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ORIGINAL ARTICLE

Design, synthesis and pharmacological evaluation of 1,2,3,4-tetrahydrobenzofuro[2,3-*c*] pyridine derivatives as p21-activated kinase 4 inhibitors for treatment of pancreatic cancer

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KEY WORDS

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Tetrahydrobenzofuro[2,3-*c*]pyridine;
Structure-activity relationship;
Allosteric inhibitor;
Pharmacokinetics properties;

Abstract The p21-activated kinase 4 (PAK4), a key regulator of malignancy, is negatively correlated with immune infiltration and has become an emergent drug target of cancer therapy. Given the lack of high efficacy PAK4 inhibitors, we herein reported the identification of a novel inhibitor **13** bearing a tetrahydrobenzofuro[2,3-*c*]pyridine tricyclic core and possessing high potency against MIA PaCa-2 and Pan02 cell lines with IC_{50} values of 0.38 and 0.50 μmol/L, respectively. This compound directly binds to PAK4 in a non-ATP competitive manner. In the mouse Pan02 model, compound **13** exhibited significant tumor growth inhibition at a dose of 100 mg/kg, accompanied by reduced levels of PAK4 and its

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Kinase selectivity;
Pancreatic cancer;
Immune infiltration

phosphorylation together with immune infiltration in mice tumor tissue. Overall, compound **13** is a novel allosteric PAK4 inhibitor with a unique tricyclic structural feature and high potency both *in vitro* and *in vivo*, thus making it worthy of further exploration.

1. Introduction

The p21-activated kinases (PAKs), a class of serine/threonine kinases, were initially considered as downstream effectors of $p21^{ras}$-related proteins[1]. There are at least six sub-types in the PAKs family which can be divided into group I (PAK1−3) and group II (PAK4−6) according to the amino acid sequence and protein structure[2]. Growing evidence has revealed that the gene amplification and protein overexpression of PAKs are common in various tumors, such as pancreatic[3,4], ovarian[5], breast[6,7], and gastric cancers[8], which may play key roles in promoting proliferation and metastasis of these tumor cells[9,10]. However, a previous study revealed that inhibition of PAK1 or PAK2 just has a narrow therapeutic window[11], suggesting the disadvantages of pan-PAK inhibitors.

Recently, PAK4, a key member of the group II PAKs, has aroused great interest due to its oncogenic and tumor immunomodulatory properties. Multiple studies have demonstrated that PAK4 is overexpressed in the esophagus[12], bladder[13], breast[7], and pancreatic cancers[4], and inhibition or knockout of PAK4 significantly suppressed the proliferation of these tumor cells. In addition, accumulating studies have shown that PAK4 is an adverse factor in the resistance and survival of pancreatic cancer[14-17]. Meanwhile, Rodriguez and coworkers[18] recently revealed that PAK4 inhibition increased T-cell infiltration, and synergistically enhanced the efficacy of PD-1 therapy in melanoma. Soon afterward, combinations of PAK4 inhibition and immunotherapy were also reported to treat glioblastoma and prostate cancer[19,20]. All these findings confirmed that PAK4 is an emergent and promising therapeutic target of cancer therapy.

At present, various PAK4 inhibitors have been disclosed (Fig. 1). PF-3758309 (**1**), developed by Pfizer, is a pan-PAKs inhibitor with a K_i value of 18.7 nmol/L for PAK4, but its clinic development was discontinued due to adverse events[2,21]. GNE-2861 (**2**), developed by Genentech Inc., is a highly selective PAK4 inhibitor with a K_i value of 3.3 nmol/L[22], but its antitumor activity *in vivo* was not reported. The aminoquinazoline derivative CZh226 (**3**) was disclosed by Hao and coworkers[23] as a potent PAK4 inhibitor with a K_i value of 9 nmol/L, nevertheless, its poor pharmacokinetic properties hindered its further development. Compounds **1**−**3** were representatives of orthosteric PAK4 inhibitors interacting in the conserved ATP-binding site.

KPT-9274 (**4**) represents the first PAK4 allosteric modulator discovered by Karyopharm Therapeutics Inc. (Fig. 1)[24]. Currently, there are two clinical trials ongoing (NCT04914845, NCT04281420)[25]. Available results have shown that **4** reduced the stability of PAK4 protein, decreased the levels of p-PAK4, and blocked the downstream signal pathway[7]. *In vivo*, this compound significantly suppressed the growth of kidney[26], breast[7], myeloid[27,28], esophagus[12], and pancreatic cancers[4]. KPT-7523 (**5**), an analogue of **4**, also showed specific binding with PAK4 and nearly no interaction with the other isoforms[27]. However, the allosteric interaction mechanism of both compounds **4** and **5** needs to be further elucidated since very limited studies have been performed[27]. In view of the potential safety concern on the early orthosteric PAK4 inhibitors and the prospective advantages of

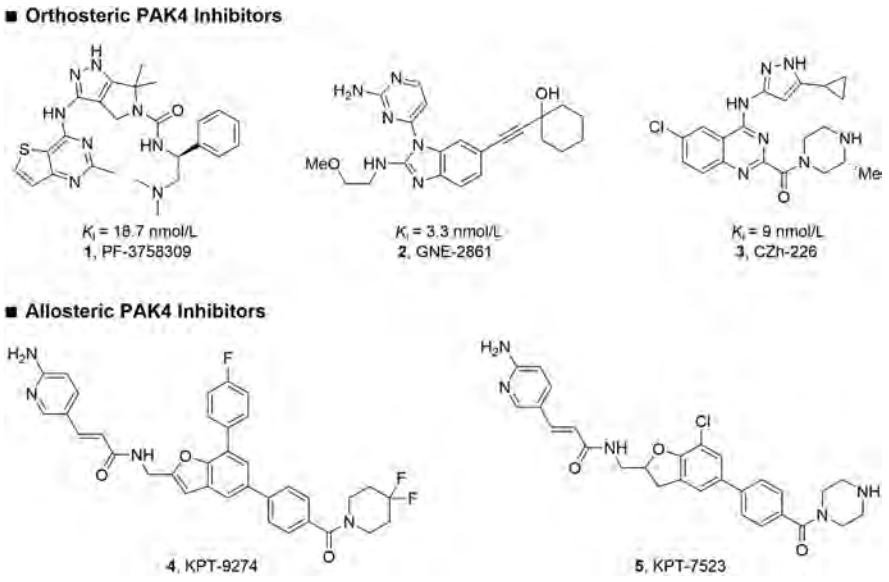


Figure 1 Representative of PAK4 inhibitors.

allosteric inhibitors, we conducted a scaffold-hopping strategy on the clinical allosteric inhibitor **4** by cyclizing its bicyclic benzofuran core into a tetrahydrobenzofuro[2,3-*c*]pyridine tricyclic core (Fig. 2). Subsequent elaboration of binding sites led to a new potent PAK4 inhibitor **13** showing promising both *in vitro* and *in vivo* activities. Herein, we reported the design, synthesis, and pharmacological characterization of these novel PAK4 inhibitors.

2. Results and discussion

2.1. Design strategy of new PAK4 inhibitors

Firstly, we conducted a literature analysis on all disclosed analogues of compound **4** and found that the benzofuran core plays a pivotal role in maintaining the activity against cancer cells[25,26], disrupting the bicyclic scaffold or replacing it with other bicyclic frameworks, such as benzothiophene or indole, negatively impacts the anti-proliferative activity. The *β*-aminopyridinyl acrylamido fragmentation is an additional important pharmacophore as the optimal substitution pattern of the furan ring. The C6 position benzamide tail and the C8 position *para*-fluorobenzene head group represent the two versatile handles for fine-tuning the activity. Based on these analyses, we decided to perform a strategic cyclization approach on the central benzofuran core of compound **4**, thus leading to a new tricyclic structure core—tetrahydrobenzofuro[2,3-*c*]pyridine (Fig. 2). In the meantime, various substitution patterns at the C1-, C6- and C8-positions were elaborated to achieve high potency and druglike PAK4 inhibitors.

2.2. Cellular activities of new compounds

Since there is no reported method to quantitatively evaluate the PAK4 allosteric inhibition, we conduct a preliminary screening of new compounds by anti-proliferative assay. All the new compounds and **4** (KPT-9274) were evaluated for their anti-proliferative activity against MIA PaCa-2 pancreatic and U-2 osteosarcoma (OS) cancer cell lines both overexpressing PAK4. As shown in Table 1, we first evaluated the activities of the tricyclic compounds **6**−**11** with or without substitution on the C1-position. Varied activities were observed for these compounds in the PAK4-overexpressing cancer cells. Compared to the submicromolar anti-proliferative effects of compound **4** in MIA PaCa-2 and U-2 OS cells (0.4 and 0.8 μmol/L), the new tricyclic compared **6** was 2- to 5-fold less potent with IC_{50} values of about 2 μmol/L against both cells. All the rest of the compounds of this subseries (**7**−**11**) showed nearly no anti-proliferative activities against the two tested cancer cells with IC_{50} values of greater than 10 μmol/L, suggesting the limited steric tolerance at the C1-site.

Since the tetrahydrobenzofuro[2,3-*c*]pyridine **6** retained reasonable potency in PAK4-expressed cancer cells, we decided to keep this tricyclic core intact and elaborate other structural components. As shown in Table 2, a series of heteroatom-substituted phenyl or heterocyclic rings were employed to replace the right-hand benzoic moiety (C6-position) with the aim to reducing the lipophilicity of the biphenyl fragment. The fluoro-substituted phenyl analogue **12** exhibited an inhibitory activity comparable to that of compound **6** in the MIA PaCa-2 cell with an IC_{50} value of 2.66 μmol/L, but reduced activity in the U-2 OS cell with an IC_{50} value over 10 μmol/L. Conversely, pyridinyl analogues **13** and **14** showed significantly enhanced potency against both cancer cells with IC_{50} values ranging between 0.38 and 0.94 μmol/L, which were nearly equally potent to the clinical compound **4**. Meanwhile, compounds **15**−**18** bearing other nitrogen-containing heterocycles were evaluated. Intriguingly, reduced potency was observed for the two-nitrogen-containing pyrimidinyl or pyrazinyl analogues **15**−**17**. However, the pyridazinyl congener **18** showed high anti-proliferative activities against both MIA PaCa-2 and U-2 OS cells with IC_{50} of 0.23 and 0.64 μmol/L, respectively, which were slightly more potent than the reference compound **4** (0.42 and 0.84 μmol/L respectively in the two cancer cells).

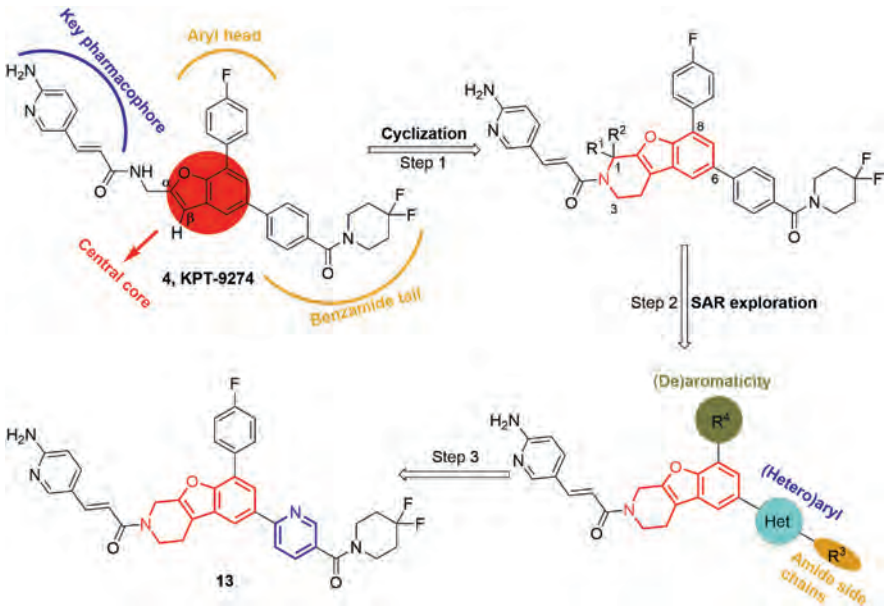
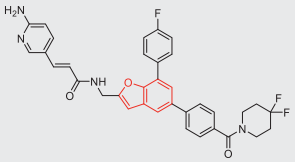
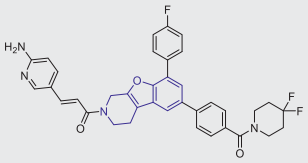
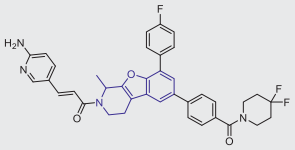
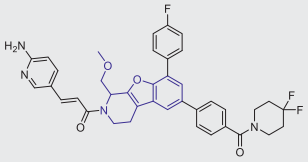
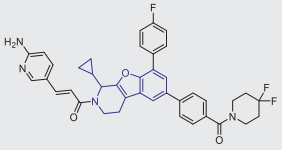
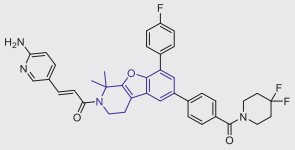
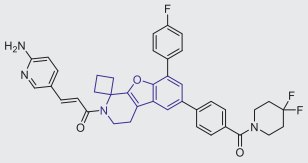


Figure 2 Design overview of new PAK4 inhibitors leading to compound **13**.

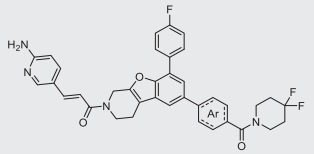
Table 1 Cellular activities of compounds **4**, **6**−**11**.

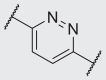
Compd.	Structure	IC_{50} (μmol/L)[a]	
		MIA PaCa-2	U-2 OS
4		0.42 ± 0.11	0.84 ± 0.42
6		2.30 ± 0.23	2.20 ± 0.05
7		>10	>10
8		>10	>10
9		>10	>10
10		>10	>10
11		>10	>10

[a]The data was calculated from three independent experiments as mean ± SD (n = 3).

Encouraged by the high potency of the tricyclic compound **18** bearing a pyridazinyl moiety within the C6 sidechain, we further optimized the terminal substituent on the C6-pyridazinyl moiety by replacing the 4,4-difluoropiperidine-1-carbonyl group with various cyclic or acyclic bioisosteric congeners. As depicted in Table 3, replacing 4,4-difluoropiperidine with morpholine, *N*-methylpiperazine, or pieridin-4-one generated compounds **19**−**21**, all retaining good potency in the two cancer cell lines, but were slightly less potent than compound **18**. In general, these compounds were more sensitive to the MIA PaCa-2 pancreatic cell than against the U-2 osteosarcoma cancer cells, especially pieridin-4-one **21** showing a 7-fold different potency in the two cell lines with IC_{50} values of 0.19 and 1.38 μmol/L, respectively. Similarly, compound **22** bearing a 4-cyano-4-methylpiperidine-1-carbonyl as the terminal moiety also showed high potency (0.13 μmol/L) in the MIA PaCa-2 but low potency (1.04 μmol/L) in the U-2 OS cells. Compound **23** with a downsized substituent 3-(difluoromethyl)azetidine and compound **24** bearing a 6,6-difluoro-3-azabicyclo[3.1.0]hexane both retained good potency with sub-micromolar IC_{50} values (0.39−1.08 μmol/L). Compounds **25**−**27** bearing an acyclic amido linkage other than cyclic amido ones retained good potency in both cells, but (3,3-difluoro-1-methylpiperidin-4-yl)carbamide **25** showed a high potency of 0.19 μmol/L and over 6-fold selectivity in the MIA PaCa-2 cell than in the U-2 OS cells (1.19 μmol/L). Unfortunately, compounds **28**−**29** containing a reversed amido linkage showed much-reduced potency in the two cells, though compound **29** retained moderate potency around 3 μmol/L.

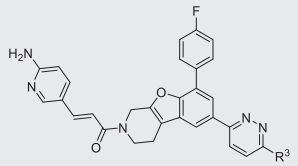
Table 2 Cellular activities of compounds **6**, **12**–**18**.

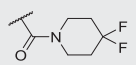
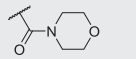
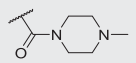
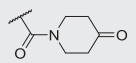
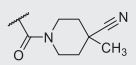
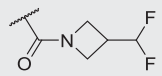
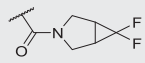
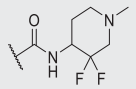
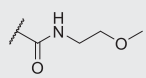
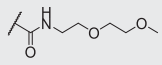
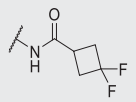
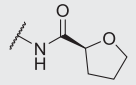


Compd.	Ar	IC_{50} (μmol/L)[a]	
		MIA PaCa-2	U-2 OS
6		2.30 ± 0.23	2.20 ± 0.05
12		2.66 ± 0.33	>10
13		0.38 ± 0.21	0.91 ± 0.44
14		0.94 ± 0.28	0.81 ± 0.24
15		4.46 ± 1.56	5.74 ± 2.86
16		1.36 ± 0.31	0.95 ± 0.23
17		2.65 ± 1.14	2.34 ± 0.01
18		0.23 ± 0.09	0.64 ± 0.33

[a]The data was calculated from three independent experiments as mean ± SD (n = 3).

Table 3 Cellular activities of compounds **18**–**29**.



Compd.	R^3	IC_{50} (μmol/L)[a]	
		MIA PaCa-2	U-2 OS
18		0.23 ± 0.09	0.64 ± 0.33
19		0.46 ± 0.24	0.73 ± 0.26
20		0.59 ± 0.47	1.65 ± 0.63
21		0.19 ± 0.02	1.38 ± 0.80
22		0.13 ± 0.01	1.04 ± 0.92
23		0.55 ± 0.08	1.08 ± 0.10
24		0.39 ± 0.08	0.76 ± 0.15
25		0.19 ± 0.02	1.19 ± 0.87
26		1.38 ± 0.17	2.52 ± 1.84
27		0.89 ± 0.32	1.10 ± 0.38
28		>10	>10
29		3.12 ± 2.22	3.83 ± 0.87

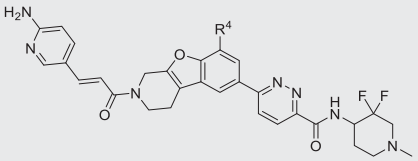
[a]The data was calculated from three independent experiments as mean ± SD (n = 3).

Next, we performed structural elaboration of 4-fluorobenzene moiety as the head group of the tricyclic skeleton. As shown in Table 4, we used compound **25** as the mode compound since it showed high anti-proliferative potency in the MIA PaCa-2 cell (IC_{50} = 0.19 μmol/L). Replacement of the 4-fluorophenyl with smaller groups, such as Cl, methyl, and cyclopropyl led to compounds **30**–**32** showing much-reduced potency. De-aromatic analogues **33** and **34** also showed a significant decrease in potency. The bioisosteric thien-3-yl analogue **35** retained good potency in the two cells with IC_{50} values of 0.65 and 1.20 μmol/L, respectively. Other substituted phenyl analogues **36**–**38** also showed reduced potency. These results suggested that the 4-fluorophenyl group in the tricyclic core is crucial for anti-proliferative activity in the PAK4-expressed cancer cells.

2.3. Anti-proliferative effects of selected compounds in pancreatic cancer cells

From the SAR studies above, several tricyclic compounds showed comparable or even higher potency than the bicyclic reference compound **4**. Meanwhile, compared to **4**, these new compounds possessed a more pronounced preference against the MIA PaCa-2 pancreatic cell than against U-2 osteosarcoma cancer cells. Since treatment of pancreatic cancer is an unmet clinical need and PAK4 has been found over-expressed in pancreatic cells, therefore, PAK4 inhibitors may provide therapeutic benefits for this most detrimental cancer. In this regard, we selected the seven potent compounds (**13**, **18**, **19**, **21**, **22**, **24**, and **25**) and tested their anti-proliferative effects against a broad spectrum of pancreatic cancer cell lines. As shown in Table 5, six pancreatic cancer cell lines (AsPC-1, Pan02, HPAF-II, PANC-1, BxPC-3, and CFPAC-1) were selected and a normal human pancreatic ductal cell line (hTERT-HPNE) was employed for comparison. All the tricyclic compounds and the reference compound **4** were significantly potent against AsPC-1, Pan02, and HPAF-II cells with submicromolar IC_{50} values, while nearly ineffective against PANC-1, BxPC-3, and CFPAC-1 cell lines, which were in accordance to the levels of PAK4 expression in these cell lines (Supporting Information

Table 4 Cellular activities of compounds **25**, **30**–**38**.



Compd.	R^4	IC_{50} (μmol/L)[a]	
		MIA PaCa-2	U-2 OS
25		0.19 ± 0.02	1.19 ± 0.87
30	-Cl	2.85 ± 0.48	3.05 ± 0.83
31	$-CH_3$	5.46 ± 1.26	6.05 ± 3.13
32		1.90 ± 0.68	3.49 ± 0.42
33		5.77 ± 0.37	5.09 ± 1.10
34		>10	5.24 ± 0.79
35		0.65 ± 0.08	1.20 ± 0.21
36		3.06 ± 0.14	5.49 ± 1.60
37		3.15 ± 0.80	4.04 ± 2.94
38		7.66 ± 0.79	6.65 ± 1.78

[a]The data was calculated from three independent experiments as mean ± SD (n = 3).

Fig. S1 and Table S1). In particular, our new tricyclic compounds displayed a 3–7-fold improvement in activity against the Pan02 cell line compared to **4**. Additionally, these compounds did not exhibit significant anti-proliferative potency against normal pancreatic ductal cells (hTERT-HPNE). These results excluded the general cytotoxicity of these new compounds.

2.4. Suppressive effects of selected compounds on PAK4 phosphorylation and downstream signaling

To investigate the PAK4 involvement in the anti-proliferative activity, we tested the effects of compounds on PAK4 phosphorylation and downstream signaling. As shown in Fig. 3, both the levels of phosphorylated PAK4 (p-PAK4) and phosphorylated β-Catenin (p-β-Catenin) were decreased following treatment with the selected compounds. Most of the selected compounds, especially compounds **13**, **18**, **19**, **21**, **22**, and **25** were superior to **4** in reducing both p-PAK4 and p-β-Catenin. The results suggested that these compounds suppressed the PAK4 signaling pathway. Given the different structural features and the high inhibitory activity both on PAK4 signaling and on cell proliferation, compounds **13**, **22**, and **25** were chosen for further investigation.

2.5. Pharmacokinetics properties and hERG inhibition of selected compounds

To validate the developmental potential of these tricyclic compounds, we tested the pharmacokinetics (PK) properties of compounds **4**, **13**, **22**, and **25** in rats after intravenous (*i.v.*) administration of 1 mg/kg and oral (*p.o.*) administration of 3 mg/kg. As shown in Table 6, compound **4** exhibited an extremely high plasma exposure (AUC_{last} = 94,401 h*ng/mL) and a very low clearance (CL_{obs} = 0.169 mL/min/kg) in intravenous administration at a dose of 1 mg/kg. Similarly, an extremely high plasma exposure (AUC_{last} = 55,493 h*ng/mL) was also observed for **4** following oral administration at a dose of 3 mg/kg. The high plasma exposure and low clearance profile of **4** support its high antitumor efficacy *in vivo* but also propose a potential safety concern on its high accumulation *in vivo*. The three new compounds displayed significant discrepancies in plasma exposure either orally or intravenously. Compound **13** bearing a piperidyl moiety showed the highest plasma exposure both orally (AUC_{last} = 1590 h·ng/mL) and intravenously (AUC_{last} = 4070 h·ng/mL) and the lowest plasma clearance (CL_{obs} = 4.06 mL/min/kg) in intravenous administration. Therefore, compound **13** has a longer half-life and an acceptable oral bioavailability of 13%. Unfortunately, compounds **22** and **25** both bearing a pyridazinyl moiety showed less optimal PK properties, especially compound **22** showing the lowest plasma exposure and nearly no oral availability. In addition, the hERG inhibitory activity of compound **13** was measured to evaluate the potential cardiotoxicity. As shown in Table 6, this compound

Table 5 Activity profile against pancreatic cancer cell lines of selected compounds[a].

Compd.	IC_{50} (μmol/L)						
	AsPC-1	Pan02	HPAF-II	PANC-1	BxPC-3	CFPAC-1	hTERT-HPNE
4	0.44 ± 0.18	1.64 ± 0.70	0.61 ± 0.25	>20	>20	>20	>20
13	0.22 ± 0.06	0.50 ± 0.14	1.16 ± 0.59	>20	>20	>20	19.2 ± 7.30
18	0.34 ± 0.22	0.25 ± 0.10	>20	>20	>20	>20	>20
19	0.87 ± 0.10	0.57 ± 0.15	0.91 ± 0.20	>20	>20	>20	>20
21	0.28 ± 0.07	0.27 ± 0.05	0.46 ± 0.11	>20	>20	18.7 ± 1.62	8.75 ± 1.33
22	0.18 ± 0.11	0.22 ± 0.06	0.40 ± 0.03	>20	>20	>20	15.4 ± 4.41
24	0.26 ± 0.12	0.30 ± 0.07	0.43 ± 0.08	>20	>20	>20	>20
25	0.27 ± 0.11	0.29 ± 0.11	0.36 ± 0.24	>20	>20	>20	>20

[a]The data was calculated from three independent experiments as mean ± SD (n = 3).

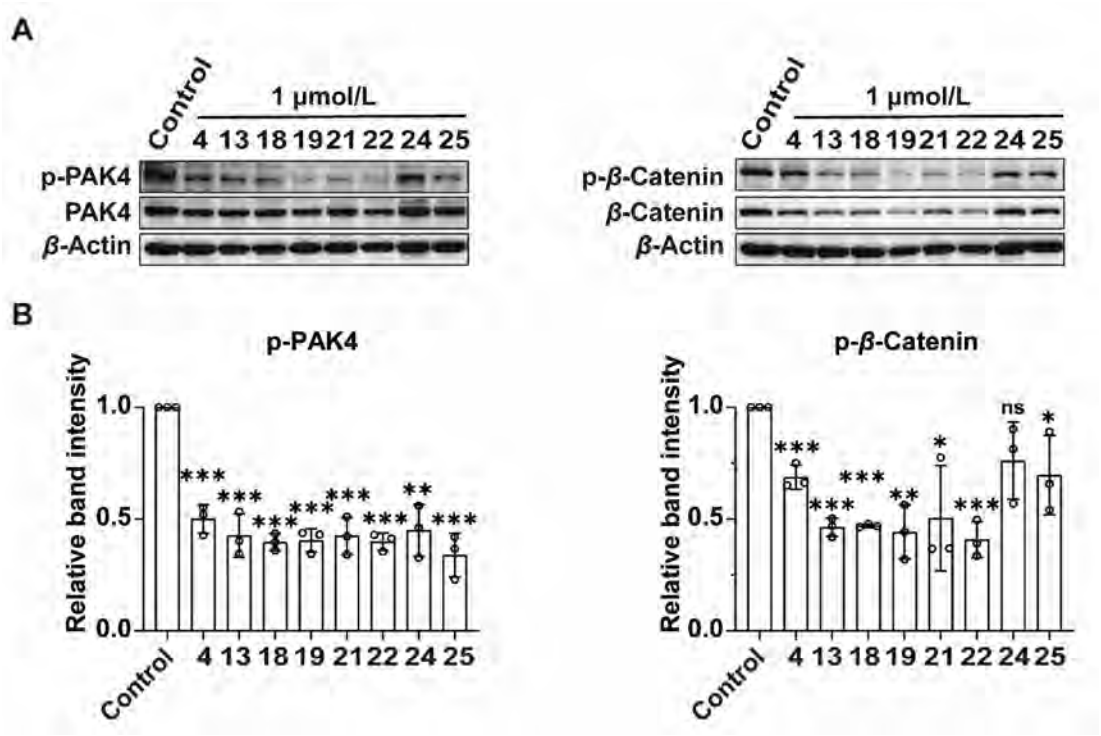


Figure 3 The inhibition effects of selected compounds on PAK4 phosphorylation and downstream signal. (A) Western blot analysis the levels of phosphorylated PAK4 (p-PAK4) and phosphorylated β-Catenin (p-β-Catenin) in MIA PaCa-2 cells after treating compounds at 1 μmol/L for 72 h. (B) Quantificational levels of p-PAK4 and p-β-Catenin in treated cells were assessed by Western blot densitometry. The ratios versus the loading control (β-Actin) at each treatment condition were normalized to that of the untreated group. Significant differences between the treated groups and the vehicle control were determined by Student's *t*-test. Data are presented as mean ± SD ($n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns, not significant).

Table 6 Pharmacokinetics parameters and hERG inhibitory activity of new compounds[a,b].

Administration	Parameter	**4**	**13**	**22**	**25**
p.o. (3 mg/kg)	$T_{1/2}$ (h)	4.59	3.47	3.35	5.52
	T_{max} (h)	4.00	4.00	0.417	3.33
	C_{max} (ng/mL)	5987	192	3.98	168
	AUC_{last} (h ng/mL)	55,493	1590	8.98	949
i.v. (1 mg/kg)	$T_{1/2}$ (h)	5.61	4.45	1.49	1.90
	AUC_{last} (h·ng/mL)	94,401	4070	388	2479
	$CL_{\_obs}$ (mL/min/kg)	0.169	4.06	42.7	6.64
	V_{ss_obs} (L/kg)	0.074	1.63	4.12	1.04
	F (%)	19.6	13.0	0.771	12.8
hERG	IC_{50} (μmol/L)	–	>40	–	–

[a]The dash line indicates no test.
[b]The data was calculated from three independent experiments as mean ± SD ($n = 3$).

showed an IC_{50} greater than 40 μmol/L in the patch-clamp assay, indicating its high safety index. Based on these results, compound **13** was elected for further mechanism and *in vivo* studies.

*2.6. Compound **13**-targeting PAK4 in a non-ATP-competitive manner*

In order to elucidate whether compound **13** directly targets PAK4, we prepared a photoaffinity-labeled probe **39** (Fig. 4A) with a structure similar to compound **13** and performed a competition assay using Western blot analysis. As shown in Fig. 4B, PAK4 protein was detected in probe-labeled products and the PAK4 protein was significantly reduced in the presence of compound **13**. A similar result was observed in the presence of the reference compound **4** (KPT-9274). These results indicated that compound **13** directly interacted with PAK4 in a similar pattern to **4**. To further confirm the interaction mode of compound **13** with PAK4, a surface plasmon resonance (SPR) assay was performed. Due to the poor aqueous solubility of compound **13**, the more aqueously soluble analogue **25** (HCl salt: 30 mg/mL) was selected to test the

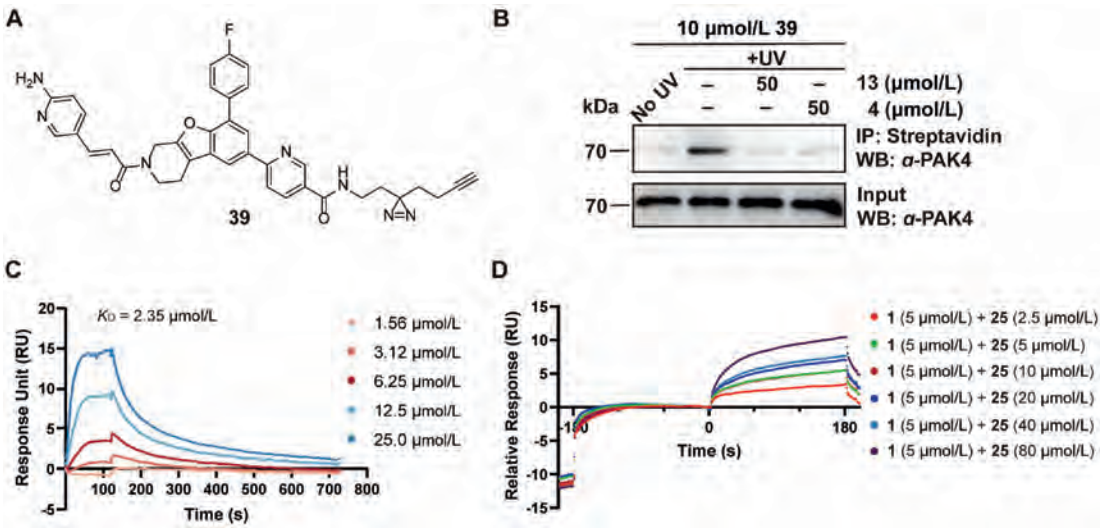


Figure 4 Compound **13** directly binds to non-ATP pocket of PAK4. (A) The structure of photoaffinity labeled probe **39**. (B) PAK4-**13** interaction by Western blot analysis in the immunoprecipitation product. No UV group was used as a negative control. (C) The binding affinity of **25**, an analogue of **13**, with PAK4 kinase domain was determined by the SPR assay. (D) PAK4 kinase domain treatment with **25** at different concentrations in the presence of supersaturated PF-3758309 (**1**) was analyzed by SPR assay.

binding affinity with the PAK4 kinase domain. Similarly, compound **5** (KPT-7523), the close analogue of **4**, was selected as the control due to its good aqueous solubility and the confirmed study on its binding to the kinase domain of PAK4 in the previous study[27]. As shown in Fig. 4C, compound **25** exhibited a medium affinity with a K_D value of 2.35 μmol/L, which is superior to that of compound **5** with a K_D value of 9.65 μmol/L (Supporting Information Fig. S2). To further investigate the binding mode of **25**, the affinity with the full-length PAK4 was determined, and the K_D value was 8.20 μmol/L (Supporting Information Fig. S3). These results suggest that compound **25** has a preferential binding affinity for the kinase domain of PAK4. Subsequently, the SPR competition assay was performed. The reported binding affinity of the orthosteric PAK4 inhibitor **1** (PF-3758309), which binds to the ATP pocket, was 4.5 nmol/L (K_D)[21], significantly more potent than that of **25**. However, the SPR signal of **25** was found to increase gradually with the increasing concentration in the presence of an excessive dose of **1** (Fig. 4D), indicating that **25** binds with PAK4 in a different manner (allosterically), rather than the catalytic pocket as of compound **1**. Taken together, the new compound **13** directly interacts with PAK4 in a non-ATP pocket of the kinase domain.

2.7. *Compound **13**-reduced the phosphorylation of PAK4 and β-Catenin in a dose-dependent manner*

To further investigate the effects of compound **13** against the PAK4 signaling pathway, Western blot analysis was conducted in the MIA PaCa-2 cell line at different concentrations. As shown in Fig. 5A, the levels of p-PAK4 (the lower band marked with an arrow, Supporting Information Fig. S4) and p-β-Catenin decreased in a dose-dependent manner after treatment of compound **13** with IC_{50} values of 0.84 and 0.73 μmol/L, respectively against p-PAK4 and p-β-Catenin (Fig. 5B), which were superior to that of compound **4** with IC_{50} values of 1.89 and 2.07 μmol/L (Supporting Information Fig. S5), respectively, in the same assay. At the concentration of 0.5 μmol/L, the level of p-β-Catenin was lower in the **13**-treated group compared to the **4**-treated group. Since the WNT/β-Catenin pathway is closely associated with pancreatic cancer progression[29,30], we then determined the levels of Cyclin D1 and c-Myc, two target proteins of the WNT/β-Catenin pathway. As shown in Fig. 5C, both the levels of Cyclin D1 and c-Myc decreased with the increasing concentrations of compound **13**. Notably, the reduction of c-Myc was more significant after treatment of compound **13** at 1 μmol/L than treatment of **4**. These results validated that compound **13** inhibited the WNT/β-Catenin pathway in a dose-dependent manner by targeting PAK4.

2.8. *Compound **13**-induced apoptosis of MIA PaCa-2 cells*

To further investigate whether compound **13** induces apoptosis in MIA PaCa-2 cells, flow cytometric analysis together with annexin V-FITC (V) and propidium iodide (PI) staining was conducted to measure the percentage of apoptotic cells after 72 h of treatment. The percentage of total apoptotic cells (annexin V+/PI + cells and annexin V+/PI− cells) increased from 2.76% to 25.9% following treatment with 0.5 μmol/L of compound **13**, and further increased to 40.3% with concentrates up to 2 μmol/L. These results indicated that compound **13** induced apoptosis in a dose-dependent manner, and was more efficient than **4** at the high concentration (Fig. 6A and B). In the meantime, we examined the expression levels of apoptosis-associated markers BCL-XL and Bim, the key downstream proteins of β-Catenin[31,32]. As shown in Fig. 6C, the anti-apoptotic protein BCL-XL decreased upon **13**-treatment. Conversely, the level of pro-apoptotic protein Bim increased in a concentration-dependent manner. Taken together, these results indicated that compound **13** exhibited anti-proliferative effects against MIA PaCa-2 cells, which was attributed to its effects on promoting apoptosis.

2.9. *Kinase selectivity of compound **13***

In view of the novel tricyclic skeleton of compound **13** distinct from the bicyclic compound **4**, an off-target effect might be a concern. In this regard, we conducted the kinase selectivity study

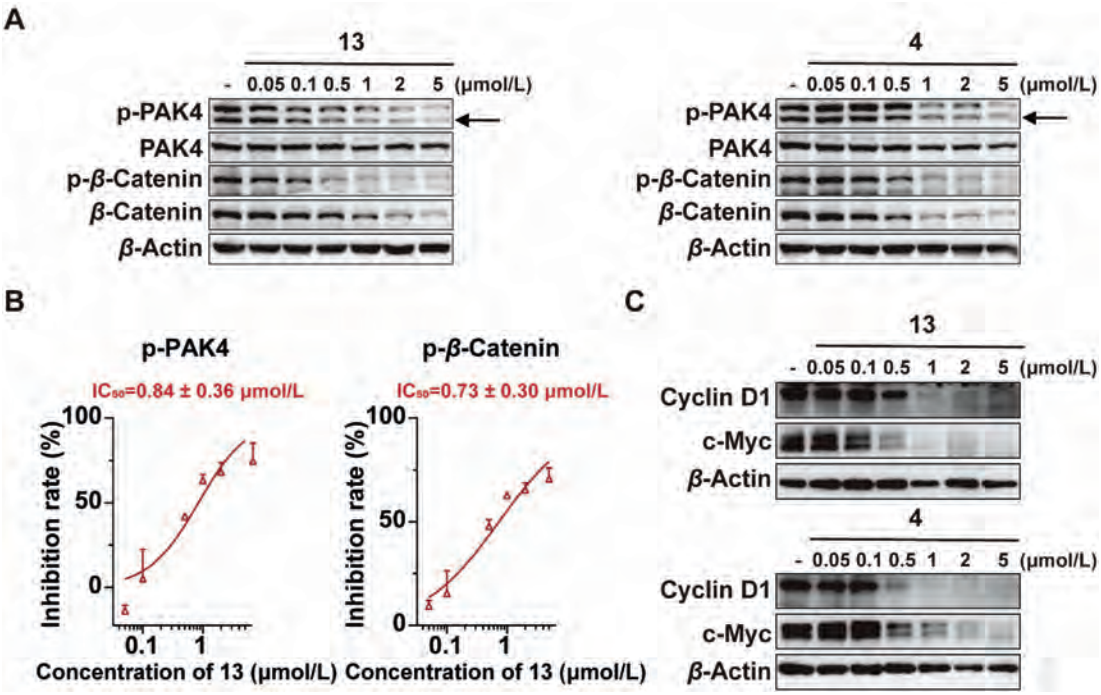


Figure 5 Compound **13** impacted the phosphorylation and downstream signaling of PAK4. (A) Western blot analysis of the levels of p-PAK4 and p-β-Catenin in MIA PaCa-2 cells after treating compound **13** or **4** at different concentrations (0, 0.05, 0.1, 0.5, 1, 2, and 5 μmol/L) for 72 h. (B) Inhibition activities of **13** against p-PAK4 and p-β-Catenin in MIA PaCa-2 cells. The inhibition rates at different concentrations were calculated according to the relative quantificational levels of p-PAK4 and p-β-Catenin compared with that of the untreated group. (C) Western blot analysis the levels of Cyclin D1 and c-Myc in MIA PaCa-2 cells after treating compound **13** or **4** at different concentrations (0, 0.05, 0.1, 0.5, 1, 2, and 5 μmol/L) for 72 h.

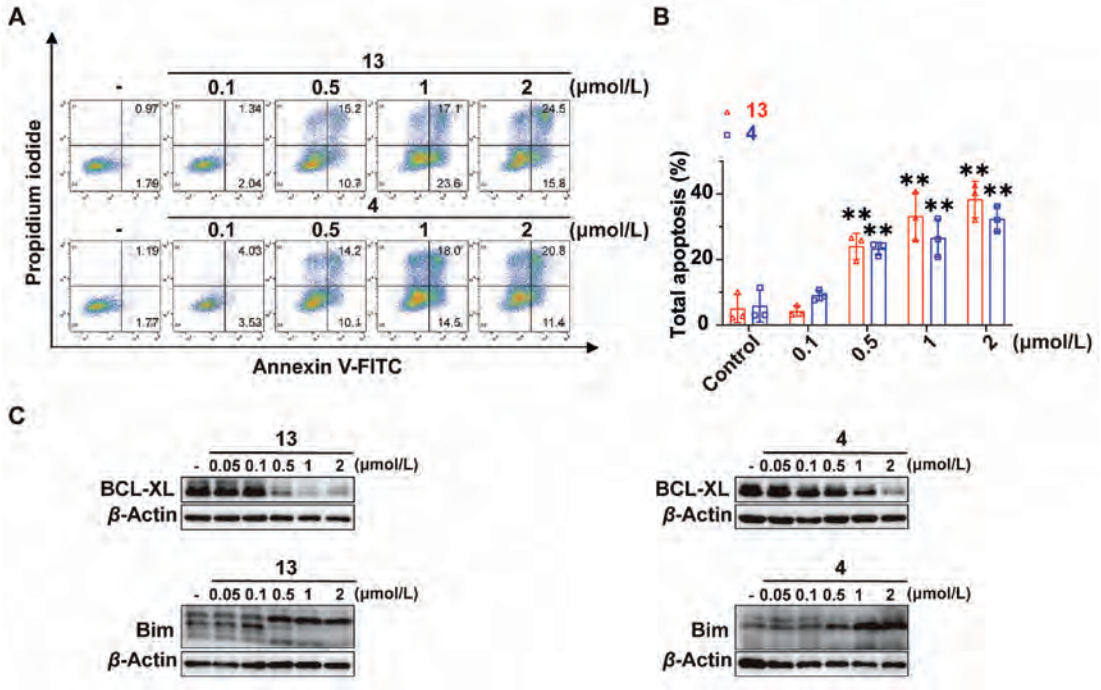


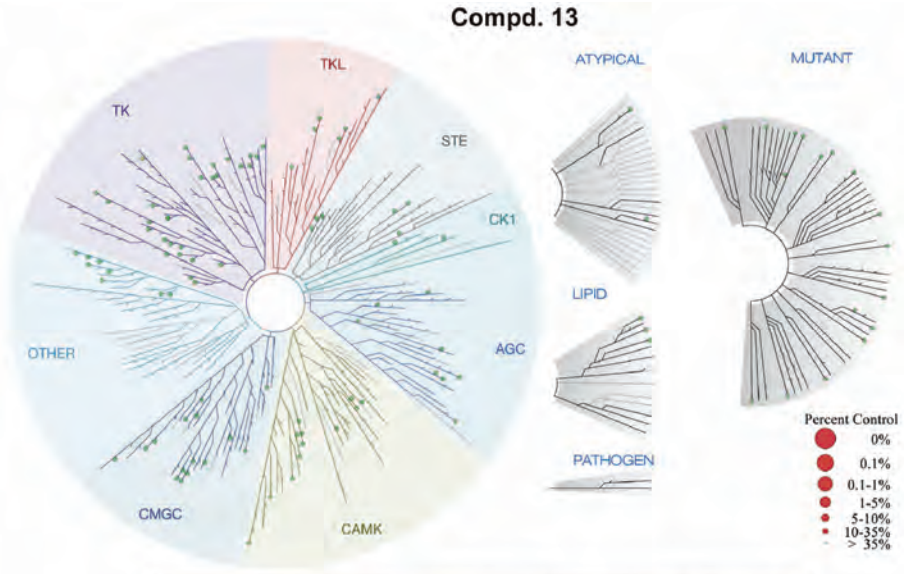
Figure 6 Compound **13** induced apoptosis in MIA PaCa-2 cells. (A) Representative scattergrams of flow cytometry analysis of MIA PaCa-2 cells after treatment with compound **13** or **4** with different concentrates (0.1, 0.5, 1, and 2 μmol/L). The cells were stained by annexin V-FITC and propidium iodide; (B) Statistical graph of total apoptosis rate; (C) Western blot analysis for expression levels of BCL-XL and Bim in MIA PaCa-2 cells treated with compound **13** or **4**. Significant differences between the treated groups and the vehicle control were determined by Student's *t*-test. Data are presented as mean ± SD ($n = 3$, $^{**}P < 0.01$).

of **13** through the KINOME*scan*™ screening platform against a panel of 97 kinases together with their mutants at the concentration of 1 μmol/L. As shown in Fig. 7 and Supporting Information Table S2, compound **13** showed no significant inhibitory effects on all the tested kinases with a percent control as low as 35% [S-score (35) = 0] at the tested concentration. Although the KINOME*scan*™ assay is to test the inhibitory effects of compounds against the catalytic activity in the ATP interaction domain of kinases and is not suitable for testing compounds interacting in the allosteric sites of kinase, the results still give useful insights on the good selectivity of compound **13** over other kinases.

*2.10. Compound **13**-suppressed the growth of tumors in vivo*

To assess the antitumor efficacy of compound **13** *in vivo*, MIA PaCa-2 cells were implanted into nude mice to establish the xenograft model. As shown in Fig. 8A and B, compound **13** exhibited antitumor efficacy *in vivo* at a dose of 100 mg/kg with a tumor growth inhibition rate (TGI) of 57.3%, and no mortality and significant body weight loss were observed during the 20-day study period. As a comparison, the antitumor effect of the reference **4** was also tested under the same conditions. Unfortunately, two mice in the **4**-treated group at the dose of 100 mg/kg died on Day 18, which might be related to its high plasma exposure and low clearance. The rest of the mice in this group achieved a tumor growth inhibition of 77.8%. Meanwhile, the levels of PAK4 and p-PAK4 were found to be reduced significantly in tumor tissues in the **13**-treatment group (Fig. 8D and E). In the tumor tissues, the downstream effector β-Catenin was also decreased (Fig. 8F). These results demonstrated that compound **13** suppressed tumor growth *in vivo* through inhibition of PAK4.

To further validate the therapeutic potential of compound **13** in pancreatic cancer, the antitumor efficacy was tested in C57BL/6 syngeneic mice bearing the Pan02 cell, another murine pancreatic cancer cell sensitive to compound **13**. As shown in Fig. 9A, compound **4** exhibited a modest tumor growth inhibition at the dose of 100 mg/kg by the end of the experiment. However, compound **13** more potently suppressed the tumor growth at the same dose without any observable loss in body weight (Fig. 9B). Subsequently, Western blot analysis of the **13**-treatment group revealed a significant reduction of PAK4 and p-PAK4 expressions in tumor tissues (Fig. 9C). Since previous reports have demonstrated a negative correlation between PAK4 levels and immune infiltration[20,33], the composition of immune cells in the Pan02 allograft in the **13**-treatment group was examined through Cytometry by Time-Of-Flight (CyTOF). As shown in Fig. 9D, treatment with compound **13** led to a significant increase in dendritic cells (DCs) and macrophages, particularly macrophages. Notably, the amount of mature DC cells (MHC II^+ DC and $CD80^+$ DC) increased significantly compared to the control group. These results suggested that compound **13** enhanced mature immune cell infiltration in pancreatic cancer. Furthermore, the antitumor $CD80^+$ macrophages were increased, whereas the tumor-promoting $CD206^+$ macrophages remained unaffected in the **13**-treated group. Additionally, the amount of immune suppressive myeloid-derived suppressor cells (MDSCs) was found to be decreased slightly in tumor tissue.



Compd	Selectivity Score Type	Number of Hits	Number of Non-Mutant Kinases	Selectivity Score
	S(35)	0	90	0
13	S(10)	0	90	0
	S(1)	0	90	0

Figure 7 Kinase selectivity of compound **13** in Eurofins DiscoverX KINOMEscan™ screening platform. The assays were performed at 1 μmol/L concentration. Upper panel: TREE*spot* interaction maps in 97 kinase targets. Lower panel: S-scores of **13** with percent control numbers less than 35, 10, and 1, respectively. S-score (number of hits/number of kinases tested) is a quantitative measure of compound selectivity.

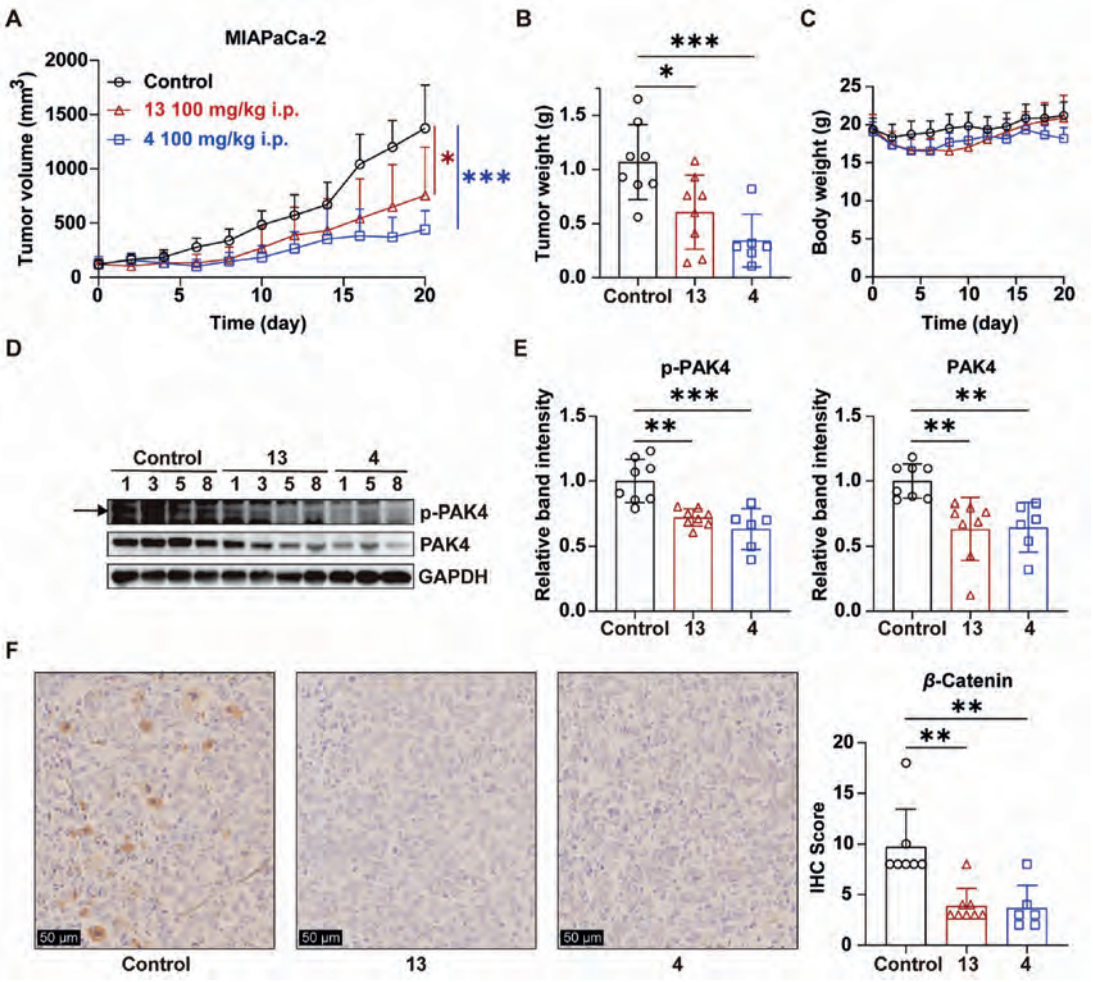


Figure 8 Compound **13** suppressed the growth of MIA PaCa-2 xenograft by targeting PAK4. (A) Recording of tumor volume during 20 days; (B) Tumor weight of each group at the endpoint; (C) Body weight of mice; (D) The levels of PAK4 and p-PAK4 in representative tumor tissue determined *via* Western blot analysis. (E) Semi-quantitative analysis of panel E; (F) The level of *β*-Catenin determined by immunohistochemistry (scale bar = 50 μm). Significant differences between the treated groups and the vehicle control were determined by two-way ANOVA and one-way ANOVA (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Intriguingly, the level of T-cell infiltration did not show an increase at the endpoint, indicating that further investigation is needed. Taken together, compound **13** improved infiltration of mature DCs and $CD80^+$ macrophages, and fostered the development of an antitumor immune microenvironment in pancreatic cancer.

2.11. Chemistry

The synthesis of tricyclic tetrahydrobenzofuro[2,3-*c*]pyridine skeleton is conducted according to the previous reports[34,35]. As shown in Scheme 1, the Suzuki coupling of bromobenzene **A1** and 4-fluorobenzeneboronic acid was conducted under $Pd(dppf)Cl_2$ and K_3PO_4 to deliver the intermediate **A2** in 59% yield. Bromination of **A2** with NBS gave the bromide **A3** in 94% yield. Nucleophilic substitution of **A3** with ethyl bromoacetate delivered the ester **A4** in 96% yield. Benzoic acid **A5** was obtained in 98% yield by treating **A4** with an aqueous solution of NaOH. Intramolecular cyclization and decarboxylation of intermediate **A5** gave 3-acetoxy-benzofuran **A6** in 73% yield. Subsequently, the ester **A6** was hydrolyzed in dilute hydrochloric acid to give benzofuran-3-one **A7** in 99% yield. **A8** was obtained in 63% yield by treating **A7** with a phosphorus ylides reagent and sodium hydride. A subsequent reduction reaction of **A8** using a borane-tetrahydrofuran complex provided the key intermediate **A9** in 53% yield. Next, treatment of amine **A9** with an appropriate aldehyde or ketone furnished the tricyclic tetrahydrobenzofuro [2,3-*c*]pyridine products **A10**−**A15** through a Mannich reaction at 50 °C or 140 °C in acidic solvents, respectively. Due to the poor solubility and the difficulty in isolation of the tricyclic compounds **A10**−**A15**, the crude products were directly subjected to NH-protection with bis(*tert*-butoxycarbonyl)oxide to generate compounds **A16**−**A21** in 24%−58% yields over two steps. The Suzuki coupling of bromides **A16**−**A21** with appropriate boronic esters under $Pd(PPh_3)_4$ and K_2CO_3 delivered precursors **A22**−**A27** in 30%−61% yields. Subsequent Boc-deprotection with trifluoroacetic acid followed by condensation with (2*E*)-3-(6-amino-3-pyridinyl)-2-propenoic acid in the presence of HATU and DIPEA provided the target compounds **6**−**11** in 18%−32% yield over two steps.

The synthesis of compounds **12**−**29** is described in Scheme 2. A coupling reaction of **A16** with bis(pinacolato)diboron delivered the borate **A28** in 47% yield. Subsequent Suzuki coupling with various

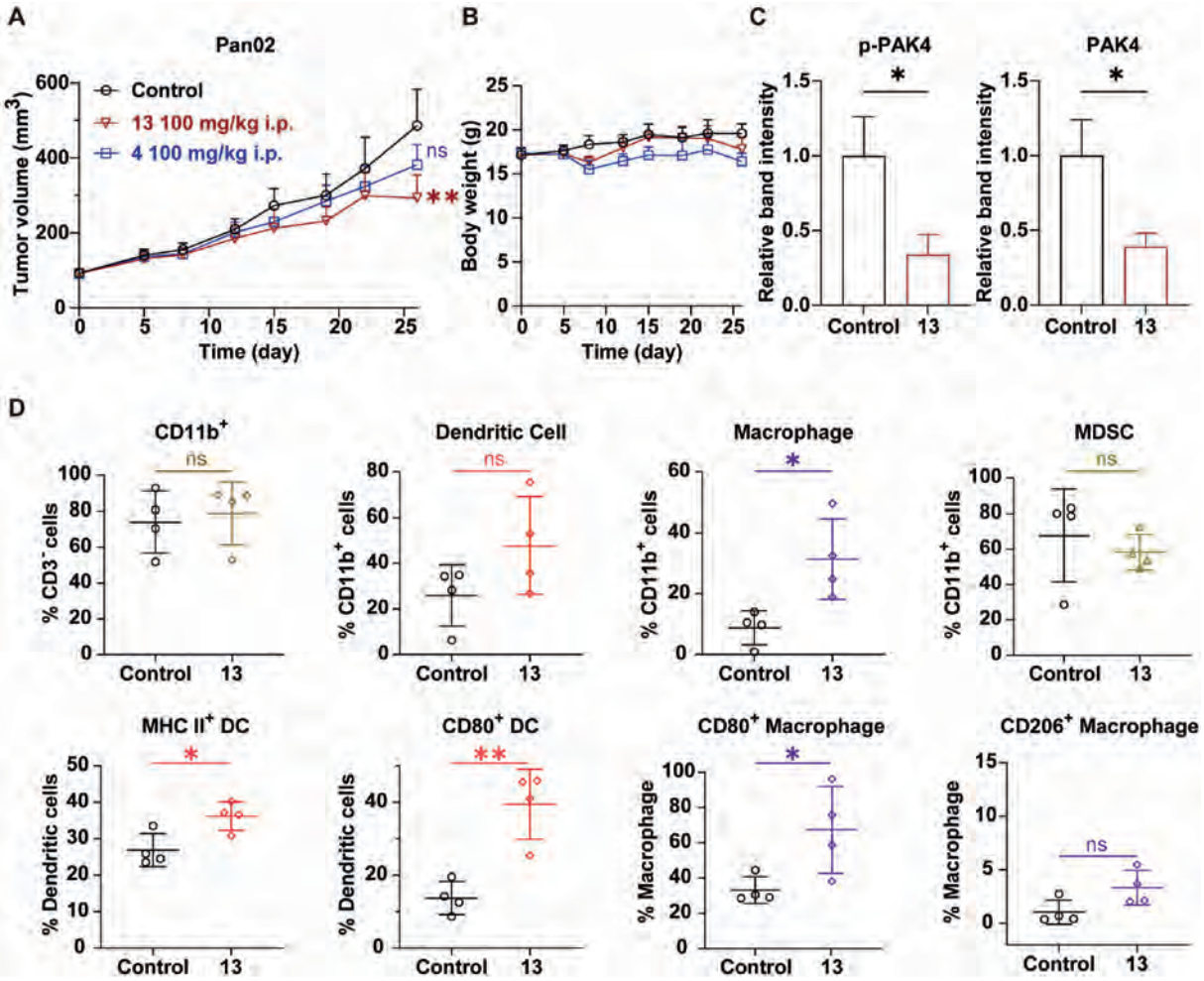
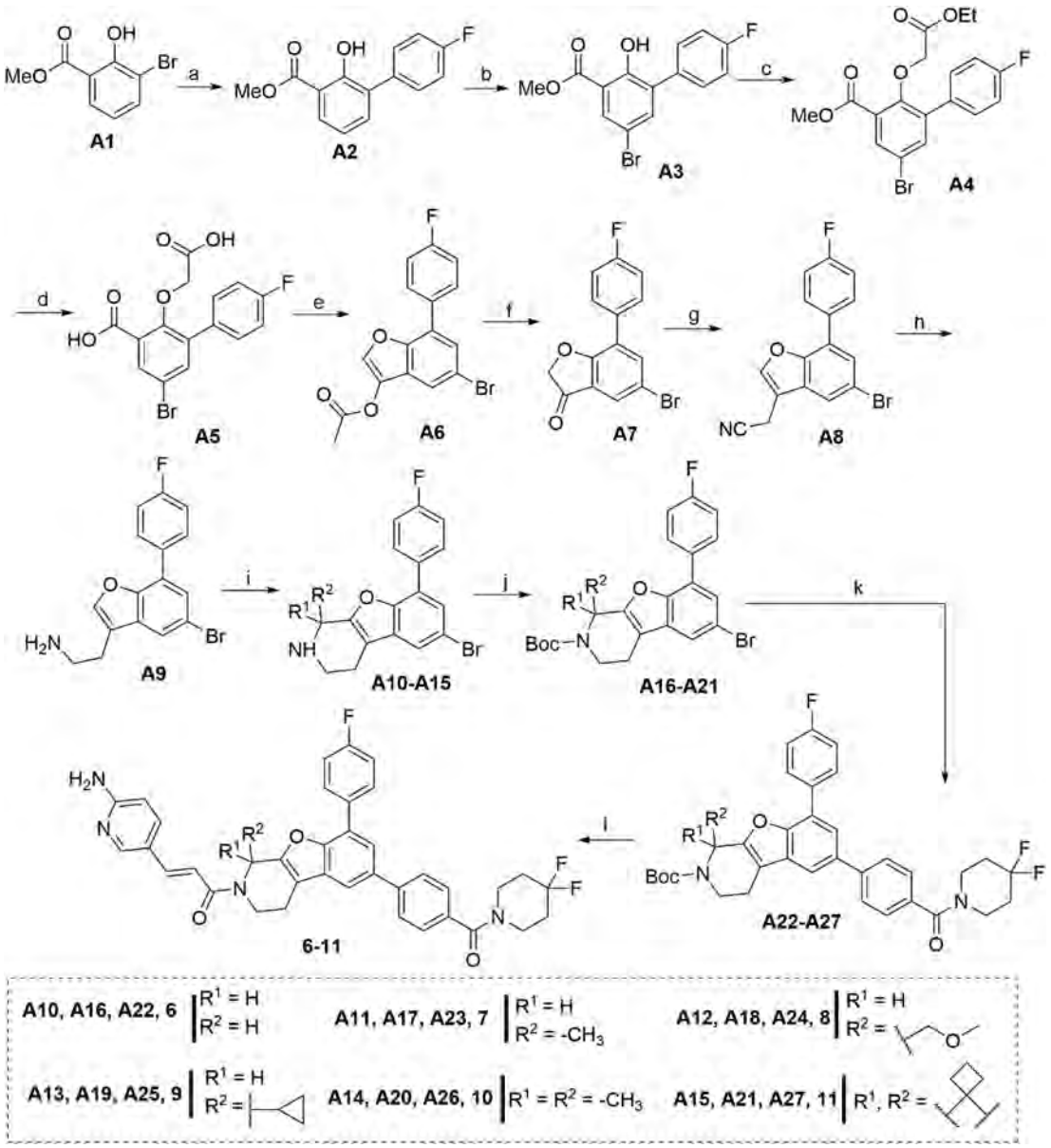


Figure 9 Compound **13** suppressed the tumor growth in the Pan02 model by targeting PAK4. (A) Recording of tumor volume during 26 days; (B) Body weight of mice during 26 days; (C) The levels of PAK4 and p-PAK4 in representative tumor tissue determined *via* Western blot analysis. (D) The percentage of $CD11b^+$, dendritic cell (DC), Macrophage, myeloid-derived suppressor cell (MDSC), MHC II^+ DC, $CD80^+$ DC, $CD80^+$ Macrophage and $CD206^+$ Macrophage were examined by CyTOF. Significant differences between the treated groups and the vehicle control were determined by two-way ANOVA and Student's *t*-test. (*$P < 0.05$, **$P < 0.01$, ns, no significant).

halogenated aryl or heteroaryl **K8**−**K14** in the presence of $Pd(PPh_3)_4$ and K_2CO_3 delivered intermediates **A29**−**A35** in 43%−83% yields. The target compounds **12**−**18** were obtained in 23%−46% yields by following similar procedures as that for compounds **6**−**11** over two steps. The synthesis of compounds **19**−**29** is outlined in Scheme 2. Suzuki coupling of boronic ester **A28** with variously substituted pyridazinyl chlorides **K15**−**K25** yielded the corresponding intermediates **A36**−**A46** in 35%−87% yields. Removal of the *N*−Boc followed by condensation with (2*E*)-3-(6-amino-3-pyridinyl)-2-propenoic acid in the presence of HATU and DIPEA in DMF provided compounds **19**−**29** in 18%−57% yields over two steps.

The synthesis of compounds **30**−**38** is described in Scheme 3. Bromination of phenol **A47** with NBS provided bromide **A48** in 96% yield, which was then subjected to nucleophilic substitution with ethyl bromoacetate to give **A49** in 98% yield. Benzoic acid **A50** was obtained in 96% yield by treating **A49** with NaOH aqueous solution. Intramolecular cyclization and decarboxylation of intermediate **A50** gave 3-acetoxy-benzofuran **A51** in 43% yield. Subsequently, the ester **A51** was hydrolyzed in dilute hydrochloric acid to give benzofuran-3-one **A52** in 98% yield. Nitrile **A53** was obtained in 62% yield by treating **A52** with a phosphorus ylide-diethyl cyanomethylphosphonate and sodium hydride. Borane reduction of **A53** in refluxing THF provided amine **A54** in 51% yield. Mannich reaction of amine **A54** and formaldehyde yielded the tricyclic **A55**, which was *N*-Boc protected to give **A56** in 40% overall yield. The key intermediate **A58** was obtained in 47% overall yield *via* treatment of **A56** with bis(pinacolato)diboron followed by Suzuki coupling with substituted pyridazinyl chlorides **K21** in the presence of $Pd(PPh_3)_4$ and K_2CO_3. Subsequent Suzuki coupling of chloride **A58** with various boronic esters under the catalysis of $Pd_2(dba)_3$ in the presence of Cs_2CO_3 in the microwave at 140 °C yielded final compounds **A59**−**A66** in 19%−82% yields. Finally, *N*-Boc-deprotection of **A58**−**A66** and subsequent amide condensation with (2*E*)-3-(6-amino-3-pyridinyl)-2-propenoic acid in the presence of HATU and DIPEA formed target compounds **30**−**38** in 21%−42% overall yields in two steps.

The synthesis of the probe compound **39** is described in Scheme 4. Suzuki coupling of boronic ester **A28** with methyl 6-bromonicotinate in the presence of $Pd(PPh_3)_4$ and K_2CO_3 delivered **A67** in 65% yield. Boc-deprotection of **A67** with trifluoroacetic acid followed by condensation with (2*E*)-3-(6-

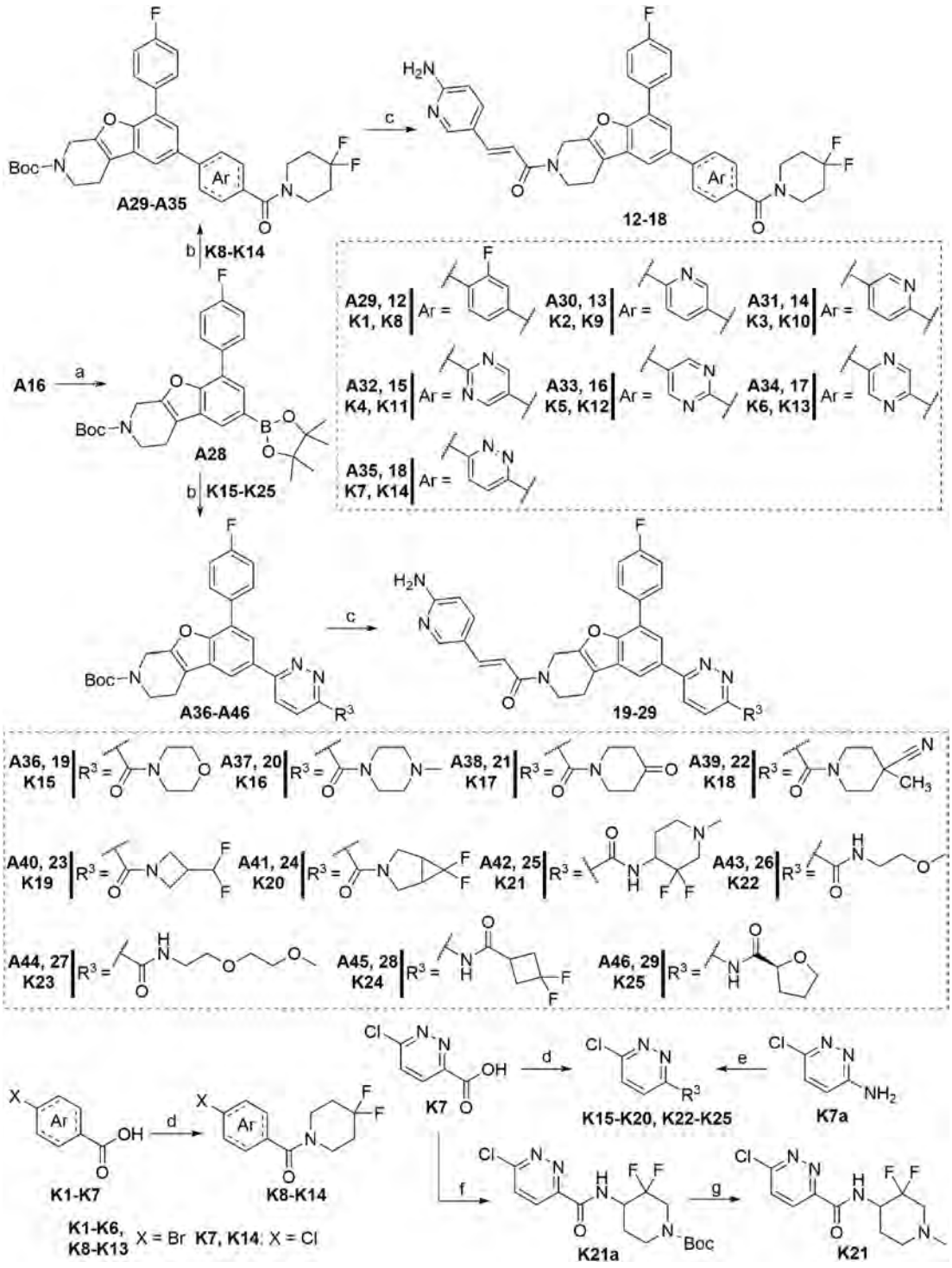


Scheme 1 Synthesis of compounds **6−11**. Reagents and conditions: (a) 4-fluorobenzeneboronic acid, $PdCl_2$(dppf), K_3PO_4, DMF, H_2O, 45 °C, 4 h, 59%; (b) NBS, CH_3CN, 60 °C, 4 h, 94%; (c) ethyl bromoacetate, K_2CO_3, acetone, reflux, 4 h, 96%; (d) 1 mol/L NaOH (aq), EtOH, reflux, 6 h, 98%; (e) Ac_2O, AcOH, AcONa, 130 °C, 8 h, 73%; (f) 1 mol/L HCl (aq), MeOH, reflux, 3 h, 99%; (g) diethyl cyanomethylphosphonate, NaH, THF, 0 °C, overnight, 63%; (h) BH_3, THF, reflux, 3 h, 53%; (i) aldehyde or ketone, HCOOH, 50 °C or 140 °C, overnight; (j) Boc_2O, DIPEA, DCM, rt, overnight, 24%−58% over two steps; (k) (4,4-difluoropiperidin-1-yl) (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanone, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane:H_2O = 3:1 reflux, 2 h, 30%−61%; (l) 1. CF_3COOH, DCM, r.t., 1 h; 2. (2*E*)-3-(6-amino-3-pyridinyl)-2-propenoic acid, HATU, DIPEA, DMF, r.t., overnight, 18%−32% over two steps.

amino-3-pyridinyl)-2-propenoic acid in the presence of HATU and DIPEA afforded pyridinyl ester **A68** in 62% yield over two steps. Hydrolysis of **A68** in LiOH gave acid **A69** followed by condensation with 2-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)ethan-1-amine to give the target compound **39** in 44% yield over two steps.

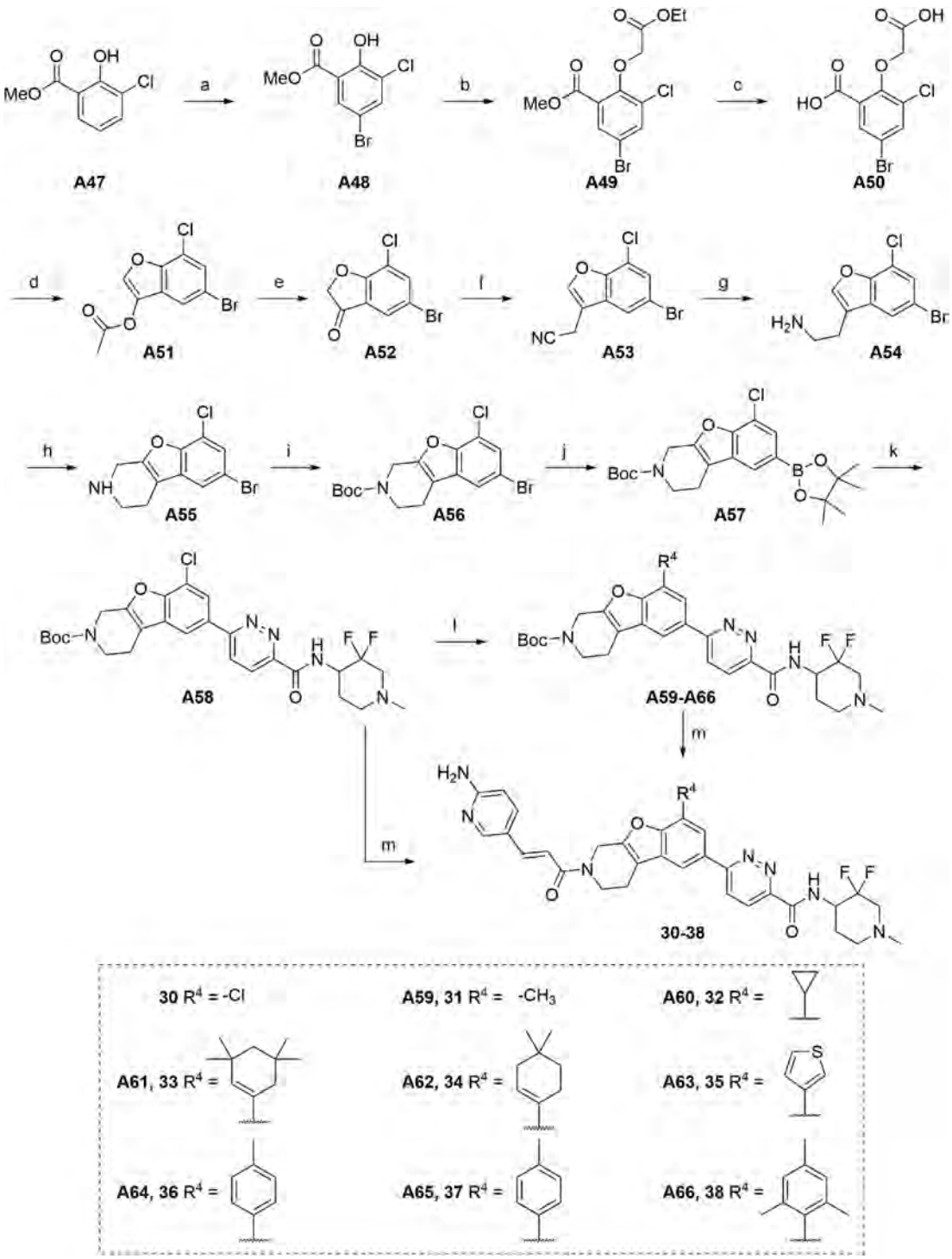
3. Conclusions

Due to its oncogenic and tumor immunomodulatory properties, PAK4 has become a promising therapeutic target for various tumors. Many small molecule PAK4 inhibitors have been disclosed, but poor antitumor efficacy and adverse safety events *in vivo* have restricted their further development. The development of allosteric inhibitors represented by KPT-9274 (**4**) raised new prospects for PAK4-targeting therapies. Inspired by the interaction mode of **4**, we conducted a scaffold hopping strategy by converting the bicyclic core of **4** into a novel tricyclic core featuring a tetrahydrobenzofuro[2,3-*c*]pyridine skeleton. Further elaboration of other structural elements of the tricyclic skeleton resulted in compound **13** exhibiting favorable PK properties and higher potency against MIA PaCa-2 and Pan02 cell lines. Furthermore,



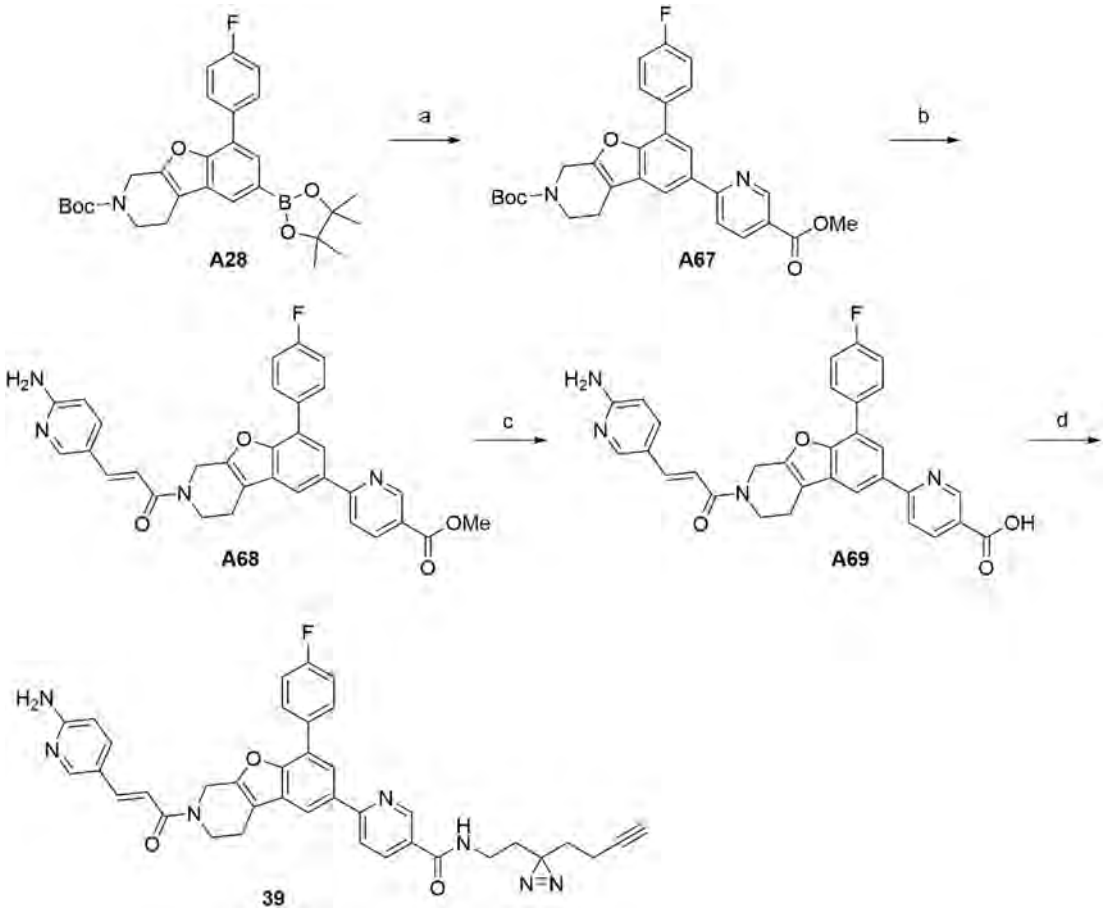
Scheme 2 Synthesis of compounds **12**−**29**. Reagents and conditions: (a) Bis(pinacolato)diboron, K_2CO_3, $Pd(dppf)Cl_2$, 100 °C, 2 h, 47%; (b) Aromatic halides, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane:H_2O = 3:1 reflux, 2 h, 35%−87%; (c) 1. CF_3COOH, DCM, r.t., 1 h, 2. (2*E*)-3-(6-Amino-3-pyridinyl)-2-propenoic acid, HATU, DIPEA, DMF, r.t., overnight, 18%−57% over two steps; (d) 1. $(COCl)_2$, DMF, DCM, r.t., 1 h; 2. amine, DIPEA, DCM, 0 °C, 1 h, 35%−90% over two steps; (e) acyl chloride, DIPEA, DCM, 0 °C, 1 h, 51%−53%; (f) *tert*-butyl 4-amino-3,3-difluoropiperidine-1-carboxylate, T_3P, Et_3N, DCM, r.t., overnight, 71%; (g) CH_2O, $NaBH_3CN$, MeOH, 0 °C− r.t., overnight, 82%.

compound **13** directly binds to the non-ATP pocket of the PAK4 kinase domain, and dose-dependently decreases the expression of p-PAK4 and its downstream effector p-β-Catenin, leading to inhibition of the WNT/β-Catenin signaling pathway. Additionally, compound **13** induced apoptosis in the MIA PaCa-2 cell lines in a dose-dependent manner. In the MIA PaCa-2 xenograft model, compound **13** displayed antitumor efficacy with a good safety index. Meanwhile, significant tumor growth inhibition was also



Scheme 3 Synthesis of compounds **30**−**38**. Reagents and conditions: (a) NBS, CH_3CN, r.t., 4 h, 96%; (b) ethyl bromoacetate, K_2CO_3, acetone, reflux, 4 h, 98%; (c) 1 mol/L NaOH(aq), EtOH, reflux, 6 h, 96%; (d) AcOAc, AcOH, AcONa, 130 °C, 8 h, 43%; (e) 1 mol/L HCl (aq), MeOH, reflux, 3 h, 98%; (f) diethyl cyanomethylphosphonate, NaH, THF, 0 °C, overnight, 62%; (g) BH_3, THF, reflux, 3 h, 51%; (h) HCHO, HCOOH, 50 °C, overnight; (i) Boc_2O, DIPEA, DCM, r.t., overnight, 40% over two steps; (j) Bis(pinacolato)diboron, K_2CO_3, $Pd(dppf)Cl_2$, 100 °C, 2 h, 90%; (k) $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane:H_2O = 3:1 reflux, 2 h, 52%; (l) borate, $Pd_2(dba)_3$, PCy_3, Cs_2CO_3, 1,4-dioxane, 140 °C, microwave, 4 h, 19%−82%; (m) 1. CF_3COOH, DCM, r.t., 1 h; 2. (2*E*)-3-(6-Amino-3-pyridinyl)-2-propenoic acid, HATU, DIPEA, DMF, r.t., overnight, 21%−42% over two steps.

observed in the Pan02 mice model, and the antitumor efficacy was associated with enhanced immune infiltration and immune activation in tumor tissue. Therefore, compound **13** is a novel PAK4 allosteric inhibitor with antitumor efficacy *in vivo* and with a good safety index, thus representing a new structural mode for further profiling. It is of note that the clinical compound **4** is also active



Scheme 4 Synthesis of compound **39**. Reagents and conditions: (a) Methyl 6-bromonicotinate, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane:H_2O = 3:1 reflux, 2 h, 65%; (b) 1. CF_3COOH, DCM, r.t., 1 h; 2. (2*E*)-3-(6-Amino-3-pyridinyl)-2-propenoic acid, HATU, DIPEA, DMF, r.t., overnight, 62% over two steps; (c) LiOH, THF, H_2O, r.t., overnight; (d) 2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)ethan-1-amine, HATU, Et_3N, DMF, r.t., 3 h, 44% over two steps.

against other targets (*e.g.*, NAMPT)[28], which might partially contribute to its antitumor efficacy, more profound investigations of compound **13** on both the selectivity in a wider range of targets and the detailed mechanism of action are necessary. These results will be reported in due course.

4. Experimental

4.1. Chemical reagents and general method

All commercially available starting materials and solvents are of reagent grade and used without further purification (Reagents and solvents were obtained from Sinopharm Chemical Reagent Co., Ltd., Shanghai; Bide Pharmatech Ltd., Shanghai; Shanghai Macklin Biochemical Technology Co., Ltd.). Column chromatography was performed using a 300−400 mesh or 200−300 mesh silica gel. Analytical TLC was carried out employing 60 F254 plates, and spots were visualized using UV (254 or 365 nm). ^1H and ^{13}C NMR spectra were recorded with the Varian-III 400 MHz NMR, Varian-III 500 MHz NMR, Varian-III 600 MHz NMR or Varian-NEO 700 MHz NMR spectrometer. Chemical shifts (δ) were reported in ppm downfield from an internal TMS standard, and *J* values were given in Hz. High-resolution mass spectra were obtained from acquity UPLC/QTOF premier mass spectrometer. HPLC spectrum was recorded for all bioassay compounds on an Agilent Technologies 1260 series LC system (Agilent ChemStationRev.A.10.02; ZORBAX-C18, 4.6 mm × 150 mm, 5 μm) with ultraviolet wavelength (UV 254 nm). The purities of these compounds (except for compound 10) were above 95%.

*Methyl 4′-fluoro-2-hydroxy-[1,1′-biphenyl]-3-carboxylate (**A2**).* To a solution of **A1** (60 g, 260 mmol) and 4-fluorobenzeneboronic acid (40 g, 286 mmol) in DMF (120 mL) and H_2O (60 mL) was added $Pd(dppf)Cl_2$ (9.6 g, 12.8 mmol) and K_3PO_4 (165 g, 779 mmol). The mixture was stirred at 45 °C for 4 h under a nitrogen atmosphere, and then poured into water. The suspension was filtered to give intermediate **A2** as a yellow solid (38 g, 59% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 11.30 (s, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.54 (dd, J = 8.3, 5.7 Hz, 2H), 7.48 (d, J = 7.2 Hz, 1H), 7.11 (t, J = 8.6 Hz, 2H), 6.94 (t, J = 7.8 Hz, 1H), 3.96 (s, 3H).

*Methyl 5-bromo-4′-fluoro-2-hydroxy-[1,1′-biphenyl]-3-carboxylate (**A3**).* To a solution of **A2** (38 g, 154 mmol) in acetonitrile (150 mL) was added NBS (30 g, 169 mmol). The mixture was stirred at 60 °C for 4 h under nitrogen atmospheres, and then cooled to room temperature and poured into ice water.

The suspension was filtered to give intermediate **A3** as a yellow solid (47 g, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.24 (s, 1H), 7.96 (d, J = 2.5 Hz, 1H), 7.58 (d, J = 2.1 Hz, 1H), 7.51 (dd, J = 8.9, 5.4 Hz, 2H), 7.11 (t, J = 8.8 Hz, 2H), 3.97 (s, 3H).

*Methyl 5-bromo-2-(2-ethoxy-2-oxoethoxy)-4′-fluoro-[1,1′-biphenyl]-3-carboxylate (**A4**).* To a solution of **A3** (47 g, 145 mmol) in acetone (200 mL) was added ethyl bromoacetate (29 g, 174 mmol) and K_2CO_3 (60 g, 434 mmol). The mixture was heated to reflux for 4 h and then the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate, washed with water and brine, dried over $NaSO_4$, and concentrated *in vacuo* to give **A4** as a yellow oil (57 g, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, J = 2.5 Hz, 1H), 7.59 (d, J = 2.6 Hz, 1H), 7.58−7.51 (m, 2H), 7.12 (t, J = 8.7 Hz, 2H), 4.17−4.10 (m, 4H), 3.91 (s, 3H), 1.20 (t, J = 7.1 Hz, 3H).

*5-Bromo-2-(carboxymethoxy)-4′-fluoro-[1,1′-biphenyl]-3-carboxylic acid (**A5**).* **A4** (57 g, 139 mmol) was added to a mixture of ethanol (300 mL) and 1 mol/L sodium hydroxide solution (300 mL). The reaction mixture was heated to 90 °C for 6 h, and then cooled to room temperature. The excessive ethanol was removed i*n vacuo*, and the pH value of the solution was adjusted to 3-4 by the addition of 1 mol/L HCl aqueous solution. The suspension was filtered to give **A5** as a white solid (50 g, 98% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.36 (s, 2H), 7.79 (d, J = 2.6 Hz, 1H), 7.69 (d, J = 2.6 Hz, 1H), 7.60 (dt, J = 18.7, 11.1 Hz, 2H), 7.29 (t, J = 8.9 Hz, 2H), 4.08 (s, 2H).

*5-Bromo-7-(4-fluorophenyl)benzofuran-3-yl acetate (**A6**).* To a solution of **A5** (50 g, 135 mmol) in a mixture of acetic acid (350 mL) and acetic anhydride (350 mL), sodium acetate (37 g, 271 mmol) was added. The mixture was heated to 130 °C overnight and then cooled to room temperature, diluted with water, and extracted with a mixture of PE and EA (V_{PE}: V_{EA} = 1:1). The organic phase was washed three times with aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated *in vacuo* to give **A6** as a white solid (34.4 g, 73% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.78 (dd, J = 8.5, 5.3 Hz, 2H), 7.67 (s, 1H), 7.55 (s, 1H), 7.19 (t, J = 8.6 Hz, 2H), 2.39 (s, 3H).

*5-Bromo-7-(4-fluorophenyl)benzofuran-3(2H)-one (**A7**).* To a solution of **A6** (34.4 g, 98.5 mmol) in MeOH (300 mL) was added 1 mol/L HCl aqueous solution (180 mL). The mixture was heated to 90 °C for 3 h, and then cooled to room temperature, diluted with water, and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give **A7** as a brown solid (29.8 g, 99% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, J = 2.1 Hz, 1H), 7.77 (d, J = 2.1 Hz, 1H), 7.70−7.65 (m, 2H), 7.20−7.16 (m, 2H), 4.73 (s, 2H).

*2-(5-Bromo-7-(4-fluorophenyl)benzofuran-3-yl)acetonitrile (**A8**).* To a solution of diethyl cyanomethylphosphonate (15.7 mL, 97.1 mmol) in THF (90 mL) was added NaH (4.7 g, 116 mmol) slowly at 0 °C. The mixture was stirred at 0 °C for 30 min, and a solution of **A7** (29.8 g, 97.1 mmol) in THF (100 mL) was added. The mixture was stirred overnight, and then diluted with water and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified using a silica gel column to afford **A8** as a pink solid (20.1 g, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81−7.71 (m, 3H), 7.68 (d, J = 1.7 Hz, 1H), 7.59 (d, J = 1.7 Hz, 1H), 7.20 (t, J = 8.6 Hz, 2H), 3.77 (s, 2H).

*2-(5-Bromo-7-(4-fluorophenyl)benzofuran-3-yl)ethan-1-amine (**A9**).* To a solution of **A8** (20.1 g, 60.9 mmol) in THF (60 mL) was added 122 mL B_2H_6 (1 mol/L in THF) at 0 °C. The mixture was stirred for 20 min, and then heated to 60 °C for 3 h and cooled to room temperature. The mixture was quenched with methanol and evaporated *in vacuo*. The residue was dissolved in ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified using a silica gel column to afford **A9** as a yellow oil (10.8 g, 53% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (dd, J = 8.6, 5.4 Hz, 2H), 7.63 (d, J = 1.9 Hz, 1H), 7.51 (d, J = 5.6 Hz, 2H), 7.16 (t, J = 8.6 Hz, 2H), 3.04 (t, J = 6.2 Hz, 2H), 2.80 (t, J = 6.4 Hz, 2H).

*General procedure A for preparation of compounds **A10−A13**.* To a solution of **A9** (0.30 mmol, 1 eq.) in formic acid (2 mL) was added aldehyde (0.33 mmol, 1.1 eq.). The mixture was heated to 50-90 °C for 1.5 h and then cooled to room temperature. The pH value of the solution was adjusted to 7-8 by the addition of 1 mol/L NaOH aqueous solution. The mixture was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give the crude products **A10—A13** without further purification.

*General procedure B for preparation of compounds **A14—A15**.* To a solution of **A9** (0.30 mmol, 1 eq.) in a mixture of toluene (2 mL) and trifluoroacetic acid (1.50 mmol, 5 eq.), was added ketone (3.00 mmol, 10 eq.). The mixture was heated to 140 °C for 4 h in the microwave, and then cooled to room temperature. The pH was adjusted to 7-8 by the addition of 1 mol/L NaOH aqueous solution. The mixture was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give the crude product **A14—A15** without further purification.

*General procedure C for preparation of compounds **A16—A21**.* To a solution of the crude product **A10—A15** obtained above (0.30 mmol, 1 eq.) in DCM (5 mL), were added di-*tert* butyl decarbonate (0.45 mmol, 1.5 eq.) and *N,N*-diisopropylethylamine (0.60 mmol, 2 eq.) at 0 °C. The mixture was stirred overnight, diluted with ethyl acetate, and washed with brine. The organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified using a silica gel column to give compounds **A16—A21**.

*Tert-butyl 6-bromo-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A16**).* White solid, 24% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79−7.73 (m, 2H), 7.53 (s, 1H), 7.46 (d, J = 1.9 Hz, 1H), 7.22−7.15 (m, 2H), 4.61 (s, 2H), 3.83−3.70 (m, 2H), 2.75−2.67 (m, 2H), 1.50 (s, 9H).

*Tert-butyl 6-bromo-8-(4-fluorophenyl)-1-methyl-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A17**).* White solid, 48% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.71 (m, 2H), 7.52 (s, 1H), 7.46 (d, J = 1.9 Hz, 1H), 7.23−7.15 (m, 2H), 5.24 (d, J = 42.1 Hz, 1H), 4.40 (d, J = 42.1 Hz, 1H), 3.08 (s, 1H), 2.82−2.69 (m, 1H), 2.60 (d, J = 15.4 Hz, 1H), 1.50 (s, 9H), 1.48 (d, J = 6.7 Hz, 3H).

*Tert-butyl 6-bromo-8-(4-fluorophenyl)-1-(methoxymethyl)-3,4-dihydrobenzofuro[2,3-c]pyridi-ne-2(1H)-carboxylate (**A18**).* White solid, 58% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.73 (m, 2H), 7.53 (s, 1H), 7.47 (s, 1H), 7.23−7.15 (m, 2H), 5.48−5.18 (m, 1H), 4.64−4.26 (m, 1H), 3.88−3.69 (m, 2H), 3.35 (s, 3H), 3.33−3.14 (m, 1H), 2.83−2.56 (m, 2H), 1.50 (s, 9H).

*Tert-butyl 6-bromo-1-cyclopropyl-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A19**).* White solid, 44% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.74 (m, 2H), 7.53 (s, 1H), 7.49 (d, J = 2.0 Hz,

1H), 7.23−7.16 (m, 2H), 4.90−4.21 (m, 2H), 3.25 (s, 1H), 2.82−2.69 (m, 1H), 2.66−2.57 (m, 1H), 1.53 (s, 9H), 0.70−0.60 (m, 4H), 0.57−0.50 (m, 1H).

*Tert-butyl 6-bromo-8-(4-fluorophenyl)-1,1-dimethyl-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H) -carboxylate (**A20**).* White solid, 25% yield over two steps. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.81−7.73 (m, 2H), 7.52 (s, 1H), 7.48 (s, 1H), 7.23−7.13 (m, 2H), 3.80 (t, J = 5.3 Hz, 2H), 2.67 (t, J = 5.4 Hz, 2H), 1.78 (s, 6H), 1.53 (s, 9H).

*Tert-butyl 6-bromo-8-(4-fluorophenyl)-3,4-dihydro-2H-spiro [benzofuro[2,3-c]pyridine-1,1′-cy-clobutane]-2-carboxylate (**A21**).* White solid, 51% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88−7.80 (m, 2H), 7.49 (s, 2H), 7.24−7.16 (m, 2H), 3.67 (t, J = 5.7 Hz, 2H), 2.92 (dt, J = 12.1, 9.8 Hz, 2H), 2.69 (t, J = 5.7 Hz, 2H), 2.59−2.48 (m, 2H), 2.24−2.08 (m, 1H), 1.98−1.84 (m, 1H), 1.53 (s, 9H).

*General procedure D for preparation of compounds **A22**—**A27***. To a solution of **A16**—**A21** (0.10 mmol, 1 eq.) in a mixture of 1,4-dioxane (0.9 mL) and H_2O (0.3 mL) was added **K3** (0.15 mmol, 1.5 eq.), $Pd(PPh_3)_4$ (0.01 mmol, 1 eq.) and K_2CO_3 (0.25 mmol, 1 eq.). The mixture was refluxed for 2 h under nitrogen. The mixture was cooled to room temperature, and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified using a silica gel column to afford compounds **A22**—**A27**.

*Tert-butyl 6-(4-(4,4-difluoropiperidine-1-carbonyl)phenyl)-8-(4-fluorophenyl)-3,4-dihydroben-zofuro[2,3-c]pyridine-2(1H)-carboxylate (**A22**).* White solid (45% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87−7.81 (m, 2H), 7.74−7.69 (m, 2H), 7.60 (s, 1H), 7.56 (d, J = 1.8 Hz, 1H), 7.54−7.50 (m, 2H), 7.25−7.18 (m, 2H), 4.64 (s, 2H), 4.07−3.51 (m, 6H), 2.80 (s, 2H), 2.20−1.96 (m, 4H), 1.51 (s, 9H).

*Tert-butyl 6-(4-(4,4-difluoropiperidine-1-carbonyl)phenyl)-8-(4-fluorophenyl)-1-methyl-3,4-di-hydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A23**).* White solid (38% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87−7.81 (m, 2H), 7.71 (d, J = 7.8 Hz, 2H), 7.60 (s, 1H), 7.56 (d, J = 1.9 Hz, 1H), 7.52 (d, J = 7.8 Hz, 2H), 7.24−7.17 (m, 2H), 5.48−5.14 (m, 1H), 4.60−4.22 (m, 1H), 3.98−3.60 (m, 4H), 3.12 (s, 1H), 2.91−2.59 (m, 2H), 2.11−1.99 (m, 4H), 1.50 (d, J = 6.4 Hz, 12H).

*Tert-butyl 6-(4-(4,4-difluoropiperidine-1-carbonyl)phenyl)-8-(4-fluorophenyl)-1-(methoxymet-hyl)-3,4-dihydrobenzofuro[2,3-c] pyridine-2(1H)-carboxylate (**A24**).* White solid (61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88−7.81 (m, 2H), 7.73−7.68 (m, 2H), 7.60 (s, 1H), 7.58−7.55 (m, 1H), 7.53−7.49 (m, 2H), 7.25−7.17 (m, 2H), 5.52−5.23 (m, 1H), 4.65−4.31 (m, 1H), 3.99−3.57 (m, 6H), 3.36 (s, 3H), 3.36−3.26 (m, 1H), 2.91−2.64 (m, 2H), 2.16−1.93 (m, 4H), 1.51 (s, 9H).

*Tert-butyl 1-cyclopropyl-6-(4-(4,4-difluoropiperidine-1-carbonyl)phenyl)-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c] pyridine-2(1H)-carboxylate (**A25**).* White solid (48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90−7.83 (m, 2H), 7.71 (d, J = 7.8 Hz, 2H), 7.63−7.57 (m, 2H), 7.51 (d, J = 7.9 Hz, 2H), 7.25−7.18 (m, 2H), 4.92−4.27 (m, 2H), 3.96−3.60 (m, 4H), 3.29 (s, 1H), 2.90−2.66 (m, 2H), 2.17−1.90 (m, 4H), 1.49 (s, 9H), 0.74−0.62 (m, 4H), 0.61−0.49 (m, 1H).

*Tert-butyl 6-(4-(4,4-difluoropiperidine-1-carbonyl)phenyl)-8-(4-fluorophenyl)-1,1-dimethyl-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A26**).* White solid (61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87−7.80 (m, 2H), 7.70 (d, J = 7.7 Hz, 2H), 7.59 (d, J = 1.7 Hz, 1H), 7.56 (d, J = 1.8 Hz, 1H), 7.50 (d, J = 7.8 Hz, 2H), 7.23−7.16 (m, 2H), 3.97−3.50 (m, 6H), 2.74 (t, J = 5.3 Hz, 2H), 2.16−1.92 (m, 4H), 1.79 (s, 6H), 1.53 (s, 9H).

*Tert-butyl 6-(4-(4,4-difluoropiperidine-1-carbonyl)phenyl)-8-(4-fluorophenyl)-3,4-dihydro-2H-spiro[benzofuro[2,3-c]pyridine-1,1′-cyclobutane]-2-carboxylate (**A27**).* White solid (30% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95−7.88 (m, 2H), 7.72 (d, J = 7.8 Hz, 2H), 7.60 (s, 1H), 7.58 (d, J = 1.9 Hz, 1H), 7.52 (d, J = 7.8 Hz, 2H), 7.25−7.18 (m, 2H), 3.96−3.46 (m, 6H), 3.01−2.89 (m, 2H), 2.77 (t, J = 5.5 Hz, 2H), 2.62−2.53 (m, 2H), 2.27−2.15 (m, 1H), 2.11−1.86 (m, 5H), 1.47 (s, 9H).

*General procedure E for preparation of compounds **6**−**11***. To a solution of **A22**—**A27** (0.08 mmol, 1 eq.) in DCM (2 mL) was added TFA (1 mL). The mixture was stirred for 1 h at room temperature and then evaporated *in vacuo*. The residue was dissolved in DMF (1 mL), and (2*E*)-3-(6-amino-3-pyridinyl)-2-propenoic acid (0.12 mmol, 1.5 eq.), HATU (0.16 mmol, 2 eq.), and DIPEA (0.24 mmol, 3 eq.) were added. The mixture was stirred overnight, diluted with ethyl acetate, and washed with brine. The organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified using a silica gel column to afford compounds **6**−**11**.

*(E)-3-(6-Aminopyridin-3-yl)-1-(6-(4-(4,4-difluoropiperidine-1-carbonyl)phenyl)-8-(4-fluorop-henyl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**6**).* White solid (32% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 8.09 (s, 1H), 7.93−7.85 (m, 3H), 7.77 (d, J = 8.2 Hz, 2H), 7.69 (s, 1H), 7.63 (s, 1H), 7.58−7.48 (m, 3H), 7.22 (t, J = 8.7 Hz, 2H), 7.04 (dd, J = 37.5, 15.3 Hz, 1H), 6.63 (d, J = 8.0 Hz, 1H), 4.97−4.83 (m, 2H), 4.04 (t, J = 5.1 Hz, 2H), 3.97−3.52 (m, 4H), 2.86 (d, J = 21.4 Hz, 2H), 2.06 (s, 4H). ^{13}C NMR (126 MHz, CD_3OD) δ 172.6, 169.0, 164.0 (d, J = 246.2 Hz), 161.6, 153.1, 152.1, 149.7, 144.6, 142.0, 137.5, 137.3, 135.0, 133.6, 131.7, 131.6, 130.5, 128.7 (2C), 128.6 (2C), 125.8, 123.8, 122.9 (t, J = 241.8 Hz), 121.8, 117.7, 116.5, 116.4, 114.4, 113.7, 110.6, 44.8, 42.2, 41.3, 40.3, 35.7−34.2 (m, 2C), 22.0 (d, J = 160.2 Hz). HR-MS (ESI) Calcd. for $C_{37}H_{32}O_3N_4F_3^+$ $[M+H]^+$, 637.2421; Found, 637.2413; purity, 97.7%.

*(E)-3-(6-Aminopyridin-3-yl)-1-(6-(4-(4,4-difluoropiperidine-1-carbonyl)phenyl)-8-(4-fluorop-henyl)-1-methyl-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**7**).* White solid (19% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.84 (s, 2H), 7.76−7.56 (m, 6H), 7.51 (dd, J = 8.1, 2.1 Hz, 2H), 7.25−7.16 (m, 2H), 6.77 (dd, J = 32.1, 15.0 Hz, 1H), 6.54−6.48 (m, 1H), 5.91 (s, 0.5H), 5.36−4.98 (m, 1H), 4.83 (d, J = 43.6 Hz, 2H), 4.33 (s, 0.5H), 4.02−3.60 (m, 4H), 3.55 (s, 0.5H), 3.10 (s, 0.5H), 2.97−2.72 (m, 2H), 2.03 (s, 4H), 1.62 (d, J = 49.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6, 166.1 (d, J = 44.6 Hz), 162.8 (d, J = 247.8 Hz), 159.3 (d, J = 10.8 Hz), 155.6, 154.0, 151.8, 149.4 (d, J = 19.5 Hz), 143.4, 140.8 (d, J = 25.5 Hz), 136.1, 133.9, 132.2, 130.5, 130.4, 129.2, 127.8 (2C), 127.7 (2C), 124.9, 123.2 (d, J = 14.6 Hz), 121.7 (t, J = 242.6 Hz), 116.8 (d, J = 49.2 Hz), 115.9, 115.8, 113.7 (d, J = 104.2 Hz), 111.3, 108.8, 49.4, 46.5, 39.9, 36.0, 35.2−33.6 (m, 2C), 21.8 (d, J = 163.1 Hz), 18.5 (d, J = 206.0 Hz). HR-MS (ESI) Calcd. for $C_{38}H_{34}O_3N_4F_3^+$ $[M+H]^+$, 651.2578; Found, 651.2574; purity, 98.1%.

*(E)-3-(6-Aminopyridin-3-yl)-1-(6-(4-(4,4-difluoropiperidine-1-carbonyl)phenyl)-8-(4-fluorop-henyl)-1-(methoxymethyl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**8**).*

White solid (24% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, J = 20.5 Hz, 1H), 7.84 (s, 2H), 7.74−7.55 (m, 6H), 7.52 (d, J = 8.2 Hz, 2H), 7.22 (t, J = 8.6 Hz, 2H), 6.91 (dd, J = 56.2, 15.4 Hz, 1H), 6.53 (t, J = 7.1 Hz, 1H), 5.97 (s, 0.5H), 5.39 (s, 0.5H), 5.09 (d, J = 9.5 Hz, 0.5H), 4.77 (s, 2H), 4.38 (d, J = 13.2 Hz, 0.5H), 4.02−3.86 (m, 3H), 3.85−3.73 (m, 2H), 3.72−3.67 (m, 0.5H), 3.67−3.48 (m, 1H), 3.40 (d, J = 46.5 Hz, 3H), 3.22−3.14 (m, 0.5H), 2.97−2.72 (m, 2H), 2.15−1.94 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.62, 167.0 (d, J = 125.4 Hz), 162.8 (d, J = 247.7 Hz), 159.1 (d, J = 14.2 Hz), 152.0, 151.8, 150.2, 149.3, 143.4 (d, J = 29.5 Hz), 140.4 (d, J = 89.3 Hz), 136.2, 134.0, 132.2, 130.5, 130.4, 129.2 (d, J = 21.0 Hz), 127.8 (2C), 127.7 (2C), 125.0, 123.4 (d, J = 45.8 Hz), 121.9 (d, J = 23.8 Hz), 121.7 (t, J = 242.6 Hz), 116.9 (d, J = 48.3 Hz), 115.9, 115.8, 115.3, 113.8 (d, J = 81.0 Hz), 108.8, 73.2, 59.6 (d, J = 28.5 Hz), 54.4, 50.5, 42.3, 36.9, 35.1−33.5 (m, 2C), 21.6 (d, J = 170.6 Hz). HR-MS (ESI) Calcd. for $C_{39}H_{36}O_4N_4F_3^+$ $[M+H]^+$, 681.2683; Found, 681.2693; purity, 95.3%.

(E)-3-(6-Aminopyridin-3-yl)-1-(1-cyclopropyl-6-(4-(4,4-difluoropiperidine-1-carbonyl)pheny-l)-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one ***(9)***. White solid (29% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 8.11 (s, 1H), 7.98−7.90 (m, 3H), 7.82 (d, J = 8.2 Hz, 2H), 7.75 (s, 1H), 7.70 (s, 1H), 7.60−7.52 (m, 3H), 7.27 (t, J = 8.7 Hz, 2H), 7.16−6.97 (m, 1H), 6.68−6.61 (m, 1H), 5.27 (d, J = 7.7 Hz, 0.5H), 5.15−4.97 (m, 0.5H), 4.90−4.86 (m, 0.5H), 4.60 (d, J = 14.2 Hz, 0.5H), 3.97−3.53 (m, 5H), 2.94−2.82 (m, 2H), 2.08 (s, 4H), 0.95−0.49 (m, 5H). ^{13}C NMR (126 MHz, CD_3OD) δ 172.7, 168.5, 164.1 (d, J = 246.3 Hz), 161.6, 154.7, 153.0, 149.6, 144.7, 142.0, 137.5, 137.3, 135.0, 133.6 (d, J = 3.3 Hz), 131.6, 131.5, 130.4, 128.7 (2C), 128.6 (2C), 125.8, 123.9, 122.9, 121.8, 117.9, 116.6, 116.4, 114.7, 113.7, 110.7, 55.6, 41.8, 35.7−34.1 (m, 2C), 23.1, 15.1, 3.8, 3.2. HR-MS (ESI) Calcd. for $C_{40}H_{36}O_3N_4F_3^+$ $[M+H]^+$, 677.2734; Found, 677.2738; purity, 96.6%.

(E)-3-(6-Aminopyridin-3-yl)-1-(6-(4-(4,4-difluoropiperidine-1-carbonyl)phenyl)-8-(4-fluorop-henyl)-1,1-dimethyl-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one ***(10)***. White solid (18% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 8.09 (d, J = 2.1 Hz, 1H), 7.95−7.90 (m, 2H), 7.88 (dd, J = 8.8, 2.3 Hz, 1H), 7.83 (d, J = 8.3 Hz, 2H), 7.76 (d, J = 1.7 Hz, 1H), 7.69 (d, J = 1.8 Hz, 1H), 7.57 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 15.5 Hz, 1H), 7.27 (t, J = 8.8 Hz, 2H), 6.96 (d, J = 15.5 Hz, 1H), 6.64 (d, J = 8.8 Hz, 1H), 3.95−3.58 (m, 6H), 2.92 (t, J = 5.2 Hz, 2H), 2.08 (s, 4H), 1.91 (s, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 172.7, 171.2, 164.0 (d, J = 246.4 Hz), 161.5, 159.6, 152.7, 149.4, 144.7, 140.2, 137.4, 137.3, 135.0, 133.7, 131.5 (2C), 130.6, 128.7 (2C), 128.6 (2C), 125.8, 123.9, 122.9 (t, J = 241.6 Hz), 121.9, 119.0, 117.9, 116.7, 116.5, 112.8, 110.6, 59.7, 44.8, 25.0 (2C), 22.4. HR-MS (ESI) Calcd. for $C_{39}H_{34}O_3N_4F_3^-$ $[M-H]^-$, 663.2588; Found, 663.2581; purity, 93.7%.

(E)-3-(6-Aminopyridin-3-yl)-1-(6-(4-(4,4-difluoropiperidine-1-carbonyl)phenyl)-8-(4-fluorop-henyl)-3,4-dihydro-2H-spiro[benzofuro[2,3-c]pyridine-1,1′-cyclobutan]-2-yl)prop-2-en-1-one ***(11)***. White solid (20% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 (d, J = 2.0 Hz, 1H), 7.95−7.89 (m, 2H), 7.70 (d, J = 8.1 Hz, 2H), 7.63 (dd, J = 8.6, 2.1 Hz, 1H), 7.59 (d, J = 1.6 Hz, 1H), 7.54−7.49 (m, 4H), 7.23 (t, J = 8.7 Hz, 2H), 6.73 (d, J = 15.4 Hz, 1H), 6.50 (d, J = 8.6 Hz, 1H), 4.77 (s, 2H), 4.02−3.51 (m, 6H), 2.98−2.88 (m, 2H), 2.80 (t, J = 5.6 Hz, 2H), 2.73 (t, J = 9.4 Hz, 2H), 2.30−2.19 (m, 1H), 2.17−1.96 (m, 5H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.7, 169.4, 162.7 (d, J = 247.5 Hz), 159.2, 158.4, 151.3, 149.3, 143.6, 139.9, 136.2, 136.1, 133.8, 132.4 (d, J = 3.1 Hz), 130.4, 130.3, 129.6, 127.8 (2C), 127.7 (2C), 124.8, 122.8, 121.7, 121.7 (t, J = 242.8 Hz), 116.5 (2C), 115.9, 115.8, 110.2, 108.7, 60.2, 42.3, 34.1 (2C), 22.6, 15.0. HR-MS (ESI) Calcd. for $C_{40}H_{36}O_3N_4F_3^+$ $[M+H]^+$, 677.2734; Found, 677.2729; purity, 98.6%.

*Tert-butyl 8-(4-fluorophenyl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroben-zofuro[2,3-c]pyridine-2(1H)-carboxylate (**A28**).* To a solution of **A16** (5.9 g, 13.3 mmol) in 1,4-dioxane (100 mL) was added KOAc (3.9 g, 39.8 mmol), $PdCl_2$(dppf) (485 mg, 0.66 mmol), and bis(pinacolato)diboron (4 g, 15.9 mmol). The mixture was refluxed for 3 h and then cooled to room temperature, diluted with water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified using a silica gel column to afford **A28** as a white solid (3.5 g, 47% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.86−7.78 (m, 3H), 7.21−7.13 (m, 2H), 4.61 (s, 2H), 3.77 (s, 2H), 2.76 (s, 2H), 1.50 (s, 9H), 1.38 (s, 12H).

*General procedure F for preparation of **A29**—**A35***. To a solution of **A28** (0.10 mmol, 1 eq.) in a mixture of 1,4-dioxane (0.9 mL) and H_2O (0.3 mL) was added **K2**−**K8** (0.15 mmol, 1.5 eq.), $Pd(PPh_3)_4$ (0.01 mmol, 1 eq.) and K_2CO_3 (0.25 mmol, 1 eq.). The mixture was refluxed for 2 h under nitrogen, and then cooled to room temperature and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified using a silica gel column to afford compounds **A29**—**A35**.

*Tert-butyl 6-(4-(4,4-difluoropiperidine-1-carbonyl)-2-fluorophenyl)-8-(4-fluorophenyl)-3,4-di-hydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A29**).* White solid (43% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86−7.80 (m, 2H), 7.62−7.55 (m, 2H), 7.51 (s, 1H), 7.32−7.24 (m, 2H), 7.23−7.16 (m, 2H), 4.69−4.60 (m, 2H), 4.01−3.55 (m, 6H), 2.79 (s, 2H), 2.15−1.87 (m, 4H), 1.51 (s, 9H).

*Tert-butyl 6-(5-(4,4-difluoropiperidine-1-carbonyl)pyridin-2-yl)-8-(4-fluorophenyl)-3,4-dihyd-robenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A30**).* White solid (52% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78−8.75 (m, 1H), 8.09 (d, J = 1.8 Hz, 1H), 8.04 (s, 1H), 7.93−7.83 (m, 4H), 4.64 (s, 2H), 4.02−3.61 (m, 6H), 2.82 (s, 2H), 2.20−1.92 (m, 4H), 1.51 (s, 9H).

*Tert-butyl 6-(6-(4,4-difluoropiperidine-1-carbonyl)pyridin-3-yl)-8-(4-fluorophenyl)-3,4-dihydr-obenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A31**).* White solid (83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (d, J = 2.2 Hz, 1H), 8.07 (dd, J = 8.1, 2.1 Hz, 1H), 7.87−7.78 (m, 3H), 7.61 (s, 1H), 7.55 (d, J = 1.7 Hz, 1H), 7.24−7.18 (m, 2H), 4.65 (s, 2H), 3.94 (t, J = 5.8 Hz, 2H), 3.86−3.76 (m, 4H), 2.81 (s, 2H), 2.22−2.06 (m, 4H), 1.51 (s, 9H).

*Tert-butyl 6-(5-(4,4-difluoropiperidine-1-carbonyl)pyrimidin-2-yl)-8-(4-fluorophenyl)-3,4-dih-ydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A32**).* White solid (81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 2H), 8.58−8.54 (m, 2H), 7.91−7.83 (m, 2H), 7.23−7.15 (m, 2H), 4.63 (s, 2H), 4.00−3.55 (m, 6H), 2.81 (s, 2H), 2.16−1.95 (m, 4H), 1.50 (s, 9H).

*Tert-butyl 6-(2-(4,4-difluoropiperidine-1-carbonyl)pyrimidin-5-yl)-8-(4-fluorophenyl)-3,4-dih-ydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A33**).* White solid (83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 2H), 7.86−7.79 (m, 2H), 7.61 (s, 1H), 7.52 (d, J = 1.8 Hz, 1H), 7.25−7.19 (m, 2H), 4.66 (s, 2H),

3.97 (t, J = 6.0 Hz, 2H), 3.81 (s, 2H), 3.56 (t, J = 5.9 Hz, 2H), 2.82 (s, 2H), 2.21−2.05 (m, 4H), 1.51 (s, 9H).

*Tert-butyl 6-(5-(4,4-difluoropiperidine-1-carbonyl)pyrazin-2-yl)-8-(4-fluorophenyl)-3,4-dihyd-robenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A34**).* White solid (78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06−9.03 (m, 2H), 8.12 (d, J = 1.7 Hz, 1H), 8.07 (s, 1H), 7.89−7.83 (m, 2H), 7.25−7.18 (m, 2H), 4.65 (s, 2H), 3.95 (t, J = 5.8 Hz, 2H), 3.87−3.76 (m, 4H), 2.82 (s, 2H), 2.21−2.07 (m, 4H), 1.51 (s, 9H).

*Tert-butyl 6-(6-(4,4-difluoropiperidine-1-carbonyl)pyridazin-3-yl)-8-(4-fluorophenyl)-3,4-dihy-drobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A35**).* White solid (80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 8.16−8.07 (m, 2H), 8.00 (d, J = 8.8 Hz, 1H), 7.89−7.84 (m, 2H), 7.25−7.18 (m, 2H), 4.65 (s, 2H), 4.02−3.93 (m, 4H), 3.81 (s, 2H), 2.81 (s, 2H), 2.29−2.10 (m, 4H), 1.51 (s, 9H).

*(E)-3-(6-Aminopyridin-3-yl)-1-(6-(4-(4,4-difluoropiperidine-1-carbonyl)-2-fluorophenyl)-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**12**).* This compound was synthesized by following the general procedure E (white solid, 33% yield over two steps). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.83 (s, 2H), 7.71−7.62 (m, 2H), 7.58 (t, J = 7.7 Hz, 2H), 7.53 (s, 1H), 7.31−7.27 (m, 2H), 7.21 (t, J = 8.6 Hz, 2H), 6.79 (dd, J = 47.4, 15.5 Hz, 1H), 6.53 (d, J = 8.6 Hz, 1H), 4.97−4.79 (m, 4H), 4.14−3.52 (m, 6H), 2.90 (s, 2H), 2.09−1.90 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.1, 166.6, 163.60, 161.2 (d, J = 231.3 Hz), 159.5 (d, J = 249.5 Hz), 159.2, 151.9, 151.3, 149.9, 149.2, 140.8, 136.3, 136.0, 132.0, 131.6 (d, J = 2.9 Hz), 130.5 (2C), 128.9, 124.8, 123.1 (2C), 121.6, 121.5 (t, J = 242.5 Hz), 118.7 (d, J = 49.5 Hz), 115.9, 115.8, 115.3 (d, J = 24.8 Hz), 113.8 (d, J = 23.4 Hz), 112.0, 108.9, 44.6, 43.9, 40.8 (d, J = 194.2 Hz), 39.3, 34.8, 33.9, 21.4 (d, J = 215.2 Hz). HR-MS (ESI) Calcd. for $C_{37}H_{31}O_3N_4F_4^+$ $[M+H]^+$, 655.2327; Found, 655.2322; HPLC purity, 95.3%.

*(E)-3-(6-Aminopyridin-3-yl)-1-(6-(5-(4,4-difluoropiperidine-1-carbonyl)pyridin-2-yl)-8-(4-flu-orophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**13**).* This compound was synthesized by following the general procedure E (white solid, 38% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (d, J = 2.0 Hz, 1H), 8.24 (s, 1H), 8.10 (d, J = 1.8 Hz, 1H), 8.04 (s, 1H), 7.92−7.83 (m, 4H), 7.70−7.61 (m, 2H), 7.21 (t, J = 8.7 Hz, 2H), 6.89−6.69 (m, 1H), 6.51 (d, J = 8.6 Hz, 1H), 4.91 (s, 2H), 4.75 (s, 2H), 4.15−3.93 (m, 2H), 3.93−3.50 (m, 4H), 2.91 (s, 2H), 2.06 (s, 4H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 168.3, 166.6, 162.8 (d, J = 247.8 Hz), 159.3, 159.1, 152.3 (d, J = 263.8 Hz), 149.9 (d, J = 62.4 Hz), 149.7, 147.9, 141.0, 136.3, 136.0 (d, J = 20.8 Hz), 134.2 (d, J = 21.2 Hz), 132.0, 130.5 (2C), 129.2, 129.0, 125.0, 123.0 (d, J = 43.8 Hz), 121.6, 121.4 (t, J = 242.4 Hz), 120.4, 117.0 (d, J = 54.6 Hz), 115.9, 115.8, 114.0 (d, J = 40.2 Hz), 113.0 (d, J = 212.4 Hz), 108.7, 44.8, 43.9 (d, J = 45.4 Hz), 40.8 (d, J = 224.3 Hz), 39.5, 34.9, 33.8, 21.5 (d, J = 253.0 Hz). HR-MS (ESI) Calcd. for $C_{36}H_{31}O_3N_5F_3^+$ $[M+H]^+$, 638.2374; Found, 638.2382; HPLC purity, 99.9%.

*(E)-3-(6-Aminopyridin-3-yl)-1-(6-(6-(4,4-difluoropiperidine-1-carbonyl)pyridin-3-yl)-8-(4-flu-orophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**14**).* This compound was synthesized by following the general procedure E (white solid, 37% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (d, J = 1.8 Hz, 1H), 8.24 (s, 1H), 8.08 (dd, J = 8.1, 2.3 Hz, 1H), 7.88−7.78 (m, 3H), 7.71−7.60 (m, 3H), 7.57 (d, J = 1.8 Hz, 1H), 7.25−7.19 (m, 2H), 6.88−6.70 (m, 1H), 6.53 (d, J = 8.6 Hz, 1H), 4.99−4.73 (m, 4H), 4.15−3.97 (m, 2H), 3.94 (t, J = 5.9 Hz, 2H), 3.81 (t, J = 5.9 Hz, 2H), 2.91 (s, 2H), 2.22−2.03 (m, 4H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 167.6, 166.7, 162.9 (d, J = 248.1 Hz), 159.2, 152.1 (d, J = 49.4 Hz), 151.8, 150.3, 149.4, 147.0, 141.0, 138.3, 136.3, 135.8, 132.9, 131.8, 130.5 (2C), 129.5, 125.4, 124.4, 123.1 (d, J = 34.4 Hz), 121.9 (t, J = 241.9 Hz), 121.6, 116.8 (d, J = 68.5 Hz), 116.0, 115.9, 113.8 (d, J = 63.3 Hz), 112.1, 108.8, 44.3, 44.0 (d, J = 53.7 Hz), 40.83 (d, J = 228.0 Hz), 39.8, 34.9 (t, J = 23.0 Hz), 34.1 (t, J = 23.3 Hz), 21.4 (d, J = 255.6 Hz). HR-MS (ESI) Calcd. for $C_{36}H_{31}O_3N_5F_3^+$ $[M + H]^+$, 638.2379; Found, 638.2375; HPLC purity, 95.8%.

*(E)-3-(6-Aminopyridin-3-yl)-1-(6-(5-(4,4-difluoropiperidine-1-carbonyl)pyrimidin-2-yl)-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**15**).* This compound was synthesized by following the general procedure E (white solid, 23% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 2H), 8.58 (d, J = 10.2 Hz, 2H), 8.24 (s, 1H), 7.96−7.85 (m, 2H), 7.74−7.59 (m, 2H), 7.24−7.16 (m, 2H), 6.88−6.69 (m, 1H), 6.52 (d, J = 8.6 Hz, 1H), 5.04−4.54 (m, 4H), 4.19−3.56 (m, 6H), 2.93 (s, 2H), 2.28−1.92 (m, 4H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.7, 165.9 (d, J = 54.9 Hz), 162.8 (d, J = 247.7 Hz), 159.3, 156.1 (2C), 154.3, 151.6, 150.2, 149.6, 141.0, 136.1, 132.3, 132.0, 130.6, 130.5, 129.2, 126.2, 124.9, 124.5, 121.6, 121.2 (t, J = 242.3 Hz), 118.9 (d, J = 74.2 Hz), 115.9, 115.7, 113.8 (d, J = 64.8 Hz), 112.6, 108.7, 44.9, 43.9 (d, J = 42.0 Hz), 40.8 (d, J = 219.8 Hz), 39.6, 34.8, 33.9, 21.5 (d, J = 253.7 Hz). HR-MS (ESI) Calcd. for $C_{35}H_{30}O_3N_6F_3^+$ $[M + H]^+$, 639.2331; Found, 639.2332; HPLC purity, 98.0%.

*(E)-3-(6-Aminopyridin-3-yl)-1-(6-(2-(4,4-difluoropiperidine-1-carbonyl)pyrimidin-5-yl)-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**16**).* This compound was synthesized by following the general procedure E (white solid, 28% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 2H), 8.23 (s, 1H), 7.87−7.80 (m, 2H), 7.71−7.58 (m, 3H), 7.54 (d, J = 1.8 Hz, 1H), 7.26−7.20 (m, 2H), 6.87−6.69 (m, 1H), 6.52 (d, J = 8.7 Hz, 1H), 4.99−4.86 (m, 2H), 4.82 (s, 2H), 4.15−3.93 (m, 4H), 3.56 (t, J = 5.9 Hz, 2H), 2.92 (s, 2H), 2.23−2.03 (m, 4H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.7, 165.3, 163.0 (d, J = 248.6 Hz), 159.7 (d, J = 123.3 Hz), 155.6 (2C), 152.6, 152.2, 150.7, 149.5, 141.1, 136.2, 134.8, 131.5, 130.5 (2C), 129.8, 129.4, 125.9, 122.7, 121.7 (t, J = 242.7 Hz), 121.5, 116.8 (d, J = 70.2 Hz), 116.1, 116.0, 113.6 (d, J = 67.3 Hz), 112.2, 108.8, 44.0 (t, J = 5.1 Hz), 43.8, 40.8 (d, J = 223.6 Hz), 39.2 (t, J = 5.3 Hz), 34.7 (t, J = 23.6 Hz), 33.9 (t, J = 23.1 Hz), 21.4 (d, J = 252.2 Hz). HR-MS (ESI) Calcd. for $C_{35}H_{30}O_3N_6F_3^+$ $[M + H]^+$, 639.2331; Found, 639.2325; HPLC purity, 97.0%.

*(E)-3-(6-Aminopyridin-3-yl)-1-(6-(5-(4,4-difluoropiperidine-1-carbonyl)pyrazin-2-yl)-8-(4-flu-orophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**17**).* This compound was synthesized by following the general procedure E (white solid, 32% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10−9.02 (m, 2H), 8.24 (s, 1H), 8.16−8.05 (m, 2H), 7.91−7.82 (m, 2H), 7.73−7.61 (m, 2H), 7.25−7.19 (m, 2H), 6.88−6.68 (m, 1H), 6.52 (d, J = 8.6 Hz, 1H), 5.01−4.67 (m, 4H), 4.15−3.92 (m, 4H), 3.84 (s, 2H), 2.93 (s, 2H), 2.22−2.03 (m, 4H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.7, 165.5, 162.9 (d, J = 247.8 Hz), 159.3, 153.6 (d, J = 23.9 Hz), 152.0, 150.5, 149.6, 146.3, 145.4, 141.1, 139.5, 136.2, 131.7, 131.4, 130.6, 130.5, 129.5, 125.5, 123.0 (d, J = 28.7 Hz), 121.7, 121.6 (d, J = 241.5 Hz), 117.2 (d,

J = 50.5 Hz), 116.0, 115.9, 113.7 (d, J = 61.2 Hz), 112.4, 108.7, 44.3, 44.0 (d, J = 49.5 Hz), 40.8 (d, J = 225.8 Hz), 40.0, 34.9 (t, J = 23.2 Hz), 34.1 (t, J = 23.2 Hz), 21.5 (d, J = 251.2 Hz). HR-MS (ESI) Calcd. for $C_{35}H_{30}O_3N_6F_3^+$ [M + H]$^+$, 639.2331; Found, 639.2336; HPLC purity, 97.9%.

*(E)-3-(6-Aminopyridin-3-yl)-1-(6-(6-(4,4-difluoropiperidine-1-carbonyl)pyridazin-3-yl)-8-(4-fluorophenyl)-3,4-dihydrobenzofuro [2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**18**).* This compound was synthesized by following the general procedure E (white solid, 46% yield over two steps). ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.29−8.19 (m, 2H), 8.18−8.06 (m, 2H), 8.01 (d, J = 9.0 Hz, 1H), 7.90−7.84 (m, 2H), 7.72−7.60 (m, 2H), 7.25−7.20 (m, 2H), 6.78 (dd, J = 59.7, 15.3 Hz, 1H), 6.52 (d, J = 8.5 Hz, 1H), 5.02−4.85 (m, 2H), 4.81 (s, 2H), 4.14−3.93 (m, 6H), 2.92 (s, 2H), 2.28−2.12 (m, 4H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.7, 165.2, 162.9 (d, J = 248.3 Hz), 159.9, 159.3, 154.2 (d, J = 195.7 Hz), 152.0, 150.5, 149.5, 141.0, 136.2, 131.7, 131.2 (d, J = 19.6 Hz), 130.6, 130.5, 129.5, 128.9, 125.5, 124.9, 123.1 (d, J = 52.4 Hz), 121.7 (t, J = 242.0 Hz), 121.6, 117.4 (d, J = 40.9 Hz), 116.0, 115.8, 113.7 (d, J = 67.9 Hz), 112.5, 108.8, 44.7 (d, J = 5.4 Hz), 43.9 (d, J = 46.3 Hz), 41.4, 40.2 (t, J = 5.2 Hz), 35.1 (t, J = 23.4 Hz), 34.1 (t, J = 23.7 Hz), 21.4 (d, J = 253.8 Hz). HR-MS (ESI) Calcd. for $C_{35}H_{30}O_3N_6F_3^+$ [M + H]$^+$, 639.2331; Found, 639.2330; HPLC purity, 95.8%.

*Tert-butyl 8-(4-fluorophenyl)-6-(6-(morpholine-4-carbonyl) pyridazin-3-yl)-3,4-dihydrobenzof-uro[2,3-c]pyridine-2(1H)-carboxylate (**A36**).* This compound was synthesized by following the general procedure F (white solid, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 8.18−8.09 (m, 2H), 8.03 (d, J = 8.8 Hz, 1H), 7.90−7.84 (m, 2H), 7.25−7.18 (m, 2H), 4.66 (s, 2H), 4.02−3.94 (m, 2H), 3.93−3.85 (m, 4H), 3.84−3.78 (m, 4H), 2.82 (s, 2H), 1.51 (s, 9H).

*Tert-butyl 8-(4-fluorophenyl)-6-(6-(4-methylpiperazine-1-carbonyl)pyridazin-3-yl)-3,4-dihydr-obenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A37**).* This compound was synthesized by following the general procedure F (white solid, 35% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 8.18−8.08 (m, 2H), 7.99 (d, J = 8.8 Hz, 1H), 7.90−7.83 (m, 2H), 7.25−7.18 (m, 2H), 4.66 (s, 2H), 3.97−3.88 (m, 4H), 3.81 (s, 2H), 2.82 (s, 2H), 2.59 (t, J = 5.2 Hz, 2H), 2.55 (t, J = 4.8 Hz, 2H), 2.36 (s, 3H), 1.51 (s, 9H).

*Tert-butyl 8-(4-fluorophenyl)-6-(6-(4-oxopiperidine-1-carbonyl)pyridazin-3-yl)-3,4-dihydrobe-nzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A38**).* This compound was synthesized by following the general procedure F (white solid, 62% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 8.16−8.02 (m, 3H), 7.89−7.83 (m, 2H), 7.24−7.16 (m, 2H), 4.64 (s, 2H), 4.21−4.10 (m, 4H), 3.79 (s, 2H), 2.80 (s, 2H), 2.71 (t, J = 6.2 Hz, 2H), 2.66 (t, J = 6.4 Hz, 2H), 1.50 (s, 9H).

*Tert-butyl 6-(6-(4-cyano-4-methylpiperidine-1-carbonyl)pyridazin-3-yl)-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A39**).* This compound was synthesized by following the general procedure F (white solid, 62% yield). ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.20 (s, 1H), 8.15−8.06 (m, 2H), 7.96 (d, J = 8.8 Hz, 1H), 7.87−7.84 (m, 2H), 7.22−7.17 (m, 2H), 4.84−4.79 (m, 1H), 4.64 (s, 2H), 4.47−4.41 (m, 1H), 3.79 (s, 2H), 3.49−3.44 (m, 1H), 3.21−3.16 (m, 1H), 2.80 (s, 2H), 2.12−2.07 (m, 1H), 2.01 (s, 3H), 2.01−1.98 (m, 1H), 1.91−1.86 (m, 1H), 1.70−1.65 (m, 1H), 1.49 (s, 9H).

*Tert-butyl 6-(6-(3-(difluoromethyl)azetidine-1-carbonyl)pyridazin-3-yl)-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A40**).* This compound was synthesized by following the general procedure F (white solid, 78.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, J = 8.8 Hz, 1H), 8.23 (d, J = 1.8 Hz, 1H), 8.16−8.07 (m, 2H), 7.90−7.84 (m, 2H), 7.25−7.18 (m, 2H), 6.07 (td, J = 56.0, 4.2 Hz, 1H), 5.03 (dd, J = 11.2, 8.6 Hz, 1H), 4.93 (dd, J = 11.2, 5.7 Hz, 1H), 4.66 (s, 2H), 4.46−4.36 (m, 1H), 4.30 (dd, J = 11.0, 5.5 Hz, 1H), 3.81 (s, 2H), 3.29−3.13 (m, 1H), 2.81 (s, 2H), 1.52 (s, 9H).

*Tert-butyl 6-(6-((1R,5S)-6,6-difluoro-1,5-dimethyl-3-azabicyclo[3.1.0]hexane-3-carbonyl)pyri-dazin-3-yl)-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A41**).* This compound was synthesized by following the general procedure F (white solid, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 8.18 (d, J = 8.9 Hz, 1H), 8.13 (s, 1H), 8.09 (d, J = 8.9 Hz, 1H), 7.89−7.83 (m, 2H), 7.24−7.17 (m, 2H), 4.65 (s, 2H), 4.58−4.50 (m, 1H), 4.43 (d, J = 12.5 Hz, 1H), 4.34 (d, J = 13.2 Hz, 1H), 4.05−3.95 (m, 1H), 3.80 (s, 2H), 2.81 (s, 2H), 2.49−2.33 (m, 2H), 1.50 (s, 9H).

*Tert-butyl 6-(6-((3,3-difluoro-1-methylpiperidin-4-yl)carbamoyl)pyridazin-3-yl)-8-(4-fluoroph-enyl)-3,4-dihydrobenzofuro [2,3-c]pyridine-2(1H)-carboxylate (**A42**).* This compound was synthesized by following the general procedure F (white solid, 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 9.5 Hz, 1H), 8.35 (d, J = 8.8 Hz, 1H), 8.25−8.11 (m, 3H), 7.90−7.84 (m, 2H), 7.24−7.17 (m, 2H), 4.65 (s, 2H), 4.57−4.40 (m, 1H), 3.80 (s, 2H), 3.23−3.13 (m, 1H), 2.94 (d, J = 11.8 Hz, 1H), 2.81 (s, 2H), 2.49−2.35 (m, 4H), 2.26 (t, J = 12.0 Hz, 1H), 2.15−2.06 (m, 1H), 1.94 (qd, J = 12.3, 3.9 Hz, 1H), 1.51 (s, 9H).

*Tert-butyl 8-(4-fluorophenyl)-6-(6-((2-methoxyethyl)carbamoyl)pyridazin-3-yl)-3,4-dihydrobe-nzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A43**).* This compound was synthesized by following the general procedure F (white solid, 81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (t, J = 5.8 Hz, 1H), 8.33 (d, J = 8.8 Hz, 1H), 8.19 (s, 1H), 8.15 (s, 1H), 8.11 (d, J = 8.8 Hz, 1H), 7.89−7.82 (m, 2H), 7.23−7.16 (m, 2H), 4.64 (s, 2H), 3.79 (s, 2H), 3.74 (q, J = 5.4 Hz, 2H), 3.60 (t, J = 5.2 Hz, 2H), 3.39 (s, 3H), 2.80 (t, J = 5.5 Hz, 2H), 1.50 (s, 9H).

*Tert-butyl 8-(4-fluorophenyl)-6-(6-((2-(2-methoxyethoxy)ethyl) carbamoyl)pyridazin-3-yl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A44**).* This compound was synthesized by following the general procedure F (white solid, 73% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (t, J = 5.6 Hz, 1H), 8.30 (d, J = 8.8 Hz, 1H), 8.14 (d, J = 1.8 Hz, 1H), 8.13−8.06 (m, 2H), 7.82 (dd, J = 8.5, 5.5 Hz, 2H), 7.20−7.11 (m, 2H), 4.60 (s, 2H), 3.68−3.58 (m, 6H), 3.52−3.48 (m, 2H), 3.34 (s, 3H), 2.76 (s, 2H), 2.39 (s, 2H), 1.45 (s, 9H).

*Tert-butyl 6-(6-(3,3-difluorocyclobutane-1-carboxamido)pyridazin-3-yl)-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A45**).* This compound was synthesized by following the general procedure F (white solid, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.79 (s, 1H), 8.77 (d, J = 9.4 Hz, 1H), 8.13 (s, 1H), 8.07 (d, J = 9.4 Hz, 1H), 7.97−7.79 (m, 3H), 7.25−7.18 (m, 2H), 4.66 (s, 2H), 4.05−3.93 (m, 1H), 3.82 (s, 2H), 3.12−2.96 (m, 2H), 2.96−2.78 (m, 4H), 1.52 (s, 9H).

*Tert-butyl (S)-8-(4-fluorophenyl)-6-(6-(tetrahydrofuran-2-carboxamido)pyridazin-3-yl)-3,4-di-hydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A46**).* This compound was synthesized by following the general procedure F (white solid, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.57 (s, 1H), 8.53 (d, J = 9.3 Hz, 1H), 8.07−7.95 (m, 2H), 7.91 (d, J = 9.3 Hz, 1H), 7.86−7.79 (m, 2H), 7.20−7.12 (m, 2H), 4.61 (s, 2H), 4.52 (dd, J = 8.5, 5.8 Hz, 1H), 4.11 (q, J = 7.0 Hz, 1H), 3.97 (q, J = 7.3 Hz, 1H), 3.76 (s, 2H), 2.77 (s, 2H), 2.44−2.31 (m, 1H), 2.24−2.12 (m, 1H), 2.05−1.87 (m, 2H), 1.49 (s, 9H).

*(E)-3-(6-Aminopyridin-3-yl)-1-(8-(4-fluorophenyl)-6-(6-(morpholine-4-carbonyl)pyridazin-3-yl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**19**).* This compound was synthesized by following the general procedure E (white solid, 37% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, J = 8.9 Hz, 1H), 8.46 (s, 1H), 8.30 (s, 1H), 8.19 (s, 1H), 8.07−7.99 (m, 3H), 7.93 (d, J = 8.8 Hz, 1H), 7.50−7.37 (m, 3H), 7.14 (dd, J = 24.5, 14.9 Hz, 1H), 6.51−6.41 (m, 3H), 4.96 (d, J = 79.5 Hz, 2H), 4.03 (d, J = 41.3 Hz, 2H), 3.74 (brs, 4H), 3.66−3.60 (m, 2H), 3.59−3.53 (m, 2H), 2.87 (d, J = 27.0 Hz, 2H). ^{13}C NMR (176 MHz, DMSO-d_6) δ 165.9 (d, J = 35.5 Hz), 164.9, 162.1 (d, J = 245.7 Hz), 160.6, 158.6, 155.1, 152.4 (d, J = 18.5 Hz), 151.8 (d, J = 16.3 Hz), 150.2, 140.5 (d, J = 16.1 Hz), 135.5, 131.6, 131.2, 130.6 (2C), 129.2, 127.8, 125.4, 124.0, 122.4, 119.4, 117.5, 115.8, 115.7, 113.0 (d, J = 24.9 Hz), 112.6 (d, J = 31.4 Hz), 108.0, 66.3, 66.0, 47.4, 42.9 (d, J = 74.7 Hz), 42.3, 40.2 (d, J = 81.7 Hz), 20.9 (d, J = 248.8 Hz). HR-MS (ESI) Calcd. for $C_{34}H_{30}O_4N_6F^+$ $[M+H]^+$, 605.2313; Found, 605.2312; HPLC purity, 99.3%.

*(E)-3-(6-Aminopyridin-3-yl)-1-(8-(4-fluorophenyl)-6-(6-(4-methylpiperazine-1-carbonyl)pyrid-azin-3-yl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**20**).* This compound was synthesized by following the general procedure E (white solid, 35% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.30−8.23 (m, 1H), 8.14−8.04 (m, 3H), 7.93−7.84 (m, 4H), 7.50 (d, J = 15.3 Hz, 1H), 7.25−7.16 (m, 2H), 7.07−6.95 (m, 1H), 6.64−6.56 (m, 1H), 4.83 (d, J = 49.0 Hz, 2H), 3.99 (s, 2H), 3.89 (s, 2H), 3.73 (t, J = 5.1 Hz, 2H), 2.84−2.72 (m, 2H), 2.69 (t, J = 5.0 Hz, 2H), 2.63 (t, J = 4.9 Hz, 2H), 2.42 (s, 3H). ^{13}C NMR (176 MHz, CD_3OD) δ 169.0, 167.1, 164.1 (d, J = 246.7 Hz), 161.8, 161.3, 156.2, 154.5, 152.7, 150.1 (d, J = 16.2 Hz), 142.2, 137.3, 133.1, 132.4, 131.7 (2C), 130.7, 129.4, 127.1, 126.1, 123.8, 121.7, 118.5, 116.6, 116.5, 114.6 (d, J = 34.8 Hz), 114.1 (d, J = 21.1 Hz), 110.5, 55.9, 55.3, 48.0, 45.8, 44.7, 43.0, 41.6 (d, J = 155.5 Hz), 22.0 (d, J = 227.8 Hz). HR-MS (ESI) Calcd. for $C_{35}H_{33}O_3N_7F^+$ $[M+H]^+$, 618.2629; Found, 618.2627; HPLC purity, 95.6%.

*(E)-1-(6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(4-fluorophenyl)-1,2,3,4-tetrahydrobenzofur-o[2,3-c]pyridin-6-yl) pyridazine-3-carbonyl)piperidin-4-one (**21**).* This compound was synthesized by following the general procedure E (white solid, 26% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30−8.21 (m, 2H), 8.17−8.05 (m, 3H), 7.92−7.84 (m, 2H), 7.74−7.61 (m, 2H), 7.26−7.19 (m, 2H), 6.88−6.70 (m, 1H), 6.52 (d, J = 8.6 Hz, 1H), 4.99−4.86 (m, 2H), 4.70 (s, 2H), 4.22−4.13 (m, 4H), 4.12−3.93 (m, 2H), 2.93 (s, 2H), 2.74 (t, J = 6.2 Hz, 2H), 2.69 (t, J = 6.4 Hz, 2H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 206.9, 166.7, 165.4, 162.9 (d, J = 248.3 Hz), 160.1, 159.3, 154.7, 153.6, 149.8, 141.1, 136.1, 131.7, 131.1, 130.6 (2C), 129.6, 129.0, 125.5, 125.0, 123.0, 121.7, 117.3, 116.0, 115.9, 113.7 (d, J = 67.8 Hz), 112.5, 108.7, 46.6, 44.0 (d, J = 68.3 Hz), 42.6, 42.0, 41.4, 41.1, 21.4 (d, J = 268.0 Hz). HR-MS (ESI) Calcd. for $C_{35}H_{30}O_4N_6F^+$ $[M+H]^+$, 617.2313; Found, 617.2308; HPLC purity, 99.4%.

*(E)-1-(6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(4-fluorophenyl)-1,2,3,4-tetrahydrobenzofur-o[2,3-c]pyridin-6-yl) pyridazine-3-carbonyl)-4-methylpiperidine-4-carbonitrile (**22**).* This compound was synthesized by following the general procedure E (white solid, 38% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 6.7 Hz, 2H), 8.17−8.06 (m, 2H), 7.98 (d, J = 8.8 Hz, 1H), 7.90−7.83 (m, 2H), 7.70−7.60 (m, 2H), 7.25−7.17 (m, 2H), 6.87−6.67 (m, 1H), 6.51 (d, J = 8.6 Hz, 1H), 4.87 (q, J = 15.5, 14.4 Hz, 5H), 4.45 (d, J = 14.0 Hz, 1H), 4.03 (d, J = 33.8 Hz, 2H), 3.53−3.41 (m, 1H), 3.20 (td, J = 13.2, 2.7 Hz, 1H), 2.91 (s, 2H), 2.14−2.08 (m, 1H), 2.05−1.98 (m, 1H), 1.90 (td, J = 12.9, 4.0 Hz, 1H), 1.69 (td, J = 13.3, 4.3 Hz, 1H), 1.48 (s, 3H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.7, 165.1, 162.9 (d, J = 248.2 Hz), 159.8, 159.3, 154.9, 153.6, 149.4, 141.0, 136.1, 131.7, 130.6, 130.5, 129.5, 128.8, 125.4, 124.9, 123.2, 122.9, 121.5, 117.3 (d, J = 15.4 Hz), 115.9, 115.8, 113.6 (d, J = 13.3 Hz), 112.4, 108.8, 45.3, 44.0 (d, J = 59.9 Hz), 41.4, 40.6, 37.2, 36.4, 33.8, 26.7, 21.4 (d, J = 260.7 Hz). HR-MS (ESI) Calcd. for $C_{37}H_{33}O_3N_7F^+$ $[M+H]^+$, 642.2629; Found, 642.2626; HPLC purity, 95.8%.

*(E)-3-(6-Aminopyridin-3-yl)-1-(6-(6-(3-(difluoromethyl)azetidine-1-carbonyl)pyridazin-3-yl)-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**23**).* This compound was synthesized by following the general procedure E (white solid, 26% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, J = 8.9 Hz, 1H), 8.24 (d, J = 14.8 Hz, 2H), 8.17−8.05 (m, 2H), 7.90−7.83 (m, 2H), 7.72−7.58 (m, 2H), 7.25−7.18 (m, 2H), 6.88−6.67 (m, 1H), 6.52 (d, J = 8.6 Hz, 1H), 6.06 (td, J = 56.1, 4.1 Hz, 1H), 5.07−4.99 (m, 1H), 4.98−4.80 (m, 5H), 4.46−4.35 (m, 1H), 4.30 (dd, J = 11.0, 5.4 Hz, 1H), 4.04 (d, J = 34.8 Hz, 2H), 3.27−3.11 (m, 1H), 2.91 (s, 2H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.7, 163.0, 162.9 (d, J = 248.1 Hz), 160.0, 159.3, 153.4 (d, J = 77.9 Hz), 152.0, 150.5, 149.5, 141.0, 136.2, 131.7, 131.2, 130.6, 130.5, 129.5, 127.7, 125.5, 124.7, 123.2 (d, J = 42.3 Hz), 121.6, 117.5 (d, J = 47.2 Hz), 116.0, 115.9 (t, J = 240.9 Hz), 115.8, 113.8 (d, J = 68.3 Hz), 112.5, 108.8, 54.4 (d, J = 6.2 Hz), 48.4 (t, J = 6.2 Hz), 43.9 (d, J = 49.3 Hz), 40.8 (d, J = 221.1 Hz), 33.2 (t, J = 23.2 Hz), 21.4 (d, J = 251.8 Hz). HR-MS (ESI) Calcd. for $C_{34}H_{28}O_3N_6F_3^+$ $[M+H]^+$, 625.2175; Found, 625.2166; HPLC purity, 97.5%.

*(E)-3-(6-Aminopyridin-3-yl)-1-(6-(6-((1R,5S)-6,6-difluoro-3-azabicyclo[3.1.0]hexane-3-carbonyl)pyridazin-3-yl)-8-(4-fluorophenyl)-3,4-dihydrobenzofuro[2,3-c]pyridin-2(1H)-yl)prop-2-en-1-one (**24**).* This compound was synthesized by following the general procedure E (white solid, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27−8.21 (m, 2H), 8.18 (d, J = 8.9 Hz, 1H), 8.16−8.10 (m, 1H), 8.08 (d, J = 8.9 Hz, 1H), 7.90−7.83 (m, 2H), 7.70−7.60 (m, 2H), 7.25−7.17 (m, 2H), 6.87−6.67 (m, 1H), 6.51 (d, J = 8.6 Hz, 1H), 4.96−4.85 (m, 2H), 4.80 (s, 2H), 4.58−4.51 (m, 1H), 4.43 (d, J = 12.5 Hz, 1H), 4.35 (d, J = 13.2 Hz, 1H), 4.14−3.94 (m, 3H), 2.90 (s, 2H), 2.48−2.34 (m, 2H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.6, 163.4, 162.8 (d. J = 248.0 Hz), 159.7, 159.3, 154.5, 153.5, 151.2 (d, J = 255.2 Hz), 149.7, 141.0, 136.0, 131.7, 131.2 (d, J = 25.2 Hz), 130.6, 130.5, 129.5, 128.7, 125.4, 124.6, 123.1 (d, J = 44.6 Hz), 121.5, 117.4 (d, J = 48.8 Hz), 115.9, 115.8, 114.0 (d, J = 61.6 Hz), 113.0 (dd, J = 294.7, 278.7 Hz), 112.9 (d, J = 174.2 Hz), 108.7, 48.7, 47.4, 43.9 (d, J = 45.8 Hz), 40.8 (d, J = 222.9 Hz), 27.2 (t, J = 11.7 Hz), 24.3 (t, J = 12.0 Hz), 21.4 (d, J = 253.0 Hz). HR-MS (ESI) Calcd. for $C_{35}H_{28}O_3N_6F_3^+$ $[M+H]^+$, 637.2175; Found, 637.2175; HPLC purity, 95.8%.

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(4-fluorophenyl)-1,2,3,4-tetrahydrobenzofur-o[2,3-c]pyridin-6-yl)-N-(3,3-difluoro-1-methylpiperidin-4-yl)pyridazine-3-carboxamide (**25**).* This compound was synthesized by following the general procedure E (white solid, 47% yield). ^{1}H NMR (700 MHz, DMSO-d_6) δ 8.98 (d, J = 9.3 Hz, 1H), 8.63 (d, J = 8.9 Hz, 1H), 8.43 (s, 1H), 8.33 (d, J = 1.8 Hz, 1H), 8.28 (d, J = 8.8 Hz, 1H), 8.19 (d, J = 7.3 Hz, 1H), 8.02 (dd, J = 8.4, 5.4 Hz, 2H), 7.92 (d, J = 8.6 Hz, 1H), 7.48−7.43 (m, 1H), 7.42 (t, J = 8.7 Hz, 2H), 7.14 (dd, J = 41.1, 15.3 Hz, 1H), 6.53−6.41 (m, 3H), 4.95 (d, J = 138.6 Hz, 2H), 4.51−4.39 (m, 1H), 4.02 (d, J = 71.2 Hz, 2H), 3.16−3.07 (m, 1H), 2.89 (s, 1H), 2.82 (d, J = 10.9 Hz, 2H),

2.43 (dd, J = 28.8, 12.0 Hz, 1H), 2.27 (s, 3H), 2.25−2.19 (m, 1H), 2.07−1.99 (m, 1H), 1.87−1.81 (m, 1H). ^{13}C NMR (176 MHz, DMSO-d_6) δ 166.0 (d, J = 33.8 Hz), 162.8 (2C), 160.7 (d, J = 246.1 Hz), 160.6, 152.5 (d, J = 18.4 Hz), 151.9 (d, J = 20.1 Hz), 150.9, 150.2, 140.5 (d, J = 16.2 Hz), 135.5, 131.6, 130.9, 130.6, 130.5, 129.2, 126.4, 125.7, 124.1, 122.5, 119.4, 119.3 (dd, J = 242.8, 4.8 Hz), 117.7, 115.8, 115.7, 113.0 (d, J = 20.2 Hz), 112.6 (d, J = 28.3 Hz), 108.0, 58.9 (dd, J = 28.0, 23.0 Hz), 52.7, 49.4 (t, J = 20.3 Hz), 44.7, 42.9 (d, J = 70.2 Hz), 40.3 (d, J = 84.1 Hz), 28.1 (d, J = 6.6 Hz), 20.9 (d, J = 248.6 Hz). HR-MS (ESI) Calcd. for $C_{36}H_{33}O_3N_7F_3^+$ [M + H]$^+$, 668.2597; Found, 668.2599; HPLC purity, 97.8%.

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(4-fluorophenyl)-1,2,3,4-tetrahydrobenzofur-o[2,3-c]pyridin-6-yl)-N-(2-methoxyethyl)pyridazine-3-carboxamide (**26**).* This compound was synthesized by following the general procedure E (white solid, 30% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (t, J = 5.5 Hz, 1H), 8.36 (d, J = 8.4 Hz, 1H), 8.23 (s, 2H), 8.20−8.09 (m, 2H), 7.91−7.84 (m, 2H), 7.72−7.59 (m, 2H), 7.21 (t, J = 8.2 Hz, 2H), 6.87−6.71 (m, 1H), 6.52 (d, J = 7.5 Hz, 1H), 5.00−4.78 (m, 4H), 4.13−3.95 (m, 2H), 3.79−3.71 (m, 2H), 3.61 (t, J = 5.4 Hz, 2H), 3.41 (s, 3H), 2.91 (s, 2H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.6, 162.9 (d, J = 248.2 Hz), 162.8, 160.7, 159.3, 153.5, 151.9, 150.7 (d, J = 87.4 Hz), 149.5, 141.0, 136.1, 131.7, 131.2 (d, J = 24.3 Hz), 130.5 (2C), 129.5, 126.4, 125.4, 124.9, 123.1 (d, J = 48.9 Hz), 121.5, 117.4 (d, J = 51.7 Hz), 115.9, 115.8, 114.1 (d, J = 54.4 Hz), 113.0 (d, J = 185.8 Hz), 108.8, 71.1, 59.0, 43.9 (d, J = 49.9 Hz), 40.8 (d, J = 223.9 Hz), 39.6, 21.4 (d, J = 253.1 Hz). HR-MS (ESI) Calcd. for $C_{33}H_{30}O_4N_6F^+$ [M + H]$^+$, 593.2313; Found, 593.2303; HPLC purity, 99.6%.

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(4-fluorophenyl)-1,2,3,4-tetrahydrobenzofur-o[2,3-c]pyridin-6-yl)-N-(2-(2-methoxyethoxy)ethyl)pyridazine-3-carboxamide (**27**).* This compound was synthesized by following the general procedure E (white solid, 18% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.15 (t, J = 5.8 Hz, 1H), 8.64 (d, J = 8.9 Hz, 1H), 8.45 (d, J = 1.8 Hz, 1H), 8.33 (d, J = 1.8 Hz, 1H), 8.27 (d, J = 8.9 Hz, 1H), 8.19 (s, 1H), 8.07−7.99 (m, 2H), 7.92 (d, J = 8.8 Hz, 1H), 7.49−7.37 (m, 3H), 7.22−7.05 (m, 1H), 6.53−6.40 (m, 3H), 4.96 (d, J = 79.8 Hz, 2H), 4.03 (d, J = 39.9 Hz, 2H), 3.65−3.51 (m, 6H), 3.49−3.43 (m, 2H), 3.25 (s, 3H), 2.87 (d, J = 26.9 Hz, 2H). ^{13}C NMR (176 MHz, DMSO-d_6) δ 165.3 (d, J = 35.0 Hz), 162.0, 160.1, 161.5 (d, J = 246.9 Hz), 159.3, 151.8, 151.3150.7, 149.6, 139.9 (d, J = 15.5 Hz), 134.8, 131.0, 130.5, 130.0 (2C), 128.6, 125.5, 125.1, 123.5, 121.9, 118.8, 117.1, 115.2, 115.1, 112.4 (d, J = 26.0 Hz), 112.0 (d, J = 33.2 Hz), 107.4, 70.7, 68.8, 68.1, 57.5, 42.3 (d, J = 72.3 Hz), 39.7 (d, J = 82.5 Hz), 38.3, 20.3 (d, J = 248.0 Hz). HR-MS (ESI) Calcd. for $C_{35}H_{34}O_5N_6F^+$ [M + H]$^+$, 637.2575; Found, 637.2568; HPLC purity, 95.5%.

*(E)-N-(6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(4-fluorophenyl)-1,2,3,4-tetrahydrobenzofur-o[2,3-c]pyridin-6-yl)pyridazin-3-yl)-3,3-difluorocyclobutane-1-carboxamide (**28**).* This compound was synthesized by following the general procedure E (white solid, 22% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (s, 1H), 8.44 (s, 2H), 8.32 (d, J = 1.8 Hz, 1H), 8.22−8.14 (m, 2H), 8.06−7.97 (m, 2H), 7.93 (d, J = 8.8 Hz, 1H), 7.50−7.32 (m, 3H), 7.22−7.05 (m, 1H), 6.54−6.38 (m, 3H), 4.94 (d, J = 80.4 Hz, 2H), 4.02 (d, J = 41.3 Hz, 2H), 3.41−3.34 (m, 1H), 2.97−2.74 (m, 6H). ^{13}C NMR (176 MHz, DMSO-d_6) δ 172.4, 165.9 (d, J = 38.0 Hz), 162.0 (d, J = 245.7 Hz), 160.6, 155.5, 154.4, 151.9 (d, J = 18.5 Hz), 151.6 (d, J = 15.6 Hz), 150.2, 140.4 (d, J = 17.5 Hz), 135.5, 131.8, 131.6, 130.6 (2C), 129.0, 126.3, 123.8, 121.7, 119.5 (dd, J = 269.2, 14.3 Hz), 119.4, 118.8, 116.6, 115.8, 115.6, 113.0, 112.5 (d, J = 10.8 Hz), 108.0, 42.9 (d, J = 71.5 Hz), 40.3 (d, J = 85.5 Hz), 37.9, 37.9 (t, J = 23.6 Hz, 2C), 27.3 (dd, J = 14.5, 5.3 Hz), 20.9 (d, J = 245.6 Hz). HR-MS (ESI) Calcd. for $C_{34}H_{28}O_3N_6F_3^+$ [M + H]$^+$, 625.2175; Found, 625.2173; HPLC purity, 97.0%.

*(S,E)-N-(6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(4-fluorophenyl)-1,2,3,4-tetrahydrobenzofu-ro[2,3-c]pyridin-6-yl)pyridazin-3-yl)tetrahydrofuran-2-carboxamide (**29**).* This compound was synthesized by following the general procedure E (white solid, 19% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 8.42−8.31 (m, 2H), 8.25 (s, 1H), 8.18−8.09 (m, 2H), 8.00−7.86 (m, 3H), 7.47−7.32 (m, 3H), 7.17−7.03 (m, 1H), 6.49−6.38 (m, 3H), 4.90 (d, J = 79.9 Hz, 2H), 4.55 (dd, J = 8.2, 5.5 Hz, 1H), 4.06−3.90 (m, 3H), 3.82 (q, J = 7.1 Hz, 1H), 2.81 (d, J = 28.0 Hz, 2H), 2.28−2.14 (m, 1H), 2.06−1.77 (m, 3H). ^{13}C NMR (176 MHz, DMSO-d_6) δ 173.0, 165.9 (d, J = 35.8 Hz), 162.0 (d, J = 245.7 Hz), 160.6, 155.7, 153.7, 151.9 (d, J = 19.0 Hz), 151.5 (d, J = 18.2 Hz), 150.2, 140.5 (d, J = 14.7 Hz), 135.4, 131.8, 131.6, 130.6, 130.5, 129.0, 126.3, 123.8, 121.8, 119.4, 118.8, 116.6, 115.8, 115.6, 113.0, 112.5 (d, J = 8.6 Hz), 108.0, 77.5, 69.0, 42.9 (d, J = 71.8 Hz), 40.2 (d, J = 83.3 Hz), 29.9, 25.2, 20.9 (d, J = 245.8 Hz). HR-MS (ESI) Calcd. for $C_{34}H_{30}O_4N_6F^+$ [M + H]$^+$, 605.2313; Found, 605.2306; HPLC purity, 95.2%.

*Compound **A58** was synthesized by following the preparation procedures of compound **A42**.*

*Methyl 5-bromo-3-chloro-2-hydroxybenzoate (**A48**).* White solid (96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.26 (s, 1H), 7.87 (d, J = 2.5 Hz, 1H), 7.66 (d, J = 2.4 Hz, 1H), 7.24 (s, 1H), 3.96 (s, 3H).

*Methyl 5-bromo-3-chloro-2-(2-ethoxy-2-oxoethoxy)benzoate (**A49**).* White solid (98% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J = 2.4 Hz, 1H), 7.69 (d, J = 2.4 Hz, 1H), 4.67 (s, 2H), 4.30 (q, J = 7.2 Hz, 2H), 3.90 (s, 3H), 1.32 (t, J = 7.2 Hz, 3H).

*5-Bromo-2-(carboxymethoxy)-3-chlorobenzoic acid (**A50**).* White solid (96% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.90 (d, J = 2.4 Hz, 1H), 7.85 (d, J = 2.4 Hz, 1H), 4.68 (s, 2H).

*5-Bromo-7-chlorobenzofuran-3-yl acetate (**A51**).* White solid (43% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.61 (s, 1H), 7.47 (s, 1H), 2.37 (s, 3H).

*5-Bromo-7-chlorobenzofuran-3(2H)-one (**A52**).* Brown solid (98% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 1.4 Hz, 1H), 7.71 (d, J = 1.4 Hz, 1H), 4.77 (s, 2H).

*2-(5-Bromo-7-chlorobenzofuran-3-yl)acetonitrile (**A53**).* Brown solid (62% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.64 (d, J = 1.7 Hz, 1H), 7.53 (d, J = 1.7 Hz, 1H), 3.74 (d, J = 1.3 Hz, 2H).

*2-(5-Bromo-7-chlorobenzofuran-3-yl)ethan-1-amine (**A54**).* Yellow oil (51% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.91 (d, J = 1.8 Hz, 1H), 7.64 (d, J = 1.8 Hz, 1H), 2.82 (t, J = 6.8 Hz, 2H), 2.70 (t, J = 6.8 Hz, 2H), 1.87 (brs, 2H).

*Tert-butyl 6-bromo-8-chloro-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A56**).* White solid (40% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.39 (s, 1H), 4.63 (s, 2H), 3.75 (s, 2H), 2.68 (s, 2H), 1.50 (s, 9H).

*Tert-butyl 8-chloro-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A57**).* White solid (90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.69 (s, 1H), 4.62 (s, 2H), 3.74 (s, 2H), 2.70 (s, 2H), 1.48 (s, 9H), 1.35 (s, 12H).

Tert-butyl 8-chloro-6-(6-((3,3-difluoro-1-methylpiperidin-4-yl)carbamoyl)pyridazin-3-yl)-3,4-dihydrobenzofuro[2,3-c]pyridine-

*2(1H)-carboxylate (**A58**).* White solid (52% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (d, J = 9.3 Hz, 1H), 8.59 (d, J = 8.7 Hz, 1H), 8.44 (s, 1H), 8.33−8.27 (m, 2H), 4.66 (s, 2H), 3.72 (s, 2H), 3.52−3.46 (m, 1H), 3.17−3.04 (m, 1H), 2.86−2.74 (m, 3H), 2.46−2.34 (m, 1H), 2.27 (s, 3H), 2.25−2.16 (m, 1H), 2.10−2.00 (m, 1H), 1.86−1.78 (m, 1H), 1.45 (s, 9H).

*General procedure G for synthesis of compounds **A59**—**A66**.* To a solution of **A58** (0.10 mmol, 1 eq.) in 1,4-dioxane (0.9 mL) was added an appropriate boronic ester (0.15 mmol, 1.5 eq.), $Pd_2(dba)_3$ (0.02 mmol, 0.2 eq.), PCy_3 (0.04 mmol, 0.4 eq.), and Cs_2CO_3 (0.25 mmol, 2.5 eq.). The mixture was heated to 140 °C for 4 h in the microwave, then cooled to room temperature and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified using a silica gel column to afford compounds **A59**—**A66**.

*Tert-butyl 6-(6-((3,3-difluoro-1-methylpiperidin-4-yl)carbamoyl)pyridazin-3-yl)-8-methyl-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A59**).* Yellow solid (35% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, J = 9.3 Hz, 1H), 8.32 (t, J = 8.9 Hz, 1H), 8.13−8.03 (m, 2H), 7.84 (s, 1H), 4.63 (s, 2H), 4.56−4.40 (m, 1H), 3.77 (s, 2H), 3.23−3.13 (m, 1H), 2.93 (d, J = 11.7 Hz, 1H), 2.76 (s, 2H), 2.58 (s, 3H), 2.49−2.36 (m, 4H), 2.26 (t, J = 12.6 Hz, 1H), 2.14−2.05 (m, 1H), 2.00−1.88 (m, 1H), 1.50 (s, 9H).

*Tert-butyl 8-cyclopropyl-6-(6-((3,3-difluoro-1-methylpiperidin-4-yl)carbamoyl)pyridazin-3-yl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A60**).* Yellow solid (50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, J = 9.5 Hz, 1H), 8.31 (d, J = 8.8 Hz, 1H), 8.06 (d, J = 8.9 Hz, 1H), 7.99 (s, 1H), 7.71−7.60 (m, 1H), 4.64 (s, 2H), 4.56−4.39 (m, 1H), 3.77 (s, 2H), 3.15 (t, J = 12.6 Hz, 1H), 2.92 (d, J = 11.8 Hz, 1H), 2.76 (s, 2H), 2.46−2.30 (m, 5H), 2.24 (t, J = 11.8 Hz, 1H), 2.13−2.04 (m, 1H), 1.98−1.85 (m, 2H), 1.50 (s, 9H), 1.14−1.06 (m, 2H), 1.04−0.97 (m, 2H).

*Tert-butyl 6-(6-((3,3-difluoro-1-methylpiperidin-4-yl)carbamoyl)pyridazin-3-yl)-8-(3,3,5,5-tetr-amethylcyclohex-1-en-1-yl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A61**).* White solid (75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, J = 9.5 Hz, 1H), 8.31 (d, J = 8.8 Hz, 1H), 8.10 (d, J = 8.9 Hz, 1H), 8.04 (s, 1H), 8.01−7.92 (m, 1H), 6.11 (s, 1H), 4.62 (s, 2H), 4.53−4.37 (m, 1H), 3.76 (s, 2H), 3.20−3.09 (m, 1H), 2.90 (d, J = 11.8 Hz, 1H), 2.75 (t, J = 5.6 Hz, 2H), 2.44−2.31 (m, 6H), 2.27−2.18 (m, 1H), 2.12−2.02 (m, 1H), 1.97−1.84 (m, 1H), 1.49 (s, 9H), 1.45 (s, 2H), 1.12 (s, 6H), 1.06 (s, 6H).

*Tert-butyl 6-(6-((3,3-difluoro-1-methylpiperidin-4-yl)carbamoyl)pyridazin-3-yl)-8-(4,4-dimeth-ylcyclohex-1-en-1-yl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A62**).* White solid (19% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 9.5 Hz, 1H), 8.33 (d, J = 8.9 Hz, 1H), 8.15−7.94 (m, 3H), 6.48 (s, 1H), 4.64 (s, 2H), 4.56−4.38 (m, 1H), 3.78 (s, 2H), 3.23−3.10 (m, 1H), 2.93 (d, J = 11.7 Hz, 1H), 2.77 (s, 2H), 2.63 (s, 2H), 2.47−2.35 (m, 4H), 2.26 (t, J = 11.4 Hz, 1H), 2.14−2.06 (m, 3H), 1.99−1.87 (m, 1H), 1.59 (t, J = 6.3 Hz, 2H), 1.50 (s, 9H), 1.02 (s, 6H).

*Tert-butyl 6-(6-((3,3-difluoro-1-methylpiperidin-4-yl)carbamoyl)pyridazin-3-yl)-8-(thiophen-3-yl)-3,4-dihydrobenzofuro [2,3-c]pyridine-2(1H)-carboxylate (**A63**).* Brown solid (77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, J = 9.5 Hz, 1H), 8.34−8.22 (m, 2H), 8.13−8.06 (m, 2H), 8.00 (dd, J = 3.0, 1.3 Hz, 1H), 7.72 (dd, J = 5.0, 1.3 Hz, 1H), 7.44 (dd, J = 5.0, 3.0 Hz, 1H), 4.65 (s, 2H), 4.54−4.37 (m, 1H), 3.78 (s, 2H), 3.21−3.11 (m, 1H), 2.91 (d, J = 11.7 Hz, 1H), 2.76 (s, 2H), 2.46−2.33 (m, 4H), 2.28−2.19 (m, 1H), 2.12−2.04 (m, 1H), 1.98−1.87 (m, 1H), 1.50 (s, 9H).

*Tert-butyl 6-(6-((3,3-difluoro-1-methylpiperidin-4-yl)carbamoyl)pyridazin-3-yl)-8-(p-tolyl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A64**).* White solid (65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, J = 9.6 Hz, 1H), 8.30 (d, J = 8.8 Hz, 1H), 8.20−8.11 (m, 2H), 8.09 (d, J = 8.8 Hz, 1H), 7.75 (d, J = 7.9 Hz, 2H), 7.29 (d, J = 7.9 Hz, 2H), 4.61 (s, 2H), 4.54−4.35 (m, 1H), 3.77 (s, 2H), 3.19−3.10 (m, 1H), 2.90 (d, J = 11.9 Hz, 1H), 2.76 (s, 2H), 2.46−2.31 (m, 7H), 2.28−2.16 (m, 1H), 2.11−2.01 (m, 1H), 1.98−1.84 (m, 1H), 1.48 (s, 9H).

*Tert-butyl 6-(6-((3,3-difluoro-1-methylpiperidin-4-yl)carbamoyl)pyridazin-3-yl)-8-(2-fluoro-4-methylphenyl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A65**).* White solid (82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, J = 9.5 Hz, 1H), 8.36−8.30 (m, 2H), 8.12 (d, J = 8.9 Hz, 1H), 8.09−8.00 (m, 1H), 7.53 (t, J = 7.8 Hz, 1H), 7.12−7.03 (m, 2H), 4.61 (s, 2H), 4.55−4.39 (m, 1H), 3.79 (s, 2H), 3.22−3.13 (m, 1H), 2.92 (d, J = 12.0 Hz, 1H), 2.81 (s, 2H), 2.48−2.33 (m, 7H), 2.30−2.21 (m, 1H), 2.14−2.05 (m, 1H), 2.00−1.87 (m, 1H), 1.49 (s, 9H).

*Tert-butyl 6-(6-((3,3-difluoro-1-methylpiperidin-4-yl)carbamoyl)pyridazin-3-yl)-8-mesityl-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A66**).* White solid (44% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 9.4 Hz, 1H), 8.39−8.35 (m, 1H), 8.34 (d, J = 8.9 Hz, 1H), 8.07 (d, J = 9.2 Hz, 1H), 7.76 (s, 1H), 7.03 (s, 2H), 4.64−4.40 (m, 3H), 3.80 (s, 2H), 3.27−3.12 (m, 1H), 2.93 (d, J = 11.7 Hz, 1H), 2.83 (s, 2H), 2.44−2.35 (m, 7H), 2.31−2.20 (m, 1H), 2.14−2.05 (m, 1H), 2.04 (s, 6H), 2.00−1.88 (m, 1H), 1.49 (s, 9H).

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-chloro-1,2,3,4-tetrahydrobenzofuro[2,3-c]pyrid-in–6-yl)-N-(3,3-difluoro-1-methylpiperidin-4-yl)pyridazine-3-carboxamide (**30**).* This compound was prepared by following the general procedure E (white solid, 21% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, J = 9.3 Hz, 1H), 8.58 (d, J = 8.9 Hz, 1H), 8.44 (s, 1H), 8.32−8.27 (m, 2H), 8.19 (s, 1H), 7.92 (dd, J = 8.7, 2.4 Hz, 1H), 7.46 (d, J = 15.1 Hz, 1H), 7.20−7.07 (m, 1H), 6.51−6.41 (m, 3H), 4.98 (d, J = 89.4 Hz, 2H), 4.54−4.36 (m, 1H), 4.01 (d, J = 35.6 Hz, 2H), 3.09 (t, J = 13.5 Hz, 1H), 2.93−2.77 (m, 3H), 2.48−2.36 (m, 1H), 2.27 (s, 3H), 2.25−2.16 (m, 1H), 2.11−1.97 (m, 1H), 1.88−1.78 (m, 1H). ^{13}C NMR (176 MHz, DMSO-d_6) δ 165.9 (d, J = 44.8 Hz), 162.7, 160.7, 159.0, 152.7, 151.2, 150.9, 150.2, 140.6, 135.4, 131.8, 129.9, 126.6, 125.8, 122.8, 119.4, 119.3 (t, J = 253.4 Hz) 117.6, 116.2, 113.70 (d, J = 61.5 Hz), 112.6 (d, J = 61.0 Hz), 108.0, 59.0 (t, J = 27.5 Hz), 52.7, 49.4 (t, J = 20.2 Hz), 44.7, 42.8 (d, J = 67.3 Hz), 40.2 (d, J = 64.1 Hz), 28.1, 20.9 (d, J = 241.9 Hz). HR-MS (ESI) Calcd. for $C_{30}H_{29}O_3N_7F_2Cl^+$ $[M + H]^+$, 608.1988; Found, 608.1990; HPLC purity, 95.9%.

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-methyl-1,2,3,4-tetrahydrobenzofuro[2,3-c]pyrid-in-6-yl)-N-(3,3-difluoro-1-methylpiperidin-*4-yl*)pyridazine-3-carboxamide (**31**).* This compound was prepared by following the general procedure E (white solid, 33% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, J = 9.5 Hz, 1H), 8.32 (d, J = 8.9 Hz, 1H), 8.23 (d, J = 2.3 Hz, 1H), 8.14 (s, 1H), 8.08 (d, J = 8.9 Hz, 1H), 7.92−7.78 (m, 1H), 7.71−7.60 (m, 2H), 6.85−6.73 (m, 1H), 6.52 (d, J = 8.6 Hz, 1H), 4.96−4.68 (m, 4H), 4.55−4.38 (m, 1H), 4.12−3.92 (m, 2H), 3.23−3.11 (m, 1H), 2.97−2.83 (m, 3H), 2.59 (s, 3H), 2.47−2.35 (m, 4H), 2.25 (t, J = 11.4 Hz, 1H), 2.15−2.06 (m, 1H), 2.00−1.87 (m, 1H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.6, 162.9, 161.2, 159.3, 155.7, 151.3, 150.2, 149.7, 140.9, 136.0, 130.3 (d, J = 31.6 Hz), 128.2, 126.5, 124.9, 124.5 (d, J = 50.6 Hz), 122.6,

121.6, 118.5 (t, J = 245.7 Hz), 116.1 (d, J = 45.5 Hz), 113.8 (d, J = 59.6 Hz), 112.4, 108.7, 60.1 (dd, J = 28.3, 23.6 Hz), 53.6, 50.1 (t, J = 20.5 Hz), 45.5, 43.9 (d, J = 30.3 Hz), 40.8 (d, J = 220.7 Hz), 29.5 (d, J = 6.2 Hz), 21.4 (d, J = 252.5 Hz), 15.30. HR-MS (ESI) Calcd. for $C_{31}H_{32}O_3N_7F_2^+$ [M + H]$^+$, 588.2535; Found, 588.2532; HPLC purity, 95.7%.

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-cyclopropyl-1,2,3,4-tetrahydrobenzofuro[2,3-c]pyridin-6-yl)-N-(3,3-difluoro-1-methylpiperidin-*4-yl*)pyridazine-3-carboxamide (**32**).* This compound was prepared by following the general procedure E (white solid, 35% yield). ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.44 (d, J = 9.5 Hz, 1H), 8.33 (d, J = 8.8 Hz, 1H), 8.24 (s, 1H), 8.09−7.99 (m, 2H), 7.74−7.61 (m, 3H), 6.87−6.73 (m, 1H), 6.52 (d, J = 8.5 Hz, 1H), 4.98−4.85 (m, 2H), 4.74 (s, 2H), 4.52−4.43 (m, 1H), 4.02 (d, J = 74.0 Hz, 2H), 3.22−3.15 (m, 1H), 2.93 (d, J = 11.9 Hz, 1H), 2.88 (s, 2H), 2.45−2.33 (m, 5H), 2.28−2.23 (m, 1H), 2.12−2.07 (m, 1H), 1.97−1.90 (m, 1H), 1.15−1.10 (m, 2H), 1.05−1.01 (m, 2H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.7, 163.0, 161.3, 159.3, 155.7, 151.3, 150.2, 149.7, 140.9, 136.1, 130.4, 129.1, 128.3, 126.5, 124.9, 121.7, 119.4, 118.5 (t, J = 245.6 Hz), 115.4 (d, J = 42.5 Hz), 113.8 (d, J = 68.0 Hz), 112.3, 108.7, 60.1 (d, J = 23.0 Hz), 53.6, 50.1 (t, J = 20.4 Hz), 45.5, 43.9 (d, J = 39.3 Hz), 40.9 (d, J = 220.9 Hz), 29.5 (d, J = 6.2 Hz), 21.4 (d, J = 251.2 Hz), 10.3, 8.4 (2C). HR-MS (ESI) Calcd. for $C_{33}H_{34}O_3N_7F_2^+$ [M + H]$^+$, 614.2691; Found, 614.2686; HPLC purity, 95.1%.

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(3,3,5,5-tetramethylcyclohex-1-en-1-yl)-1,2,3,4-tetrahydrobenzofuro[2,3-c]pyridin-6-yl)-N-(3,3-difluoro-1-methylpiperidin-4-yl)pyridazine-3-carb-oxamide (**33**).* This compound was prepared by following the general procedure E (white solid, 34% yield). ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.45 (d, J = 9.5 Hz, 1H), 8.35 (d, J = 8.8 Hz, 1H), 8.25 (d, J = 2.3 Hz, 1H), 8.13 (d, J = 8.8 Hz, 1H), 8.11−7.93 (m, 2H), 7.70 (dd, J = 8.6, 2.4 Hz, 1H), 7.66 (d, J = 15.3 Hz, 1H), 6.87−6.74 (m, 1H), 6.53 (d, J = 8.6 Hz, 1H), 6.15 (s, 1H), 4.99−4.64 (m, 4H), 4.53−4.43 (m, 1H), 4.03 (d, J = 70.9 Hz, 2H), 3.22−3.13 (m, 1H), 2.93 (d, J = 12.0 Hz, 1H), 2.91−2.83 (m, 2H), 2.46−2.38 (m, 4H), 2.36 (s, 2H), 2.29−2.24 (m, 1H), 2.13−2.07 (m, 1H), 1.98−1.90 (m, 1H), 1.49 (s, 2H), 1.16 (s, 6H), 1.09 (s, 6H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.7, 163.0, 161.3, 159.3, 153.9, 151.3, 150.2, 149.5, 140.9, 138.4, 136.1, 130.4 (d, J = 27.6 Hz), 129.4, 129.2, 129.0, 126.5, 125.0, 121.9 (d, J = 65.1 Hz), 121.6, 118.5 (t, J = 245.7 Hz), 116.4 (d, J = 40.4 Hz), 113.9 (d, J = 72.7 Hz), 112.0, 108.8, 60.1 (dd, J = 28.3, 23.4 Hz), 53.6, 50.1 (t, J = 20.5 Hz), 49.6, 45.5, 44.0 (d, J = 41.6 Hz), 42.0, 40.8 (d, J = 223.9 Hz), 33.5, 31.7 (2C), 31.1, 30.1 (2C), 29.5 (d, J = 6.2 Hz), 21.3 (d, J = 247.6 Hz). HR-MS (ESI) Calcd. for $C_{40}H_{46}O_3N_7F_2^+$ [M + H]$^+$, 710.3630; Found, 710.3632; HPLC purity, 97.8%.

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(4,4-dimethylcyclohex-1-en-1-yl)-1,2,3,4-tetrahy -drobenzofuro[2,3-c] pyridin-6-yl)-N-(3,3-difluoro-1-methylpiperidin-4-yl)pyridazine-3-carboxami-de (**34**).* This compound was prepared by following the general procedure E (white solid, 30% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 9.5 Hz, 1H), 8.34 (d, J = 8.8 Hz, 1H), 8.23 (s, 1H), 8.18−7.94 (m, 2H), 7.73−7.60 (m, 2H), 6.88−6.71 (m, 1H), 6.56−6.47 (m, 2H), 4.99−4.76 (m, 4H), 4.57−4.40 (m, 1H), 4.03 (d, J = 36.2 Hz, 2H), 3.24−3.13 (m, 1H), 2.99−2.83 (m, 3H), 2.65 (s, 2H), 2.49−2.35 (m, 4H), 2.26 (t, J = 11.9 Hz, 1H), 2.06−1.85 (m, 4H), 1.61 (t, J = 6.3 Hz, 2H), 1.03 (s, 6H). ^{13}C NMR (176 MHz, DMSO-d_6) δ 166.0 (d, J = 30.9 Hz), 162.8, 160.7, 160.1, 152.6, 151.3, 150.8, 150.2, 140.5 (d, J = 15.6 Hz), 135.5, 130.9, 130.3, 128.8, 128.2, 128.1, 126.8, 126.4, 125.5, 120.9, 119.4, 119.3 (dd, J = 242.5, 4.9 Hz), 116.7, 113.2, 112.9 (d, J = 36.7 Hz), 112.4 (d, J = 28.9 Hz), 108.0, 59.0 (dd, J = 28.9, 23.6 Hz), 52.7, 49.3 (t, J = 20.1 Hz), 44.7, 42.9 (d, J = 65.5 Hz), 40.3 (d, J = 89.0 Hz), 35.2, 28.2 (d, J = 6.5 Hz), 28.0 (2C), 25.1, 20.8 (d, J = 255.6 Hz). HR-MS (ESI) Calcd. for $C_{38}H_{42}O_3N_7F_2^+$ [M + H]$^+$, 682.3317; Found, 682.3312; HPLC purity, 95.7%.

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(thiophen-3-yl)-1,2,3,4-tetrahydrobenzofuro[2,3-c]pyridin-6-yl)-N-(3,3-difluoro-1-methylpiperidin-4-yl)pyridazine-3-carboxamide (**35**).* This compound was prepared by following the general procedure E (white solid, 36% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 9.5 Hz, 1H), 8.37 (d, J = 8.8 Hz, 1H), 8.35−8.27 (m, 1H), 8.25 (d, J = 2.3 Hz, 1H), 8.20 (s, 1H), 8.15 (d, J = 8.8 Hz, 1H), 8.05 (d, J = 3.8 Hz, 1H), 7.76 (dd, J = 5.0, 1.3 Hz, 1H), 7.72−7.62 (m, 2H), 7.49 (dd, J = 5.0, 3.0 Hz, 1H), 6.88−6.73 (m, 1H), 6.53 (d, J = 8.6 Hz, 1H), 4.96 (s, 2H), 4.74 (s, 2H), 4.57−4.41 (m, 1H), 4.16−3.95 (m, 2H), 3.25−3.14 (m, 1H), 2.99−2.85 (m, 3H), 2.48−2.35 (m, 4H), 2.27 (t, J = 11.8 Hz, 1H), 2.15−2.06 (m, 1H), 2.02−1.87 (m, 1H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.7, 162.9, 161.0, 159.3, 153.4, 151.8, 150.4, 149.7, 141.0, 136.1, 135.9, 130.8 (d, J = 26.6 Hz), 129.5, 127.1, 126.6, 126.1, 125.0, 124.2, 122.1 (d, J = 62.0 Hz), 121.7, 121.2, 118.5 (d, J = 245.2 Hz), 116.8, 113.8 (d, J = 66.5 Hz), 112.4, 108.7, 60.1 (dd, J = 28.5, 23.4 Hz), 53.6, 50.2 (t, J = 20.5 Hz), 45.6, 44.0 (d, J = 50.7 Hz), 40.8 (d, J = 226.1 Hz), 29.5 (d, J = 6.3 Hz), 21.4 (d, J = 255.0 Hz). HR-MS (ESI) Calcd. for $C_{34}H_{32}O_3N_7F_2S^+$ [M + H]$^+$, 656.2255; Found, 656.2258; HPLC purity, 96.0%.

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(p-tolyl)-1,2,3,4-tetrahydrobenzofuro[2,3-c]pyri-din-6-yl)-N-(3,3-difluoro-1-methylpiperidin-*4-yl*)pyridazine-3-carboxamide (**36**).* This compound was prepared by following the general procedure E (white solid, 35% yield). ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.46 (d, J = 9.5 Hz, 1H), 8.36 (d, J = 8.8 Hz, 1H), 8.31−8.11 (m, 4H), 7.80 (d, J = 7.7 Hz, 2H), 7.72−7.59 (m, 2H), 7.35 (d, J = 7.8 Hz, 2H), 6.88−6.68 (m, 1H), 6.52 (d, J = 8.5 Hz, 1H), 4.91 (d, J = 39.9 Hz, 2H), 4.73 (s, 2H), 4.54−4.43 (m, 1H), 4.05 (d, J = 73.4 Hz, 2H), 3.22−3.14 (m, 1H), 2.97−2.86 (m, 3H), 2.45 (s, 3H), 2.43−2.37 (m, 4H), 2.26 (t, J = 11.7 Hz, 1H), 2.13−2.08 (m, 1H), 1.99−1.91 (m, 1H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.7, 162.9, 161.1, 159.3, 153.8, 151.8, 150.3, 149.7, 141.0, 138.4, 136.1, 132.7, 130.8, 129.6 (2C), 129.5, 128.7 (2C), 126.6, 126.5, 125.0, 123.0 (d, J = 56.3 Hz), 121.6, 118.5 (t, J = 245.6 Hz), 117.1 (d, J = 44.1 Hz), 113.7 (d, J = 65.9 Hz), 112.4, 108.8, 60.0 (d, J = 23.9 Hz), 53.6, 50.1 (t, J = 20.6 Hz), 45.5, 43.9 (d, J = 49.4 Hz), 40.8 (d, J = 225.5 Hz), 29.5 (d, J = 5.8 Hz), 21.4, 21.3 (d, J = 256.5 Hz). HR-MS (ESI) Calcd. for $C_{37}H_{36}O_3N_7F_2^+$ [M + H]$^+$, 664.2848; Found, 664.2845; HPLC purity, 97.0%.

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(2-fluoro-4-methylphenyl)-1,2,3,4-tetrahydrobe-n-zofuro[2,3-c]pyridin-6-yl)-N-(3,3-difluoro-1-methylpiperidin-4-yl)pyridazine-3-carboxamide (**37**).* This compound was prepared by following the general procedure E (white solid, 42% yield). ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.45 (d, J = 9.3 Hz, 1H), 8.39−8.30 (m, 2H), 8.22 (s, 1H), 8.12 (d, J = 8.9 Hz, 1H), 8.10−7.99 (m, 1H), 7.72−7.59 (m, 2H), 7.54 (s, 1H), 7.11 (d, J = 8.1 Hz, 1H), 7.07 (d, J = 11.6 Hz, 1H), 6.87−6.67 (m, 1H), 6.51 (s, 1H), 4.98−4.65 (m, 4H), 4.53−4.42 (m, 1H), 4.03 (d, J = 72.1 Hz, 2H), 3.18 (s, 1H), 2.98−2.84 (m, 3H), 2.44 (s, 3H), 2.43−2.36 (m, 4H), 2.30−2.21

(m, 1H), 2.14−2.06 (m, 1H), 1.98−1.90 (m, 1H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.6, 162.9, 161.0, 159.8 (t, J = 248.8 Hz), 159.3, 154.1, 151.9, 150.3, 149.6, 141.1, 140.9, 136.0, 131.3, 130.6 (d, J = 35.9 Hz), 129.2, 126.6, 125.2, 125.0, 124.7 (d, J = 38.1 Hz), 121.6, 121.1, 120.3, 118.5 (t, J = 245.6 Hz), 117.9 (d, J = 41.9 Hz), 116.8 (d, J = 22.3 Hz), 113.7 (d, J = 59.0 Hz), 112.4, 108.7, 60.0 (t, J = 26.1 Hz), 53.6, 50.1 (t, J = 20.0 Hz), 45.5, 43.9 (d, J = 43.8 Hz), 40.8 (d, J = 230.8 Hz), 29.5, 21.4, 21.3 (d, J = 250.3 Hz). HR-MS (ESI) Calcd. for $C_{37}H_{35}O_3N_7F_3^+$ $[M + H]^+$, 682.2753; Found, 682.2755; HPLC purity, 98.4%.

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-mesityl-1,2,3,4-tetrahydrobenzofuro[2,3-c]pyrid-in-6-yl)-N-(3,3-difluoro-1-methylpiperidin-4-yl)pyridazine-3-carboxamide (**38**).* This compound was prepared by following the general procedure E (white solid, 38% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, J = 9.5 Hz, 1H), 8.39 (s, 1H), 8.33 (d, J = 8.8 Hz, 1H), 8.21 (s, 1H), 8.07 (d, J = 8.8 Hz, 1H), 7.85−7.54 (m, 3H), 7.02 (s, 2H), 6.87−6.61 (m, 1H), 6.51 (d, J = 8.6 Hz, 1H), 4.92−4.69 (m, 4H), 4.54−4.39 (m, 1H), 4.15−3.91 (m, 2H), 3.24−3.09 (m, 1H), 2.99−2.83 (m, 3H), 2.48−2.40 (m, 1H), 2.39 (s, 3H), 2.37 (s, 3H), 2.26 (t, J = 11.9 Hz, 1H), 2.12−2.07 (m, 1H), 2.04 (s, 6H), 2.00−1.90 (m, 1H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.6, 162.9, 161.2, 159.3, 154.4, 151.9, 150.3, 149.6, 140.9, 138.1, 136.8 (2C), 136.0 (d, J = 33.8 Hz), 132.1, 130.6 (d, J = 32.6 Hz), 129.0, 128.5 (2C), 126.5, 125.6, 125.0, 124.8, 121.6, 118.4 (t, J = 245.7 Hz) 117.4 (d, J = 49.9 Hz), 113.7 (d, J = 53.4 Hz), 112.4, 108.8, 60.0 (dd, J = 28.9, 23.2 Hz), 53.6, 50.1 (t, J = 20.4 Hz), 45.5, 43.9 (d, J = 48.6 Hz), 40.7 (d, J = 242.6 Hz), 29.4 (d, J = 6.2 Hz), 22.1, 21.3, 20.7 (2C). HR-MS (ESI) Calcd. for $C_{39}H_{40}O_3N_7F_2^+$ $[M + H]^+$, 692.3161; Found, 692.3165; HPLC purity, 95.2%.

*Tert-butyl 8-(4-fluorophenyl)-6-(5-(methoxycarbonyl)pyridin-2-yl)-3,4-dihydrobenzofuro[2,3-c]pyridine-2(1H)-carboxylate (**A67**).* Replacing **K8** with methyl 6-bromonicotinate. This compound was prepared by following the general procedure F (white solid, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.36 (d, J = 8.3 Hz, 1H), 8.13 (s, 1H), 8.07 (s, 1H), 7.92−7.84 (m, 3H), 7.24−7.18 (m, 2H), 4.64 (s, 2H), 3.98 (s, 3H), 3.80 (s, 2H), 2.82 (s, 2H), 1.51 (s, 9H).

*Methyl (E)-6-(2-(3-(6-aminopyridin-3-yl)acryloyl)-8-(4-fluorophenyl)-1,2,3,4-tetrahydrobenz-ofuro[2,3-c]pyridin-6-yl) nicotinate (**A68**).* This compound was prepared by following the general procedure E (white solid, 62% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.36 (d, J = 8.4 Hz, 1H), 8.23 (s, 1H), 8.14 (s, 1H), 8.08 (s, 1H), 7.94−7.82 (m, 3H), 7.71−7.58 (m, 2H), 7.25−7.16 (m, 2H), 6.87−6.68 (m, 1H), 6.52 (d, J = 8.5 Hz, 1H), 4.99−4.73 (m, 4H), 4.13−3.90 (m, 5H), 2.91 (s, 2H).

*(E)-6-(2-(3-(6-Aminopyridin-3-yl)acryloyl)-8-(4-fluorophenyl)-1,2,3,4-tetrahydrobenzofu-ro[2,3-c]pyridin-6-yl)-N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)nicotinamide (**39**).* To a solution of **A68** (38 mg, 0.07 mmol) in a mixed solvent (0.6 mL THF, 0.2 mL H_2O), LiOH H_2O (5 mg, 0.12 mmol) was added. The mixture was stirred at room temperature overnight, and acidified with 4 mol/L hydrochloric methanol solution. After evaporation of the solvent, the crude product **A69** was obtained and dissolved in DMF (1 mL). 2-(3-(But-3-yn-1-yl)-3*H*-diazirin-3-yl)ethan-1-amine (14 mg, 0.10 mmol), HATU (53 mg, 0.14 mmol) and Et_3N (48 μL, 0.35 mmol) were added. The mixture was stirred at room temperature for 3 h, diluted with ethyl acetate, and washed with brine. The organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified using a silica gel column to afford **39** (white solid, 20 mg, 44% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (d, J = 2.2 Hz, 1H), 8.21 (s, 1H), 8.17 (dd, J = 8.3, 2.3 Hz, 1H), 8.03 (s, 1H), 8.00 (s, 1H), 7.87−7.78 (m, 3H), 7.69−7.57 (m, 2H), 7.18 (t, J = 8.6 Hz, 2H), 6.86−6.68 (m, 2H), 6.50 (d, J = 8.6 Hz, 1H), 4.84 (s, 4H), 3.98 (d, J = 37.5 Hz, 2H), 3.37 (q, J = 6.4 Hz, 2H), 2.84 (s, 2H), 2.08−2.00 (m, 3H), 1.86 (t, J = 6.6 Hz, 2H), 1.70 (t, J = 7.1 Hz, 2H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.7, 165.8, 162.7 (d, J = 247.9 Hz), 159.9, 159.4, 153.0, 150.6 (d, J = 232.8 Hz), 149.6, 148.10, 141.0, 136.1, 136.0 (d, J = 15.8 Hz), 134.1 (d, J = 33.6 Hz), 132.0, 130.5, 130.4, 129.1, 128.1, 124.7, 123.0 (d, J = 54.6 Hz), 121.5, 120.1, 117.0 (d, J = 61.1 Hz), 115.8, 115.7, 114.0 (d, J = 33.6 Hz), 112.9 (d, J = 202.7 Hz), 108.7, 82.9, 69.7, 43.9 (d, J = 40.2 Hz), 40.8 (d, J = 224.6 Hz), 35.2, 32.5, 32.2, 27.1, 21.4 (d, J = 249.9 Hz), 13.3. HR-MS (ESI) Calcd. for $C_{38}H_{33}O_3N_7F^+$ $[M + H]^+$, 654.2629; Found, 654.2619; HPLC purity, 98.0%.

4.2. Biology

4.2.1. Cell lines and cell culture

All cell lines were cultured in a humidified atmosphere in 5% CO_2 at 37 °C. MIA PaCa-2, PANC-1, BxPC-3 cells were purchased from American type culture collection (ATCC, Manassas, VA, USA). AsPC-1, HPAF-II, hTERT-HPNE cells were purchased from Cobioer Biosciences Co., Ltd. (Nanjing, China). Pan02 cells were purchased from Shanghai Zishi (Shanghai, China). CFPAC-1 cells were purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China). U-2 OS cells were obtained from National Collection of Authenticated Cell Cultures.

MIA PaCa-2 cells were cultured in DMEM medium with 10% fetal bovine serum, 2.5% horse serum, and 1% penicillin-streptomycin. AsPC-1 cells and BxPC-3 cells were cultured in RPMI-1640 medium with 10% fetal bovine serum and 1% penicillin-streptomycin. Pan02 cells and PANC-1 cells were cultured in DMEM medium with 10% fetal bovine serum and 1% penicillin-streptomycin. HPAF-II cells were cultured in MEM medium with 10% fetal bovine serum, 1% NEAA, 1% NaP, and 1% penicillin-streptomycin. CFPAC-1 cells were cultured in IMDM medium with 10% fetal bovine serum, and 1% penicillin-streptomycin. hTERT-HPNE cells were cultured in DMEM medium with 25% M3 medium, 5% fetal bovine serum, and 10 ng/mL EGF. U-2 OS cells were cultured in McCoy's 5a medium with 10% fetal bovine serum and 1% penicillin-streptomycin.

4.2.2. Cell proliferation assay

Cells were seeded in 96-well plates at a density of 1500 cells per well overnight and subsequently treated compounds at different concentrations. After 72 h of incubation, the anti-proliferation effects of compounds were evaluated through Cell Counting Kit-8 (Life iLab, #D3100L4053, Shanghai, China). The OD values were detected using the SPECTRAmax PLUS (Molecular Devices). The IC_{50} Value was calculated *via* the software of the instrument. The experiments were repeated at least three times.

4.2.3. Western blot analysis

MIA PaCa-2 cells were seeded in 6-well plates overnight and treated with the indicated dose of tested compounds for 72 h. The treated cells were lysed with 2% (*w*/*v*) SDS, 1% glycerol, 0.01% Bromophenol blue, and 62.5 mmol/L Tris-HCl (pH: 6.8) and denatured at 100 °C for 20 min. The lysates were loaded onto 10% SDS-PAGE (Epizyme, PG004, Shanghai, China) and transferred to nitrocellulose membranes. Membranes were blocked with 5% skim milk-TBST for 1 h at room temperature and blotted with

primary antibodies. Primary antibodies used in this article include: β-Catenin (D10A8) XP® Rabbit mAb (Cell Signaling Technology, 8480s, Cambridge, MA, USA), phospho-β-catenin (Ser675) (Cell Signaling Technology, 4176s), PAK4 (Proteintech, 14685-1-AP, Rosemont, IL, USA), PAK4(Ser474) (Biovision, A1973-100, Cambridge, UK), Cyclin D1 (92G2) Rabbit mAb (Cell Signaling Technology, 2978S), c-Myc (E5Q6W) Rabbit mAb (Cell Signaling Technology, 18583S), Bim (C34C5) Rabbit mAb (Cell Signaling Technology, 2933S), Bcl-xL (54H6) Rabbit mAb (Cell Signaling Technology, 2764S), beta Actin Monoclonal Antibody (2D4H5) (Proteintech, 66009-1-IG).

4.2.4. Photoaffinity labeling assay

MIA PaCa-2 cells were washed with cold PBS and resuspended in protein lysis buffer (NP40 lysis buffer, Beyotime, P0013F, Shanghai, China). The cells were sonicated for 20 s and centrifuged for 30 min at 15,000×*g* at 4 °C. The supernatant was collected and the protein concentration was adjusted to 1 mg/mL by using the BCA Protein Quantification Kit (Yeasen Biotechnolog Co., Ltd., 20201ES76, Shanghai, China). The samples were treated with different compounds on a shaker for 60 min at room temperature. Then the samples were irradiated under UV light (365 nm) for 30 min on ice. The following reagents were added sequentially for the click reaction: TCEP-HCl (50 mmol/L), $CuSO_4$ (50 mmol/L), TBTA (1.7 mmol/L), and Biotin-N_3 (1.25 mmol/L). After the click reaction, the samples were rotated for 2 h at room temperature. The proteins were then precipitated by cold acetone for 2 h and washed by methanol. The samples were resuspended with pull-down buffer (0.2% SDS/PBS) and the protein concentrations were normalized. Before streptavidin beads (Thermo Fisher Scientific, Pierce™ Streptavidin Magnetic Beads, 88,816, Waltham, MA, USA) were added, 50 μL of each sample was left behind as the input for the Western blot. The streptavidin beads were added to samples, and the mixtures were incubated for 4 h with rotation under room temperature. The pull-down proteins were analyzed by Western blotting.

4.2.5. Surface plasma resonance (SPR) assay

The experiment was conducted with Biacore 8K instrument (GE Healthcare, Uppsala, Sweden). The recombinant PAK4 kinase domain (300−591) was coupled on CM5 chip (Cytiva, Washington, D.C., USA). The test compound **25** (HCl salt) was firstly dissolved in H_2O as 125 μmol/L stock, and then diluted in PBST buffer as a mobile phase flowing through PAK4 coupled chip. The dissociation constant was calculated in kinetic analysis mode using Biacore evaluation software. The final figure was displayed using GraphPad (version 8.0).

4.2.6. SPR A-B-A competition assay

Using the same PAK4 kinase domain immobilized CM5 chip, SPR A-B-A competition assay was performed following "ABA" method in the same instrument. Compounds **1** and **25** were firstly dissolved in DMSO as 20 mmol/L stocks, and then diluted in a PBST running buffer containing 5% DMSO. First, Compound **1** (5 μmol/L) was injected over the surface of the chip for 180 s. Then the same concentration of compound 1 was injected with different concentration gradient **25** (2.5−80 μmol/L) for 180 s. The final figure was displayed using GraphPad (version 8.0).

4.2.7. Apoptosis assay

Cell apoptosis was evaluated using the Annexin V-FITC/PI Apoptosis Detection Kit (Vazyme, Nanjing, China). MIA PaCa-2 cells were seeded in 6-well plates overnight at a density of 5×10^4/well and treated with different concentrations of compound **13** and **4** for 72 h. After the incubation, the treated cells were trypsinized and stained with Annexin V-FITC and PI for 10 min. The apoptotic cells were analyzed using a CytoFLEX (Beckman Coulter, Brea, CA, USA).

4.2.8. In vivo study

Animal experiments were conducted under the guidance of the Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica (approval number: 2023-01-DJ-73). To examine the *in vivo* antitumor activity of **13**, 5×10^6 MIA PaCa-2 cells were implanted subcutaneously into the right flanks of nude mice. After the tumor volume reached 50−100 mm^3, the mice were randomly assigned into the Control group ($n = 8$) and treatment groups ($n = 8$). Compounds **13** and **4** were formulated in 5% DMSO, 60% PEG400, and 35% saline. The control group was given vehicle (5% DMSO, 60% PEG400, and 35% saline) only, and the treatment groups were treated with compounds **13** and **4** (100 mg/kg, q.d.) intraperitoneally. Tumor volumes were examined three times per week by measuring two perpendicular dimensions using a caliper.

Pan02 cells were injected subcutaneously into the right flanks of syngeneic C57BL/6 mice at a density of 5×10^6 per mouse. After the tumor volume reached 50−100 mm^3, the mice were randomly assigned into the Control group ($n = 8$) and treatment groups ($n = 8$). The control group was given vehicle (5% DMSO, 60% PEG400, and 35% saline) only, and the treatment groups were treated with compounds **13** and **4** (100 mg/kg) intraperitoneally three times per five days. Body weights and tumor volumes were measured twice per week. The tumor volume was calculated as Eq. (1):

$$\text{Tumor volume} = \left(\text{Length} \times \text{Width}^2\right) / 2 \quad (1)$$

4.2.9. Immunohistochemistry

Tumor-bearing mice were sacrificed after treatment. The tumor tissue was fixed *via* 4% paraformaldehyde for over 24 h. Then, the tumor samples were transferred to 70% ethanol and embedded in paraffin wax. Immunohistochemistry experiments were conducted by Shanghai Zuocheng Biotechnology. Images were analyzed by NDP.scan3.2.15. β-Catenin (D10A8) XP® Rabbit mAb (Cell Signaling Technology, 8480s) was used in this study. Expression level of β-Catenin was assessed using the multiplicative score method.

4.2.10. CyTOF

Tumor tissue was dissociated enzymatically (Miltenyi Biotec, 130-096-730, Cologne, Germany) to obtain a single-cell suspension. The single-cell suspension was filtered and centrifuged at 300×*g*. The $CD45^+$ cells were isolated through CD45 MicroBeads (Miltenyi Biotec, 130-110-618). Surface markers were stained by the antibody cocktail for 30 min at 4 °C. Cells were resuspended with Maxpar Cell Staining Buffer (CSB, Fluidigm, San Francisco, CA, USA) and incubated with 0.5 μmol/L cisplatin (Fluidigm) for 5 min. After centrifugation at 500×*g* for 6 min, the cells were stained with 500 μL of 50 nmol/L Ir for 1 h at 4 °C. Add 1.5 mL CSB to the samples and gently mix the cells after centrifugation at 500×*g* for 6 min. The samples were then loaded onto the Helios sample loader for data acquisition. Data were collected with Cytobank 7.3.0 (Beckman Coulter) and analyzed using FlowJo (V.10.0).

4.2.11. Statistical analysis

Statistical data are expressed as means ± standard deviation (SD). Comparisons between different groups were performed with Student's *t*-test or variance (ANOVA) as appropriate using GraphPad Prism (ver. 8.0). Values of $P < 0.05$ were considered statistically significant ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

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Author contributions

Yang Li: Investigation, Conceptualization, Methodology, Data curation, Formal analysis, Writing − original draft, Writing − review and editing. Yan Fang: Investigation, Conceptualization, Methodology, Data curation, Formal analysis, Writing − original draft, Writing − review and editing. Xiaoyu Chen: Methodology, Data curation, Formal analysis, Writing − review and editing. Linjiang Tong, Methodology, Data curation. Fang Feng, Methodology, Data curation. Qianqian Zhou, Methodology, Data curation. Shulun Chen, Methodology, Data curation. Jian Ding: Supervision, Resources. Hua Xie: Supervision, Resource, Writing − review and editing. Ao Zhang: Supervision, Resource, Writing − review and editing.

Conflicts of interest

The authors declare no competing financial interest.

Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2024.10.002.

References

1. Manser E, Leung T, Salihuddin H, Zhao ZS, Lim L. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. *Nature* 1994;**367**:40−6.
2. Rudolph J, Crawford JJ, Hoeflich KP, Wang W. Inhibitors of p21-activated kinases (PAKs). *J Med Chem* 2015;**58**:111−29.
3. Wang J, Zhu Y, Chen J, Yang Y, Zhu L, Zhao J, et al. Identification of a novel PAK1 inhibitor to treat pancreatic cancer. *Acta Pharm Sin B* 2020;**10**:603−14.
4. Aboukameel A, Muqbil I, Senapedis W, Baloglu E, Landesman Y, Shacham S, et al. Novel p21-activated kinase 4 (PAK4) allosteric modulators overcome drug resistance and stemness in pancreatic ductal adenocarcinoma. *Mol Cancer Ther* 2017;**16**:76−87.
5. Siu MK, Chan HY, Kong DS, Wong ES, Wong OG, Ngan HY, et al. p21-Activated kinase 4 regulates ovarian cancer cell proliferation, migration, and invasion and contributes to poor prognosis in patients. *Proc Natl Acad Sci U S A* 2010;**107**:18622−7.
6. Ong CC, Gierke S, Pitt C, Sagolla M, Cheng CK, Zhou W, et al. Small molecule inhibition of group I p21-activated kinases in breast cancer induces apoptosis and potentiates the activity of microtubule stabilizing agents. *Breast Cancer Res* 2015;**17**:59.
7. Rane C, Senapedis W, Baloglu E, Landesman Y, Crochiere M, Das-Gupta S, et al. A novel orally bioavailable compound KPT-9274 inhibits PAK4, and blocks triple negative breast cancer tumor growth. *Sci Rep* 2017;**7**:42555.
8. Aburatani T, Inokuchi M, Takagi Y, Ishikawa T, Okuno K, Gokita K, et al. High expression of p21-activated kinase 5 protein is associated with poor survival in gastric cancer. *Oncol Lett* 2017;**14**:404−10.
9. Cao F, Yin LX. PAK1 promotes proliferation, migration and invasion of hepatocellular carcinoma by facilitating EMT *via* directly up-regulating Snail. *Genomics* 2020;**112**:694−702.
10. Wang M, Gao Q, Chen Y, Li Z, Yue L, Cao Y. PAK4, a target of miR-9-5p, promotes cell proliferation and inhibits apoptosis in colorectal cancer. *Cell Mol Biol Lett* 2019;**24**:58.
11. Rudolph J, Murray LJ, Ndubaku CO, O'Brien T, Blackwood E, Wang W, et al. Chemically diverse group I p21-activated kinase (PAK) inhibitors impart acute cardiovascular toxicity with a narrow therapeutic window. *J Med Chem* 2016;**59**:5520−41.
12. Jiang YY, Lin DC, Mayakonda A, Hazawa M, Ding LW, Chien WW, et al. Targeting super-enhancer-associated oncogenes in oesophageal squamous cell carcinoma. *Gut* 2017;**66**:1358−68.
13. Chandrashekar DS, Chakravarthi BVSK, Robinson AD, Anderson JC, Agarwal S, Balasubramanya SAH, et al. Therapeutically actionable PAK4 is amplified, overexpressed, and involved in bladder cancer progression. *Oncogene* 2020;**39**:4077−91.
14. Mohammad RM, Li Y, Muqbil I, Aboukameel A, Senapedis W, Baloglu E, et al. Targeting Rho GTPase effector p21 activated kinase 4 (PAK4) suppresses p-Bad-microRNA drug resistance axis leading to inhibition of pancreatic ductal adenocarcinoma proliferation. *Small GTPases* 2019;**10**:367−77.
15. Xu L, Faruqu FN, Lim YM, Lim KY, Liam-Or R, Walters AA, et al. Exosome-mediated RNAi of PAK4 prolongs survival of pancreatic cancer mouse model after loco-regional treatment. *Biomaterials* 2021;**264**:120369.
16. Ma Y, Nikfarjam M, He H. The trilogy of p21 activated kinase, autophagy and immune evasion in pancreatic ductal adenocarcinoma. *Cancer Lett* 2022;**548**:215868.
17. Khan HY, Nagasaka M, Aboukameel A, Alkhalili O, Uddin MH, Bannoura SF, et al. Anticancer efficacy of KRASG12C inhibitors is potentiated by PAK4 inhibitor KPT9274 in preclinical models of KRASG12C-mutant pancreatic and lung cancers. *Mol Cancer Ther* 2023;**22**:1422−33.
18. Abril-Rodriguez G, Torrejon DY, Liu W, Zaretsky JM, Nowicki TS, Tsoi J, et al. PAK4 inhibition improves PD-1 blockade immunotherapy. *Nat Cancer* 2020;**1**:46−58.
19. Ma W, Wang Y, Zhang R, Yang F, Zhang D, Huang M, et al. Targeting PAK4 to reprogram the vascular microenvironment and improve CAR-T immunotherapy for glioblastoma. *Nat Cancer* 2021;**2**:83−97.
20. Su S, You S, Wang Y, Tamukong P, Quist MJ, Grasso CS, et al. PAK4 inhibition improves PD1 blockade immunotherapy in prostate cancer by increasing immune infiltration. *Cancer Lett* 2023;**555**:216034.
21. Murray BW, Guo C, Piraino J, Westwick JK, Zhang C, Lamerdin J, et al. Small-molecule p21-activated kinase inhibitor PF-3758309 is a potent inhibitor of oncogenic signaling and tumor growth. *Proc Natl Acad Sci U S A* 2010;**107**:9446−51.
22. Staben ST, Feng JA, Lyle K, Belvin M, Boggs J, Burch JD, et al. Back pocket flexibility provides group II p21-activated kinase (PAK) selectivity for type I 1/2 kinase inhibitors. *J Med Chem* 2014;**57**:1033−45.
23. Hao C, Zhao F, Song H, Guo J, Li X, Jiang X, et al. Structure-based design of 6-chloro-4-aminoquinazoline-2-carboxamide derivatives as potent and selective p21-activated kinase 4 (PAK4) inhibitors. *J Med Chem* 2018;**61**:265−85.

24. Wang H, Song P, Gao Y, Shen L, Xu H, Wang J, et al. Drug discovery targeting p21-activated kinase 4 (PAK4): a patent review. *Expert Opin Ther Pat* 2021;**31**:977−87.
25. Li Y, Lu Q, Xie C, Yu Y, Zhang A. Recent advances on development of p21-activated kinase 4 inhibitors as anti-tumor agents. *Front Pharmacol* 2022;**13**:956220.
26. Abu Aboud O, Chen CH, Senapedis W, Baloglu E, Argueta C, Weiss RH. Dual and specific inhibition of NAMPT and PAK4 by KPT-9274 decreases kidney cancer growth. *Mol Cancer Ther* 2016;**15**:2119−29.
27. Fulciniti M, Martinez-Lopez J, Senapedis W, Oliva S, Lakshmi Bandi R, Amodio N, et al. Functional role and therapeutic targeting of p21-activated kinase 4 in multiple myeloma. *Blood* 2017;**129**: 2233−45.
28. Mitchell SR, Larkin K, Grieselhuber NR, Lai TH, Cannon M, Orwick S, et al. Selective targeting of NAMPT by KPT-9274 in acute myeloid leukemia. *Blood Adv* 2019;**3**:242−55.
29. Samant C, Kale R, Bokare A, Verma M, Pai KSR, Bhonde M. PAK4 inhibition significantly potentiates Gemcitabine activity in PDAC cells *via* inhibition of WNT/β-catenin, p-ERK/MAPK and p-AKT/PI3K pathways. *Biochem Biophys Rep* 2023;**35**:101544.
30. Raineri F, Bourgoin-Voillard S, Cossutta M, Habert D, Ponzo M, Houppe C, et al. Nucleolin targeting by N6L inhibits WNT/β-catenin pathway activation in pancreatic ductal adenocarcinoma. *Cancers* 2021;**13**:2986.
31. Zhang J, Liu J, Li H, Wang J. β-Catenin signaling pathway regulates cisplatin resistance in lung adenocarcinoma cells by upregulating Bcl-xl. *Mol Med Rep* 2016;**13**:2543−51.
32. Kim DY, Ryu YS, Lee ES, Koh DI, Moon JH, Jung SA, et al. DGG-300273, a novel WNT/β-catenin inhibitor, induces apoptotic cell death by activating ROS-Bim signaling in a WNT-dependent manner in colon cancer cells. *Invest New Drug* 2022;**41**:105−14.
33. Gajewski TF, Fessler J. PAK4 as a cancer immune-evasion target. *Nat Cancer* 2020;**1**:18−9.
34. Liu R, Qi J, Wang H, Fan L, Zhang P, Yu J, et al. Transformation of a dopamine D2 receptor agonist to partial agonists as novel antipsychotic agents. *J Med Chem* 2023;**66**:6274−87.
35. Gharat LA, Gopalan B, Khairatkar-Joshi N, inventors; Glenmark Pharmaceuticals S.A., assignee. *Novel heterocyclic compounds, their preparation, and their use as PDE4 inhibitors for treating inflammatory and allergic disorders*. June 22, 2006. WO2006064355A2.