization at the C-3 position of the imidazo[1,2-a]pyridine core via adding a hydrophilic group is a feasible way to enhance the enzymatic and anti-proliferative activities.

4. Experimental section

4.1 Chemistry and chemical methods

All reagents and solvents were commercially available without further purification. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a 400 Bruker NMR spectrometer with tetramethylsilane (TMS) as an internal standard. All chemical shifts are reported in ppm (δ) and coupling constants (*J*) are in hertz (Hz). All the melting points were determined on a Beijing micromelting-point apparatus and thermometer was uncorrected. High-resolution exact mass measurements were performed using electrospray ionization (positive mode) on a quadrupole time-of-flight (QTOF) mass spectrometer (microTOF-Q, Bruker Inc.).

*4.1.1. N-(5-bromo-2-methoxypyridin-3-yl)-4-fluorobenzenesulfonamide (**2a**)*

To a solution of 5-bromo-2-methoxypyridin-3-amine (**1**) (2.01 g, 10 mmol) in pyridine (50 ml) at 0 ºC, 4-fluorophenylsulfonyl chloride (2.14 g, 11 mmol) was added. Then the mixture was stirred at room temperature for 24 h. Pyridine was removed under reduced pressure and adding water (100 ml), extracted with

ethyl acetate (3 × 100 ml), the organic layer was washed with water (50 ml), dried with Na_2SO_4 and evaporated to give compound **2a** as a white solid (3.22 g, 89.4% yield). mp152-154 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.22 (s, 1H, NH), 8.08 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.83 (dd, *J* = 8.8, 5.2 Hz, 2H, Ar-H), 7.72 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.43 (t, *J* = 8.8 Hz, 2H, Ar-H), 3.64 (s, 3H, OCH_3). ESI-MS: m/z 385.1 $[M+H+Na]^+$.

*4.1.2. N-(5-bromo-2-methoxypyridin-3-yl)-2,4-difluorobenzenesulfonamide (**2b**)*

This compound was synthesized according to the procedure described in **2a**.

85.6% yield. mp 163-165 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.46 (s, 1H, NH), 8.13 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.83-7.74 (m, 2H, Ar-H), 7.62-7.52 (m, 1H, Ar-H), 7.24 (td, *J* = 2.0, 8.5 Hz, 1H, Ar-H), 3.62 (s, 3H, OCH_3). ESI-MS: m/z 376.9 $[M-H]^+$.

*4.1.3. 4-fluoro-N-(2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)benzenesulfonamide (**3a**)*

A solution of the **2a** (1.80 g, 5 mmol), bis(pinacolato)diborane (1.27 g, 5 mmol), $Pd(dppf)_2Cl_2$ (0.18 g, 0.25 mmol) and KOAc (1.47 g, 15 mmol) in anhydrous DMF (30 ml) under N_2 was stirred at 100 ºC for 8 h. DMF was removed under reduced pressure and add water (100 ml), extracted with ethyl acetate (3 × 100 ml), the organic layer was washed with water (20 ml), dried with Na_2SO_4 and evaporated to give compound **3a** as a white solid (1.52 g, 74.5% yield). mp 149-151 ºC. ^{1}H NMR (400 MHz, DMSO) δ 9.92 (s, 1H, NH), 8.18 (s, 1H, Ar-H), 7.73 (m, 3H, Ar-H), 7.40 (t, *J* = 8.7 Hz, 2H, Ar-H), 3.62 (s, 3H, OCH_3), 1.29 (s, 12H, CH_3). ESI-MS: m/z 409.3 $[M+H]^+$.

4.1.4.

*2,4-difluoro-N-(2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)benzenesulfonamide (**3b**)*

This compound was synthesized according to the procedure described in **3a**.

78.8% yield. mp 171-173 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.19 (s, 1H, NH), 8.21 (d, *J* = 1.5 Hz, 1H, Ar-H), 7.72 (d, *J* = 1.5 Hz, 1H, Ar-H), 7.71-7.67 (m, 1H, Ar-H), 7.59-7.54 (m, 1H, Ar-H), 7.20 (td, *J* = 2.0, 8.6 Hz, 1H, Ar-H), 3.62 (s, 3H, OCH_3), 1.30 (s, 12H, CH_3). ESI-MS: m/z 427.3 $[M+H]^+$

*4.1.5. 2-methoxy-3-nitro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (**5**)*

This compound was synthesized according to the procedure described in **3a**.

72.4% yield, mp107-109 ºC. ^{1}H NMR (400 MHz, DMSO) δ 8.64 (d, *J* = 1.6 Hz, 1H, Ar-H), 8.45 (d, *J* = 1.6 Hz, 1H, Ar-H), 4.07 (s, 3H, OCH_3), 1.33 (s, 12H, CH_3). ESI-MS: m/z 281.3 $[M+H]^+$.

*4.1.6. ethyl 6-(6-methoxy-5-nitropyridin-3-yl)imidazo[1,2-a]pyridine-3-carboxylate (**6**)*

A solution of the **5** (1.40 g, 5 mmol), ethyl 6-bromoimidazo[1,2-a]pyridine-3-carboxylate (1.34 g, 5 mmol), $Pd(dppf)_2Cl_2$ (0.18 g, 0.25 mmol) andCs_2CO_3 (3.26 g, 10 mmol) in DMF (30 ml) under an atmosphere of N_2 was stirred at 90 ºC for 8 h. DMF was removed under reduced pressure and the residue was purified through a column chromatography on silica with chloroform/methanol (V:V 50:1) as a white solid (3.06 g, 89.5% yield). mp 217-219 ºC.^{1}H NMR (400 MHz, CDCl3) δ 9.60 (s, 1H, Ar-H), 8.68 (d, *J* = 2.4 Hz, 1H, Ar-H), 8.52 (d, *J* =

2.4 Hz, 1H, Ar-H), 8.37 (s, 1H, Ar-H), 7.92 (d, *J* = 9.3 Hz, 1H, Ar-H), 7.64 (dd, *J* = 9.3, 1.8 Hz, 1H, Ar-H), 4.46 (q, *J* = 7.1 Hz, 2H, CH_2), 4.20 (s, 3H, CH_3), 1.45 (t, *J* = 7.1 Hz, 3H, CH_3). ESI-MS: m/z 343.2 $[M+H]^+$.

*4.1.7. ethyl 6-(5-amino-6-methoxypyridin-3-yl)imidazo[1,2-a]pyridine-3-carboxylate (**7**)*

A solution of the **6** (2.57 g, 7.5 mmol), iron dust (4.2 g, 75 mmol) and HCl/H_2O (50 ml, V:V 1:5) was stirred at 80 ºC for 4 h. Add water (100 ml), the pH of the mixture was adjusted to 8 using saturated aqueous $NaHCO_3$. Extracted with ethyl acetate (3 × 200 ml), the organic layer was washed with water (50 ml), dried with Na_2SO_4 and evaporated to give compound 2 as a white solid (1.76 g, 75.4% yield). mp 113-115 ºC.^{1}H NMR (400 MHz, DMSO) δ 9.36 (s, 1H, Ar-H), 8.31 (s, 1H, Ar-H), 7.88 (d, *J* = 9.3 Hz, 1H, Ar-H), 7.83 (dd, *J* = 9.3, 1.6 Hz, 1H, Ar-H), 7.72 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.20 (d, *J* = 2.2 Hz, 1H, Ar-H), 5.25 (s, 2H, NH_2), 4.38 (q, *J* = 7.1 Hz, 2H, CH^2), 3.93 (s, 3H, CH_3), 1.36 (t, *J* = 7.1 Hz, 3H, CH_3). ESI-MS: m/z 313.3 $[M+H]^+$.

*4.1.8. ethyl 2-(6-bromo-4-oxoquinazolin-3(4H)-yl)acetate (**9a**)*

A solution of the 6-bromoquinazolin-4(3H)-one (0.44 g, 2 mmol), ethyl chloroacetate (0.147 g, 2.4 mmol), K_2CO_3(0.28 g, 4 mmol) and DMF(10 ml) was stirred at 6ºC for 12 h. DMF was removed under reduced pressure and the residue was purified through a column chromatography on silica with chloroform/methanol (V:V 50:1) as a white solid (0.59 g, 95.2% yield). mp 137-139ºC.^{1}H NMR (400 MHz, DMSO) δ 8.43 (s, 1H, Ar-H), 8.24 (d, *J* = 2.3 Hz, 1H, Ar-H), 8.03 (dd, *J* = 8.7, 2.3 Hz, 1H, Ar-H), 7.68 (d, *J* = 8.7 Hz, 1H, Ar-H), 4.84 (s, 2H, CH_2), 4.18 (q, *J* = 7.1 Hz, 2H, CH_2), 1.22 (t, *J* = 7.1 Hz, 3H, CH_3). ESI-MS: m/z 308.9 $[M-H]^+$.

Compounds **9b-g** were synthesized according to the procedure described in **9a**.

*4.1.9. 6-bromo-3-(3-morpholinopropyl)quinazolin-4(3H)-one (**9b**)*

92.4% yield, mp 84-86 ºC. ^{1}H NMR (400 MHz, DMSO) δ 8.44 (s, 1H, Ar-H), 8.23 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.97 (dd, *J* = 8.7, 2.3 Hz, 1H, Ar-H), 7.64 (d, *J* = 8.7 Hz, 1H, Ar-H), 4.03 (t, *J* = 6.8 Hz, 2H, CH_2), 3.45 (s, 4H, CH_2), 2.32 (t, *J* = 6.6 Hz, 2H, CH_2), 2.27 (s, 4H, CH_2), 1.87 (p, *J* = 6.6 Hz, 2H, CH_2). ESI-MS: m/z 352.2 $[M+H]^+$.

*4.1.10. 3-(3-(1H-pyrazol-1-yl)propyl)-6-bromoquinazolin-4(3H)-one (**9c**)*

83.6% yield, mp 181-183ºC. ^{1}H NMR (400 MHz, DMSO) δ 8.40 (s, 1H, Ar-H), 8.22 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.97 (dd, *J* = 8.7, 2.4 Hz, 1H, Ar-H), 7.75 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.69 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.63 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.43 (dd, *J* = 3.9, 1.4 Hz, 2H, Ar-H), 6.22 (dt, *J* = 4.0, 2.0 Hz, 2H, Ar-H), 4.20 (t, *J* = 7.3 Hz, 2H, CH_2), 3.99 (t, *J* = 7.0 Hz, 2H, CH_2), 2.23 (p, *J* = 6.9 Hz, 2H, CH_2). ESI-MS: m/z 355.2 $[M+Na]^+$.

*4.1.11. 3-(3-(1H-imidazol-1-yl)propyl)-6-bromoquinazolin-4(3H)-one (**9d**)*

78.8% yield, mp 151-153 ºC. ^{1}H NMR (400 MHz, DMSO) δ 8.39 (s, 1H, Ar-H), 8.23 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.98 (dd, *J* = 8.7, 2.3 Hz, 1H, Ar-H), 7.70 (s, 1H, Ar-H), 7.64 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.23 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 4.06 (t, *J* = 7.2 Hz, 2H, Ar-H), 4.00 (dd, *J* = 13.4, 6.4 Hz, 2H, CH_2), 2.18 (p, *J* = 7.1 Hz, 2H, CH_2). ESI-MS: m/z 333.1 $[M+H]^+$.

*4.1.12. 6-bromo-3-(4-morpholino-4-oxobutyl)quinazolin-4(3H)-one (**9e**)*

91.8% yield, mp 50-52 ºC. ^{1}H NMR (400 MHz, DMSO) δ 8.40 (s, 1H, Ar-H), 8.23 (d, *J* = 2.2 Hz, 1H,

Ar-H), 7.97 (dd, *J* = 8.7, 2.3 Hz, 1H, Ar-H), 7.63 (d, *J* = 8.7 Hz, 1H, Ar-H), 4.01 (t, *J* = 6.8 Hz, 2H, Ar-H), 3.55 (dd, *J* = 12.7, 8.3 Hz, 4H, CH_2), 3.40 (dd, *J* = 10.3, 5.0 Hz, 4H, CH_2), 2.39 (m, 2H, CH_2), 1.95 (t, *J* = 6.8 Hz, 2H, CH_2). ESI-MS: m/z 380.2 $[M+H]^+$.

*4.1.13. 6-bromo-3-(5-morpholino-5-oxopentyl)quinazolin-4(3H)-one (**9f**)*

90.3% yield, mp 52-54 ºC. ^{1}H NMR (400 MHz, DMSO) δ 8.46 (s, 1H, Ar-H), 8.23 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.97 (dd, *J* = 8.7, 2.3 Hz, 1H, Ar-H), 7.64 (d, *J* = 8.7 Hz, 1H, Ar-H), 4.00 (t, *J* = 7.1 Hz, 2H, Ar-H), 3.52 (dd, *J* = 9.4, 4.8 Hz, 4H, CH_2), 3.42 (brs, 4H, CH_2), 2.35 (t, *J* = 7.4 Hz, 2H, CH_2), 1.81 – 1.65 (m, 2H, CH_2), 1.52 (dd, *J* = 15.0, 7.5 Hz, 2H, CH_2). ESI-MS: m/z 394.2 $[M+H]^+$.

*4.1.14. 6-bromo-3-(3-(2-oxopyridin-1(2H)-yl)propyl)quinazolin-4(3H)-one (**9g**)*

82.5% yield, mp148-150 ºC. ^{1}H NMR (400 MHz, DMSO) δ 8.47 (s, 1H, Ar-H), 8.23 (d, *J* = 1.5 Hz, 1H, Ar-H), 7.99 - 7.97 (m, 1H, Ar-H), 7.70 (d, *J* = 5.5 Hz, 1H, Ar-H), 7.64 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.41-7.37 (m, 1H, Ar-H), 6.36 (d, J = 9.0 Hz, 1H, Ar-H), 6.21 (t, J = 6.3 Hz, 1H, Ar-H), 4.02 (t, *J* = 6.7 Hz, 2H, CH_2), 3.95 (t, *J* = 6.9 Hz, 2H, CH_2), 2.11- 2.04 (m, 2H, CH_2). ESI-MS: m/z 382.1 $[M+Na]^+$.

*4.1.15. ethyl 5-(5-(4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-1H-indole-2-carboxylate (**1a**)*

A solution of the **3a** (0.204 g, 0.5 mmol), ethyl 5-bromo-1H-indole-2-carboxylate (0.134 g, 0.5 mmol), $Pd(dppf)_2Cl_2$ (0.018 g, 0.025 mmol) and Cs_2CO_3 (0.33 g, 0.56 mmol) in DMF (10 ml) under an atmosphere of N_2 was stirred at 90 ºC for 4 h. DMF was removed under reduced pressure and the residue was purified through a column chromatography on silica with chloroform/methanol (V:V 20:1) as a white solid (0.12 g, 51.2% yield). mp 108-110 ºC. ^{1}H NMR (400 MHz, DMSO) δ 12.00 (s, 1H, NH), 10.02 (s, 1H, NH), 8.27 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.87 – 7.80 (m, 4H, Ar-H), 7.55 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.49 (dd, *J* = 8.6, 1.4 Hz, 1H, Ar-H), 7.43 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.22 (d, *J* = 1.5 Hz, 1H, Ar-H), 4.36 (q, *J* = 7.1 Hz, 2H, CH_2), 3.66 (s, 3H, CH_3), 1.36 (t, *J* = 7.1 Hz, 3H, CH_3).^{13}C NMR (100 MHz, DMSO) δ 164.8 (*J* = 249.7 Hz), 161.7, 156.4, 141.6, 137.4, 137.1, 137.0, 132.3, 131.1, 130.3 (d, *J* = 9.6 Hz), 129.2, 128.7, 127.8, 124.2, 120.6, 120.2, 116.7 (d, *J* = 22.8 Hz), 113.8, 108.5, 61.0, 53.8, 14.8. HRMS: m/z 470.1181 $[M+H]^+$.

Compounds **1b-f** was synthesized according to the procedure described in **1a**.

*4.1.16. ethyl 6-(5-(4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)imidazo[1,2-a]pyridine-2-carboxylate (**1b**)*

72.6% yield, mp 222-224 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.11 (s, 1H, NH), 8.90 (s, 1H, Ar-H), 8.57 (s, 1H, Ar-H), 8.32 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.94 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.85 (dd, *J* =5.2, 8.7 Hz, 2H, Ar-H), 7.73 (d, *J* = 9.5 Hz, 1H, Ar-H), 7.65 (dd, *J* = 1.3, 9.5Hz, 1H, Ar-H), 7.43 (t, *J* = 8.8 Hz, 2H, Ar-H), 4.34 (q, *J* = 7.1 Hz, 2H, CH_2), 3.66 (s, 3H, CH_3), 1.34 (t, *J* = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO) δ 164.8 (d, *J* = 249.6 Hz), 163.0, 157.2, 144.2, 141.8, 137.1, 136.5, 132.0, 130.3 (d, *J* = 9.7 Hz), 126.7, 126.3, 124.9, 123.3, 121.1, 118.9, 118.6, 116.7 (d, *J* = 22.7 Hz), 60.75, 53.94, 14.71. HRMS: m/z 471.1126 $[M+H]^+$.

*4.1.17. ethyl 6-(5-(4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)imidazo[1,2-a]pyridine-3-carboxylate (**1c**)*

83.1% yield, mp168-170 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.17 (s, 1H, NH), 9.34 (s, 1H, Ar-H), 8.33 (d, *J* = 2.3 Hz, 1H, Ar-H), 8.32 (s, 1H, Ar-H), 7.93 – 7.83 (m, 5H, Ar-H), 7.44 (t, *J* = 8.8 Hz, 2H, Ar-H), 4.41 (q, *J* = 7.1 Hz, 2H, CH_2), 3.72 (s, 3H, CH_2), 1.39 (t, *J* = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO) δ 164.9 (d, *J* = 250.1 Hz), 160.4, 157.1, 147.5, 141.9, 141.7, 136.9, 131.2, 130.3 (d, *J* = 9.6 Hz), 128.0, 126.3, 124.7, 124.6, 121.3, 118.3, 116.8(d, *J* = 22.7 Hz), 116.2, 60.80, 54.04, 14.75. HRMS: m/z 471.1131 $[M+H]^+$.

*4.1.18. ethyl 6-(6-methoxy-5-(methylsulfonamido)pyridin-3-yl)imidazo[1,2-a]pyridine-3-carboxylate (**1d**)*

65.9% yield, mp 208-210 ºC. ^{1}H NMR (400 MHz, DMSO) δ 9.45 (s, 1H, NH), 9.38 (s, 1H, Ar-H), 8.36 (d, *J* = 2.2 Hz, 1H, Ar-H), 8.33 (s, 1H, Ar-H), 7.94 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 7.90 (d, *J* = 1.6 Hz, 1H, Ar-H), 4.39 (q, *J* = 7.1 Hz, 2H, CH_2), 4.00 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 1.37 (t, *J* = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO) δ 160.3, 156.9, 147.5, 142.0, 141.1, 130.6, 128.3, 126.5, 125.0, 124.7, 122.2, 118.2, 116.2, 60.8, 54.4, 41.2, 14.7. HRMS: m/z 391.1066 $[M+H]^+$.

*4.1.19. ethyl 6-(5-(butylsulfonamido)-6-methoxypyridin-3-yl)imidazo[1,2-a]pyridine-3-carboxylate (**1e**)*

78.4% yield, mp 147-149 ºC. ^{1}H NMR (400 MHz, DMSO) δ 9.48 (s, 1H, NH), 9.36 (s, 1H, Ar-H), 8.35 (s, 1H, Ar-H), 8.31 (s, 1H, Ar-H), 7.94 (s, 1H, Ar-H), 7.88 (q, *J* = 9.5 Hz, 2H, Ar-H), 4.39 (q, *J* = 7.0 Hz, 2H, CH_2), 4.00 (s, 3H, CH_3), 3.25 – 3.11 (m, 2H, CH_2), 1.83 – 1.68 (m, 2H, CH_2), 1.46 – 1.36 (m, 5H, CH_2 and CH_3), 0.89 (t, *J* = 7.3 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO) δ 160.3 , 156.9, 147.4, 141.9, 141.1, 130.9, 128.1, 126.4, 124.9, 124.5, 122.1, 118.2, 116.2, 60.8, 54.3, 52.5, 25.6, 21.3, 14.7, 14.0. HRMS: m/z 433.1560 $[M+H]^+$.

*4.1.20 ethyl 6-(5-(2,4-difluorophenylsulfonamido)-6-methoxypyridin-3-yl)imidazo[1,2-a]pyridine-3-carboxylate (**1f**)*

76.3% yield, mp 159-161 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.43 (s, 1H, NH), 9.35 (s, 1H, Ar-H), 8.38 (d, *J* = 2.3 Hz, 1H, Ar-H), 8.32 (s, 1H, Ar-H), 7.97 – 7.74 (m, 4H, Ar-H), 7.65 – 7.54 (m, 1H, Ar-H), 7.24 (td, *J* = 1.9, 8.5 Hz, 1H, Ar-H), 4.40 (q, *J* = 7.1 Hz, 2H, CH_2), 3.70 (s, 3H, CH_3), 1.38 (t, *J* = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO) δ 165.6 (dd, *J* = 12.2, 252.9 Hz), 160.3, 159.8 (dd, *J* = 13.3, 256.1 Hz), 158.1, 147.4, 142.7, 141.9, 133.7, 132.3 (d, *J* = 10.6 Hz), 128.0, 126.4, 125.4 (dd, *J* = 3.5, 14.2 Hz), 124.6, 124.5, 120.5, 118.2, 116.2, 112.4 (dd, *J* = 22.4, 3.5 Hz), 106.3 (t, *J* = 26.1 Hz), 60.8, 54.0, 14.7. HRMS: m/z 489.1022 $[M+H]^+$.

*4.1.21. 6-(5-(2,4-difluorophenylsulfonamido)-6-methoxypyridin-3-yl)imidazo[1,2-a]pyridine-3-carboxylic acid (**1g**)*

1f was dissolved in EtOH-H_2O-NaOH (1.5 mol/L) and refluxed for 0.5 h, then acidified to pH 4-5 with HCl (2 mol/L) to afford white solid precipitate, following by wash and dry, to afford **1g**. 94.0% yield, mp 217-219 ºC. ^{1}H NMR (400 MHz, DMSO) δ 13.20 (s, 1H), 10.42 (s, 1H, NH), 9.42 (s, 1H, Ar-H), 8.38 (s, 1H, Ar-H), 8.29 (s, 1H, Ar-H), 7.91-7.69 (m, 4H, Ar-H), 7.59 (t, *J* = 9.2 Hz, 1H, Ar-H), 7.23 (t, *J* = 8.4 Hz, 1H, Ar-H), 3.69 (s, 3H, CH_3).^{13}C NMR (100 MHz, DMSO) δ 167.4, 165.6 (dd, J = 11.6, 252.5 Hz), 159.9 (dd, *J* = 13.4, 270.9 Hz), 158.1, 147.4, 142.7, 141.9, 133.7, 132.4 (d, *J* = 10.8 Hz), 127.8, 126.6, 125.4 (dd, *J* = 3.5, 14.2 Hz), 124.7, 124.3, 120.5, 118.2, 116.9, 112.4 (dd, *J* = 3.5, 22.4 Hz), 106.3(t, J = 25.8 Hz), 54.0. HRMS: m/z

461.0727 $[M+H]^+$.

*4.1.22.6-(5-(2,4-difluorophenylsulfonamido)-6-methoxypyridin-3-yl)-N-(2-(dimethylamino)ethyl)imidazo[1,2-a] pyridine-3-carboxamide (**1h**)*

A solution of the **1f** (0.23 g, 0.5 mmol), N,N-dimethylethane-1,2-diamine (0.044 g, 0.5 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 0.14 g, 0.75mmol), N-hydroxybenzotrizole (HOBt, 0.21 g, 0.5 mmol), and N-diisopropylethylamine (DIPEA, 0.26ml, 1.5 mmol) in anhydrous THF (10 ml) was stirred for 24 h. THF was removed under reduced pressure and the residue was purified through a column chromatography on silica with chloroform/methanol (V:V 20:1) as a white solid. 78.9% yield, mp 256-258 ºC. ^{1}H NMR (400 MHz, DMSO) δ 9.62 (s, 1H, NH), 8.60 (t, *J* = 5.3 Hz, 1H, Ar-H), 8.36 (s, 1H, Ar-H), 8.11 (s, 1H, Ar-H), 7.86 (dd, *J* = 8.3, 15.1 Hz, 1H, Ar-H), 7.79 (d, *J* = 9.3 Hz, 1H, Ar-H), 7.77 – 7.66 (m, 2H, Ar-H), 7.46 (t, *J* = 9.0 Hz, 1H, Ar-H), 7.21 (t, *J* = 7.7 Hz, 1H, Ar-H), 3.72 (s, 3H, CH_3), 3.52 (dd, *J* = 5.9, 11.9 Hz, 2H, CH_2), 2.75 (t, *J* = 6.3 Hz, 2H, CH_2), 2.44 (s, 6H, CH_3×2). ^{13}C NMR (100 MHz, DMSO) δ 165.6 (dd, *J* = 12.0, 252.8 Hz), 160.8, 159.7 (dd, *J* = 12.7, 269.2 Hz), 158.2, 145.2, 142.8, 135.8, 133.7, 132.3(d, *J* = 10.7 Hz), 128.4, 126.3 , 125.4 (dd, *J* = 13.5, 4.4 Hz), 125.0 , 124.5, 120.5, 119.0, 117.1, 112.5 (dd, *J* = 3.7, 21.7 Hz), 106.4 (t, *J* = 25.7 Hz), 56.4, 54.0, 43.0, 34.4. HRMS: m/z 531.1631 $[M+H]^+$.

Compounds **1i-m** were synthesized according to the procedure described in **1h**.

*4.1.23.6-(5-(2,4-difluorophenylsulfonamido)-6-methoxypyridin-3-yl)-N-(2-morpholinoethyl)imidazo[1,2-a]pyri dine-3-carboxamide (**1i**)*

74.5% yield, mp 114-116 ºC. ^{1}H NMR (400 MHz, DMSO) δ 9.67 (s, 1H, NH), 8.56 (s, 1H, Ar-H), 8.37 (s, 1H, Ar-H), 8.32 (s, 1H, Ar-H), 7.86 (s, 1H, Ar-H), 7.85 – 7.79 (m, 2H, Ar-H), 7.75 (d, *J* = 9.3 Hz, 1H, Ar-H), 7.57 (t, *J* = 9.1 Hz, 1H, Ar-H), 7.24 (t, *J* = 7.9 Hz, 1H, Ar-H), 3.70 (s, 3H, CH_3), 3.61 (s, 4H, CH_2×2), 3.48 (d, *J* = 6.1 Hz, 2H, CH_2), 2.51 (s, 4H, CH_2×2). ^{13}C NMR (100 MHz, DMSO) δ 165.5 (dd, *J* = 11.3, 252.0 Hz), 160.6, 159.8 (dd, *J* = 13.3, 255.6 Hz), 157.9, 146.4, 142.0, 137.6, 133.0, 132.4 (d, *J* = 10.6 Hz), 126.7(2C), 125.7(dd, *J* = 3.4, 14.7 Hz), 124.8, 123.7, 121.1, 119.1, 117.9, 112.4 (dd, *J* = 3.3, 22.0 Hz), 106.3 (t, *J* = 26.1 Hz), 66.5, 57.9, 53.9, 53.7, 36.2. HRMS: m/z 573.1727 $[M+H]^+$.

4.1.24.

*6-(5-(2,4-difluorophenylsulfonamido)-6-methoxypyridin-3-yl)-N-(3-morpholinopropyl)imidazo[1,2-a]pyridine-3 -carboxamide (**1j**)*

76.0% yield, mp 100-102 ºC. ^{1}H NMR (400 MHz, DMSO) δ 9.67 (s, 1H, NH), 8.58 (t, *J* = 5.2 Hz, 1H, Ar-H), 8.35 (d, *J* = 8.2 Hz, 1H, Ar-H), 8.31 (d, *J* = 1.9 Hz, 1H, Ar-H), 7.88 – 7.79 (m, 2H, Ar-H), 7.77 –7.68 (m, 1H, Ar-H), 7.62 – 7.52 (m, 1H, Ar-H), 7.28 – 7.20 (m, 1H, Ar-H), 3.69 (s, 3H, CH_3), 3.60 (s, 4H, CH_2×2), 3.35 (dt, *J* = 6.5, 15.4 Hz, 4H, CH_2×2), 2.44 (d, *J* = 6.4 Hz, 4H, CH_2×2), 1.83 - 1.68 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO) δ 165.4 (dd, *J* = 11.9, 252.1 Hz), 160.6, 159.8 (dd, *J* = 12.9, 255.3 Hz), 157.9, 146.3, 141.7, 137.5, 132.7, 132.4 (d, *J* = 10.6 Hz), 126.7, 126.6, 125.8 (dd, *J* = 4.1, 15.1 Hz), 124.7, 123.7, 121.5, 119.1,

117.9, 112.3 (dd, J = 3.3, 22.2 Hz), 106.3 (t, *J* = 26.2 Hz), 66.4, 56.2, 53.9, 53.6, 37.3, 26.4. HRMS: m/z 587.1865 $[M+H]^+$.

*4.1.25.1-(6-(5-(2,4-difluorophenylsulfonamido)-6-methoxypyridin-3-yl)imidazo[1,2-a]pyridine-3-carbonyl)piperidin-4-yl benzoate (**1k**)*

67.2% yield, mp 129-131 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.42 (s, 1H, NH), 9.06 (s, 1H, Ar-H), 8.37 (d, *J* = 2.3 Hz, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 8.03 (d, *J* = 1.2 Hz, 1H, Ar-H), 7.91 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.82 (dt, *J* = 6.9, 14.8 Hz, 2H, Ar-H), 7.75 (dd, *J* = 1.7, 9.4 Hz, 1H, Ar-H), 7.68 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.58 (dt, *J* = 5.0, 15.3 Hz, 3H, Ar-H), 7.24 (td, *J* = 2.1, 8.5 Hz, 1H, Ar-H), 5.38 – 5.24 (m, 1H, CH), 4.12 – 3.98 (m, 2H, CH_2), 3.87 – 3.73 (m, 2H, CH_2), 3.69 (s, 3H, CH_3), 2.20 – 2.06 (m, 2H, CH_2), 1.88 (ddd, *J* = 5.2, 8.7, 11.4 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO) δ 165.6 (dd, *J* = 11.5, 252.6 Hz), 165.5, 160.5, 159.8 (dd, *J* = 12.9, 255.9 Hz), 158.1, 146.1, 142.7, 137.6, 134.0, 133.8, 132.4 (d, *J* = 10.5 Hz), 130.4, 129.7, 129.2, 126.8, 125.5 (dd, *J* = 14.2, 3.4 Hz), 125.3, 123.1, 120.4, 118.5, 117.8, 112.4 (dd, *J* = 3.2, 22.2 Hz), 106.32 (t, *J* = 25.8 Hz), 70.3, 53.9, 30.9, 25.4. HRMS: m/z 648.1742 $[M+H]^+$.

4.1.26.

*1-(6-(5-(2,4-difluorophenylsulfonamido)-6-methoxypyridin-3-yl)imidazo[1,2-a]pyridine-3-carbonyl)piperidin-4-yl benzoate (**1l**)*

73.2% yield, mp 89-91 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.39 (s, 1H, NH), 9.02 (s, 1H, Ar-H), 8.36 (d, *J* = 2.3 Hz, 1H, Ar-H), 8.02 (s, 1H, Ar-H), 7.89 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.84 – 7.76 (m, 2H, Ar-H), 7.73 (dd, *J* = 1.7, 9.4 Hz, 1H, Ar-H), 7.63 – 7.54 (m, 1H, Ar-H), 7.23 (td, *J* = 2.1, 8.5 Hz, 1H, Ar-H), 4.85 (d, *J* = 3.5 Hz, 1H, Ar-H), 4.08 (dt, *J* = 3.9, 9.1 Hz, 2H, CH_2), 3.82 (d, *J* = 3.5 Hz, 1H, CH), 3.67 (s, 3H, CH_3), 3.52 – 3.37 (m, 2H, CH_2), 1.91 – 1.80 (m, 2H, CH_2), 1.57 – 1.42 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO) δ165.6 (dd, *J* = 11.2, 252.2 Hz), 160.2, 159.8 (dd, *J* = 13.3, 256.0 Hz), 158.0, 146.0, 143.3, 137.4, 134.0 , 132.4 (d, *J* = 10.4 Hz), 126.8, 126.7, 125.5 (dd, *J* = 3.9, 14.6 Hz), 125.2, 123.0, 120.4, 118.6, 117.8, 112.4 (dd, *J* = 2.9, 22.2 Hz), 106.32 (t, *J* = 26.3 Hz), 66.0, 53.9, 34.7. HRMS: m/z 544.1457 $[M+H]^+$.

4.1.27.

*6-(5-(2,4-difluorophenylsulfonamido)-6-methoxypyridin-3-yl)-N-(pyridin-4-ylmethyl)imidazo[1,2-a]pyridine-3-carboxamide (**1m**)*

69.5% yield, mp 91-93 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.43 (s, 1H, NH), 9.64 (s, 1H, Ar-H), 9.54 (t, *J* = 5.8 Hz, 1H, Ar-H), 8.84 (d, *J* = 6.1 Hz, 2H, Ar-H), 8.60 (s, 1H, Ar-H), 8.36 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.93 (td, *J* = 3.9, 11.5 Hz, 4H, Ar-H), 7.87 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.79 (dt, *J* = 7.5, 14.9 Hz, 1H, Ar-H), 7.64 – 7.53 (m, 1H, Ar-H), 7.19 (dd, *J* = 5.3, 11.6 Hz, 1H, Ar-H), 4.81 (d, *J* = 5.6 Hz, 2H, CH2), 3.68 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO) δ 165.6 (dd, *J* = 11.3, 252.0 Hz), 160.6, 159.8 (dd, *J* = 13.8, 255.6 Hz), 158.1, 144.8, 143.1 (2C), 142.7, 135.1, 133.5, 132.4 (d, *J* = 10.8 Hz), 129.2, 126.1, 125.4 (d, *J* = 3.7 Hz), 125.3, 125.1 (2C), 124.9, 120.5, 118.8, 116.7, 112.4 (dd, *J* = 22.3, 3.2 Hz), 106.6, 106.3 (t, *J* = 25.7 Hz), 54.0, 42.2. HRMS: m/z 551.1317 $[M+H]^+$.

Compounds **10a-g** were synthesized according to the procedure described in **1a**.

*4.1.28. ethyl 2-(6-(5-(2,4-difluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)acetate (**10a**)*

81.2% yield, mp 208-210 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.37 (s, 1H, H), 8.45 (d, *J* = 1.8 Hz, 1H, Ar-H), 8.42 (s, 1H, Ar-H), 8.28 (s, 1H, Ar-H), 8.15 (dd, *J* = 8.4, 1.4 Hz, 1H, Ar-H), 7.98 (d, *J* = 1.7 Hz, 1H, Ar-H), 7.82 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.79 – 7.75 (m, 1H, Ar-H), 7.59 (m, 1H, Ar-H), 7.22 (m, 1H, Ar-H), 4.87 (s, 2H, CH_2), 4.20 (q, *J* = 7.0 Hz, 2H, CH_2), 3.68 (s, 3H, CH_3), 1.24 (t, *J* = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO) δ 168.36, 165.6 (dd, *J* = 11.6, 252.6 Hz), 160.5, 159.8 (dd, *J* = 13.5, 256.0 Hz), 158.1, 148.6, 147.8, 143.0, 135.6, 134.3, 133.3, 132.3 (d, *J* = 10.8 Hz), 128.8, 125.5 (dd, *J* = 3.8, 14.3 Hz), 123.5, 122.2, 120.3, 112.4 (dd, *J* = 3.2, 22.3 Hz), 106.3 (t, *J* = 25.9 Hz), 61.8, 54.0, 47.8, 14.5. HRMS: m/z 531.1159 $[M+H]^+$.

*4.1.29.2,4-difluoro-N-(2-methoxy-5-(3-(3-morpholinopropyl)-4-oxo-3,4-dihydroquinazolin-6-yl)pyridin-3-yl)benzenesulfonamide (**10b**)*

77.8% yield, mp 179-181 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.37(s, 1H, NH), 8.55 (s, 1H, Ar-H), 8.52 – 8.37 (m, 2H, Ar-H), 8.09 (d, *J* = 2.1 Hz, 1H, Ar-H), 8.06 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.83 – 7.68 (m, 2H, Ar-H), 7.64 – 7.50 (m, 1H, Ar-H), 7.20 (dd, *J* = 5.2, 11.9 Hz, 1H, Ar-H), 3.63 (s, 3H, CH_3), 3.62 – 3.53 (m, 6H, CH_2×3), 2.48 – 2.32 (m, 6H, CH_2×3), 1.86 (p, *J* = 6.8Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO) δ165.4 (dd, *J* = 11.8, 252.2 Hz), 159.9, 159.8 (dd, *J* = 13.3, 255.9 Hz), 158.3, 155.8, 148.9, 142.9, 135.2, 133.8, 132.3 (d, *J* = 10.1 Hz), 131.1, 129.4, 128.8, 125.8 (dd, *J* = 3.1, 14.5 Hz), 120.5 (2C), 115.7, 112.4 (dd, *J* = 3.0, 22.0 Hz), 106.2 (t, *J* = 25.7 Hz), 66.5, 56.4, 53.8, 53.7, 26.8, 25.8. HRMS: m/z 571.1905 $[M]^+$.

*4.1.30.N-(5-(3-(3-(1H-pyrazol-1-yl)propyl)-4-oxo-3,4-dihydroquinazolin-6-yl)-2-methoxypyridin-3-yl)-2,4-difluorobenzenesulfonamide (**10c**)*

80.2% yield, mp 182-184 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.37 (s, 1H, H), 8.43 (d, *J* = 2.2 Hz, 1H, Ar-H), 8.39 (s, 1H, Ar-H), 8.28 (d, *J* = 2.0 Hz, 1H, Ar-H), 8.10 (dd, *J* = 2.1, 8.5 Hz, 1H, Ar-H), 7.97 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.84 – 7.74 (m, 3H, Ar-H), 7.64 – 7.54 (m, 1H, Ar-H), 7.45 (d, *J* = 1.3 Hz, 1H, Ar-H), 7.23 (td, *J* = 2.0, 8.5 Hz, 1H, Ar-H), 6.24 (t, *J* = 1.9 Hz, 1H, Ar-H), 4.22 (t, *J* = 6.9 Hz, 2H, CH_2), 4.03 (t, *J* = 6.9 Hz, 2H, CH_2), 3.68 (s, 3H, CH_3), 2.26 (p, *J* = 6.9 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO) δ165.6 (dd, *J* = 11.1, 252.3 Hz), 160.7, 159.8 (dd, *J* = 13.4, 256.0 Hz), 158.0, 148.7, 147.8, 142.8, 139.1, 135.2, 134.2, 132.8, 132.3 (d, *J* = 11.4 Hz), 130.3, 128.9, 128.6, 125.5 (dd, *J* = 3.5, 14.4 Hz), 123.5, 122.5, 120.3, 112.4 (dd, *J* = 3.3, 21.9 Hz), 106.3 (d, *J* = 26.2 Hz), 105.6, 54.0, 49.0, 44.4, 30.0. HRMS: m/z 553.1464 $[M+H]^+$.

*4.1.31.N-(5-(3-(3-(1H-imidazol-1-yl)propyl)-4-oxo-3,4-dihydroquinazolin-6-yl)-2-methoxypyridin-3-yl)-2,4-difluorobenzenesulfonamide (**10d**)*

75.8% yield, mp 191-193 ºC. ^{1}H NMR (400 MHz, DMSO) δ 8.40 (d, *J* = 2.3 Hz, 1H, Ar-H), 8.38 (s, 1H, Ar-H), 8.28 (d, *J* = 2.1 Hz, 1H, Ar-H), 8.10 (dd, *J* = 8.5, 2.2 Hz, 1H, Ar-H), 7.95 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.83 – 7.71 (m, 3H, Ar-H), 7.61 – 7.52 (m, 1H, Ar-H), 7.26 (s, 1H, Ar-H), 7.22 (td, *J* = 2.2, 8.6 Hz, 1H, Ar-H), 6.93

(s, 1H, Ar-H), 4.08 (t, *J* = 7.1 Hz, 2H, CH_2), 4.02 (t, *J* = 6.9 Hz, 2H, CH_2), 3.68 (s, 3H, CH_3), 2.30 – 2.13 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO) δ165.4 (dd, *J* = 10.9, 251.7 Hz), 160.7, 159.8 (d, *J* = 10.5, 253.5 Hz), 158.0, 148.5, 147.8, 142.3, 137.6, 135.4, 133.6, 132.8, 132.3 (d, *J* = 11.2 Hz), 128.9, 128.6, 128.5, 125.7 (dd, *J* = 2.9, 14.6 Hz), 123.5, 122.5, 121.0, 119.8, 112.3 (dd, *J* = 3.3, 21.9 Hz), 106.3 (t, *J* = 26.5 Hz), 53.9, 44.2, 44.1, 30.6. HRMS: m/z 553.1474 $[M+H]^+$.

*4.1.32.2,4-difluoro-N-(2-methoxy-5-(4-oxo-3-(3-(2-oxopyridin-1(2H)-yl)propyl)-3,4-dihydroquinazolin-6-yl)pyridin-3-yl)benzenesulfonamide (**10e**)*

74.2% yield, mp 118-120 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.39 (s, 1H, NH), 8.46 (s, 1H, Ar-H), 8.42 (d, *J* = 2.2 Hz, 1H, Ar-H), 8.28 (d, *J* = 1.9 Hz, 1H, Ar-H), 8.10 (dd, *J* = 8.5, 2.1 Hz, 1H, Ar-H), 7.96 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.83 – 7.75 (m, 2H, Ar-H), 7.73 (dd, *J* = 1.5, 6.7 Hz, 1H, Ar-H), 7.63 – 7.54 (m, 1H, Ar-H), 7.40 (ddd, *J* = 1.9, 6.6, 8.8 Hz, 1H, Ar-H), 7.22 (td, *J* = 1.9, 8.6 Hz, 1H, Ar-H), 6.38 (d, *J* = 9.0 Hz, 1H, Ar-H), 6.22 (t, *J* = 6.2 Hz, 1H, Ar-H), 4.06 (t, *J* = 6.8 Hz, 2H, CH_2), 3.98 (t, *J* = 7.1 Hz, 2H, CH_2), 3.68 (s, 3H, CH_3), 2.19 – 2.05 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO) δ 165.6 (dd, *J* = 11.8, 252.6 Hz), 162.8, 161.9, 160.6, 159.8 (d, *J* = 13.9, 252.3 Hz), 158.0, 148.6, 147.8, 142.7, 140.3, 139.4, 135.2, 134.1, 132.7, 132.3 (d, *J* = 11.0 Hz), 128.9, 128.6, 125.5 (dd, *J* = 3.7, 14.1 Hz), 123.5, 122.5, 120.3, 120.1, 112.3 (dd, *J* = 3.5, 22.2 Hz), 106.3 (t, *J* = 26.1 Hz), 105.9, 53.9, 46.5, 44.3, 29.1. HRMS: m/z 580.1477 $[M+H]^+$.

*4.1.33. 4-difluoro-N-(2-methoxy-5-(3-(4-morpholino-4-oxobutyl)-4-oxo-3,4-dihydroquinazolin-6-yl)pyridin-3-yl) benzenesulfonamide (**10f**)*

80.1% yield, mp 113-115 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.36 (s, 1H, NH), 8.43 (d, *J* = 2.3 Hz, 1H, Ar-H), 8.39 (s, 1H, Ar-H), 8.28 (d, *J* = 2.1 Hz, 1H, Ar-H), 8.10 (dd, *J* = 2.2, 8.5 Hz, 1H, Ar-H), 7.96 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.83 – 7.73 (m, 2H, Ar-H), 7.64 – 7.54 (m, 1H, Ar-H), 7.22 (td, *J* = 2.0, 8.4 Hz, 1H, Ar-H), 4.04 (t, *J* = 6.8 Hz, 2H, CH_2), 3.68 (s, 3H, CH_3), 3.60 – 3.53 (m, 2H, CH_2), 3.53 – 3.48 (m, 2H, CH_2), 3.41 (dd, *J* = 4.9, 11.4 Hz, 4H, CH_2×2), 2.41 (t, *J* = 7.2 Hz, 2H, CH_2), 1.98 (p, *J* = 7.0 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO) δ 170.4, 165.6 (dd, *J* = 11.5, 252.4 Hz), 160.6, 159.8 (dd, *J* = 13.6, 256.3 Hz), 158.0, 148.7, 147.8, 142.8, 135.2, 134.1, 132.7, 132.3 (d, *J* = 11.0 Hz), 128.9, 128.6, 125.4 (dd, *J* = 3.4, 14.6 Hz), 123.6, 122.5, 120.3, 112.3 (dd, *J* = 3.2, 22.3 Hz), 106.3 (t, *J* = 25.8 Hz), 55.4, 53.9, 46.3, 45.8, 41.9, 29.5. HRMS: m/z 600.1741 $[M+H]^+$.

*4.1.34.4-difluoro-N-(2-methoxy-5-(3-(5-morpholino-5-oxopentyl)-4-oxo-3,4-dihydroquinazolin-6-yl)pyridin-3-yl) benzenesulfonamide (**10g**)*

72.7% yield, mp 136-138 ºC. ^{1}H NMR (400 MHz, DMSO) δ 10.36 (s, 1H, NH), 8.44 (s, 1H, Ar-H), 8.43 (d, *J* = 2.2 Hz, 1H, Ar-H), 8.28 (d, *J* = 1.8 Hz, 1H, Ar-H), 8.10 (dd, *J* = 2.0, 8.5 Hz, 1H, Ar-H), 7.97 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.85 – 7.73 (m, 2H, Ar-H), 7.65 – 7.52 (m, 1H, Ar-H), 7.31 – 7.15 (m, 1H, Ar-H), 4.03 (t, *J* = 6.8 Hz, 2H, CH_2), 3.68 (s, 3H, CH_3), 3.52 (m, 4H, CH_2×2), 3.42 (m, 4H, CH_2×2), 2.36 (t, *J* = 7.3 Hz, 2H, CH_2), 1.81 – 1.66 (m, 2H, CH_2), 1.54 (dd, *J* = 7.5, 14.7 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO) δ 171.0, 165.6 (dd, *J* = 12.5, 252.6 Hz), 160.5, 159.8 (dd, *J* = 14.6, 255.9 Hz), 158.0, 148.7, 147.8, 142.8, 135.3, 134.1, 132.8,

132.3 (d, *J* = 10.6 Hz), 128.9, 128.6, 125.4 (dd, *J* = 3.8, 14.4 Hz), 123.5, 122.4, 120.3, 112.3 (dd, *J* = 3.3, 22.1 Hz), 106.2 (t, *J* = 26.0 Hz), 53.9, 46.3, 45.8, 41.9, 31.9, 28.8, 22.1. HRMS: m/z 614.1896 $[M+H]^+$.

4.2 Biological assay methods

4.2.1. Cell culture

HCT-116, A549, SK-HEP-1, SNU638, MDA-MB-231 cells and HUVEC were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were grown in DMEM (SNU-638, HUVEC and SK-HEP-1) or RPMI1640 (HCT-116, A549 and MDA-MB-231 cells) supplemented with 10% FBS and antibiotics-antimycotics (PSF; 100 units/mL penicillin G sodium, 100 μg/mL streptomycin, and 250 ng/mL amphotericin B) in a humidified incubator containing 5% CO_2 at 37 °C.

4.2.2. Antiproliferative activity

The cell viability was evaluated using the sulforhodamine B (SRB) cellular protein-staining method with minor modifications. Briefly, cells were treated with various concentrations of compounds in 96-well plates and incubated at 37 ºC in a humidified atmosphere with 5% CO_2 for 72 h. After treatment, the cells were fixed with 10% TCA solution, and cell viability was determined with a sulforhodamine B (SRB) assay. The percentage of cell-growth inhibition was calculated using the formulae below. The IC_{50} values were calculated using a non-linear regression analysis (percent growth versus concentration).

$$\%\ \text{growth inhibition} = 100 - 100 \times (OD_{sample} - OD_{Day0}) / (OD_{neg\ control} - OD_{Day0})$$

4.2.3. PI3K enzymatic activity assay

The PI3K kinase assay was measured by PI3 Kinase Activity/Inhibitor Assay Kit (EMD Millipore, #17-493) following the manufacturer's protocols.

4.2.4. Molecular docking studies

The crystal structure of PI3Kα (PDB entry code: 4ZOP) in complex with 4Q2 was used for molecular modeling. The AutoDock 4.2 was used to perform docking calculations. Polar hydrogens and partial charges were added for PI3Kα protein using the Kollman United Atom charges with Sybyl 6.9.1, and energy minimization was made employing both steepest descent and conjugate gradients protocols. Atomic solvation parameters and fragmental volumes for the proteins were assigned using the addsol utility in the AutoDock 4.2 program. A 60×60×60 Å grid box with a grid spacing of 0.375 Å was generated for the receptor. Affinity grid fields were generated using the auxiliary program AutoGrid 4.0. The Lamarckian genetic algorithm (LGA) was used to find the appropriate binding positions, orientations, and conformations of the ligands. The optimized AutoDocking parameters are as follows: the maximum number of energy evaluations was increased to 25,000,000 per run; the iterations of Solis & Wets local search was 3000; the number of individuals in population was 300 and the number of generations was 100. Results differing by less than 2 Å in a positional root mean square deviation (RMSD) were clustered together. In each group, the lowest binding energy

configuration with the highest % frequency was selected as the group representative. All other parameters were maintained as default. Accelrys Discovery Studio Visualizer 4.0 was used for graphic display.

4.2.5. Western blot analysis

Preparation of whole-cell protein lysates and western blot analysis were described previously [36]. Total cell lysates were prepared in RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS). The protein concentration was determined, and equal amounts of protein samples were subjected to 10% SDSPAGE. Separated proteins were transferred to PVDF membranes (Millipore, Bedford, MA, USA) and probed with the indicated antibodies. Exposures were obtained using ImageQuant LAS 4000 biomolecular imager (GE Healthcare).

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Figure legends

Fig.1. RTK/PI3K/Akt signaling cascade in cancer.

Fig.2. The structures of PI3K inhibitors.

Fig.3. The design strategy based on HS-173 and GSK2126458

Fig.4. Docking mode of **1i** and HS-173 into protein crystal structure of PI3Kα. (A) Key interactions of HS-173 in the active site of PI3Kα. (B) Key interactions of compound **1i** in the active site of PI3Kα. (C) The binding pose of **1i** and HS-173 in the active site of PI3Kα. **1i** was highlighted with yellow. (D) Binding modes of **1i** and HS-173 in the binding pocket of PI3Kα. The ligands are shown in stick model, while the proteins are shown in surface model for better visualization in the three dimensional combination models. **1i** was highlighted with yellow.

Fig.5. Effect of compound **1i** on PI3K pathway in HCT-116 cells. (A) Compound **1i** decreased phospho-Akt (S473) in a dose-dependent manner. (B) Compound **1i** is more effective than HS-173 in reducing the levels of phospho-Akt (S473). Cells were treated with 0.1% DMSO, 0.5μM compound **1i** or HS-173 for 24 h.

Scheme 1. (a) benzenesulfonyl chloride, pyridine, rt, 24 h; (b) bis(pinacolato)diborane, $Pd(dppf)_2Cl_2$, AcOK, DMF, 100 ºC, 8 h; (c) $Pd(dppf)_2Cl_2$, Cs_2CO_3, DMF/ H_2O, 90 ºC, 8 h; (d) bis(pinacolato)diborane, $Pd(dppf)_2Cl_2$, AcOK, DMF, 100 ºC, 8 h; (e) $Pd(dppf)_2Cl_2$, Cs_2CO_3, DMF, 90ºC, 8h; (f) Fe, HCl/H_2O, 80 ºC , 4 h; (g) sulfonyl chloride, pyridine, rt, 24 h; (h) NaOH/Ethanol/ H_2O, 80 ºC, 0.5 h, then HCl (2 mol/L); (i) DIPEA, HOBt, EDCI, THF,12 h, rt.

Scheme 2. (a) chlorinated compounds, K_2CO_3, DMF, 60 ºC, 12 h; (b) **3b**, $Pd(dppf)_2Cl_2$, Cs_2CO_3, DMF/ H_2O, 90ºC, 8h.

Highlights

- Imidazo [1, 2-a] pyridine and quinazolin-4(3H)-one derivatives were synthesized.
- Their antiproliferative activities against cancer cell lines were evaluated.
- 1i exhibited an IC_{50} value of 10nM against HCT116 and SNU638 cells.
- The compounds 1b, 1i and 10b were evaluated for their PI3K inhibitory activity and selectivity.
- Compound **1i** is considered as a potent PI3Kα inhibitor with IC_{50} of 0.5nM.