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Discovery of (*S*)-2-amino-*N*-(5-(6-chloro-5-(3-methylphenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (CHMFL-PI3KD-317) as a potent and selective phosphoinositide 3-kinase delta (PI3Kδ) inhibitor

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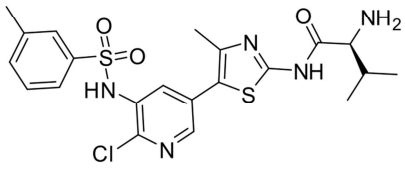
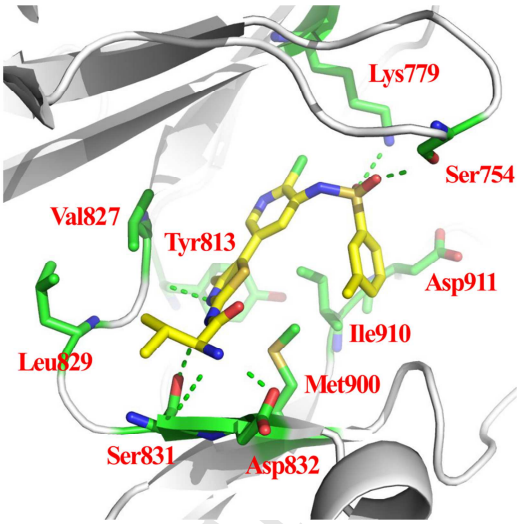


Compound 15i (CHMFL-PI3KD-317)

	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
Biochemical IC_{50} (nM)	62.6	354.7	284	6.0
Cellular EC_{50} (nM)	>3000	>3000	>3000	4.3



Discovery of (*S*)-2-amino-*N*-(5-(6-chloro-5-(3-methylphenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (CHMFL-PI3KD-317) as a Potent and Selective Phosphoinositide 3-kinase Delta (PI3Kδ) Inhibitor

Xiaofei Liang[1,3,8], *Feng Li*[1,2,8], *Cheng Chen*[1,2,8], *Zongru Jiang*[1,2,8], *Aoli Wang*[1,3,8], *Xiaochuan Liu*[1,3], *Juan Ge*[4], *Zhenquan Hu*[1,3], *Kailin Yu*[1,3], *Wenliang Wang*[1,2], *Fengming Zou*[1,3], *Qingwang Liu*[4], *Beilei Wang*[1,2], *Li Wang*[1,2], *Shanchun Zhang*[3,5], *Yuxin Wang*[7], *Qingsong Liu*[1,2,3,4,6,7*], *Jing Liu*[1,3,4,7*]

1. High Magnetic Field Laboratory, Key Laboratory of High Magnetic Field and Ion Beam Physical Biology, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, Anhui, 230031, P. R. China
2. University of Science and Technology of China, Hefei, Anhui 230036, P. R. China
3. CHMFL-HCMTC Target Therapy Joint Laboratory, 350 Shushanhu Road, Hefei, Anhui 230031, P. R. China
4. Precision Targeted Therapy Discovery Center, Institute of Technology Innovation, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, Anhui 230088, P. R. China
5. Hefei Cosource Medicine Technology Co. LTD., 358 Ganquan Road, Hefei, Anhui 230031, P. R. China
6. Institute of Physical Science and Information Technology, Anhui University, Hefei, Anhui 230601, P. R. China

7. Precision Medicine Research Laboratory of Anhui Province, Hefei, Anhui, 230088, P. R. China
8. These authors contribute equally

AUTHOR INFORMATION

Corresponding Authors

* E-mail address: qsliu97@hmfl.ac.cn (Q. Liu), E-mail address: jingliu@hmfl.ac.cn (J. Liu).

ABSTRACT

PI3Kδ, which is mainly expressed in leukocytes, plays a critical role in B-cell receptor mediated signaling pathway and has been extensively studied as a drug discovery target for B cell malignances such as AML, CLL etc. In this manuscript, we report the discovery, SAR optimization and pharmacological evaluation of a novel series of aminothiazole-pyridine containing PI3Kδ inhibitors. Among them compound **15i** (CHMFL-PI3KD-317) displays an IC_{50} of 6 nM against PI3Kδ in the ADP-Glo biochemical assays. It also exhibits over 10-1500 fold selectivity over other class I, II and III PIKK family isoforms. In addition, in the cellular context, **15i** can selectively and potently inhibit PI3Kδ mediated phosphorylation of Akt T308 but not PI3Kα, β, γ mediated Akt phosphorylation. **15i** also exhibits an excellent selectivity profile in the protein kinases including 468 kinases/mutants at the concentration of 1 μM. **15i** has acceptable pharmacokinetic properties and can dose-dependently inhibit the tumor growth of AML cell line MOLM14 inoculated xenograft mouse model. The high selectivity and potency makes **15i** a potential valuable addition to the current PI3Kδ armory.

Keywords

PI3Kδ, Kinase inhibitor, Acute myeloid leukemia, Structure-activity relationship

Abbreviations used

PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PI4K, phosphatidylinositol 4-kinase; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; MCL, mantel cell lymphoma; TGI, tumor growth inhibition; HATU, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]-pyridinium 3-oxid hexafluorophosphate; DIPEA, *N*,*N*-diisopro-pylethylamine; DMF, dimethylformamide; DCM, dichloromethane.

1. Introduction

Phosphoinositide 3-kinase delta (PI3Kδ) is a lipid kinase, which belongs to a large family of phosphoinositide 3-kinases. This family of kinases is classified into four classes (I, II, III and IV) based on their sequence homology. Class I is further divided into class IA and class IB based on their different regulatory subunits and upstream activators[1]. Class IA contains three isoforms (PI3Kα, PI3Kβ and PI3Kδ) with the respective p110 catalytic subunit (p110α, p110β and p110δ) bound to the p85 regulatory subunit[2], which are activated by receptor tyrosine kinases (RTKs). Class IB is activated by G protein-coupled receptors (GPCRs), which consists of PI3Kγ with p110γ bound to p101 or p84[3]. Class I PI3K catalyzes the phosphorylation of phosphatidylinositol 4,5-bisphosphate to produce the cellular secondary messenger phosphatidyinositol 3,4,5-triphosphate, subsequently initiating a cascade of downstream events to modulate a variety of biological processes including cell growth, proliferation, survival and differention[4-7]. Among these, PI3Kα and PI3Kβ are ubiquitously expressed in all of the mammalian tissues while PI3Kδ and PI3Kγ are predominately expressed in leukocytes[8-10]. PI3Kδ has been shown to be involved in B-cell related malignances such as chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), follicular lymphoma (FL) and small lymphocytic lymphoma (SLL). Therefore it has been extensively studied as a therapeutic target in hematologic malignances.

CAL-101 (**1**, Idelalisib, Figure 1) is the first selective PI3Kδ inhibitor that has been approved by FDA for relapsed FL after two lines of therapy, relapsed CLL in combination with rituximab and relapsed SLL after two lines of therapy[11]. More recently, BAY 80-6946 (**2**, Copanlisib), which is a pan-PI3K inhibitor with preferential activity against PI3Kα and PI3Kδ, was approved by FDA for relapsed follicular lymphoma[12]. Several other small molecules that inhibit the PI3Kδ activity, including AMG-319 (**3**)[13], TGR-1202 (**4**)[14-15], GSK-2269557 (**5**)[16] and UCB5857 (**6**, Seletalisib)[17], are currently in clinical trials for the treatment of different cancers or respiratory conditions and psoriasis/Sjögrens syndrome. In this manuscript, we describe the discovery and optimization of a novel aminothiazole scaffold of PI3Kδ selective inhibitors and their potential application in the treatment of B-cell malignances.

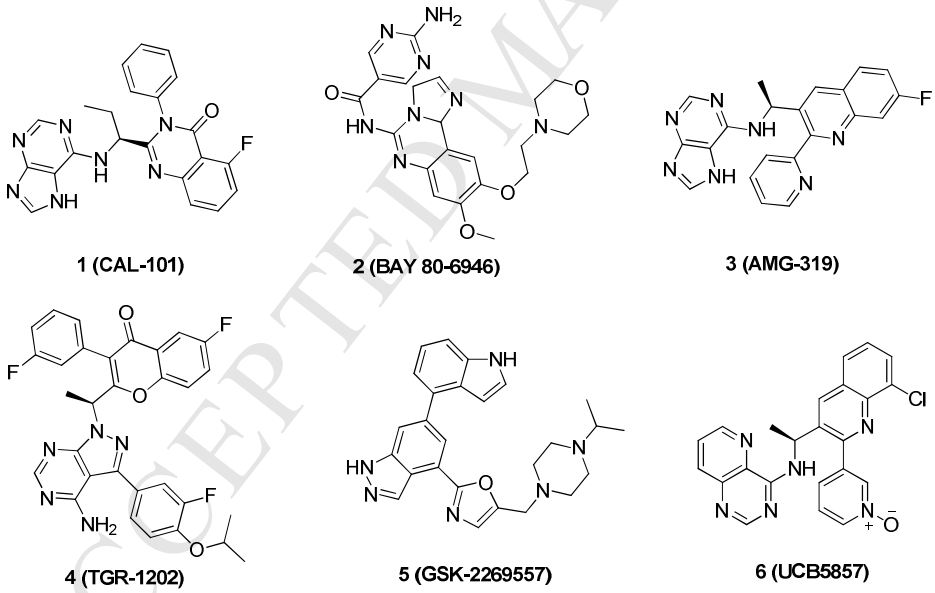


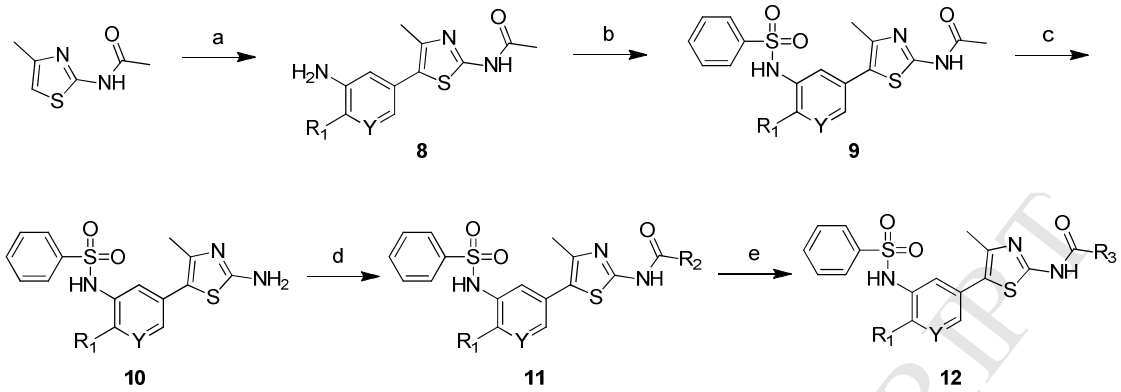
Figure 1. Chemical structures of representative PI3Kδ inhibitors.

2. Results and discussion

2.1. Chemistry

General procedures to synthesize compounds **11a** and **12a-s** were shown in scheme 1 which were similar to methods described previously[18-19]. Heck reaction of aryl bromides and *N*-(4-methylthiazol-2-yl)acetamide provided the intermediates **8a-d**[20-23], which were sulfonated to give **9a-d**[20-24]. The intermediates **9a-d** were then hydrolyzed under acidic condition to yield **10a-e**. Intermediates **11a-t** were obtained from **10a-e** and R_2-substituted carboxylic acid under amide coupling condition. The following *N*-Boc deprotection reaction of intermediates **11b-t** provided the final products **12a-s**.

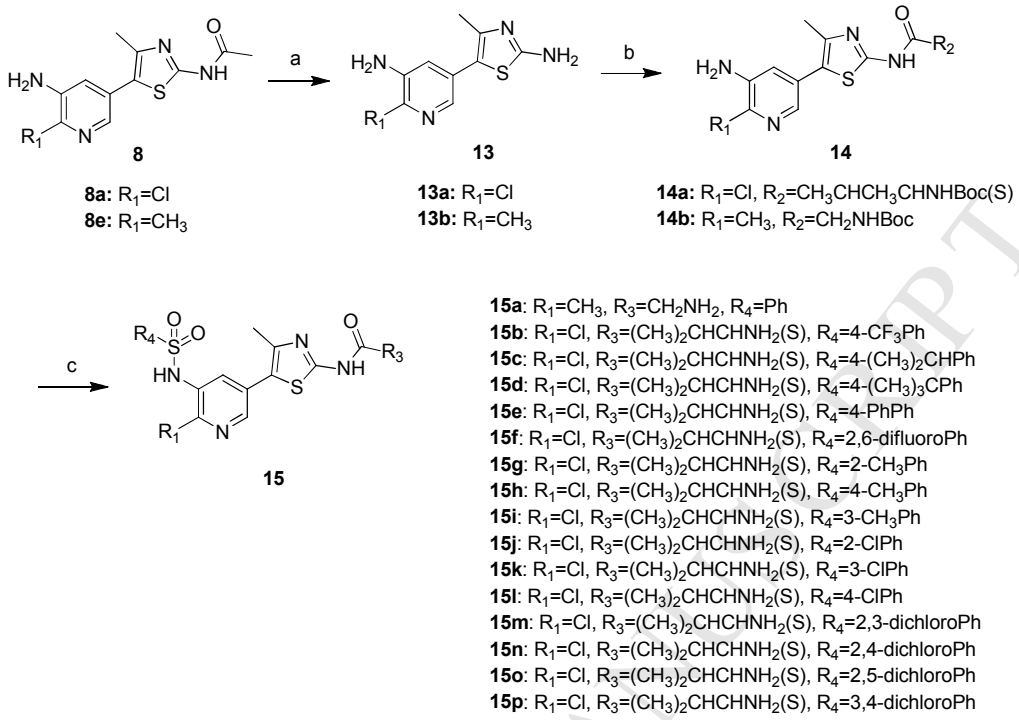
The syntheses of **15a-p** were carried out in 4 steps as depicted in scheme 2. Compounds **8a** and **8e** were hydrolyzed under acidic condition to provide the intermediates **13a-b**, which were coupled with R_2-substituted carboxylic acid to give the intermediates **14a-b**. Then **14a-b** were sulfonated under basic condition followed by the *N*-Boc deprotection to afford the final products **15a-p**.



8a-10a: R_1=Cl, Y=N
8b-10b: R_1=OCH_3, Y=N
8c-10c: R_1=H, Y=N
8d-10d: R_1=Cl, Y=CH
10e: R_1=OH, Y=N
11a: R_1=Cl, Y=N, R_2=CH_2CH_3
11b: R_1=Cl, Y=N, R_2=CH_2NHBoc
11c: R_1=OCH_3, Y=N, R_2=CH_2NHBoc
11d: R_1=OH, Y=N, R_2=CH_2NHBoc
11e: R_1=H, Y=N, R_2=CH_2NHBoc
11f: R_1=Cl, Y=CH, R_2=CH_2NHBoc
11g: R_1=Cl, Y=N, R_2=$CH_3CHNHBoc$(R)
11h: R_1=Cl, Y=N, R_2=$CH_3CHNHBoc$(S)
11i: R_1=Cl, Y=N, R_2=$(CH_3)_2CNHBoc$
11j: R_1=Cl, Y=N, R_2=(cyclpropane)CNHBoc
11k: R_1=Cl, Y=N, R_2=$CH(CH_2OH)NHBoc$(S)
11l: R_1=Cl, Y=N, R_2=$CH_3CH(OH)CHNHBoc$(2S,3R)
11m: R_1=Cl, Y=N, R_2=$CH_3CH_2CHNHBoc$(S)
11n: R_1=Cl, Y=N, R_2=$CH_3CH_2CHNHBoc$(R)
11o: R_1=Cl, Y=N, R_2=$CH_3CHCH_3CHNHBoc$(S)
11p: R_1=Cl, Y=N, R_2=$CH_3CHCH_3CHNHBoc$(R)
11q: R_1=Cl, Y=N, R_2=(cyclpropane)CHNHBoc
11r: R_1=Cl, Y=N, R_2=$(CH_3)_2CHCH_2CHNHBoc$(S)
11s: R_1=Cl, Y=N, R_2=$(CH_3CH_2)CH_3CHCHNHBoc$(S)
11t: R_1=Cl, Y=N, R_2=$PhCH_2CHNHBoc$(S)

12a: R_1=Cl, Y=N, R_3=CH_2NH_2
12b: R_1=OMe, Y=N, R_3=CH_2NH_2
12c: R_1=OH, Y=N, R_3=CH_2NH_2
12d: R_1=H, Y=N, R_3=CH_2NH_2
12e: R_1=Cl, Y=CH, R_3=CH_2NH_2
12f: R_1=Cl, Y=N, R_3=$CHCH_3NH_2$(R)
12g: R_1=Cl, Y=N, R_3=$CHCH_3NH_2$(S)
12h: R_1=Cl, Y=N, R_3=$(CH_3)_2CNH_2$
12i: R_1=Cl, Y=N, R_3=(cyclpropane)CNH_2
12j: R_1=Cl, Y=N, R_3=$CH(CH_2OH)NH_2$(S)
12k: R_1=Cl, Y=N, R_3=$CH_3CH(OH)CHNH_2$(2S,3R)
12l: R_1=Cl, Y=N, R_3=$CH_3CH_2CHNH_2$(S)
12m: R_1=Cl, Y=N, R_3=$CH_3CH_2CHNH_2$(R)
12n: R_1=Cl, Y=N, R_3=$CH_3CHCH_3CHNH_2$(S)
12o: R_1=Cl, Y=N, R_3=$CH_3CHCH_3CHNH_2$(R)
12p: R_1=Cl, Y=N, R_3=(cyclpropane)$CHNH_2$
12q: R_1=Cl, Y=N, R_3=$(CH_3)_2CHCH_2CHNH_2$(S)
12r: R_1=Cl, Y=N, R_3=$(CH_3CH_2)CH_3CHCHNH_2$(S)
12s: R_1=Cl, Y=N, R_3=$PhCH_2CHNH_2$(S)

Scheme 1. Reagents and conditions: (a) aryl bromide, $Pd(OAc)_2$, *t*-Bu_3P, Cs_2CO_3, 130 °C, 14 h; (b) sulfonyl chloride, pyridine, 50 °C, 2 h; (c) for **10a-d**: 6 M HCl, EtOH, 100 °C, 6 h; for **10e**: 6 M HCl, EtOH, 100 °C, 12 h; (d) carboxylic acid, HATU, DIPEA, DMF, rt, 12 h; (e) 4 M HCl in MeOH, MeOH, rt, 2 h.



Scheme 2. Reagents and conditions: (a) 6 M HCl, EtOH, 100 °C, 6 h; (b) carboxylic acid, HATU, DIPEA, DMF, 0 °C to rt, 14 h; (c) i: sulfonyl chloride, pyridine, 50 °C, 6 h; ii: 4 M HCl in MeOH, MeOH, rt, 2 h.

2.2. Structure-activity relationship (SAR)

The aminothiazol-pyridine compound **7** (Figure 2A) has been reported as a multi-target inhibitor against PI3Kα, PI4Kα, PI4Kβ and PIP5Kγ[22], another closely structure compound was also developed as selective phosphatidylinositol 4 kinase IIIβ for antiviral[25]. However, we found that it could also potently inhibit PI3Kδ, PI3Kβ and PI3Kγ (IC_{50} = 1 nM, 1.28 nM and 6 nM respectively). After careful analysis of the molecular modeling results, we envisioned that **7** adopts a flat conformation in the ATP binding pocket and occupies a deeper binding pocket that is potentially accessible by ATP[26-28] (Figure 2B). The aminothiazole forms two hydrogen bonds with Val828 in the hinge binding region[29-30] (Figure 2B). The nitrogen of pyridine forms a

hydrogen bond with the side chain of Tyr813 (Figure 2C). The sulfonamide moiety forms two hydrogen bonds with the side chain of residues Asp911 and Ser754 in the P-loop (Figure 2C). Alignment of PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ revealed that although the structures of these lipid kinases are highly similar in the ATP binding pocket, there are indeed several amino acid residues that are different among them. These residues include Val 827 in PI3Kδ (α, β, δ=Val, γ=Ile), Ser831 (α, β, δ, γ=Ala) and Leu829 (α=Arg, β=Ser, γ=Lys) adjacent to the hinge binding area, which provide potential opportunities to explore these regions to achieve the selectivity (Figure 2B). Based on these analyses, we decided to optimize three regions of the molecule including the central pyridine ring (in blue), the sulfonamide moiety (in cyan) and the acetamide moiety (in magenta) as shown in Figure 2A to obtain a full spectrum of the SAR with protein enzymatic ADP-Glo assay as the primary readout.

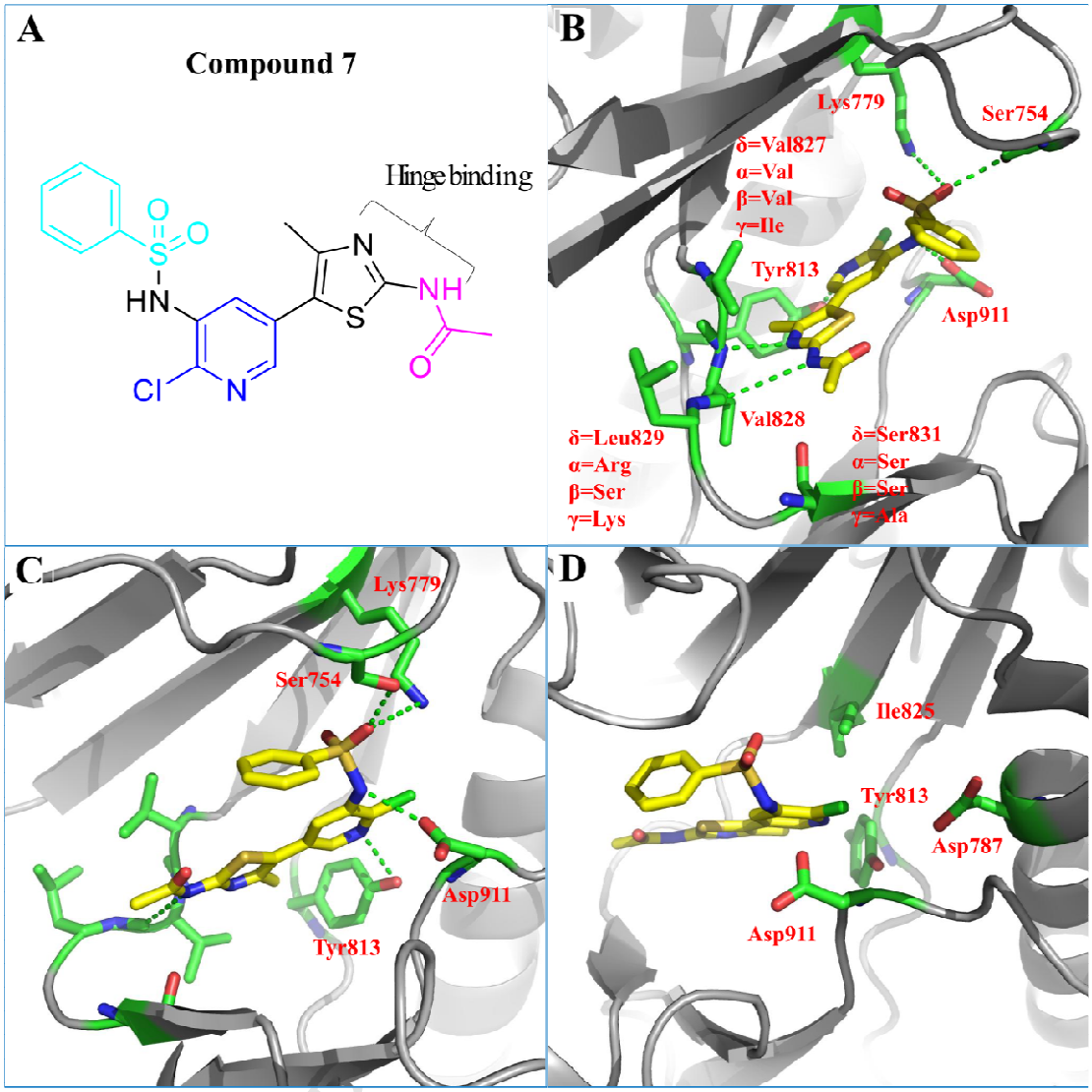
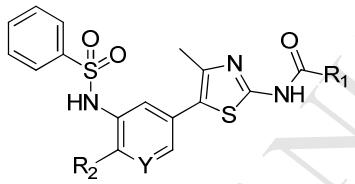


Figure 2. Illustration of the SAR exploration rationale. (A) Structure of compound **7**. (B) - (D) Docking of compound **7** into PI3Kδ kinase (PDB: 5DXU).

The acetyl group of **7** was replaced with either a propionyl group (**11a**) or aminoacetyl group (**12a**), which all led to activity loss against PI3Kδ (Table 1). We then paid attention to the R_2 moiety. The docking results showed that the chloro substituent directed into a affinity pocket, which was formed with the side chains of Tyr813, Ile825, Asp911 and Asp787 residues (Figure 2D). Replacement of the Cl atom with a methoxy group (**12b**) retained the potency against

PI3Kδ and gained an increase in selectivity over PI3Kα, PI3Kβ and PI3Kγ compared to **12a**. Further changes at R_2 position with the hydroxyl group (**12c**), methyl group (**15a**) or hydrogen atom (**12d**) all led to activity loss against PI3Kδ. Switching the nitrogen atom of the pyridine ring in **12a** to carbon (**12e**) at Y position caused loss of potency against PI3Kδ, which reconfirmed the docking results that the pyridine ring forms a critical hydrogen bond with Tyr813[22].

Table 1. SAR results of R_1/R_2/Y modifications (data represents IC_{50} values in nM)[a].



Compd.	R_1	R_2	Y	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
7	Me	Cl	N	2.1	1.28	6	1
11a	Et	Cl	N	2.3	6.6	17	6.8
12a	CH_2NH_2	Cl	N	2.3	17	28	25.8
12b	CH_2NH_2	OMe	N	222	290	303	21
12c	CH_2NH_2	OH	N	1017	1539	236	109.4
15a	CH_2NH_2	Me	N	>10000	8769	>10000	420.4
12d	CH_2NH_2	H	N	604.5	422.1	1590	375.9

12e	CH_2NH_2	Cl	C	1448	>10000	1207	2134

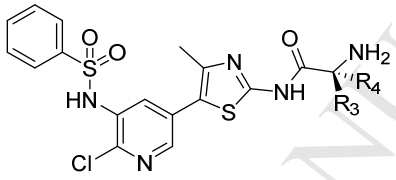
[a]Average of triplet testings.

Because of the sulfonamide, Cl and pyridine moieties are critical for the binding, we then further explored the aminoacetyl moiety (R_3, R_4) and the phenyl group (R_5) (Table 2 and Table 3). Introduction of a methyl group (**12f**, **12g**) to the R_3/R_4 position of **12a** enhanced both inhibitory activity against PI3Kδ and selectivity over other PI3Ks. Unfortunately, the dimethyl substitute (**12h**) at this position resulted in both activity and selectivity loss. Introduction of a cyclopropane ring (**12i**) retained the potency against PI3Kδ and increased the selectivity over PI3Kγ (>500 fold). Installment of a methyl hydroxyl group (**12j**) and 2-hydroxy ethyl substituent (**12k**) group at R_3 both resulted in activity loss against PI3Kδ. Interestingly, introduction of an ethyl group (**12l-12m**) led to both good activity against PI3Kδ and selectivity over other PI3Ks. In addition, the S-isomer configuration (**12l**) displayed better activity against PI3Kδ than the R-isomer configuration (**12m**). Similarly, the S-isomer of isopropyl substituent (**12n**)[23] also displayed stronger potency against PI3Kδ and selectivity over other PI3Ks than the R-isomer (**12o**). The racemic cyclopropyl substituent (**12p**) resulted in slight decrease of the activity against PI3Kδ but better selectivity over PI3Kγ (>100 fold). The isobutane moiety (**12q**) retained the potency against PI3Kδ and selectivity over PI3Ks. However, the 2-methylbutane moiety (**12r**) and larger group at this position such as benzyl group (**12s**) both led to activity loss against PI3Kδ.

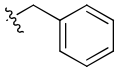
When R_3 and R_4 moieties were fixed as in **12n**, para-substituted benzene derivatives at R_5 position (**15b-15e**) all caused activity loss to PI3Kδ (Table 3). This suggested that the hydrophobic pocket created by Met900, Ile910 and Asp911 in PI3Kδ could only tolerate

medium-size hydrophobic moiety. Introduction of a methyl or chloro substitute to the benzene ring at the 2-position (**15g**, **15j**), 3-position (**15i**, **15k**) and 4-position (**15h**, **15l**) all resulted in similar or slightly better activities against PI3Kδ and much better selectivity over PI3Kγ compared with **12n**. Interestingly, the dichlorobenzene substitutes (**15m-15o**) and difluorobenzene substitute (**15f**) at R_5 retained the potency against PI3Kδ and the selectivity over PI3Kγ except **15p**.

Table 2. SAR results of R_3 and R_4 modifications (data represents IC_{50} values in nM)[a.]

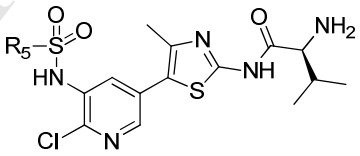


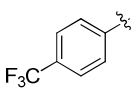
Compd.	R_3	R_4	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
12f	Me	H	7.5	19.5	47	3
12g	H	Me	15.6	27.5	62	2.1
12h	Me	Me	29.5	23.4	31	53.9
12i		-	<10	50	708	1.3
12j		H	8.2	85.6	41	17.4
12k		H	28	57.2	751	751

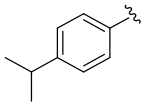
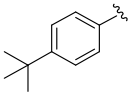
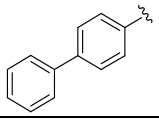
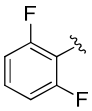
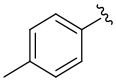
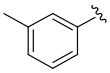
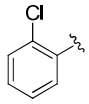
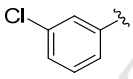
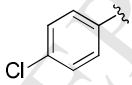
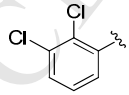
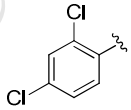
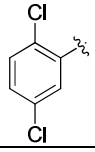
12l	Et	H	41.2	82.1	255.7	1.4
12m	H	Et	66	480.5	408	11.5
12n		H	62.1	98.8	120.5	5.3
12o	H		328.5	228.8	1036	135.2
12p	 (rac)	H	52.8	124.2	2628	20.5
12q		H	33.9	119.3	456.7	8.6
12r		H	75.9	84.2	893.1	100
12s		H	78.2	148.5	358.3	156.5

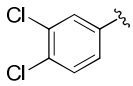
[a]Average of triplet testings

Table 3. SAR results of R_5 modifications (data represents IC_{50} values in nM)[a.]



Compd.	R_5	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
15b		17.7	386	256	87.8

15c		59.8	317.1	602.1	25.6
15d		129.1	766.4	1898	100.4
15e		1224	1761	1591	291.5
15f		105.8	62.5	118.1	6.2
15g		43	243.7	245.5	11.5
15h		22.8	155.9	193.7	3.2
15i		62.6	284	202.7	6.0
15j		14.8	34.7	110	3.3
15k		27.7	307.5	596.3	10
15l		48.3	201.5	146	12.3
15m		53.5	28.4	64	2
15n		19.9	92.3	140	2
15o		24.7	7.3	76	1

15p		73.2	109.3	170	15

[a]Average of triplet testings

2.3. Biochemical and cellular property evaluation

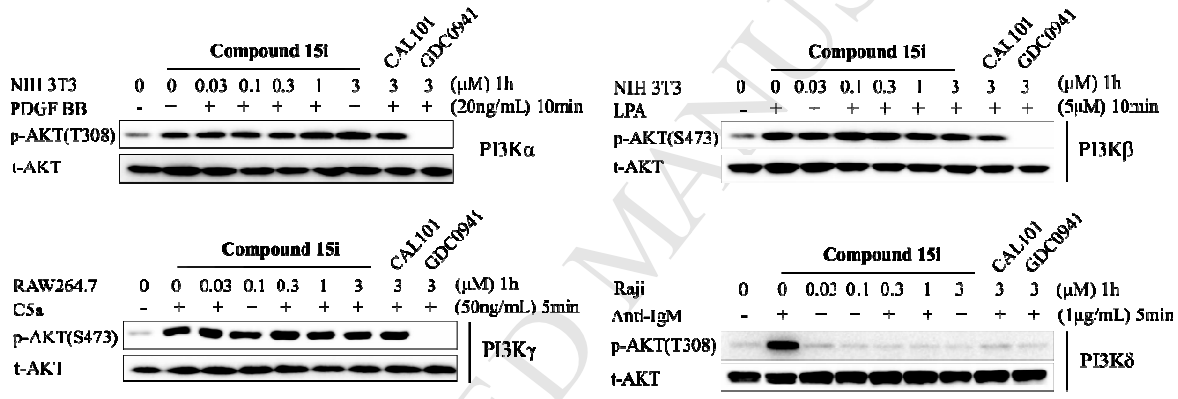
Since a number of compounds displayed good inhibitory activity against PI3Kδ as well as selectivity over other class I PI3K isoforms, we then chose a panel of them (**15g-15i**) for further selectivity testing among class I/II/III and PI4KIII family proteins (Table 4). The selective PI3Kδ inhibitor **1** and pan-PI3K inhibitor GDC-0941 (**16**)[31] were used as control compounds. The results revealed that compounds **15g** and **15h** exhibited selectivity among class I PI3K isoforms and PI4KIII except PI3Kα. Compound **15i** inhibited PI3Kδ with an IC_{50} of 6 nM, meanwhile it exhibited 10-200 fold selectivity over PI3Kα, PI3Kβ, PI3Kγ, VPS34, PI4KIIIA and PI4KIIIB. These data suggested that overall compound **15i** achieved the best selectivity.

Table 4. Activities of a panel of selected PI3Kδ inhibitors against class I/II/III PI3K and PI4KIII (data represents IC_{50} values in nM)[a]

Compd.	P3IKα	P3IKβ	P3IKδ	P I3Kγ	PIK3C2A	PIK3C2B	VPS34	PI4KIIIA	PI4KIIIB
1	391.3	845.5	5.9	67	>10000	>10000	4417	>10000	>10000
15g	43.0	243.7	11.5	245.5	2675	1096	834	130.6	114.5
15h	22.8	155.9	3.23	193.7	1518	247.9	405	163.7	68
15i	62.6	284	6.0	202.7	>10000	882.3	1801.7	574.1	300.2
16	6.5	181	15.7	110.5	6039	900.9	2837	>10000	>10000

[a]Average of triplet testings.

In order to further confirm the selectivity of **15i** among the class I PI3Ks, we then examined its inhibitory efficacy in the cellular background (Figure 3). Upon anti-IgM stimulation, the PI3Kδ-mediated Akt T308 phosphorylation in Raji cells[32] was inhibited by **15i** with an EC_{50} value of 4.3 nM. However, **15i** did not exhibit apparent inhibitory effect on Akt phosphorylation controlled by PI3Kα (NIH3T3 cell, PDGFBB stimulation), PI3Kβ (NIH3T3 cell, LPA stimulation) and PI3Kγ (RAW264.7 cell, C5a stimulation) (EC_{50}>3000 nM). These cellular data combining with the biochemical assay results demonstrated that **15i** was a highly potent and selective PI3Kδ inhibitor.



Drug/class I PI3K cellular EC50 (nM)	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
Compound 15i	>3000	>3000	>3000	4.3

Figure 3. Inhibitory efficacy of compound **15i** against class I PI3K in cellular background.

We then moved forward to examine the selectivity of **15i** profile against other protein kinases. In the DiscoverX KINOMEscan selectivity testing platform, **15i** (at 1 μM) exhibited high selectivity among 468 kinases/mutants tested with a S score(1)=0.0 (Figure 4 and Supplemental table 1). Only PI3Kδ was hit with a percent control number less than 1 and none of other kinases was affected.

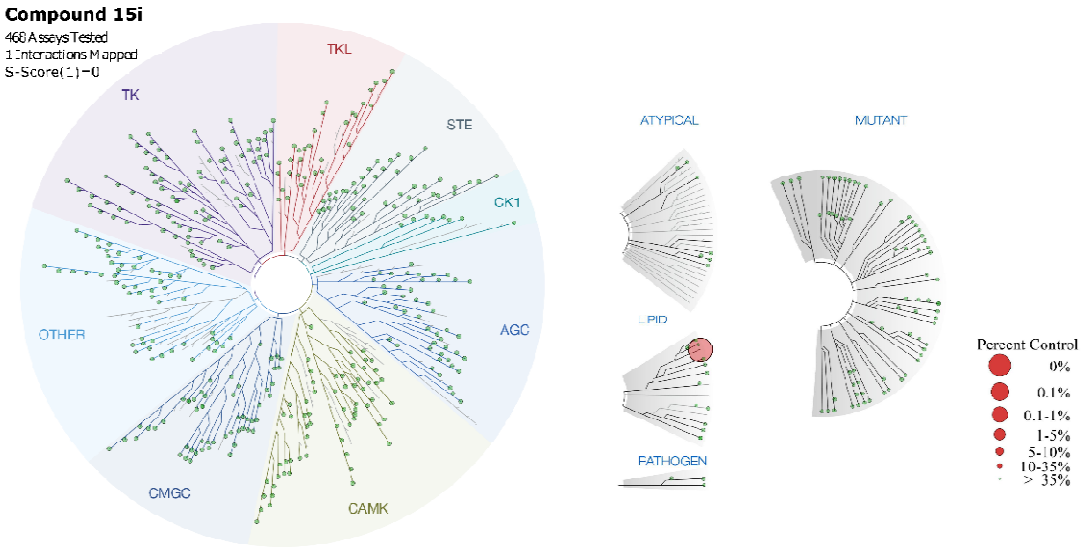


Figure 4. Kinome-wide selectivity profiling of compound **15i** with DiscoverX KINOMEscan technology.

In order to better understand the binding mode of compound **15i**, we then docked it into PI3Kδ (PDB ID: 5DXU) (Figure 5). The results showed that the aminothiazole moiety formed two hydrogen bonds with and Ser831 (Figure 5A). The NH_2 group of **15i** formed two hydrogen bonds with the side chain of Asp832 and backbone of Ser831 (Figure 5B). The two oxygens of the sulfonamide moiety also formed hydrogen bonds with the side chain of conserved residue Lys779 located in the roof β-sheet and Ser754 of P-loop (Figure 5B). Furthermore, the isopropyl moiety formed close hydrophobic interaction with the side chain of Val827 and Leu829 (Figure 5C), which could explain the activity difference between **12n** and **12r**/**12s**. In addition, the side chains of Met900, Ile910 and Asp911 formed a small hydrophobic pocket, which could accommodate the tolyl group of **15i**, and this could explain the reason why **15d** and **15e** started to lose the activity to PI3Kδ.

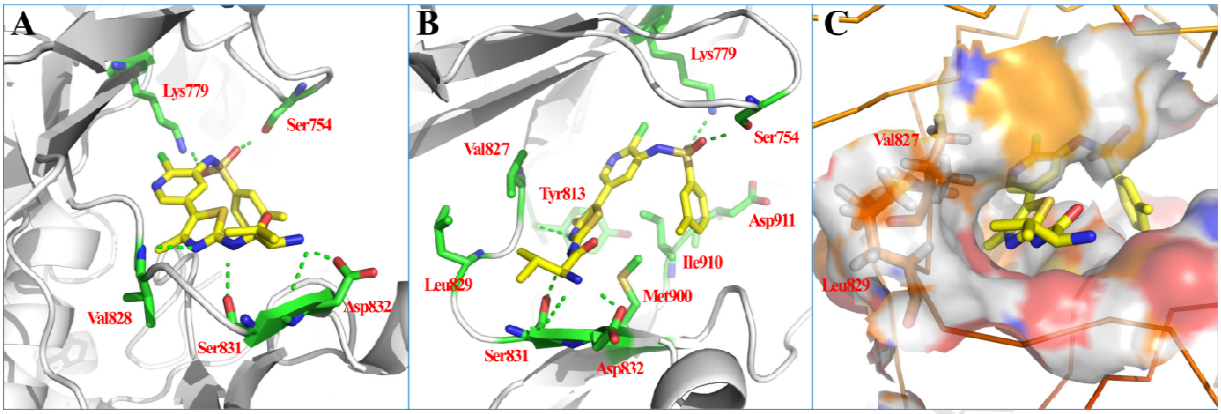


Figure 5. Binding mode illustration of compound **15i** with PI3Kδ (PDB ID: 5DXU) in (A) and (B) catoon mode and (B) solid phase.

Since PI3Kδ is specifically over-expressed/aberrantly activated in a variety of B-cell malignances such as CLL and AML, we then screened compound **15i** against a panel of B-cell malignancies derived cancer cell lines using compounds **1** and **16** as positive control (Table 4). **15i** exhibited moderate antiproliferative effects against most of the cell lines including AML and ALL cell lines with GI_{50} values between 3.0-4.8 μM. The well-established highly selective PI3Kδ inhibitor **1** didn't exhibit apparent inhibitory against most of the cell lines. In contrast, the pan-PI3K inhibitor **16** displayed more potent antiproliferative efficacies than **15i** and **1**. This again might reflect the fact that PI3Kδ inhibitor might contribute to the antitumor efficacy through microenvironment rather than killing the cells directly[33].

Table 5. The antiproliferative effects of compounds **1, 16** and **15i** against a panel of B-cell malignances related cancer cell lines (data represents GI_{50} values in μM)[a].

Cell line	Cell type	Compd. **1** (GI_{50}: μM)	Compd. **16** (GI_{50}: μM)	Compd. **15i** (GI_{50}: μM)
PF382	ALL	>10	0.14±0.01	3.5±0.8

NALM-6	ALL	>10	0.15±0.03	4.0±0.9
MV4-11	AML	>10	0.88±0.06	4.8±0.2
MOLM-14	AML	6.4±1.0	0.21±0.15	3.3±0.2
MOLM-13	AML	1.7±0.4	0.048±0.012	3.0±0.4

[a]Average of triplet testings.

2.4. In vivo PK/PD evaluation

We next evaluated the pharmacokinetics profile of compound **15i** in Sprague-Dawley rats (Table 5). At an oral dose of 10 mg/kg, **15i** exhibited good systemic exposure (AUC_{0-t}=1216 h·ng/mL, C_{max}=2063 ng/mL), favorable oral bioavailability (F%=22.8%), acceptable half-life ($T_{1/2}$=3.28 h) and fast absorption (T_{max}=0.25 h). These data suggested that **15i** might be suitable for oral administration for the in vivo efficacy study.

Table 6. Pharmacokinetic profile of compound **15i** in Sprague-Dawley Rats

	IV (1 mg/kg)	PO (10 mg/kg)
AUC_{0-t} (h·ng/mL)	549±68	1216±222
$AUC_{0-\infty}$ (h·ng/mL)	551±69	1257±209
C_{max} (ng/mL)	2467±142	2063±736
T_{max} (h)	0.033±0	0.25±0
Vz (mL/kg)	6210±421	37248±12023
Cl (mL/h/kg)	1833±225	8103±1317
MRT_{0-t} (h)	0.33±0.03	1.18±0.41
$MRT_{0-\infty}$ (h)	0.38±0.04	1.72±1.00

$T_{1/2}$ (h)	2.36±0.20	3.28±1.20
F %	-	22.8%±3.8%

Mean±SD, n=3.

Based on the favorable potency, selectivity and pharmacokinetics profile of compound **15i**, we finally tested its in vivo antitumor efficacy in the MOLM-14 cell-inoculated xenograft animal model. All dosages (25, 50 and 100 mg/kg/day) of **15i** were well tolerated with no mortality and no significant body weight loss observed (Figure 6 A). After 14 days of continuous treatment, **15i** dose-dependently inhibited the growth of the MOLM14 tumor at 25, 50 and 100 mg/kg/day dosages (Figure 6 B-C). **15i** displayed obvious antitumor efficacy with a TGI of 77% at 100 mg/kg/day dosage which was better than **1** at the same dosage. In addition, the immunohistochemistry (IHC) stain revealed that the tumor proliferation was effectively inhibited (Ki-67 lane, blue arrow, Figure 6 D) and significant apoptosis was induced (TUNEL lane, blue arrow, Figure 6 D) in the tumor.

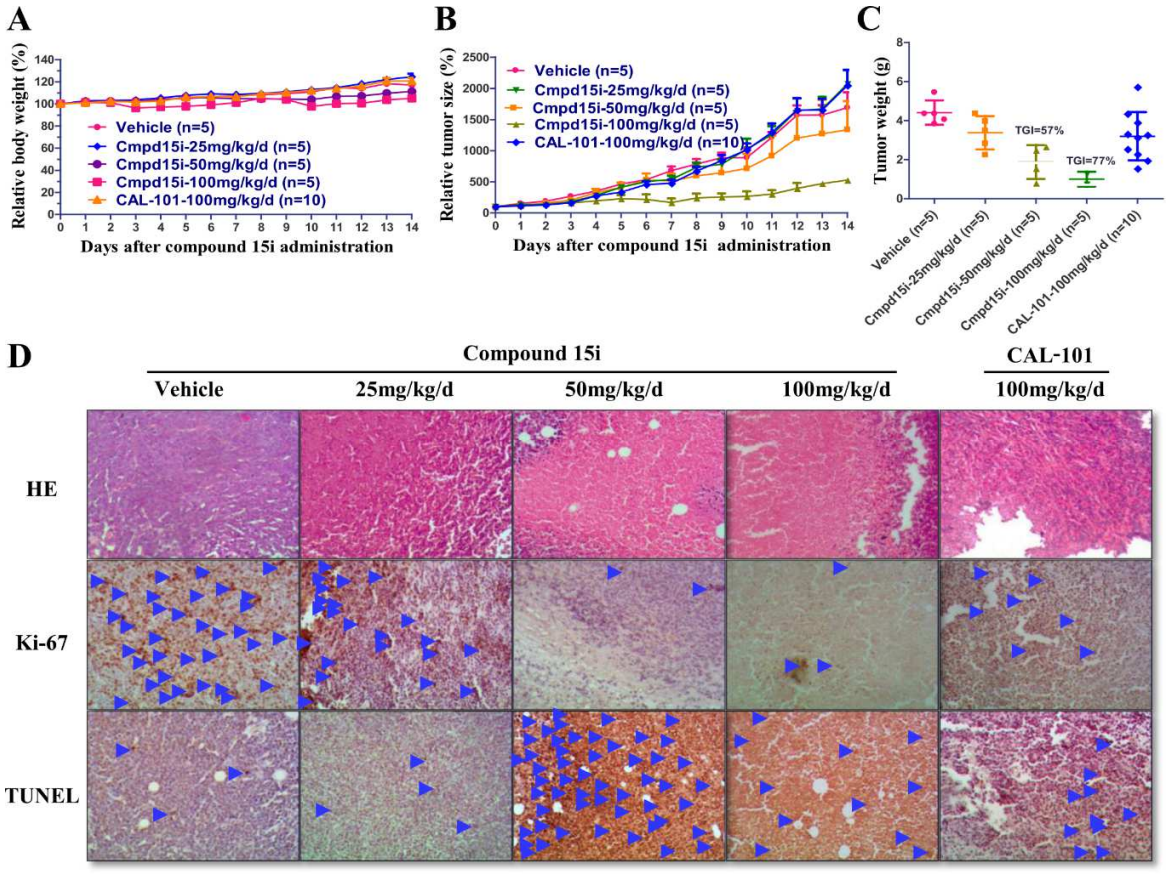


Figure 6. Efficacy of compound **15i** on MOLM-14 cell-inoculated xenograft mouse model. Female nu/nu mice bearing an established control group and MOLM-14 tumor xenografts were treated with **15i** at 25 mg/kg/day, 50 mg/kg/day, 100 mg/kg/day or vehicle. Daily oral administration was initiated when MOLM-14 tumors had reached a size of 300 mm^3. Each group contained 5 animals. Data = mean ± SD. (A) Body weight measurement from MOLM-14 xenograft mice after **15i** administration. Initial body weight was set as 100%. (B) Tumor size measurement from MOLM-14 xenograft mice after **15i** administration. Initial tumor size was set as 100%. (C) Comparison of the final tumor weight in each group after a 14-day treatment period. (D) Representative micrographs of hematoxylin and eosin (HE), Ki-67 and TUNEL staining of tumor tissues with **15i** treatment compared to the vehicle group and **1**.

3. Conclusions

In summary, starting from a pan-PI3K inhibitor **7**, through a focused medicinal chemistry approach guided by structure-aided drug design, we have discovered a aminothiaozle-pyridine scaffold based compound **15i** via 5 steps of chemical syntheses (24% overall yield). **15i** had an IC_{50} value of 6 nM against PI3Kδ, and it exhibited 10 to 300 fold selectivity against isoforms of PI3Ks and PI4Ks as well as excellent selectivity over other protein kinases. **15i** potently inhibited the phosphorylation of Akt with an EC_{50} of 4.3 nM in anti-IgM-stimulated PI3Kδ active Raji cell line, but displayed far less activity against PI3Kα, PI3Kβ and PI3Kγ. In addition, **15i** exhibited better antiproliferative effects against the majority of B-cell related malignancies that were tested (ALL and AML) compared to **1**. The suitable pharmacokinetic profile and good in vivo antitumor efficacy suggested that **15i** might be a potential drug candidate for B-cell related malignances.

4. Experimental section

4.1. Chemistry. All reagents and solvents were purchased from commercial sources and were used as received unless specified otherwise, or prepared as described in the literature. All moisture sensitive reactions were carried out using dry solvents under ultrapure argon protection. Glassware was dried in an oven at 140 °C for at least 12 h prior to use and then assembled quickly while hot, sealed with rubber septa, and allowed to cool under a stream of argon. Reactions were stirred magnetically using Teflon-coated magnetic stirring bars. Commercially available disposable syringes were used for transferring the reagents and solvents. LC/MS were performed on an Agilent 6224 TOF using an ESI source coupled to an Agilent 1260 Infinity HPLC system operating in reverse mode with an Agilent XDB-C18 column (4.6 × 50 mm, 1.8 μm) using a water/acetonitrile (each with 0.2% (v/v) formic acid) gradient at a flow rate at 0.4

mL/min. ^{1}H and ^{13}C spectra were recorded with a Bruker 400 MHz NMR spectrometer and referenced to deuterated methanol (CD_3OD), deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$). Chemical shifts are expressed in ppm. In the NMR tabulation, s indicates singlet; d, doublet; t, triplet; q, quartet and m, multiplet. Flash column chromatography was conducted using silica gel (Silicycle40−64 μm). The purities of all final compounds were determined to be above 95% by HPLC.

4.1.1. Compounds **8a-d** were prepared following the synthetic procedure of **8a-c** as described previously[20-23].

*4.1.1.1 N-(5-(3-Amino-4-chlorophenyl)-4-methylthiazol-2-yl)acetamide (**8d**).* Yield 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.21 (s, 1H), 6.91 (s, 1H), 6.61 (s, 1H), 5.48 (s, 2H), 2.33 (s, 3H), 2.14 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 168.84, 155.39, 145.35, 142.22, 132.04, 129.79, 124.01, 117.33, 116.54, 115.44, 22.90, 16.62. LC-MS (ESI, m/z): 282.0453 $[M+H]^+$.

4.1.2. Compounds **9a-d** were prepared following the synthetic procedure of **9a-b** as described previously[20-24].

*4.1.2.1. N-(4-Methyl-5-(5-(phenylsulfonamido)pyridin-3-yl)thiazol-2-yl)acetamide (**9c**).* Yield 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 10.76 (s, 1H), 8.35 (s, 1H), 8.25 (s, 1H), 7.82 (s, 2H), 7.63 (s, 1H), 7.59 (s, 2H), 7.51 (s, 1H), 2.25 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (100MHz, $CDCl_3$) δ 169.05, 156.53, 144.56, 144.40, 140.18, 139.42, 134.82, 133.80, 130.00, 129.12, 127.17, 126.51, 119.83, 22.89, 16.41. LC-MS (ESI, m/z): 389.0764 $[M+H]^+$.

*4.1.2.2. N-(5-(4-Chloro-3-(phenylsulfonamido)phenyl)-4-methylthiazol-2-yl)acetamide (**9d**).* Yield 64%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.16 (s, 1H), 10.18 (s, 1H), 7.79 (s, 2H), 7.64 (s, 1H), 7.58 (s, 2H), 7.43 (s, 1H), 7.25 (s, 2H), 2.23 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (100 MHz,

DMSO-d_6) δ 168.94, 155.99, 143.35, 140.70, 134.36, 133.51, 132.20, 130.85, 129.79, 127.86, 127.54, 127.13, 126.76, 122.45, 22.89, 16.43. LC-MS (ESI, m/z): 422.0392 $[M+H]^+$.

4.1.3. Compounds **10b-e** were prepared following the synthetic procedure of **10a**.

*4.1.3.1. N-(5-(2-Amino-4-methylthiazol-5-yl)-2-chloropyridin-3-yl)benzenesulfonamide (**10a**).* To a solution of **9a** (211 mg, 0.5 mmol, 1.00 eq.) in EtOH (4 mL), 6 M HCl (0.7 mL) was added at room temperature under argon. Then it was heated to reflux for 6 h. The reaction mixture was cooled to room temperature and filtered to afford the product **10a** as yellow solid (169 mg, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (s, 1H), 8.32 (s, 1H), 7.78 (d, J = 7.1 Hz, 2H), 7.69 (d, J = 6.7 Hz, 1H), 7.65 (s, 1H), 7.61 (d, J = 7.3 Hz, 2H), 2.21 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.27, 146.02, 145.36, 140.17, 134.71, 133.89, 131.30, 130.00, 127.28, 126.52, 111.42, 99.99, 13.11. LC-MS (ESI, m/z): 381.0225 $[M+H]^+$.

*4.1.3.2. N-(5-(2-Amino-4-methylthiazol-5-yl)-2-methoxypyridin-3-yl)benzenesulfonamide (**10b**).* Yield 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.76 (s, 2H), 7.56 (s, 3H), 7.25 (s, 1H), 3.67 (s, 3H), 2.12 (s, 3H). LC-MS (ESI, m/z): 377.0741 $[M+H]^+$.

*4.1.3.3. N-(5-(2-Amino-4-methylthiazol-5-yl)pyridin-3-yl)benzenesulfonamide (**10c**).* Yield 89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (s, 2H), 7.90 (s, 1H), 7.87 (s, 3H), 7.65 (d, J = 6.7 Hz, 1H), 7.61 (s, 1H), 7.59 (s, 1H), 7.58 (s, 1H), 2.21 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.39, 141.13, 140.32 , 139.12, 136.50, 135.05, 133.97, 130.06, 129.34, 128.14, 127.26, 111.51, 13.14. LC-MS (ESI, m/z): 347.0624 $[M+H]^+$.

*4.1.3.4. N-(5-(2-Amino-4-methylthiazol-5-yl)-2-chlorophenyl)benzenesulfonamide (**10d**).* Yield 90%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 9.71 (s, 2H), 7.74 (s, 2H), 7.65 (s, 1H), 7.59 (s, 2H), 7.49 (s, 1H), 7.24 (s, 1H), 7.17 (s, 1H), 2.15 (s, 3H). ^{13}C NMR (100 MHz, DMSO-

d_6) δ 167.87, 140.51, 134.66, 133.62, 132.70, 131.23, 129.83, 129.41, 129.37, 127.51, 127.14, 126.80, 114.83, 13.07. LC-MS (ESI, m/z): 380.0298 $[M+H]^+$.

*4.1.4. N-(5-(2-Amino-4-methylthiazol-5-yl)-2-hydroxypyridin-3-yl)benzenesulfonamide (**10e**).* To a solution of **9b** (211 mg, 0.5 mmol, 1.00 eq.) in EtOH (4 mL), 6 M HCl (0.7 mL) was added at room temperature under argon. Then it was heated to reflux for 12 h. The reaction mixture was cooled to room temperature and filtered to afford the product **10e** as yellow solid (65 mg, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (s, 1H), 7.63 (s, 2H), 7.50 (s, 3H), 7.25 (s, 1H), 2.07 (s, 3H). LC-MS (ESI, m/z): 363.0582 $[M+H]^+$.

4.1.5. Compounds **11a** and **11c-t** were prepared following the synthetic procedure of **11b**.

*4.1.5.1. tert-Butyl(2-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-2-oxoethyl)carbamate (**11b**).* To a solution of **10a** (40 mg, 0.1 mmol, 1.00 eq.) in anhydrous DMF (2 mL), Boc-glycine (33 mg, 0.15 mmol, 1.50 eq.), HATU (57 mg, 1.50 eq.) and DIPEA (0. 89 mL, 0.50 mmol, 5.0 eq.) were added at 0 °C under argon. The reaction mixture was stirred at 0 °C for 1 h, and then it was allowed to warm to room temperature for 20 h. The resulting mixture was concentrated to dryness. The residue was diluted with water (30 mL) and then extracted with EtOAc (3×30 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-1.5 %) to give the title compound **11b** as yellow solid (44 mg, 76%). ^{1}H NMR (400 MHz, CDCl3) δ 8.19 (s, 1H), 8.01 (s, 1H), 7.87 (s, 1H), 7.85 (s, 1H), 7.63 (t, J = 7.4 Hz, 1H), 7.53 (t, J = 7.5 Hz, 2H), 4.14 (s, 2H), 2.38 (s, 3H), 1.48 (s, 9H). LC-MS (ESI, m/z): 538.0952 $[M+H]^+$.

*4.1.5.2. N-(5-(6-Chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)propionamide (**11a**).* Yield 70%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 2.2 Hz, 1H), 8.03 (d, *J* = 2.2 Hz, 1H), 7.88 (s, 1H), 7.86 (d, *J* = 1.3 Hz, 1H), 7.64 (t, *J* = 7.5 Hz, 1H), 7.54 (t, *J* = 7.7 Hz, 2H), 2.55 (q, *J* = 7.5 Hz, 2H), 2.39 (s, 3H), 1.31 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.69, 157.02, 144.57, 144.37, 140.57, 138.51, 133.96, 130.53, 129.55, 129.50, 129.01, 127.21, 120.05, 29.49, 16.11, 9.07. LC-MS (ESI, m/z): 437.0498 $[M+H]^+$.

*4.1.5.3. tert-Butyl(2-((5-(6-methoxy-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-2-oxoethyl)carbamate (**11c**).* Yield 76%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.21 (s, 1H), 10.10 (s, 1H), 8.01 (s, 1H), 7.81 (s, 2H), 7.73 – 7.51 (m, 4H), 3.86 (s, 2H), 3.70 (s, 3H), 2.25 (s, 3H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.16, 156.39, 156.01, 155.61, 143.31, 142.42, 140.60, 133.45, 131.98, 129.66, 127.15, 122.12, 121.20, 120.23, 78.74, 54.00, 43.46, 28.68, 16.12. LC-MS (ESI, m/z): 534.1476 $[M+H]^+$.

*4.1.5.4. tert-Butyl(2-((5-(6-hydroxy-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-2-oxoethyl)carbamate (**11d**).* Yield 77%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 2H), 9.80 (s, 1H), 7.92 (s, 2H), 7.78 – 7.54 (m, 3H), 7.33 (s, 1H), 7.21 (s, 2H), 3.85 (s, 2H), 2.20 (s, 3H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.02, 156.90, 156.36, 154.98, 142.69, 140.22, 133.63, 129.72, 129.12, 128.35, 127.28, 126.33, 120.24, 109.78, 78.75, 43.48, 28.65, 15.98. LC-MS (ESI, m/z): 520.1316 $[M+H]^+$.

*4.1.5.5. tert-Butyl(2-((4-methyl-5-(5-(phenylsulfonamido)pyridin-3-yl)thiazol-2-yl)amino)-2-oxoethyl)carbamate (**11e**).* Yield 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 2H), 7.85 (d, *J* = 6.7 Hz, 2H), 7.54 (d, *J* = 6.1 Hz, 1H), 7.46 (s, 3H), 4.19 (s, 2H), 2.24 (s, 3H), 1.47 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.72, 156.44, 156.20, 145.17, 143.96, 140.58, 138.90, 134.03,

133.40, 129.35, 128.94, 128.07, 127.15, 121.43, 81.22, 50.75, 28.32, 15.97. LC-MS (ESI, m/z): 504.1366 $[M+H]^+$.

*4.1.5.6. tert-Butyl(2-((5-(4-chloro-3-(phenylsulfonamido)phenyl)-4-methylthiazol-2-yl)amino)-2-oxoethyl)carbamate (**11f**).* Yield 81%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 10.21 (s, 1H), 7.79 (d, *J* = 7.5 Hz, 2H), 7.69 – 7.63 (m, 1H), 7.59 (t, *J* = 7.1 Hz, 2H), 7.47 (d, *J* = 8.1 Hz, 1H), 7.31 – 7.24 (m, 2H), 7.21 (t, *J* = 5.4 Hz, 1H), 3.87 (d, *J* = 5.3 Hz, 2H), 2.24 (s, 3H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.17, 162.76, 156.36, 143.49, 140.72, 134.43, 133.51, 132.07, 130.82, 129.72, 127.94, 127.47, 127.13, 126.77, 122.64, 78.72, 38.69, 28.64, 16.42. LC-MS (ESI, m/z): 537.1043 $[M+H]^+$.

*4.1.5.7. tert-Butyl(R)-(1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-1-oxopropan-2-yl)carbamate (**11g**).* Yield 74%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.17 (s, 1H), 7.98 (d, *J* = 1.8 Hz, 1H), 7.83 (s, 1H), 7.82 (s, 1H), 7.65 – 7.59 (m, 1H), 7.52 (t, *J* = 7.7 Hz, 2H), 4.37 (d, *J* = 6.4 Hz, 1H), 2.38 (s, 3H), 1.45 (d, *J* = 5.4 Hz, 9H), 1.43 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 172.32, 169.59, 156.83, 144.80, 144.52, 142.44, 139.59, 133.32, 132.84, 131.32, 129.17, 128.91, 126.91, 119.80, 80.00, 50.14, 27.58, 17.25, 15.43. LC-MS (ESI, m/z): 552.1161 $[M+H]^+$.

*4.1.5.8. tert-butyl(S)-(1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-1-oxopropan-2-yl)carbamate (**11h**).* Yield 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 10.54 (s, 1H), 8.32 (s, 1H), 7.80 (s, 2H), 7.72 – 7.52 (m, 4H), 7.28 (s, 1H), 4.25 (s, 1H), 2.28 (s, 3H), 1.36 (s, 9H), 1.28 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.90, 156.76, 155.67, 145.60, 145.21, 144.03, 140.36, 134.22, 133.76, 131.24, 129.93, 128.87, 127.12, 118.89, 78.70, 50.21, 28.63, 17.96, 16.32. LC-MS (ESI, m/z): 552.1154 $[M+H]^+$.

*4.1.5.9. tert-Butyl(1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-2-methyl-1-oxopropan-2-yl)carbamate (**11i**).* Yield 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.02 (s, 1H), 7.86 (d, *J* = 7.9 Hz, 2H), 7.62 (t, *J* = 7.0 Hz, 1H), 7.53 (d, *J* = 7.2 Hz, 2H), 2.37 (s, 3H), 1.60 (s, 6H), 1.44 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.90, 154.92, 144.82, 144.34, 138.72, 133.83, 130.82, 129.50, 129.32, 129.15, 127.22, 120.05, 57.11, 29.71, 28.27, 16.05. LC-MS (ESI, m/z): 566.1286 $[M+H]^+$.

*4.1.5.10. tert-Butyl(1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)carbamoyl)cyclopropyl)carbamate (**11j**).*Yield 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.16 (s, 1H), 8.03 (d, *J* = 7.4 Hz, 2H), 7.83 (d, *J* = 7.1 Hz, 1H), 7.74 (t, *J* = 7.2 Hz, 2H), 2.58 (s, 3H), 1.69 (s, 9H), 1.47 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.96, 158.11, 145.45, 143.54, 140.51, 134.30, 133.82, 132.25, 130.12, 129.79, 127.80, 120.50, 61.13, 30.17, 28.56, 16.12, 14.30. LC-MS (ESI, m/z): 564.1153 $[M+H]^+$.

*4.1.5.11. tert-Butyl(S)-(1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate (**11k**).* Yield 76%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (d, *J* = 3.9 Hz, 1H), 8.43 (d, *J* = 8.4 Hz, 1H), 8.21 (s, 1H), 7.96 (d, *J* = 1.9 Hz, 1H), 7.83 (d, *J* = 7.7 Hz, 1H), 7.65 (t, *J* = 7.2 Hz, 1H), 7.56 (s, 1H), 4.44 (s, 1H), 3.98 – 3.82 (m, 2H), 2.37 (s, 3H), 1.46 (d, *J* = 13.2 Hz, 9H). ^{13}C NMR (100 MHz, CD_3OD) δ 169.97, 156.82, 150.81, 144.56, 142.51, 139.86, 135.15, 133.27, 131.21, 129.13, 128.51, 126.84, 120.72, 119.62, 79.76, 61.69, 56.59, 27.29, 14.79. LC-MS (ESI, m/z): 568.1105 $[M+H]^+$.

*4.1.5.12. tert-Butyl((2S,3R)-1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-3-hydroxy-1-oxobutan-2-yl)carbamate (**11l**).* Yield 77%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 1.6 Hz, 1H), 7.93 (s, 1H), 7.85 (t, *J* = 6.5 Hz, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.7 Hz, 2H), 4.69 – 4.59 (m, 1H), 4.46 (d, *J* = 5.9 Hz, 1H), 2.35 (s, 3H), 1.52 (s, 9H),

1.48 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.61, 166.38, 156.33, 155.74, 144.80, 144.22, 139.51, 137.80, 133.91, 129.14, 127.23, 122.03, 120.38, 114.92, 81.27, 66.88, 59.34, 28.30, 18.93, 15.95. LC-MS (ESI, m/z): 582.1251 $[M+H]^+$.

*4.1.5.13. tert-Butyl(S)-(1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-1-oxobutan-2-yl)carbamate (**11m**).* Yield 84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.01 (s, 1H), 7.86 (d, *J* = 7.2 Hz, 2H), 7.63 (d, *J* = 6.8 Hz, 1H), 7.54 (d, *J* = 7.1 Hz, 2H), 4.45 (s, 1H), 2.39 (s, 3H), 2.07 – 1.76 (m, 2H), 1.48 (s, 9H), 1.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.69, 156.09, 155.97, 144.96, 144.49, 140.39, 138.49, 133.93, 130.53, 129.53, 129.27, 129.02, 127.22, 120.26, 80.88, 55.83, 28.24, 22.68, 16.04, 14.00. LC-MS (ESI, m/z): 566.1275 $[M+H]^+$.

*4.1.5.14. tert-Butyl(R)-(1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-1-oxobutan-2-yl)carbamate (**11n**).* Yield 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.02 (s, 1H), 7.86 (d, *J* = 6.7 Hz, 2H), 7.63 (d, *J* = 6.3 Hz, 1H), 7.54 (d, *J* = 6.5 Hz, 2H), 4.43 (s, 1H), 2.39 (s, 3H), 2.07 – 1.77 (m, 2H), 1.49 (s, 9H), 1.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.60, 156.03, 155.96, 144.97, 144.49, 140.35, 138.46, 133.95, 130.51, 129.54, 129.20, 129.03, 127.22, 120.27, 81.08, 55.83, 28.45, 22.68, 16.05, 14.01. LC-MS (ESI, m/z): 566.1283 $[M+H]^+$.

*4.1.5.15. tert-Butyl(S)-(1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (**11o**).* Yield 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, *J* = 1.6 Hz, 1H), 8.02 (d, *J* = 1.8 Hz, 1H), 7.86 (d, *J* = 7.6 Hz, 2H), 7.62 (d, *J* = 7.5 Hz, 1H), 7.53 (t, *J* = 7.7 Hz, 2H), 4.31 (s, 1H), 2.39 (s, 3H), 2.33 (dd, *J* = 13.1, 6.6 Hz, 1H), 1.49 (s, 9H), 1.05 (d, *J* = 6.8 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.21,

155.91, 144.96, 144.44, 140.32, 138.52, 133.91, 130.54, 129.51, 129.13, 129.02, 127.22, 120.27, 38.64, 30.56, 28.30, 19.26, 17.70, 16.00. LC-MS (ESI, m/z): 580.1466 $[M+H]^+$.

*4.1.5.16. tert-Butyl(R)-(1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (**11p**).* Yield 84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 8.00 (s, 1H), 7.85 (d, *J* = 7.7 Hz, 2H), 7.67 – 7.58 (m, 1H), 7.52 (t, *J* = 7.3 Hz, 2H), 4.38 (s, 1H), 2.38 (s, 3H), 2.33 – 2.22 (m, 1H), 1.47 (s, 9H), 1.04 (d, *J* = 6.4 Hz, 3H), 1.00 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.60, 156.10, 144.94, 144.52, 140.48, 138.62, 133.86, 130.54, 129.49, 129.01, 127.20, 120.19, 80.65, 59.98, 30.66, 28.02, 19.37, 16.19. LC-MS (ESI, m/z): 580.1458 $[M+H]^+$.

*4.1.5.17. tert-Butyl(2-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-1-cyclopropyl-2-oxoethyl)carbamate (**11q**).* Yield 79%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.02 (s, 1H), 7.87 (s, 2H), 7.61 (d, *J* = 18.0 Hz, 1H), 7.53 (s, 2H), 3.86 (s, 1H), 2.39 (s, 3H), 1.47(s, 9H), 1.25(s, 1H), 0.90 (s, 2H), 0.66 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.18, 170.22, 155.97, 144.95, 144.52, 140.50, 138.52, 133.92, 130.54, 129.56, 129.53, 129.03, 127.22, 120.27, 31.61, 28.31, 22.68, 16.04, 14.16, 13.70, 3.83. LC-MS (ESI, m/z): 578.1310 $[M+H]^+$.

*4.1.5.18. tert-Butyl(S)-(1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**11r**).* Yield 80%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 10.52 (s, 1H), 8.33 (s, 1H), 7.79 (s, 2H), 7.68 (s, 2H), 7.60 (s, 2H), 7.21 (s, 1H), 4.26 (s, 1H), 2.29 (s, 3H), 1.64 (s, 1H), 1.54 (s, 1H), 1.37 (s, 9H), 1.30 (s, 1H), 0.88 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.84, 156.71, 155.91, 145.60, 145.23, 144.01, 140.36, 134.22, 133.76, 131.24, 129.93, 128.87, 127.10, 118.89, 78.68, 53.27, 28.63, 24.81, 23.36, 21.80, 16.32. LC-MS (ESI, m/z): 594.1615 $[M+H]^+$.

*4.1.5.19. tert-Butyl((2S)-1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-3-methyl-1-oxopentan-2-yl)carbamate (**11s**).* Yield 81%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (s, 1H), 10.50 (s, 1H), 8.33 (s, 1H), 7.79 (s, 2H), 7.69 (d, *J* = 11.2 Hz, 2H), 7.59 (s, 2H), 7.14 (s, 1H), 4.11 (s, 1H), 2.29 (s, 3H), 1.78 (s, 1H), 1.46 (s, 1H), 1.33 (s, 9H), 1.18 (s, 1H), 0.82 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.95, 156.38, 155.97, 145.60, 145.28, 144.00, 140.39, 134.22, 133.74, 131.25, 129.92, 128.85, 127.10, 118.93, 78.71, 59.19, 36.55, 28.61, 25.12, 16.31, 15.64, 11.16. LC-MS (ESI, m/z): 594.1623 $[M+H]^+$.

*4.1.5.20. tert-Butyl(S)-(1-((5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**11t**).* Yield 77%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.56 (s, 1H), 10.54 (s, 1H), 8.34 (s, 1H), 7.80 (d, *J* = 5.1 Hz, 2H), 7.67 (s, 2H), 7.60 (s, 2H), 7.36 (s, 3H), 7.28 (s, 2H), 7.20 (s, 1H), 4.46 (s, 1H), 3.01 (s, 1H), 2.85 (s, 1H), 2.29 (s, 3H), 1.27 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.86, 156.63, 155.84, 145.62, 145.26, 144.07, 140.28, 138.07, 134.26, 133.78, 131.27, 129.95, 129.76, 128.85, 128.56, 127.12, 126.91, 118.98, 78.74, 56.52, 37.42, 28.49, 16.25. LC-MS (ESI, m/z): 628.1463 $[M+H]^+$.

4.1.6. Compounds **12b-s** were prepared following the synthetic procedure of **12a**.

*4.1.6.1. 2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)acetamide (**12a**).* To a solution of **11b** (35 mg, 0.08 mmol, 1.00 eq.) in MeOH (1 mL), 4 M HCl in MeOH (1 mL) was added at room temperature. The reaction mixture was stirred at room temperature for 2 h. Evaporation of the solvent provided the white solid product (26 mg, 95%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (s, 1H), 8.01 (d, *J* = 8.7 Hz, 1H), 7.82 (d, *J* = 7.0 Hz, 2H), 7.66 (s, 1H), 7.57 (d, *J* = 6.8 Hz, 2H), 4.07 (s, 2H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 165.05, 156.69, 144.65, 143.83, 143.12, 139.87, 133.18, 129.12, 129.08, 128.27, 126.83, 126.80, 119.86, 40.86, 14.41. LC-MS (ESI, m/z): 438.0469 $[M+H]^+$.

*4.1.6.2. 2-Amino-N-(5-(6-methoxy-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)acetamide (**12b**).* Yield 92%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.86 (s, 1H), 7.72 (s, 2H), 7.71 (s, 1H), 7.52 (t, *J* = 6.6 Hz, 1H), 7.45– 7.43 (m, 2H), 4.05 (s, 2H), 3.70 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 163.93, 155.54, 140.41, 138.29, 131.37, 129.28, 127.24, 125.47, 125.31, 120.18, 118.67, 51.65, 39.54, 17.61. LC-MS (ESI, m/z): 434.0915 $[M+H]^+$.

*4.1.6.3. 2-Amino-N-(5-(6-hydroxy-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)acetamide (**12c**).* Yield 95%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.83 (d, *J* = 6.0 Hz, 2H), 7.67 – 7.41 (m, 4H), 7.19 (s, 1H), 4.03 (s, 2H), 2.23 (s, 3H). LC-MS (ESI, m/z): 420.0753 $[M+H]^+$.

*4.1.6.4. 2-Amino-N-(4-methyl-5-(5-(phenylsulfonamido)pyridin-3-yl)thiazol-2-yl)acetamide (**12d**).* Yield 91%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.59 (s, 1H), 8.51 (s, 1H), 8.18 (s, 1H), 7.90 (s, 2H), 7.61 (s, 1H), 7.54 (s, 2H), 3.99 (s, 2H), 2.33 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 163.68, 156.13, 145.70, 137.22, 137.17, 133.89, 132.31, 132.03, 131.52, 128.01, 127.97 125.44, 116.32, 39.27, 13.38. LC-MS (ESI, m/z): 404.0796 $[M+H]^+$.

*4.1.6.5. 2-Amino-N-(5-(4-chloro-3-(phenylsulfonamido)phenyl)-4-methylthiazol-2-yl)acetamide (**12e**).* Yield 96%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 1H), 8.60 (s, 3H), 7.77 (s, 2H), 7.65 (s, 1H), 7.57 (s, 2H), 7.47 (s, 1H), 7.27 (s, 1H), 7.18 (s, 1H), 3.91 (s, 2H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.89, 155.17, 143.48, 140.64, 134.42, 133.63, 131.68, 131.06, 129.89, 128.44, 127.84, 127.16, 126.99, 123.14, 49.15, 16.36. LC-MS (ESI, m/z): 437.0445 $[M+H]^+$.

*4.1.6.6. (R)-2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)propenamide (**12f**).* Yield 96%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (s, 2H), 8.56 (s, 1H), 8.29 (s, 1H), 7.76 (s, 2H), 7.64 (s, 1H), 7.56 (s, 2H), 4.19 (s, 1H), 2.15 (s, 3H), 1.43 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 169.35, 156.09, 145.89, 144.91, 140.24, 134.78, 134.32, 133.88,

131.21, 130.02, 128.46, 127.20, 119.38, 48.73, 17.46, 16.33. LC-MS (ESI, m/z): 452.0621 $[M+H]^+$.

*4.1.6.7. (S)-2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)propenamide (**12g**).* Yield 95%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.24 (s, 1H), 7.98 (s, 1H), 7.82 (d, *J* = 7.5 Hz, 2H), 7.66 (t, *J* = 7.1 Hz, 1H), 7.56 (t, *J* = 7.4 Hz, 2H), 4.30 (d, *J* = 6.4 Hz, 1H), 2.40 (s, 3H), 1.67 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 168.85, 157.21, 144.65, 143.35, 143.22, 139.85, 133.29, 133.20, 131.37, 129.09, 128.20, 126.81, 119.91, 49.37, 15.98, 14.27. LC-MS (ESI, m/z): 452.0633 $[M+H]^+$.

*4.1.6.8. 2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-2-methylpropanamide (**12h**).*Yield 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (s, 2H), 8.31 (s, 1H), 7.78 (s, 2H), 7.62 (s, 4H), 2.26 (s, 3H), 1.64 (s, 6H). LC-MS (ESI, m/z): 466.0749 $[M+H]^+$.

*4.1.6.9. 1-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)cyclopropane-1-carboxamide (**12i**).* Yield 90%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 7.78 (s, 2H), 7.69-7.65 (m, 2H), 7.61 (s, 2H), 2.20 (s, 3H), 1.59 (s, 1H), 1.50 (s, 1H), 1.36 (d, *J* = 12.5 Hz, 1H), 1.23 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.56, 161.08, 145.66, 145.34, 144.58, 140.27, 134.26, 133.85, 131.29, 129.89, 128.38, 127.11, 117.26, 36.37, 16.58, 13.78. LC-MS (ESI, m/z): 464.0631 $[M+H]^+$.

*4.1.6.10. (S)-2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-hydroxypropanamide (**12j**).* Yield 95%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.77 (s, 1H), 8.48 (d, *J* = 8.0 Hz, 1H), 8.27 (s, 1H), 8.00 (s, 1H), 7.83 (d, *J* = 6.3 Hz, 1H), 7.67 (s, 1H), 7.57 (d, *J* = 4.7 Hz, 1H), 4.39 (s, 1H), 4.11 (s, 2H), 2.44 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 150.68, 144.81, 143.62, 139.85, 133.50, 133.23, 131.47, 129.13, 128.80, 127.53, 126.83, 120.97, 59.98, 55.50, 13.73. LC-MS (ESI, m/z): 468.0585 $[M+H]^+$.

*4.1.6.11. (2S,3R)-2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-hydroxybutanamide (**12k**).* Yield 94%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.26 (s, 1H), 8.01 (s, 1H), 7.83 (d, *J* = 7.3 Hz, 2H), 7.67 (t, *J* = 6.8 Hz, 1H), 7.57 (t, *J* = 7.5 Hz, 2H), 4.35 – 4.26 (m, 1H), 4.12 (d, *J* = 8.7 Hz, 1H), 2.43 (s, 3H), 1.39 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 166.72, 157.30, 144.68, 143.37, 142.66, 139.86, 133.41, 133.19, 131.42, 129.08, 127.91, 126.79, 120.00, 65.75, 59.00, 18.93, 13.98. LC-MS (ESI, m/z): 482.0714 $[M+H]^+$.

*4.1.6.12. (S)-2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)butanamide (**12l**).* Yield 91%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (s, 1H), 7.98 (s, 1H), 7.83 (s, 2H), 7.65 (s, 1H), 7.56 (d, *J* = 5.9 Hz, 2H), 4.15 (s, 1H), 2.39 (s, 3H), 2.01 (s, 2H), 0.90 (s, 3H).^{13}C NMR (100 MHz, CD_3OD) δ 166.39, 155.09, 143.05, 142.82, 141.42, 138.34, 131.69, 131.66, 129.81, 127.56, 127.01, 125.29, 118.34, 52.97, 27.84, 13.09, 6.56. LC-MS (ESI, m/z): 466.0759 $[M+H]^+$.

*4.1.6.13. (R)-2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)butanamide (**12m**).* Yield 94%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.14 (s, 1H), 7.88 (s, 1H), 7.71 (s, 2H), 7.50 (s, 3H), 4.09 (s, 1H), 2.31 (s, 3H), 1.18 (s, 2H), 1.00 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 166.53, 155.53, 143.23, 141.77, 141.64, 138.33, 131.89, 131.68, 129.87, 127.59, 126.52, 125.30, 118.50, 52.95, 22.96, 17.64, 6.65. LC-MS (ESI, m/z): 466.0762 $[M+H]^+$.

*4.1.6.14. (S)-2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**12n**).* Yield 92%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.26 (s, 1H), 8.00 (s, 1H), 7.84 (s, 2H), 7.67 (d, *J* = 3.2 Hz, 1H), 7.57 (s, 2H), 4.03 (s, 1H), 2.42 (s, 4H), 1.20 – 1.07 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 166.02, 155.13, 143.14, 142.64, 141.50, 138.41, 131.83, 131.68, 129.92, 127.50, 126.85, 125.42, 118.42, 57.14, 28.81, 16.03, 14.94, 12.96. LC-MS (ESI, m/z): 480.0915 $[M+H]^+$.

*4.1.6.15. (R)-2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**12o**).* Yield 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (s, 2H), 8.34 (s, 1H), 7.79 (s, 2H), 7.68 (s, 2H), 7.60 (s, 2H), 3.97 (s, 1H), 2.28 (s, 4H), 0.98 (s, 6H).^{13}C NMR (100 MHz, DMSO-d_6) δ 167.98, 155.78, 145.63, 144.51, 140.34, 134.37, 133.72, 133.17, 131.31, 129.95, 128.49, 127.12, 119.58, 57.87, 30.37, 18.68, 18.43, 16.33. LC-MS (ESI, m/z): 480.0947 $[M+H]^+$.

*4.1.6.16. 2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-2-cyclopropylacetamide (**12p**).* Yield 96%. 1H NMR (400 MHz, CD_3OD) δ 8.11 (s, 1H), 7.85 (s, 1H), 7.68 (d, J = 6.9 Hz, 2H), 7.54 – 7.49 (m, 1H), 7.42 (d, J = 6.8 Hz, 2H), 3.49 (d, J = 9.1 Hz, 1H), 2.28 (s, 3H), 0.77 (s, 5H). ^{13}C NMR (100 MHz, CD_3OD) δ 165.95, 155.58, 143.15, 141.89, 141.17, 138.30, 131.85, 131.65, 129.87, 127.54, 126.26, 125.25, 118.64, 56.05, 12.49, 10.85, 2.18, 1.40. LC-MS (ESI, m/z): 478.0766 $[M+H]^+$.

*4.1.6.17. (S)-2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-4-methylpentanamide (**12q**).* Yield 91%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 3H), 8.35 (s, 1H), 7.80 (s, 2H), 7.69 (s, 2H), 7.60 (s, 2H), 4.09 (s, 1H), 2.30 (s, 3H), 1.70 (s, 3H), 0.93 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.02, 156.02, 146.01, 144.64, 140.29, 134.61, 133.72, 131.24, 129.94, 128.48, 127.19, 125.99, 119.48, 51.44, 24.19, 22.95, 22.59, 16.20. LC-MS (ESI, m/z): 494.1105 $[M+H]^+$.

*4.1.6.18. (2S)-2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylpentanamide (**12r**).* Yield 94%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (s, 1H), 8.76 (s, 3H), 8.33 (s, 1H), 7.80 (s, 2H), 7.65 (s, 2H), 7.58 (s, 2H), 4.06 (s, 1H), 2.25 (s, 3H), 2.00 (s, 1H), 1.58 (s, 1H), 1.15 (s, 1H), 0.93 (s, 3H), 0.85 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ

168.06, 155.82, 145.83, 144.58, 140.29, 134.64, 133.79, 131.23, 129.86, 128.49, 127.14, 126.09, 119.52, 56.80, 36.66, 24.65, 16.19, 14.90, 11.49. LC-MS (ESI, m/z): 494.1025 $[M+H]^+$.

*4.1.6.19. (S)-2-Amino-N-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-phenylpropanamide (**12s**).* Yield 93%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (s, 1H), 7.80 (s, 2H), 7.65 (s, 2H), 7.59 (s, 3H), 7.28 (s, 5H), 4.41 (s, 1H), 3.24 (s, 2H), 2.25 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.97, 155.91, 145.57, 144.55, 140.44, 134.93, 134.39, 133.73, 131.50, 129.93, 129.36, 129.05, 128.46, 127.76, 127.14, 126.05, 119.63, 54.06, 37.08, 16.24. LC-MS (ESI, m/z): 528.0953 $[M+H]^+$.

4.1.7. Compound **13b** were prepared following the synthetic procedure of **13a**.

*4.1.7.1. 5-(5-Amino-6-chloropyridin-3-yl)-4-methylthiazol-2-amine (**13a**)* To a solution of **8a** (2. 00 g, 0.71 mmol, 1.00 eq.) in EtOH (40 mL) was added 6 M HCl solution (7 mL) at room temperature. Then the reaction mixture was heated to reflux for 6 h. The resulting mixture was concentrated to dryness. The residue was diluted with EtOH and filtered to afford **13a** as yellow solid (146 mg, 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s, 2H), 7.65 (s, 1H), 7.29 (s, 1H), 2.27 (s, 3H). LC-MS (ESI, m/z): 241.0337 $[M+H]^+$.

*4.1.7.2. 5-(5-Amino-6-methylpyridin-3-yl)-4-methylthiazol-2-amine (**13b**).* Yield 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 2H), 7.86 (s, 1H), 7.51 (s, 1H), 2.26 (s, 3H). LC-MS (ESI, m/z): 221.0869 $[M+H]^+$.

4.1.8. Compounds **14b** were prepared following the synthetic procedure of **14a**.

*4.1.8.1. tert-Butyl(S)-(1-((5-(5-amino-6-chloropyridin-3-yl)-4-methylthiazol-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (**14a**)* To a solution of **13a** (80 mg, 0.21 mmol, 1.00 eq.) in anhydrous DMF (2 mL) was added Boc-L-Valine (46 mg, 0.21 mmol, 1.00 eq.), HATU (80 mg, 0.21 mmol, 1.00 eq.) and DIPEA (0. 17 mL, 1.00 mmol, 5.0 eq.) at 0 °C under argon. The

reaction mixture was stirred at 0 °C for 1 h then it was allowed to warm to room temperature for 14 h. The resulting mixture was concentrated to dryness. The residue was diluted with water (30 mL) and extracted with EtOAc (3×30 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-1.5 %) to give the product **14a** as yellow solid (65 mg, 69%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.67 (s, 1H), 7.23 (s, 1H), 4.21 (s, 1H), 2.36 (s, 3H), 2.12 (d, J = 6.2 Hz, 1H), 1.42 (s, 9H), 0.99 (d, J = 5.7 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 169.99, 155.24, 154.42, 142.34, 139.87, 134.12, 132.90, 127.46, 120.28, 119.50, 77.72, 58.56, 29.40, 25.91, 16.88, 15.65. LC-MS (ESI, m/z): 439.1466 $[M+H]^+$.

*4.1.8.2. tert-Butyl(2-((5-(5-amino-6-methylpyridin-3-yl)-4-methylthiazol-2-yl)amino)-2-oxoethyl)carbamate (**14b**).* Yield 79%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (s, 1H), 7.93 (s, 1H), 3.90 (s, 2H), 2.44 (s, 3H), 2.18 (s, 3H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) δ 168.80, 166.74, 155.76, 148.11, 142.71, 142.39, 130.67, 130.35, 126.16, 112.41, 78.03, 41.97, 25.83, 17.38, 13.12. LC-MS (ESI, m/z):378.1611$[M+H]^+$.

4.1.9. Compounds **15a** and **15c-p** were prepared following the synthetic procedure of **15b**.

*4.1.9.1. (S)-2-Amino-N-(5-(6-chloro-5-((4-(trifluoromethyl)phenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15b**)* To a solution of **14a** (79 mg, 0.18 mmol, 1.00 eq.) in anhydrous pyridine (3 mL) was added 4-(trifluoromethyl)benzenesulfonyl chloride (86 mg, 0.35 mmol, 2.00 eq.) at room temperature under argon. Then the reaction mixture was stirred at room temperature for 6 h. The resulting mixture was concentrated to give the crude product, which was added 1 mL MeOH and 1 mL of 4 M HCl (in MeOH) at room temperature. The resulting mixture was stirred at room temperature for 2 h, which was concentrated and purified

by flash chromatography (eluting with MeOH in DCM 0-10 %) to offer **15b** as yellow solid (177 mg, 93 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (s, 3H), 8.37 (s, 1H), 7.98 (s, 5H), 7.70 (s, 1H), 3.99 (s, 1H), 2.24 (s, 4H), 0.97 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.97, 155.84, 146.51, 145.48, 145.24, 144.20, 135.85, 130.67, 128.67, 128.22, 127.15, 127.12, 126.60, 119.41, 57.73, 30.35, 18.67, 18.53, 16.13. LC-MS (ESI, m/z): 548.0834 $[M+H]^+$.

*4.1.9.2. 2-Amino-N-(4-methyl-5-(6-methyl-5-(phenylsulfonamido)pyridin-3-yl)thiazol-2-yl)acetamide (**15a**).* Yield 90%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.03 (s, 1H), 10.34 (s, 1H), 8.42 (s, 1H), 8.24 (s, 2H), 8.05 (s, 1H), 7.83 (s, 2H), 7.57 (s, 3H), 2.24 (s, 3H). LC-MS (ESI, m/z): 418.0973 $[M+H]^+$.

*4.1.9.3. (S)-2-Amino-N-(5-(6-chloro-5-((4-isopropylphenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15c**).* Yield 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (s, 3H), 8.34 (s, 1H), 7.72 (s, 2H), 7.64 (s, 1H), 7.49 (s, 2H), 3.92 (s, 1H), 2.98 (s, 1H), 2.26 (s, 4H), 1.20 (s, 6H), 0.99 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.91, 155.78, 154.73, 145.65, 145.25, 144.51, 137.73, 134.20, 131.40, 128.44, 127.85, 127.39, 119.56, 57.71, 33.84, 30.36, 23.86, 18.55 (d, J = 18.8 Hz), 16.20. LC-MS (ESI, m/z): 522.1386 $[M+H]^+$.

*4.1.9.4. (S)-2-Amino-N-(5-(5-((4-(tert-butyl)phenyl)sulfonamido)-6-chloropyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15d**).* Yield 94%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.72 (s, 3H), 8.32 (s, 1H), 7.73 (s, 2H), 7.58 (d, J = 7.8 Hz, 3H), 4.01 (s, 1H), 2.20 (s, 4H), 1.24 (s, 9H), 0.97 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.89, 156.92, 155.75, 145.60, 144.51, 141.87, 137.38, 133.96, 131.42, 128.48, 127.13, 126.74, 119.60, 57.70, 35.34, 31.16, 30.36, 18.54 (d, J = 17.9 Hz), 16.18. LC-MS (ESI, m/z): 536.1534 $[M+H]^+$.

*4.1.9.5. (S)-N-(5-(5-([1,1'-Biphenyl]-4-sulfonamido)-6-chloropyridin-3-yl)-4-methylthiazol-2-yl)-2-amino-3-methylbutanamide (**15e**).* Yield 94%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 3H),

8.36 (s, 1H), 7.90 (s, 4H), 7.75 (s, 3H), 7.52 (s, 3H), 3.92 (s, 1H), 2.29 (s, 3H), 2.21(s, 1H), 0.98 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.94, 155.80, 145.82, 145.33, 145.19, 144.52, 139.09, 138.67, 134.47, 131.28, 129.62, 129.12, 128.53, 128.06, 127.88, 127.55, 119.56, 57.73, 30.36, 18.67, 18.45, 16.17. LC-MS (ESI, m/z): 556.1273 $[M+H]^+$.

*4.1.9.6. (S)-2-Amino-N-(5-(6-chloro-5-((2,6-difluorophenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15f**).* Yield 93%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (s, 1H), 7.94 (s, 1H), 7.58 (s, 1H), 7.04 (s, 2H), 3.92 (s, 1H), 2.30 (s, 3H), 1.89 (s, 1H), 1.02 (d, *J* = 13.4 Hz, 6H).^{13}C NMR (100 MHz, CD_3OD) δ 165.98, 159.25, 156.81, 154.96, 143.80, 142.62, 141.73, 134.44, 132.56, 129.13, 126.97, 118.29, 111.56, 57.11, 28.80, 16.10, 15.01, 12.72. LC-MS (ESI, m/z): 516.0750 $[M+H]^+$.

*4.1.9.7. (S)-2-Amino-N-(5-(6-chloro-5-((2-methylphenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15g**).* Yield 90%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (s, 1H), 10.66 (s, 1H), 8.69 (s, 3H), 8.33 (s, 1H), 7.71 (s, 2H), 7.45 (s, 1H), 7.37 (s, 2H), 3.96 (s, 1H), 2.65 (s, 3H), 2.28 (s, 4H), 0.98 (s, 6H). LC-MS (ESI, m/z): 494.1062 $[M+H]^+$.

*4.1.9.8. (S)-2-Amino-N-(5-(6-chloro-5-((4-methylphenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15h**).* Yield 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (s, 1H), 10.48 (s, 1H), 8.60 (s, 2H), 8.33 (s, 1H), 7.66 (s, 3H), 7.40 (s, 2H), 3.94 (s, 1H), 2.38 (s, 3H), 2.29 (s, 3H), 2.22 (s, 1H), 0.98 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.87, 155.81, 145.68, 145.30, 144.60, 144.29, 137.38, 134.49, 131.47, 130.36, 128.46, 127.25, 119.62, 57.65, 30.36, 21.54, 18.59 (d, *J* = 16.3 Hz), 16.20. LC-MS (ESI, m/z): 494.1096 $[M+H]^+$.

*4.1.9.9. (S)-2-Amino-N-(5-(6-chloro-5-((3-methylphenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15i**).* Yield 90%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.61 (t, *J* = 5.7 Hz, 3H), 8.35 (d, *J* = 2.3 Hz, 1H), 7.65 (d, *J* = 2.3 Hz, 1H), 7.64 (s, 1H), 7.60 (dd,

J = 5.2, 3.8 Hz, 1H), 7.52 – 7.48 (m, 2H), 2.37 (s, 3H), 2.28 (s, 3H), 2.23 (dt, *J* = 13.4, 6.6 Hz, 1H), 0.99 (dd, *J* = 6.9, 3.7 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.88, 155.73, 145.67, 145.17, 144.53, 140.15, 139.61, 134.45, 134.14, 131.32, 129.87, 128.38, 127.36, 124.36, 119.51, 57.65, 30.35, 21.42, 18.63 (d, *J* = 19.3 Hz), 16.28. LC-MS (ESI, m/z): 494.1074 $[M+H]^+$.

*4.1.9.10. (S)-2-amino-N-(5-(6-chloro-5-((2-chlorophenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15j**).* Yield 91%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.99 (s, 1H), 10.85 (s, 1H), 8.69 (s, 3H), 8.38 (s, 1H), 7.94 (s, 1H), 7.75 (s, 1H), 7.71 (s, 2H), 7.52 (s, 1H), 2.29 (s, 3H), 2.22 (s, 1H), 0.98 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.95, 155.80, 146.23, 145.34, 145.18, 137.92, 135.74, 135.34, 132.60, 131.50, 131.10, 130.95, 128.58, 128.34, 119.42, 57.66, 30.35, 18.59 (d, *J* = 16.7 Hz), 16.22. LC-MS (ESI, m/z): 514.0562 $[M+H]^+$.

*4.1.9.11. (S)-2-Amino-N-(5-(6-chloro-5-((3-chlorophenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15k**).* Yield 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 1H), 8.76 (s, 3H), 8.37 (s, 1H), 7.84 (s, 1H), 7.75 (s, 2H), 7.66 (s, 2H), 4.02 (s, 1H), 2.27 (s, 4H), 0.98 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.93, 155.79, 155.66, 146.31, 145.10, 142.21, 135.38, 134.46, 133.75, 132.05, 130.88, 128.62, 126.80, 125.91, 119.43, 57.66, 30.36, 18.59 (d, *J* = 16.8 Hz), 16.19. LC-MS (ESI, m/z): 514.0551 $[M+H]^+$.

*4.1.9.12. (S)-2-Amino-N-(5-(6-chloro-5-((4-chlorophenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15l**).* Yield 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (s, 3H), 8.37 (s, 1H), 7.77 (s, 2H), 7.68 (s, 3H), 3.98 (s, 1H), 2.29 (s, 3H), 2.22 (s, 1H), 0.98 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.93, 155.83, 146.26, 145.41, 144.88, 139.22, 138.69, 135.42, 130.89, 130.10, 129.12, 128.62, 119.51, 57.77, 30.37, 18.68, 18.42, 16.19. LC-MS (ESI, m/z): 514.0533 $[M+H]^+$.

*4.1.9.13. (S)-2-Amino-N-(5-(6-chloro-5-((2,3-dichlorophenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15m**).* Yield 91%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (s, 3H), 8.41 (s, 1H), 7.99 (s, 1H), 7.91 (s, 1H), 7.78 (s, 1H), 7.54 (s, 1H), 3.93 (s, 1H), 2.30 (s, 3H), 2.22 (s, 1H), 0.98 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.95, 155.85, 146.62, 145.56, 145.41, 140.11, 136.39, 135.46, 134.92, 130.66, 129.95, 129.78, 129.16, 128.68, 119.35, 57.68, 30.35, 18.56 (d, J = 18.6 Hz), 16.20. LC-MS (ESI, m/z): 548.0137 $[M+H]^+$.

*4.1.9.14. (S)-2-amino-N-(5-(6-chloro-5-((2,4-dichlorophenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15n**).* Yield 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (s, 1H), 10.97 (s, 1H), 8.55 (s, 3H), 8.41 (s, 1H), 7.93 (d, J = 12.8 Hz, 2H), 7.80 (s, 1H), 7.63 (s, 1H), 3.92 (s, 1H), 2.32 (s, 3H), 2.22 (s, 1H), 0.98 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.93, 155.83, 146.66, 145.54, 145.40, 141.81, 139.22, 137.07, 136.48, 132.84, 132.55, 131.99, 130.59, 128.48, 119.39, 57.69, 30.36, 18.57 (d, J = 19.5 Hz), 16.17. LC-MS (ESI, m/z): 548.0169 $[M+H]^+$.

*4.1.9.15. (S)-2-Amino-N-(5-(6-chloro-5-((2,5-dichlorophenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15o**).* Yield 94%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (s, 3H), 8.41 (s, 1H), 7.90 (s, 1H), 7.79 (s, 3H), 3.95 (s, 1H), 2.31 (s, 3H), 2.22 (s, 1H), 0.98 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.93, 155.87, 146.71, 145.57, 145.42, 139.62, 136.48, 135.00, 134.41, 132.66, 130.59, 130.34, 128.69, 119.37, 57.70, 30.36, 18.55 (d, J = 19.4 Hz), 16.12. LC-MS (ESI, m/z): 548.0173 $[M+H]^+$.

*4.1.9.16. (S)-2-Amino-N-(5-(6-chloro-5-((3,4-dichlorophenyl)sulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)-3-methylbutanamide (**15p**).* Yield 93%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (s, 1H), 8.81 (s, 1H), 8.58 (s, 3H), 8.40 (s, 1H), 7.99 (s, 1H), 7.89 (s, 1H), 7.74 (s, 2H), 3.92 (s, 1H), 2.31 (s, 3H), 2.23 (s, 1H), 0.97 (s, 6H). LC-MS (ESI, m/z): 548.0129 $[M+H]^+$.

4.2. Biology

4.2.1. PI3K Isoforms ADP-Glo Assay. The inhibitory activities of inhibitors against PI3K isoforms were determined by ADP-Glo assay. Briefly, the optimized enzyme concentrations were chosen as follows: PI3Kα 0.16 μg/mL, PI3Kβ 6 μg/mL, PI3Kδ 2.2 μg/mL, PI3Kγ 12 μg/mL, PI3KC2α 13 μg/mL, PI3KC2β 20 μg/mL, Vps34 2.2 μg/mL, PI4KIIIα13 μg/mL, PI4KIIIβ 2.2 μg/mL (Invitrogen, USA). In all cases, 2.5 μL samples of kinase was incubated with the inhibitor for 1 h at room temperature in reaction buffer followed by addition of ATP/substrate mixture. Each reaction was initiated by the addition of 2.5μL mixture of optimized concentrations of ATP and the substrate (100 μM PIP2: PS for PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ, 100 μM PI: PS for VPS34, PI4Kα and PI4Kβ, 100 μM PI for PI3KC2α and PI3KC2β, respectively). The ATP concentration was chosen as follows: 10 μM ATP for PI3Kα and PI3Kβ, 50 μM ATP for detection of the other seven kinases. The assay was conducted for 1 h at 37 °C before addition of 5 μL ADP-Glo reagent and incubation for 40 min at room temperature. 10 μL Kinase detection reagent was added and incubated for 30 min at room temperature before the luminescence signal was read with an envision Perkin Elmer plate reader.

4.2.2. PI3K Isoforms Cellular Selectivity Assay. NIH-3T3 (ATCC), RAW264.7 (ATCC) and Raji (ATCC) cells were seeded in a 6-well tissue culture plate and starved for 24 h, then incubated with DMSO, serially diluted compound **15i** and 3μM CAL-101, 3μM GDC-0941 for 1 h followed by 20 ng/ml PDGF-BB for 10 min, 5 μM LPA for 10 min, 50 ng/ml C5a for 5min and 1μg/ml anti-IgM for 5 min. All cells were washed in 1×PBS buffer and lysed in cell lysis buffer at 4 °C for 30 min. The lysates were cleared by centrifugation and the protein concentrations were measured by BCA analysis (Beyotime, China). Lysates containing 50 μg of total proteins were mixed with 5× loading buffer and heated in a metal bath for 10 min at a temperature of 100

°C. We separated the protein bands of different sizes using SDS-PAGE (10%) electrophoresis, and then transferred the protein to nitrocellulose membrane form polyacrylamide gel. Intensity of the AKT phosphorylation bands was determined using ImageJ 1.42q (NIH, USA) and normalized to total AKT (loading control).

4.2.3. Molecular Docking. Docking simulations were performed using Induced Fit Docking (IFD) protocol as implemented in Schrodinger release 2017. Prior to docking, the PI3K-delta receptor structure (PDB ID: 5DXU) was prepared using the Protein Preparation Wizard at pH 7.4 and with force field OPLS3. The ligands were prepared using LigPrep in possible states at pH 7.41.0. The best docking poses were visually inspected from the top 20% ranking poses of IFD score.

4.2.4. Cell Lines and Cell Culture. The human cancer cell lines, Pfeiffer, U937, KU812, PF382, NALM-6 and Raji were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). MV4-11, MOLM-13 and MOLM-14 were provided by Dr. Scott Armstrong, Dana Farber Cancer Institute (DFCI), Boston, MA. MEC-1, MEC-2 and SU-DHL-2 were purchased from Cobioer Biosciences CO., LTD (Nanjing, China). MV4-11 was cultured in IMDM media (Corning, USA) with 10% FBS and supplemented with 2% L-glutamine and 1% pen/strep. The rest of the cell lines were cultured in RPMI 1640 media (Corning, USA) with 10% fetal bovine serum (FBS) and supplemented with 2% L-glutamine 1% penicillin/streptomycin. All cell lines were maintained in culture media at 37 ºC with 5% CO_2.

4.2.5. Proliferation Study. Cells were grown in 96-well culture plates (3000/well). The compounds of various concentrations were added into the plates. DMSO concentrations were kept constant and did not exceed 0.1% of the total volume. Cell proliferation was determined after treatment with compounds for 72 h. Cell viability was measured using the CellTiter–Glo

assay (Promega, USA), according to the manufacturer's instructions, and luminescence was measured in a multi-label reader (Envision, PerkinElmer, USA). Data were normalized to control groups (DMSO) and represented by the mean of three independent measurements with standard error <20%. GI_{50} values were calculated using Prism 5.0 (GraphPad Software, San Diego, CA).

4.2.6. Pharmacokinetic Study. This study protocol was approved by the Animal Ethics Committee of Hefei Institutes of Physical Science, Chinese Academy of Sciences (Hefei, China). The male Sprague–Dawley rats (190–210 g) were provided by Laboratory Animal Center of Anhui Medical University (Hefei, China). The animals were housed in an air-conditioned animal room at a temperature of 23 ± 2°C and a relative humidity of 50 ± 10% and allowed free access to tap water and lab diet. The rats were acclimatized to the facilities for one week and then fasted for 12 h with free access to water prior to the experiment.

The six rats were randomly and equally divided into two groups for one compound's pharmacokinetic study. One group was injected with i.v. formulation at a dose of 1 mg/kg, and the other three groups were treated by oral administration of p.o. formulation at doses of 10 mg/kg. The p.o. formulation (2 mg/mL) is consisted of 10 mg compound dissolved in 0.5 mL of dimethyl sulfoxide and 4.5 mL of 5% glucose water. The i.v. formulation is made with 0.5 mL of the p.o. formulation and 4.5 mL of 5% glucose water. About 300 μL of blood samples were collected into heparinized tubes at 2, 5, 15, 30, 60, 120, 240, 360, 540 and 720 min after intravenous injection and at 5, 15, 30, 60, 90, 120, 240, 360, 540 and 720 min after oral administration. 100 μL of plasma was harvested by centrifuging the blood sample at 4 °C and 5000 rpm for 3 min, and then stored at −80 °C until analysis. An aliquot of 100 μL of each plasma sample was mixed with 20 μL of internal standard working solution (200 ng/mL of caffeine). Methanol (400 μL) was then added for precipitation. After vortexing for 5 min and

centrifuging at 14,000 rpm for 10 min, 5 μL of the supernatant was injected for LC–MS/MS analysis.

The pharmacokinetic parameters were analyzed through noncompartment model using WinNonlin 6.1 software (Pharsight Corporation, Mountain View, USA), including half-life (T1/2), plasma concentration at 0 min (C0), the peak of the plasma concentration (Cmax), the time to peak of the plasma concentration (Tmax), the area under the plasma concentration–time curve during the period of observation (AUC0–t), the area under the plasma concentration–time curve from zero to infinity (AUC0−∞), clearance (CL), apparent volume of distribution (Vd) and the mean residence time (MRT). The oral bioavailability (F) is calculated according to the following equation: F=AUC0−∞(oral)/AUC0−∞(iv)×Dose(iv)/Dose(oral)×100%.

4.2.7. MOLM-14 Xenograft Tumor Models. Four-week old female nu/nu mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). All animals were housed in a specific pathogen-free facility and used according to the animal care regulations of Hefei Institutes of Physical Science Chinese Academy of Sciences. Prior to implantation, cells were harvested during exponential growth. Ten million MOLM-14 cells in PBS were formulated as a 1:1 mixture with Matrigel (BD Biosciences) and injected into the subcutaneous space on the right flank of nu/nu mice. Daily oral administration was initiated when tumors had reached a size of 200 to 400 mm^3. Animals were then randomized into treatment groups of 5 mice each for efficacy studies. Compound **15i** was delivered daily in a HKI solution (0.5% Methocellulose/0.4% Tween 80 in ddH_2O) by orally gavage. A range of doses of **15i** or its vehicle were administered as indicated in figure legends. Body weight and tumor growth were measured daily after **15i** treatment. Tumor volumes were calculated as

follows: tumor volume (mm^3)=[(W^2 × L)/2] in which width (W) is defined as the smaller of the two measurements and length (L) is defined as the larger of the two measurements.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. X.L., F.L., C.C., Z.J. and A.W. contributed equally to this work.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at XXXXXX.

Notes

The authors declare the following competing financial interest(s): Dr. Shanchun Zhang is a shareholder of Hefei Cosource Medicine Technology Co. LTD.

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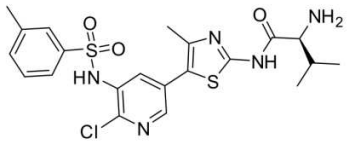
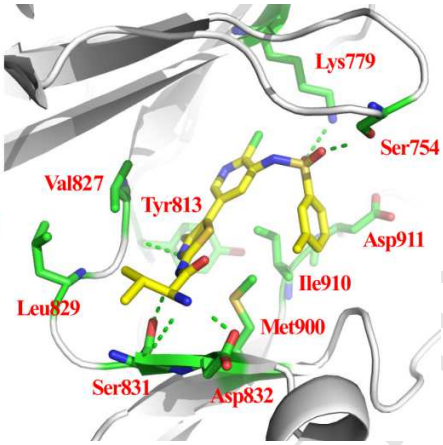


Compound 15i (CHMFL-PI3KD-317)

	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
Biochemical IC_{50} (nM)	62.6	354.7	284	6.0
Cellular EC_{50} (nM)	>3000	>3000	>3000	4.3



Highlights

- A selective and potent PI3Kδ kinase inhibitor **15i** was discovered.
- **15i** exhibits an excellent selectivity profile in the protein kinases.
- **15i** selectively and potently inhibits PI3Kδ mediated phosphorylation of Akt T308.
- **15i** has acceptable PK properties and inhibits the tumor growth of MOLM14.