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Discovery of a PROTAC targeting ALK with *in vivo* activity

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ABSTRACT

Anaplastic lymphoma kinase (ALK) was involved in the development of various cancer types. Although several ALK inhibitors have been advanced to clinical trials, the emergence of drug resistance has limited the clinical application of them. To overcome the drug resistance, proteolysis targeting chimeras (PROTACs) could be an alternative strategy. In this study, a series of ALK degraders were designed and synthesized. The degraders were developed through the conjugation of LDK378 and CRBN E3 ubiquitin ligase ligands. Among all the molecules, compound **B3** showed potent selective inhibitory activity to ALK and can decrease the cellular levels of ALK fusion proteins in a concentration- and time-dependent manner in H3122 cell line. Meanwhile, **B3** showed improved anticancer activity *in vitro* comparing with LDK378 and the antiproliferative activity to xenograft tumor model was acceptable. All the results demonstrated that ALK degrader **B3** with *in vitro* and *in vivo* anti-cancer activities was valuable for further investigation.

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1. Introduction

Lung cancer has become the leading cause of cancer deaths in the urban population in China, among them, non-small cell lung cancer (NSCLC) accounts for 80% [1]. 75% of the patients were diagnosed in the middle or advanced stage, and the 5-year survival rate is low [2]. ALK was first discovered in a subtype of anaplastic large cell lymphoma (ALCL) [3], after that, several other ALK fusion partners have been described in multiple malignancies [4]. It has been proved that ALK is a strong driver gene enhancing the development of various cancers [5]. The incidence of ALK gene rearrangement in NSCLC is 3–7%, most of which were fused with EML4 [2]. It was reported that the inhibition of EML4-ALK can lead to the reduction of cancer loading [6]. Although the incidence of ALK rearrangement in NSCLC is significantly lower than that of ALCL or inflammatory myofibroblastic tumor (IMT) [7], given to the significant morbidity of lung cancer worldwide, NSCLC patients constitute the largest group of ALK rearrangement.

Crizotinib and ceritinib (LDK378) are potent ALK inhibitors in clinical trials [8,9] (Fig. 1). NCCN recommended crizotinib as first-line treatment for NSCLC patients with ALK rearrangements, and ceritinib for the treatment of recurrence or intolerance after treated with crizotinib. However, drug resistance of ceritinib has been observed in clinical trials [10], suggesting that the subsequent development of ALK inhibitors is needed to overcome drug resistance in clinical trials.

PROTAC method is a chemical knockdown strategy in which a heterobifunctional molecule recruits a specific protein target to an E3 ubiquitin ligase, resulting in the target's ubiquitination and degradation [11]. PROTACs behave catalytically in their ability to induce the ubiquitination of proteins, which is a key advantage over small molecule inhibitors [12]. To date, PROTAC method has been successfully applied in the degradation of variety of targets such as BRD4 [13–16], RIPK2 [11], Akt [17], HDAC6 [18–20], BCR-ABL [21], BCL-X_L [22,23], Smad3 [24], STAT3 [25,26], CDK9 [27], Estrogen Receptor α [28], TANK-Binding Kinase 1 [29], Sirt2 [30] and Tau [31]. Among the targets of PROTACs, kinases attracted much attention for the predominant regulatory roles in nearly every aspect of cell biology [32].

In recent years, degraders of ALK also have been reported (Fig. 2): Gray et al. reported TL13-112 can induce the degradation of

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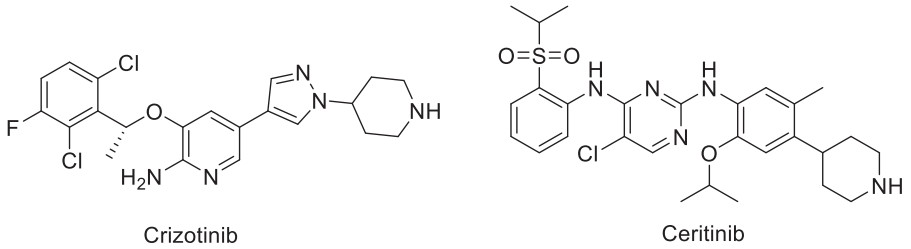


Fig. 1. Chemical structures of Crizotinib and Ceritinib.

ALK at half-maximal degrading concentration (DC_{50}) value of 10 nM [33]; Hwang's group reported TD-004 can significantly reduce the amount of ALK protein over 90% in SU-DHL-1 at 1 μM [34]; Two potent ALK degraders (5 and 6) were reported by Jin's group [35]; Jiang's group reported SIAIS117 with DC_{50} of 51 nM in H2228 cell line [36]. It is noteworthy that ALK degraders in Fig. 2 did not share much similarity in linker part, the phenomenon may suggest that the optional linker may be different varying on different ALK inhibitors and E3 ligase ligands utilized.

As far as we learned, most of the reported PROTAC molecules lack of oral bioactivity, which limited the clinical application [37,38]. In this study, we describe our efforts in the discovery of potent ALK degraders utilizing CRBN E3 ligase and resulted in a promising compound with *in vitro* and *in vivo* anticancer activities.

2. Results and discussion

2.1. Molecular design

In this work, a series of PROTACs targeting ALK were designed and synthesized. LDK378 was selected as the part of ALK inhibitor because of the success of previously reported works. Among the reported ALK degraders, only Hwang's group reported TD004 with *in vivo* anticancer activities [34]. TD004 was designed by linking Von Hippel-Lindau (VHL) E3 ligase ligand with LDK378. However, we suggested it is possible to get orally active molecules by utilizing CRBN ligands to recruit the E3 ligase and careful optimization of linker part.

To get novel PROTACs with *in vivo* bioactivities, different linkers and CRBN E3 ligase ligands were utilized. Different type and length of linkers were applied to discuss the structure activity relationship (SAR). Based on the x-ray co-crystal structure of ALK kinase domain and LDK378 (pdb: 4MKC) [39], nitrogen atom of piperidine is solvent exposed and therefore can serve as proper attachment site for a linker, which is consistent with the reported works.

2.2. Chemistry

The synthetic route of target compounds was summarized in Scheme 1. The compounds in this work shared similar synthetic methods: the preparation of target molecules was started from LDK378, michael addition reaction of methyl acrylate and the subsequently hydrolysis under basic conditions provided intermediate **K2**. After that, the intermediate product was connected with CRBN ligands through different linkers to generate the corresponding compounds.

Reagents and conditions: (a): Chloroacetyl chloride, THF, reflux, 5h, yield 70%; (b) Acryloyl chloride, THF, reflux, 5h, yield 63%; (c) K_2CO_3, *tert*-Butyl bromoacetate, DMF, rt, 15h; CF_3COOH, 1h, yield 70%; (d) DIPEA, NPM, 90 °C, 10 h, yield 52–68%; (e) K_2CO_3, THF, 66 °C, 5h, yield 61–72%; (f) Triethylamine, THF, 90 °C, 12 h, yield 56–73%; (g) HATU, DIPEA, DMF, rt, yield 35–60%; (h) Methyl acrylate, Triethylamine, MeOH, rt, 8h; NaOH, MeOH/H2O,10h, yield 95%; (i) HATU, DIPEA, DMF, rt, 15h, yield 21–51%.

2.3. In vitro bioassays

The binding affinities of the compounds to ALK were evaluated through mobility shift assay with ATP at concentration of Km

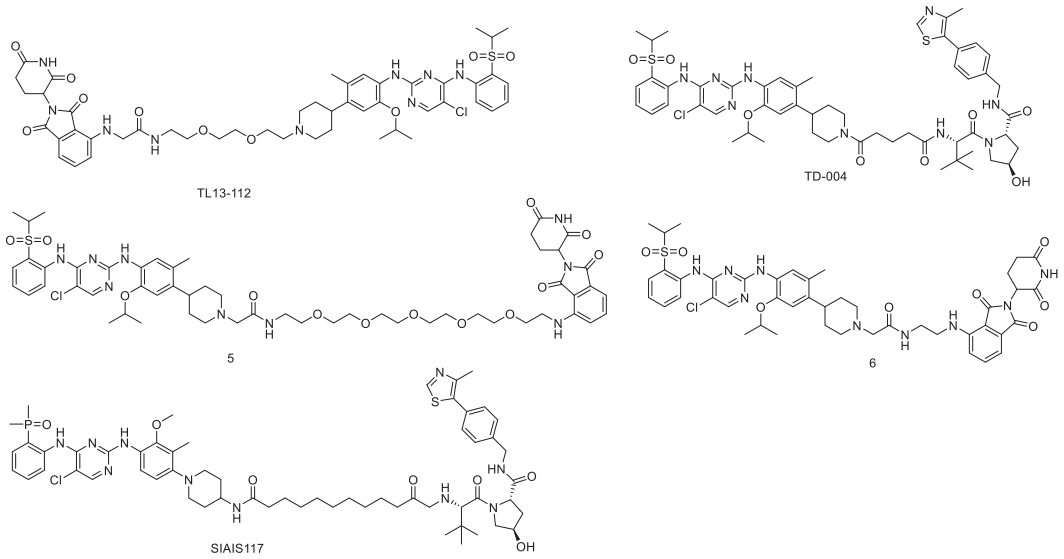
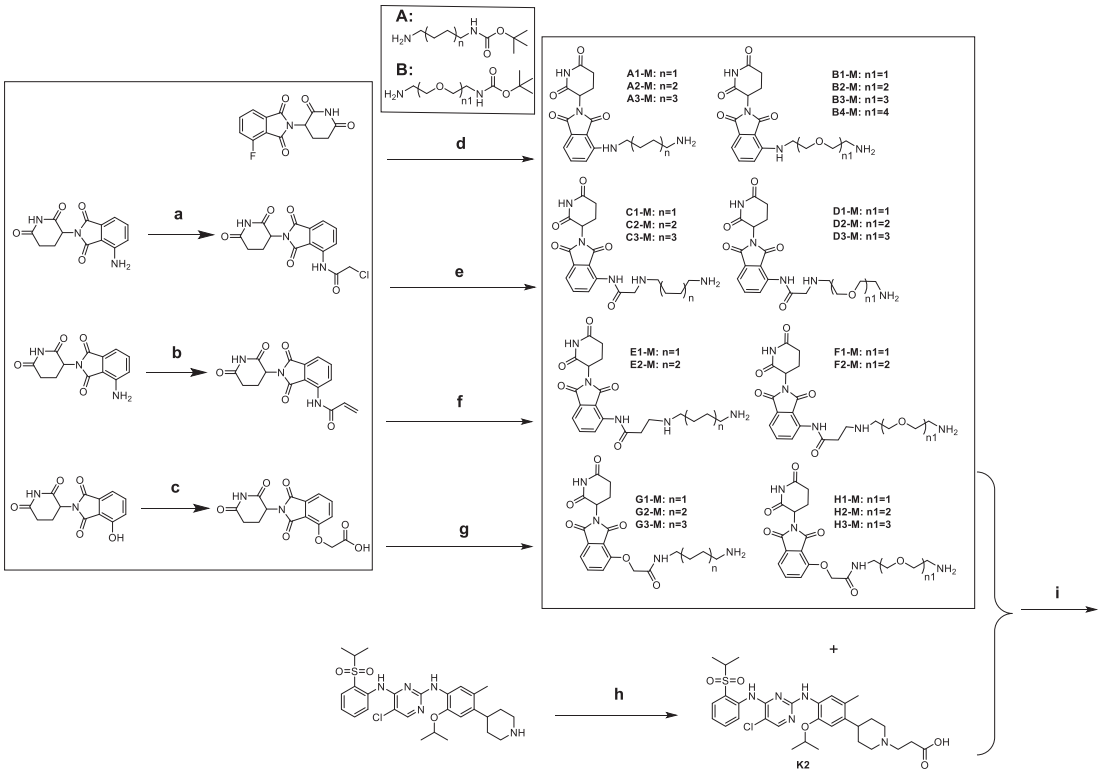


Fig. 2. Chemical structures of the previously reported ALK degraders.



Scheme 1. Syntheses of ALK PROTACs.

(30.3 μM). The IC_{50} values were summarized in Table 1. Most of the degraders exhibited inhibitory activities at low IC_{50} values demonstrating that the linkers applied in this study were feasible to maintain the ALK inhibitory activity of the synthesized degraders. Additionally, the type of linker may have little affection on the binding affinity of the molecules to ALK: as can be depicted in Table1, in most cases, the molecules with chains of **B** can show better inhibitory activities comparing with other ones, which may result from the better flexibility and solubility provided by the chains.

To evaluate the antiproliferative effect of degraders against cancer cells, we performed MTT assay for 72h of synthesized compounds on several tumor cell lines, which including H3122 (NSCLC cell line), H2228 (NSCLC cell line), H1299 (NSCLC cell line), A549 (lung cancer cell line) and Hela (human cervical cancer cell). The IC_{50} values of the degraders were summarized in Table 2 and Supplementary Table 1. Two of the molecules (**B2**, **B3**) showed better anti-proliferation effect than LDK378 in H3122 cells. In addition, as can be demonstrated in kinase assay, none of the two molecules (**B2**, **B3**) showed superior inhibitory activity against ALK than LDK378, indicating that the potent anti-cancer activity may due to the degradation-inducing activity. The phenomenon was consistent with the reported ALK degraders.

The toxicity of inhibitors has always aroused great concern in drug development. The biologically active molecules were tested against normal human liver cell line LO2 by MTT for 72h to learn the toxicities. As a result, all the compounds showed less inhibitory activities to the cells comparing with LDK378 (Fig. 3), indicating that the degraders of ALK do not increase the toxicity to normal cells. The *in vitro* bioassays of the compounds showed that the cells with ALK-fusion proteins were more sensitive to ALK inhibitors or degraders than other cells [39,40].

Table 1
The IC_{50} (nM) value of compounds to ALK.

NO.	IC_{50}	NO.	IC_{50}
A1	19.3 ± 1.52	E1	1.5 ± 0.09
A2	15.8 ± 1.31	E2	12.6 ± 0.93
A3	19.8 ± 1.73	E3	8.2 ± 0.71
B1	10.2 ± 0.96	F1	1.4 ± 0.06
B2	2.3 ± 0.22	F2	1.9 ± 0.09
B3	1.6 ± 0.12	G1	15.8 ± 1.06
B4	11.4 ± 0.73	G2	2.1 ± 0.16
C1	8.3 ± 0.76	G3	5.4 ± 0.14
C2	50.9 ± 2.81	H1	3.6 ± 0.22
C3	12.8 ± 0.92	H2	1.9 ± 0.11
D1	4.6 ± 0.15	H3	1.5 ± 0.09
D2	4.8 ± 0.17	K2	3.9 ± 0.12
D3	5.4 ± 0.13	LDK378	0.81 ± 0.07

2.4. B3 effectively induce ALK degradation

To evaluate the extent of ALK degradation induced by PROTACs, western blot was conducted. H3122 was selected because the expression of ALK fusion proteins and the cell line was highly sensitive in cytotoxicity assay [39].

Table 2
Antiproliferative activity of the compounds (IC_{50}: μM).

NO.	H3122	H2228	H1299	A549	HeLa
B2	0.7 ± 0.05	3.5 ± 0.21	7.59 ± 0.34	2.6 ± 0.11	2.23 ± 0.09
B3	**0.3 ± 0.02**	**0.9 ± 0.03**	**2.84 ± 0.23**	**1.6 ± 0.09**	**1.18 ± 0.06**
C1	3.52 ± 0.35	8.7 ± 0.56	>20	13.38 ± 0.85	8.15 ± 0.62
C2	1.1 ± 0.04	3.1 ± 0.14	>20	5.13 ± 0.38	4.36 ± 0.25
C3	2.66 ± 0.06	15.1 ± 0.67	>20	>20	7.67 ± 0.28
D1	1.83 ± 0.18	5.74 ± 0.26	7.23 ± 0.38	6.73 ± 0.24	5.78 ± 0.21
D2	2.34 ± 0.11	4.51 ± 0.23	10.86 ± 0.92	5.81 ± 0.32	5.86 ± 0.25
F1	4.86 ± 0.24	5.2 ± 0.26	12.28 ± 0.74	10.9 ± 0.59	7.98 ± 0.37
G1	1.3 ± 0.11	>20	9.33 ± 0.13	>20	6.99 ± 0.49
LDK378	1.1 ± 0.21	1.3 ± 0.14	2.92 ± 0.19	1.32 ± 0.06	0.94 ± 0.04

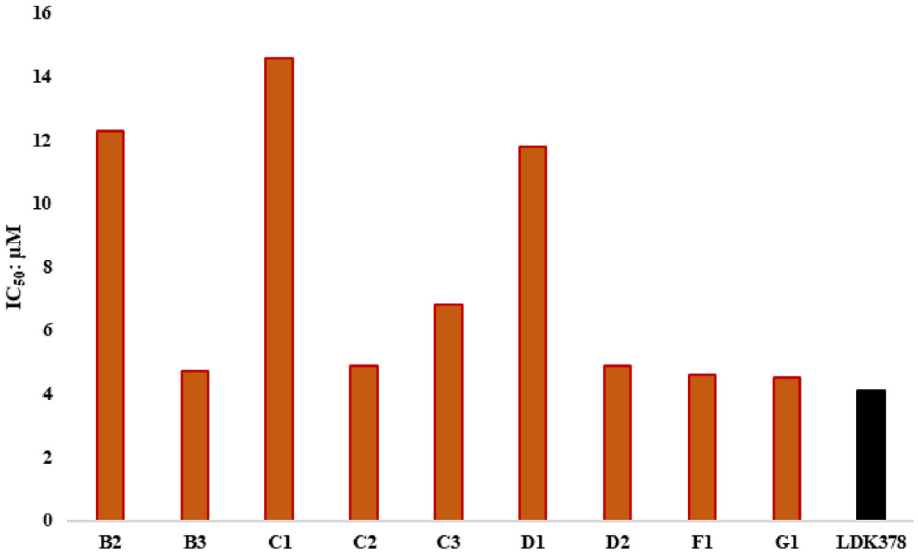


Fig. 3. Antiproliferative activities of compounds to human normal cell line LO2.

To determine if **B2** and **B3** were ALK degraders, we treated H3122 cells with increasing concentrations of **B2**/**B3** for 8 h (h) and the lysates were subjected to western blot analyses with antibodies for EML4-ALK and GAPDH. LDK378 was utilized as control in this assay. As depicted in Fig. 4, **B2** and **B3** can degrade ALK in a dose dependent manner. At the concentration of 50 nM, **B2** and **B3** effectively degraded ALK in contrast with LDK378. Moreover, **B3** demonstrated a more potent ability to degrade ALK comparing with **B2**, at the concentration of 200 nM, **B3** could degrade all the ALK protein in H3122.

Next, we conducted time and concentration-course studies to assess the degradation of ALK fusing proteins and the inhibition of signaling pathways by compound **B3** in H3122 cell line (Fig. 5). After 16 h treatment of **B3** at concentration of 100 nM, significant amount (>80%) of EML4-ALK degradation was achieved, meanwhile, maximum degradation of EML4-ALK degradation was not observed until treatment of **B3** for 24 h. At the same time, p-ALK was significantly inhibited after 24 h treatment, while the level of p-STAT3 was slightly downregulated after treatment. In order to identify the degradation activity of **B3** in different concentrations, ALK degradation assay was conducted with a fixed incubation time of 24 h. The results revealed that **B3** could induce the degradation of ALK in a concentration dependent manner, at a concentration of 50 nM, maximum degradation of EML4-ALK degradation was achieved. Meanwhile, the level of p-ALK and p-STAT3 was also downregulated in a concentration dependent manner. In summary, it can be concluded that **B3** can decrease the cellular levels of ALK fusion proteins in a concentration- and time-dependent manner. The results indicated that **B3** is a potent ALK degrader comparable with the reported ones.

2.5. Selectivity of B3

Among all the molecules, **B3** showed significant increase in *in vitro* bioactivity in contrary with LDK378 while keeping low toxicity to normal cells, therefore this molecule was selected for further investigation. **B3** could potently inhibit ALK with low IC_{50} value, meanwhile, high selectivity may be the important feature in the design of a safe and effective drug. In this study, to learn the selectivity of **B3**, several reported main targets of LDK378 (IGF1R, INSR, FLT3 and FGFR2) were selected [41]. As a result, **B3** showed high selectivity to ALK (Fig. 6), maintaining moderate inhibition activity to IGF1R and INSR. In order to learn if **B3** could induce the degradation of IGF1R and INSR in H3122, western blot was conducted with concentrations of **B3** from 0 to 500 nM. The result showed that no significant degradation was observed (Fig. S1), demonstrating high selectivity of **B3** to ALK in inhibition and degradation activities.

2.6. Bioactivity of B3 to ALK mutant cell lines

To assay the possible anti-drug resistant activity of **B3,** ALK mutant cell lines BaF3-ALK-L1196M and BaF3-ALK-G1202R were employed. As shown in Fig. 7, to BaF3-ALK-L1196M cells**, B3** was slightly more active than LDK378, and BaF3-ALK-G1202R was less sensitive to **B3** comparing with LDK378, which demonstrated that **B3** may be a potential treatment for ALK mutant cancer cells.

2.7. Pharmacokinetics evaluation of B3 in rats

We performed a pharmacokinetics (PK) analysis in rats before assessing the antitumor activity of **B3** *in vivo*, and the results were listed in Fig. 8. In this experiment, a single dose of **B3** at 1 mg/kg

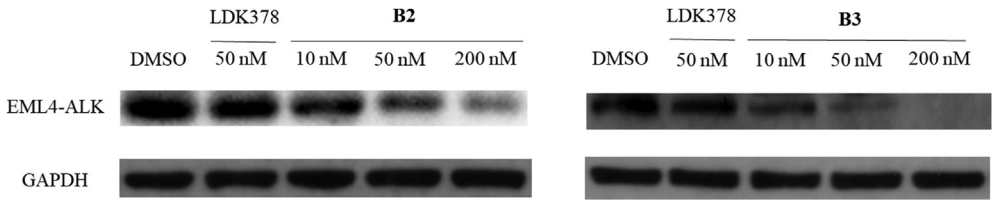


Fig. 4. **B2** and **B3** could significantly reduce the ALK protein levels in a concentration-dependent manner.

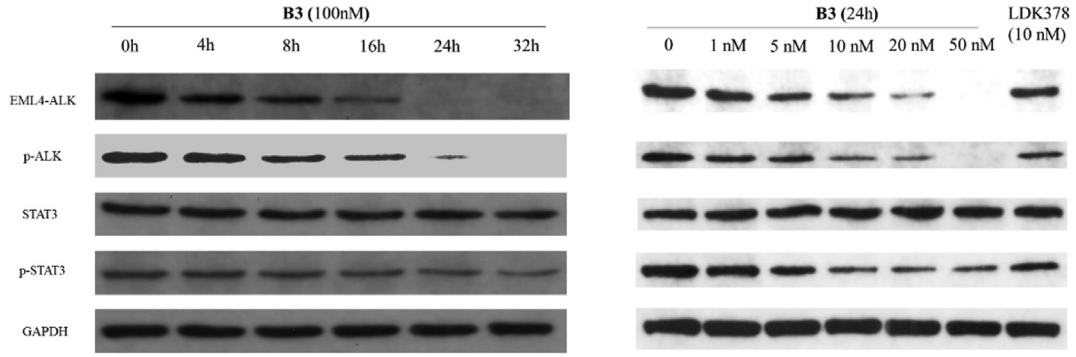


Fig. 5. **B3** could significantly reduce the ALK protein levels and inhibit the ALK down-stream signaling in a time and concentration-dependent manner.

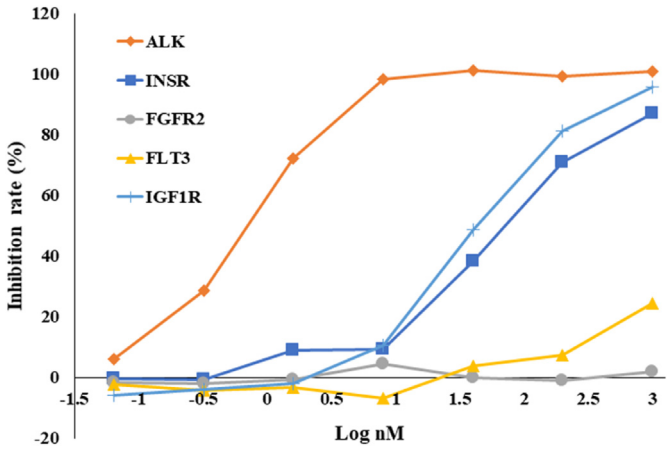


Fig. 6. Selectivity of **B3** against ALK, IGF1R, INSR, FLT3 and FGFR2.

was taken by intravenous injection followed by plasma level quantitation. The data showed that **B3** with a half-life of 4.09 h (n = 3) has a concentration of 10 ng/mL in plasma at 10 h. Based on the acceptable half-life and concentration in plasma, we suggest **B3** could be an effective candidate for *in vivo* cancer treatment.

2.8. In vivo antiproliferative activity of compound B3

The anti-tumor activity *in vivo* of **B3** was conducted, BALB/c nude mice bearing H3122 xenograft tumors were treated with **B3** by intragastric administration for 15 days. As can be depicted in Fig. 9, the growth of xenograft tumors can be inhibited by **B3** in a dose-dependent manner. Tumor growth inhibitions (TGIs) of 37% and 48% were observed in the H3122 xenograft model at dose of 25 mg/kg and 50 mg/kg, respectively. LDK378 as positive control in this assay have a TGI of 49% at dose of 25 mg/kg. In addition, **B3** did not cause significant weight loss and toxicity during the treatment period, suggesting a safe candidate for further investigation.

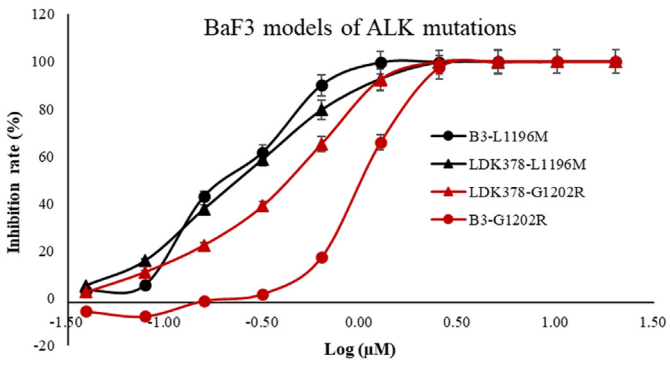


Fig. 7. Bioactivity of **B3** to BaF3 models of ALK mutations.

3. Conclusions

In this work, by linking LDK378 to CRBN ligands through different linkers, a series of PROTACs targeting ALK were designed and synthesized. Among all the molecules, **B3** could effectively induce the degradation of ALK in H3122 cell line, which is comparable with the reported ALK degraders. In addition, **B3** displayed a clear advantage in inhibiting the growth of cell line H3122 comparing with LDK378 and the *in vivo* anticancer activity is acceptable. In summary, this is a novel reported ALK PROTAC molecule that can inhibit the growth of NSCLC *in vitro* and *in vivo*.

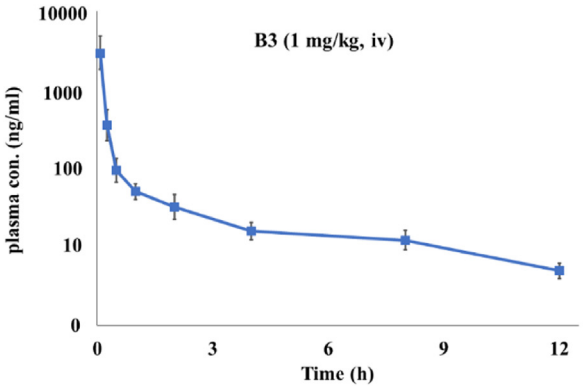
4. Experimental section

4.1. Chemistry

Unless otherwise noted, all reagents and solvents obtained from commercial sources were used without further purification. Flash column chromatography was performed using silica gel from Qingdao Haiyang. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX 400 spectrometer and were calibrated using TMS or residual deuterated solvent as an internal reference ($CDCl_3$: ^{1}H, δ = 7.26 ppm; ^{13}C, δ = 77.16 ppm, DMSO-d_6 2.50 ppm, CD_3OD 3.31 ppm). All of the target compounds were examined by HPLC, and the purity of the biologically tested compounds was ≥95%.

4.1.1. Synthesis of *K2*

To a solution of LDK378 (330 mg, 0.6 mmol) in MeOH (50 mL), methyl acrylate (78 mg, 0.9 mmol) and Triethylamine (240 mg, 2.4 mmol) were added. The resulting reaction mixture was stirred at room temperature for 8 h and monitored by TLC. Upon completion, the methanol was removed by vacuum distillation, the reaction was then extracted with EtOAc, the organic layer was washed twice with water, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography, and the product was obtained as white solid. The white solid (320 mg, 0.5 mmol) was then suspended in 1:1 v/v MeOH/H_2O (50 mL), to this solution was added NaOH (200 mg, 5 mmol). The resulting reaction mixture was stirred at room temperature for 8 h and monitored by TLC. Upon completion, the methanol was removed by vacuum distillation, and the pH of water layer was adjusted to 2 with dilute hydrochloric acid. The reaction was then extracted with EtOAc, the organic layer was washed twice with water, dried over anhydrous



Parameters	Mean
AUC_{all} (ng*h/ml)	1322
AUC_{inf} (ng*h/ml)	1409
$t_{1/2}$ (h)	4.09
CL (ml/min/kg)	16.3
V_{ss} (ml/kg)	1335
MRT_{inf} (h)	1.37

Fig. 8. PK study of **B3** in rats (n = 3 per group).

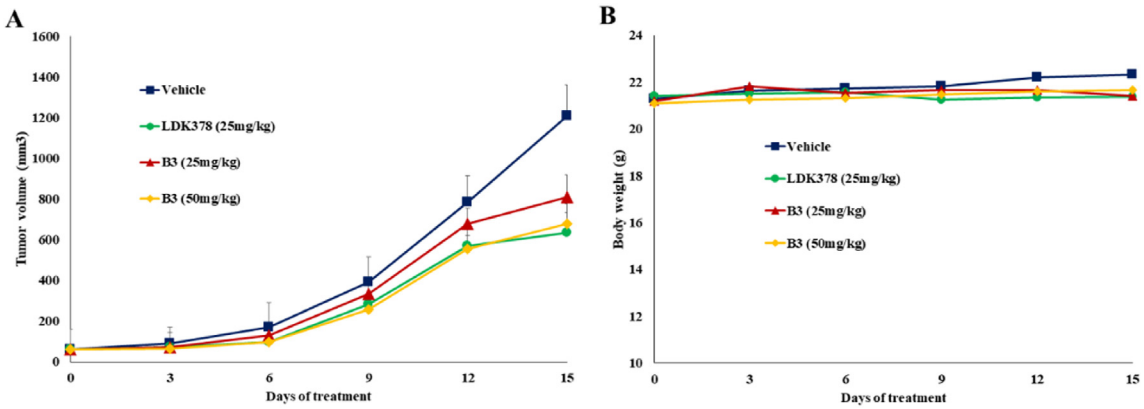


Fig. 9. Pharmacodynamic profile of **B3** *in vivo*. (A) Growth inhibitory effect of **B3** on established H3122 xenografts in female BALB/c nude mice (N = 6 per group); (B) Body weight of the mice during the dosage period.

sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography, and the product was obtained as white powder (yield 95%). HRMS (DART-TOF) calculated for $C_{31}H_{41}ClN_5O_5S$ $[M + H]^+$ *m/z* 630.2517, found 630.2508.

*4.1.2. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butyl)propenamide (**A1**)*

A1-M was prepared as reported [42]. Next, to a solution of A1-M (90 mg, 0.2 mmol) in DMF (10 mL), **K2** (63 mg, 0.1 mmol), HATU (45.6 mg, 0.12 mmol) and DIPEA (38.7 mg, 0.3 mmol) were added. The resulting reaction mixture was stirred at room temperature for 15 h and monitored by TLC. Upon completion, the reaction was then quenched with water and extracted with EtOAc. The organic layer was washed twice with water, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography to afford target molecule as yellow powder (yield 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.45 (s, 1H, CONHCO), 8.51 (d, *J* = 8.3, 1H, Ar−H), 8.09 (s, 1H, Ar-NH), 7.94 (s, 1H, Ar−H), 7.90−7.78 (m, 1H, Ar−H), 7.53 (m, 3H, Ar−H, Ar-NH), 7.41 (dd, *J* = 15.8, 8.3, 1H, Ar−H), 7.21−7.10 (m, 1H, Ar−H), 7.00 (d, *J* = 7.1, 1H, Ar−H), 6.81 (d, *J* = 8.5, 1H, Ar−H), 6.70 (s, 1H, Ar−H), 4.94−4.71 (m, 1H, COCHN), 4.47 (dt, *J* = 11.9, 5.9, 1H, OCH), 3.39−3.05 (m, 4H, N-CH2), 2.96−2.29 (m, 8H, CH2), 2.08 (s, 3H, Ar-CH3), 1.84−1.53 (m, 8H, CH2), 1.37−1.22 (m, 12H, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.83, 171.20, 169.59, 168.67, 167.55, 157.42, 155.37, 146.87, 144.80, 138.48, 136.23, 134.62, 132.48, 131.30, 128.11, 127.17, 124.92, 123.59, 123.16, 120.82, 116.72, 111.63, 111.06, 110.06, 105.96, 71.88, 60.40, 55.49, 53.86, 48.91, 42.16, 31.71, 26.93, 26.59, 22.23, 15.37. HRMS (DART-TOF) calculated for $C_{48}H_{59}ClN_9O_8S$ $[M + H]^+$ *m/z* 956.3896, found 956.3958.

*4.1.3. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)propenamide (**A2**)*

The title compound was obtained from *tert*-butyl (6-aminohexyl)carbamate following similar synthesis procedure of **A1** (yellow powder, yield 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.46 (s, 1H, CONHCO), 8.51 (d, *J* = 8.3, 1H, Ar−H), 8.09 (s, 1H, Ar-NH), 7.89 (s, 1H, Ar−H), 7.85 (dd, *J* = 8.0, 1.5, 1H, Ar−H), 7.79 (s, 1H, Ar-NH), 7.62 (s, 1H, Ar−H), 7.56−7.49 (m, 1H, Ar−H), 7.43−7.36 (m, 1H, Ar−H), 7.16 (d, *J* = 7.9, 1H, Ar−H), 7.00 (d, *J* = 7.1, 1H, Ar−H), 6.78 (d, *J* = 8.5, 1H, Ar−H), 6.68 (s, 1H, Ar−H), 4.83 (dd, *J* = 12.3, 5.3, 1H, COCHN), 4.53−4.37 (m, 1H, OCH), 3.29−3.07 (m, 4H, NCH2), 2.86−2.58 (m, 8H, CH2), 2.19 (s, 3H, Ar-CH3), 2.13−2.01 (m, 4H, CH2), 1.59 (dt, *J* = 13.9, 6.8, 4H, CH2), 1.23 (m, 12H, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.98, 171.40, 169.60, 168.91, 167.67, 157.47, 155.38, 155.19, 146.92, 144.97, 138.48, 136.12, 134.62, 132.54, 131.27, 127.82, 126.98, 124.87, 123.61, 123.10, 121.15, 116.57, 111.41, 110.96, 109.98, 105.80, 71.59, 60.40, 55.48, 54.14, 53.81, 48.98, 42.50, 39.12, 37.58, 32.38, 31.93, 31.52, 29.69, 29.44, 29.06, 26.81, 26.67, 22.80,

22.69, 22.12, 18.90, 15.36, 14.20, 14.12. HRMS (DART-TOF) calculated for $C_{50}H_{63}ClN_9O_8S$ $[M + H]^+$ *m/z* 984.4209, found 984.4210.

*4.1.4. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)propanamide (**A3**)*

The title compound was obtained from *tert*-butyl (8-aminooctyl)carbamate following similar synthesis procedure of **A1** (yellow powder, yield 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.53 (s, 1H, CONHCO), 8.58 (d, *J* = 8.3, 1H, Ar−H), 8.20 (s, 1H, Ar-NH), 7.95 (s, 1H, Ar−H), 7.92 (dd, *J* = 8.0, 1.4, 1H, Ar−H), 7.76 (s, 1H, Ar−H), 7.63−7.55 (m, 1H, Ar−H), 7.51−7.44 (m, 1H, Ar−H), 7.27−7.21 (m, 2H, Ar−H, Ar-NH), 7.07 (d, *J* = 7.1, 1H, Ar−H), 6.86 (d, *J* = 8.6, 1H, Ar−H), 6.73 (s, 1H, Ar−H), 4.91 (dd, *J* = 12.2, 5.3, 1H, COCHN), 4.58−4.45 (m, 1H, OCH), 3.36−3.22 (m, 6H, N-CH2), 2.95−2.64 (m, 8H, CH2), 2.09 (s, 3H, Ar-CH3), 1.90−1.56 (m, 8H, CH2), 1.40−1.29 (m, 20H, CH2, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.17, 171.49, 169.57, 169.01, 167.72, 157.47, 155.40, 155.09, 146.96, 145.07, 138.47, 137.17, 136.05, 134.63, 132.57, 131.25, 127.68, 126.83, 124.87, 123.64, 123.09, 121.38, 116.53, 111.29, 110.79, 109.95, 105.74, 71.43, 60.39, 55.48, 54.19, 53.77, 48.96, 42.40, 39.18, 37.76, 32.62, 32.29, 31.55, 29.70, 29.46, 29.19, 29.05, 27.01, 26.75, 22.77, 22.07, 22.03, 21.04, 18.92, 15.37, 15.35, 14.20. HRMS (DART-TOF) calculated for $C_{52}H_{67}ClN_9O_8S$ $[M + H]^+$ *m/z* 1012.4522, found 1012.4512.

*4.1.5. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)propenamide (**B1**)*

The title compound was obtained from *tert*-butyl (2-(2-aminoethoxy)ethyl)carbamate following similar synthesis procedure of **A1** (yellow powder, yield 46%).^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.49 (s, 1H, CONHCO), 8.57 (d, *J* = 8.3, 1H, Ar−H), 8.15 (s, 1H, Ar-NH), 7.97 (s, 1H, Ar−H), 7.92 (dd, *J* = 7.9, 1.3, 1H, Ar−H), 7.67−7.58 (m, 1H, Ar−H), 7.54 (s, 1H, Ar−H), 7.53−7.47 (m, 1H, Ar−H), 7.24 (d, *J* = 7.8, 1H, Ar−H), 7.13 (d, *J* = 7.1, 1H, Ar−H), 6.86 (d, *J* = 8.5, 1H, Ar−H), 6.77 (s, 1H, Ar−H), 4.89 (dd, *J* = 11.9, 5.6, 1H, COCHN), 4.61−4.46 (m, 1H, OCH), 3.80−3.21 (m, 12H, OCH2, NCH2), 2.90−2.45 (m, 9H, CH2, Ar-CH), 2.24−2.10 (m, 5H, Ar-CH3, CH2), 1.37−1.30 (m, 12H, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.53, 171.83, 171.14, 170.11, 167.47, 157.51, 155.33, 146.75, 144.73, 138.48, 136.32, 134.67, 132.51, 131.24, 127.70, 127.15, 124.88, 123.71, 123.13, 120.76, 116.79, 111.92, 111.32, 110.49, 105.69, 71.81, 70.16, 68.31, 60.39, 55.46, 54.54, 53.88, 53.22, 48.91, 41.67, 39.17, 37.72, 32.56, 31.74, 31.48, 29.69, 23.65, 22.27, 22.26, 21.04, 18.93, 15.37, 14.20. HRMS (DART-TOF) calculated for $C_{48}H_{59}ClN_9O_9S$ $[M + H]^+$ *m/z* 972.3845, found 972.3887.

*4.1.6. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl) propenamide (**B2**)*

The title compound was obtained from *tert*-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl) carbamate following similar synthesis procedure of **A1** (yellow powder, yield 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.51 (s, 1H, CONHCO), 8.57 (d, *J* = 8.3, 1H, Ar−H), 8.15 (s, 1H, Ar-NH), 8.00 (s, 1H, Ar−H), 7.93 (dd, *J* = 8.0, 1.4, 1H, Ar−H), 7.62 (dd, *J* = 12.4, 3.2, 2H, Ar−H, Ar-NH), 7.52−7.45 (m, 1H, Ar−H), 7.27−7.21 (m, 1H, Ar−H), 7.08 (d, *J* = 7.0, 1H, Ar−H), 6.90 (d, *J* = 8.5, 1H, Ar−H), 6.82 (s, 1H, Ar−H), 6.52 (t, *J* = 5.4, 1H, Ar−H), 4.98−4.90 (m, 1H, COCHN), 4.60 (dt, *J* = 12.0, 6.0, 1H, OCH), 3.72 (t, *J* = 5.2, 2H, CH2), 3.69−3.58 (m, 8H, CH2), 3.55 (t, *J* = 5.0, 2H, CH2), 3.44 (td, *J* = 10.5, 5.2, 5H, CH2, CH), 3.26 (dt, *J* = 13.7, 6.8, 1H, CH), 2.91−2.69 (m, 6H, CH2), 2.18−2.08 (m, 5H, CH2, Ar-CH3), 1.85 (d, *J* = 12.5, 4H, CH2), 1.33 (dd, *J* = 10.9, 6.4, 12H, CH3).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.26, 172.05, 169.54, 169.45, 167.62, 157.45, 155.35, 155.29, 146.73, 144.80, 138.46, 136.17, 134.68, 132.52, 131.28, 127.96, 127.18, 124.85, 123.59, 123.19, 120.88, 116.82, 111.73, 110.95, 110.30, 71.74, 70.70, 70.03, 69.83, 69.12, 60.40, 55.50, 53.82, 48.91, 42.20, 39.44, 31.38, 22.95, 22.21, 21.05, 18.92, 15.37, 14.20. HRMS (DART-TOF) calculated for $C_{50}H_{63}ClN_9O_{10}S$ $[M + H]^+$ *m/z* 1016.4107, found 1016.4116.

*4.1.7. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy) ethoxy)ethoxy) ethyl)propenamide (**B3**)*

The title compound was obtained from *tert*-butyl (2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl)carbamate following similar synthesis procedure of **A1** (yellow powder, yield 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.51 (s, 1H, CONHCO), 8.57 (d, *J* = 8.4, 1H, Ar−H), 8.16 (s, 1H, Ar-NH), 7.99 (s, 1H, Ar−H), 7.92 (d, *J* = 7.9, 1H, Ar−H), 7.83 (s, 1H, Ar-NH), 7.67−7.54 (m, 2H, Ar−H), 7.46 (t, *J* = 7.7, 1H, Ar−H), 7.27−7.20 (m, 1H, Ar−H), 7.06 (d, *J* = 7.0, 1H, Ar−H), 6.89 (d, *J* = 8.6, 1H, Ar−H), 6.77 (s, 1H, Ar−H), 6.46 (d, *J* = 5.1, 1H, Ar-NH), 4.93 (dd, *J* = 11.7, 5.7, 1H, COCHN), 4.56 (dt, *J* = 12.0, 6.0, 1H, OCH), 3.78−3.52 (m, 12H, OCH2), 3.45 (d, *J* = 4.7, 4H, NCH2), 3.25 (dd, *J* = 13.4, 6.7, 3H, NCH2, Ar-CH), 2.95−2.65 (m, 6H, CH2), 2.59−2.33 (m, 4H, CH2), 2.17−2.11 (m, 4H, Ar-CH3, SO2CH), 1.80 (d, *J* = 21.0, 4H, CH2), 1.41−1.28 (m, 12H, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.74, 172.07, 171.16, 169.44, 169.41, 167.65, 157.46, 155.34, 155.27, 146.75, 144.83, 138.46, 136.14, 134.69, 132.45, 131.26, 127.85, 127.12, 124.81, 123.59, 123.17, 120.93, 116.89, 111.67, 110.96, 110.26, 105.79, 71.69, 70.43, 70.27, 70.07, 69.96, 69.36, 60.39, 55.50, 54.08, 53.79, 48.90, 42.22, 39.14, 37.22, 31.81, 31.39, 22.75, 22.19, 21.04, 18.91, 15.36, 14.20. HRMS (DART-TOF) calculated for $C_{52}H_{67}ClN_9O_{11}S$ $[M + H]^+$ *m/z* 1060.4369, found 1060.4349.

*4.1.8. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecyl) propenamide (**B4**)*

The title compound was obtained from *tert*-butyl (14-amino-3,6,9,12-tetraoxatetradecyl)carbamate following similar synthesis procedure of **A1** (yellow powder, yield 47%). ^{1}H NMR (400 MHz, MeOD) δ = 8.35 (d, *J* = 8.5, 1H, Ar−H), 8.05 (d, *J* = 1.5, 1H, Ar−H), 7.82 (d, *J* = 8.0, 1H, Ar−H), 7.66 (t, *J* = 6.0, 1H, Ar−H), 7.60−7.54 (m, 1H, Ar−H), 7.43 (ddd, *J* = 9.6, 8.5, 6.0, 1H, Ar−H), 7.26 (t, *J* = 7.7, 1H, Ar−H), 7.03−6.88 (m, 2H, Ar−H), 6.74 (d, *J* = 3.8, 1H, Ar−H), 6.20−6.08 (m, 1H, Ar-NH), 5.00−4.90 (m, 1H, COCHN), 4.56−4.43 (m, 1H, OCH), 3.66−3.25 (m, 20H, OCH2, N-CH2), 2.92−2.33 (m, 8H, CH2), 2.09−1.99 (s, 3H, Ar-CH3), 1.27−1.14 (m, 12H, CH3). HRMS (DART-TOF) calculated for $C_{54}H_{71}ClN_9O_{12}S$ $[M + H]^+$ *m/z* 1104.4631, found 1104.4629.

*4.1.9. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(4-((2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethyl)amino)butyl) propenamide (**C1**)*

To a solution of Pomalidomide (546 mg, 2 mmol) in THF (100 mL), Chloroacetyl chloride (452 mg, 4 mmol) was added. The

resulting reaction mixture was stirred at 66 °C for 5 h and monitored by TLC. Upon completion, EtOAc (200 mL) was added and the mixture was washed with water (3 x 100 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography, and the product was obtained as white solid (yield 70%). Then the product (70 mg, 0.2 mmol) was dissolved in THF (10 mL), to this solution tert-butyl (4-aminobutyl)carbamate (113 mg, 0.6 mmol) and K_2CO_3 (83 mg, 0.6 mmol) was added and stirred at 66 °C for 5 h and monitored by TLC. Upon completion, the reaction was then quenched with 30 mL H_2O and extracted with EtOAc. The organic layer was washed twice with water, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography, and the product was obtained as white solid (yield 69%). Then the product of this step was dissolved in 5 mL trifluoroacetic acid and stirred at room temperature for 5 h. Upon completion, the reaction was then quenched with DCM (10 mL) and the solution was removed by vacuum distillation to get C1-M (brown oil). C1-M was applied in synthesis without further purification. Next, to a solution of C1-M (92 mg, 0.2 mmol) in DMF (10 mL), **K2**(63 mg, 0.1 mmol), HATU (45.6 mg, 0.12 mmol) and DIPEA (38.7 mg, 0.3 mmol) were added. The resulting reaction mixture was stirred at room temperature for 15 h and monitored by TLC. Upon completion, the reaction was then quenched with water and extracted with EtOAc. The organic layer was washed twice with water, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography to afford target molecule as yellow powder (yield 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.51 (s, 1H, CONHCO), 8.89 (d, J = 8.4, 1H, Ar−H), 8.57 (d, J = 8.3, 1H, Ar−H), 8.16 (s, 1H, Ar-NH), 8.01 (d, J = 4.5, 1H, Ar−H), 7.93 (d, J = 7.8, 1H, Ar−H), 7.75−7.65 (m, 1H, Ar−H), 7.65−7.59 (m, 1H, Ar−H), 7.55 (d, J = 6.7, 1H, Ar−H), 7.25 (d, J = 9.3, 3H, Ar−H, Ar-NH), 6.80−6.72 (m, 1H, Ar−H), 5.06−4.86 (m, 1H, COCHN), 4.55 (dd, J = 12.0, 5.9, 1H, OCH), 3.30 (ddd, J = 20.4, 15.4, 7.6, 6H, NCH2), 3.00−2.61 (m, 7H, CH2, NH), 2.21−2.13 (s, 3H, Ar-CH3), 1.97−1.49 (m, 4H, CH2), 1.38−1.29 (m, 12H, CH3). HRMS (DART-TOF) calculated for $C_{50}H_{62}ClN_{10}O_9S$ $[M + H]^+$ m/z 1013.4110, found 1013.4082.

4.1.10. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(6-((2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethyl)amino)hexyl) propenamide (**C2**)

The title compound was obtained from *tert*-butyl (6-aminohexyl)carbamate following similar synthesis procedure of **C1** (yellow powder, yield 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.12 (s, 1H, CONHAr), 9.44 (s, 1H, CONHCO), 8.83 (d, J = 8.4, 1H, Ar−H), 8.50 (d, J = 8.2, 1H, Ar−H), 8.09 (s, 1H, Ar-NH), 7.92 (s, 1H, Ar−H), 7.86 (d, J = 7.8, 1H, Ar−H), 7.63 (t, J = 7.7, 1H, Ar−H), 7.58−7.44 (m, 3H, Ar−H, Ar-NH), 7.18 (d, J = 11.8, 2H, Ar−H), 6.70 (s, 1H, Ar−H), 4.90 (dd, J = 11.6, 5.1, 1H, COCHN), 4.47 (dt, J = 11.7, 5.8, 1H, OCH), 3.35 (q, J = 17.6, 2H, CH2), 3.27−3.06 (m, 5H, CH2, CH), 2.95−2.51 (m, 10H, CH2), 2.19 (s, 3H, Ar-CH3), 1.47 (ddd, J = 22.2, 16.0, 7.8, 6H, CH2), 1.26 (dd, J = 13.5, 6.4, 12H, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.01, 171.73, 168.63, 168.33, 166.98, 157.45, 155.29, 144.78, 138.48, 137.08, 136.13, 134.63, 131.52, 131.28, 127.88, 127.11, 125.16, 124.94, 123.63, 123.13, 120.87, 118.43, 116.02, 111.01, 105.86, 100.00, 71.74, 60.40, 55.47, 54.10, 53.81, 53.53, 50.44, 49.27, 39.24, 37.59, 32.42, 31.57, 29.96, 29.54, 26.96, 26.77, 22.74, 22.22, 18.92, 15.37, 14.20. HRMS (DART-TOF) calculated for $C_{52}H_{66}ClN_{10}O_9S$ $[M + H]^+$ m/z 1041.4423, found 1041.4438.

4.1.11. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(8-((2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethyl)amino)octyl) propenamide (**C3**)

The title compound was obtained from *tert*-butyl (8-aminooctyl)carbamate following similar synthesis procedure of **C1** (yellow powder, yield 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.52 (s, 1H, CONHCO), 8.58 (d, J = 8.3, 1H, Ar−H), 8.17 (d, J = 6.8, 1H, Ar−H), 8.17 (d, J = 6.8, 1H, Ar−H), 8.01 (d, J = 9.4, 1H, Ar−H), 7.93 (d, J = 7.9, 1H, Ar−H), 7.71 (dt, J = 12.6, 6.4, 1H, Ar−H), 7.66−7.50 (m, 3H, Ar−H, Ar-NH), 7.24 (d, J = 7.7, 1H, Ar−H), 4.96 (dd, J = 12.0, 5.1, 1H, COCHN), 4.52 (dd, J = 12.0, 5.9, 1H, OCH), 3.57−3.38 (m, 2H, CH2), 3.33−3.13 (m, 5H, CH2, NH), 2.95−2.40 (m, 10H, CH2), 2.15 (s, 3H, Ar-CH3), 1.90−1.70 (m, 4H, CH2), 1.32 (ddd, J = 26.8, 14.8, 6.7, 24H, CH2, CH3). HRMS (DART-TOF) calculated for $C_{54}H_{70}ClN_{10}O_9S$ $[M + H]^+$ m/z 1069.4736, found 1069.4791.

4.1.12. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(2-(2-((2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethyl)amino)ethoxy) ethyl) propenamide (**D1**)

The title compound was obtained from *tert*-butyl (2-(2-aminoethoxy)ethyl)carbamate following similar synthesis procedure of **C1** (yellow powder, yield 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.18 (s, 1H, CONH), 9.51 (s, 1H, CONHCO), 8.87 (d, J = 8.5, 1H, Ar−H), 8.58 (d, J = 8.3, 1H, Ar−H), 8.14 (d, J = 11.3, 1H, Ar−H), 7.99 (s, 1H, Ar-NH), 7.92 (dd, J = 7.9, 1.3, 1H, Ar−H), 7.75−7.66 (m, 1H, Ar−H), 7.65−7.58 (m, 1H, Ar−H), 7.58−7.50 (m, 2H, Ar−H, Ar-NH), 7.25 (d, J = 7.6, 1H, Ar−H), 6.77 (s, 1H, Ar−H), 4.96 (dd, J = 12.1, 5.4, 1H, COCHN), 4.60−4.48 (m, 1H, OCH), 3.78−3.53 (m, 4H, OCH2), 3.53−3.38 (m, 4H, NCH2), 3.34−3.07 (m, 2H, CH2), 2.96−2.61 (m, 8H, CH2), 2.47 (t, J = 6.4, 2H, CH2), 2.15 (s, 3H, Ar-CH3), 1.88−1.61 (m, 4H, CH2), 1.38−1.30 (m, 12H, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.72, 171.80, 171.45, 168.55, 168.50, 166.89, 157.47, 155.34, 144.76, 138.49, 137.02, 136.23, 134.65, 131.47, 131.27, 127.85, 127.25, 125.14, 124.87, 123.62, 123.12, 120.87, 118.53, 116.03, 111.18, 105.83, 71.83, 70.23, 69.83, 60.40, 55.47, 54.12, 53.99, 53.83, 53.30, 49.57, 49.28, 39.02, 37.83, 32.45, 31.42, 29.69, 22.83, 22.69, 22.26, 21.04, 18.92, 15.37, 14.20, 14.12. HRMS (DART-TOF) calculated for $C_{50}H_{60}ClN_{10}O_{10}S$ $[M + H]^+$ m/z 1029.4060, found 1029.4071.

4.1.13. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(2-(2-(2-((2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethyl) amino) ethoxy)ethoxy)ethyl)propenamide (**D2**)

The title compound was obtained from *tert*-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate following similar synthesis procedure of **C1** (yellow powder, yield 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.21 (s, 1H, CONH), 9.50 (s, 1H, CONHCO), 8.88 (d, J = 8.5, 1H, Ar−H), 8.57 (d, J = 8.4, 1H, Ar−H), 8.14 (s, 1H, Ar-NH), 7.97 (s, 1H, Ar−H), 7.92 (d, J = 7.5, 2H, Ar−H), 7.75−7.66 (m, 1H, Ar−H), 7.66−7.57 (m, 2H, Ar−H, Ar-NH), 7.55 (d, J = 7.2, 1H, Ar−H), 7.24 (d, J = 7.6, 1H, Ar−H), 6.78 (s, 1H, Ar−H), 4.96 (dd, J = 12.1, 5.3, 1H, COCHN), 4.54 (dt, J = 12.1, 6.0, 1H, OCH), 3.77−3.38 (m, 14H, OCH2, NCH2), 3.26 (dt, J = 13.7, 6.8, 1H, CH), 3.13 (d, J = 11.2, 2H, CH2), 2.97−2.61 (m, 4H, CH2), 2.45 (t, J = 6.5, 2H, CH2), 2.15 (d, J = 6.4, 7H, CH2, Ar-CH3), 1.75 (d, J = 11.4, 6H, CH2), 1.38−1.29 (m, 12H, CH3).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.51, 171.91, 171.40, 168.49, 168.45, 166.92, 157.47, 155.34, 155.26, 144.80, 138.48, 137.41, 137.08, 136.19, 134.66, 131.45, 131.26, 127.70, 127.09, 125.15, 124.86, 123.63, 123.11, 120.88, 118.45, 116.01, 111.12, 105.76, 71.67, 70.55, 70.23, 70.06, 69.97, 55.47, 54.20, 53.91, 53.18, 49.40, 49.30, 38.97, 37.95, 32.85, 32.52, 31.52, 29.69, 22.71, 22.22, 18.93, 15.36, 14.20. HRMS (DART-TOF) calculated for $C_{52}H_{66}ClN_{10}O_{11}S$ $[M + H]^+$ *m/z* 1073.4322, found 1073.4313.

*4.1.14. Synthesis of 14-(3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl) amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)propanamido)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-6,9,12-trioxa-3-azatetradecanamide (**D3**)*

The title compound was obtained from *tert*-butyl (2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl)carbamate following similar synthesis procedure of **C1** (yellow powder, yield 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.51 (s, 1H, CONHCO), 8.77 (d, *J* = 7.7, 1H, Ar−H), 8.56 (d, *J* = 8.2, 1H, Ar−H), 8.15 (s, 1H, Ar-NH), 7.99 (s, 1H, Ar−H), 7.92 (d, *J* = 8.1, 1H, Ar−H), 7.68 (d, *J* = 7.3, 1H, Ar−H), 7.65−7.59 (m, 1H, Ar−H), 7.55 (d, *J* = 11.0, 1H, Ar−H), 7.52 (s, 1H, Ar-NH), 7.29−7.21 (m, 4H, Ar−H, Ar-NH), 6.74 (s, 1H, Ar−H), 4.63−4.51 (m, 1H, OCH), 3.64 (m, 12H, OCH2), 3.39 (m, 4H, NCH2), 2.84 (m, 6H, CH2), 2.57 (m, 4H, CH2), 2.13 (m, 5H, CH2, Ar-CH3), 1.32 (dd, *J* = 11.0, 6.4, 12H, CH3). HRMS (DART-TOF) calculated for $C_{54}H_{70}ClN_{10}O_{12}S$ $[M + H]^+$ *m/z* 1117.4584, found 1117.4648.

*4.1.15. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(4-((3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3-oxopropyl)amino)butyl) propenamide (**E1**)*

The title compound was obtained from Acryloyl chloride and *tert*-butyl (4-aminobutyl)carbamate following similar synthesis procedure of **C1** (yellow powder, yield 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.51 (s, 1H, CONHCO), 8.81−8.65 (m, 1H, Ar−H), 8.56 (t, *J* = 9.4, 1H, Ar−H), 8.20−8.10 (m, 1H, Ar−H), 8.08−7.98 (m, 1H, Ar−H), 7.93 (d, *J* = 7.5, 1H, Ar−H), 7.71 (d, *J* = 5.2, 1H, Ar−H), 7.67−7.48 (m, 3H, Ar−H, Ar-NH), 7.24 (s, 1H, Ar−H), 6.80 (d, *J* = 10.0, 1H, Ar−H), 4.96 (s, 1H, COCHN), 4.57 (s, 1H, OCH), 3.77 (dd, *J* = 22.6, 16.6, 2H, CH2), 3.60−3.12 (m, 6H, CH2), 2.81 (dd, *J* = 91.0, 52.5, 8H, CH2), 2.14 (s, 3H, Ar-CH3), 2.09−1.52 (m, 8H, CH2), 1.40−1.28 (m, 12H, CH3). HRMS (DART-TOF) calculated for $C_{51}H_{64}ClN_{10}O_9S$ $[M + H]^+$ *m/z* 1027.4267, found 1027.4261.

*4.1.16. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(6-((3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3-oxopropyl)amino)hexyl) propenamide (**E2**)*

The title compound was obtained from *tert*-butyl (6-aminohexyl)carbamate following similar synthesis procedure of **E1** (yellow powder, yield 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.52 (s, 1H, CONHCO), 8.57 (t, *J* = 8.6, 1H, Ar−H), 8.16 (d, *J* = 1.8, 1H, Ar−H), 8.05 (d, *J* = 13.6, 1H, Ar−H), 7.93 (dd, *J* = 8.0, 1.5, 1H, Ar−H), 7.62 (t, *J* = 7.9, 1H, Ar−H), 7.56 (d, *J* = 4.2, 1H, Ar−H), 7.36−7.25 (m, 4H, Ar−H, Ar-NH), 6.74 (d, *J* = 8.0, 1H, Ar−H), 4.60 (td, *J* = 12.5, 6.3, 1H, OCH), 4.05−3.95 (m, 1H, CH), 3.77 (d, *J* = 13.4, 1H, CH), 3.34−2.89 (m, 6H, CH2), 2.84−2.67 (m, 1H, CH), 2.20 (s, 3H, Ar-CH3), 1.94−1.49 (m, 8H, CH2), 1.40−1.21 (m, 16H, CH2, CH3). HRMS (DART-TOF) calculated for $C_{53}H_{68}ClN_{10}O_9S$ $[M + H]^+$ *m/z* 1055.4580, found 1055.4595.

*4.1.17. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(2-(2-(2-((3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3-oxopropyl)amino)ethoxy) ethyl)propenamide (**F1**)*

The title compound was obtained from *tert*-butyl (2-(2-aminoethoxy)ethyl)carbamate following similar synthesis procedure of **E1** (yellow powder, yield 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.52 (s, 1H, CONHCO), 8.79 (d, *J* = 8.2, 1H, Ar−H), 8.58 (d, *J* = 8.0, 1H, Ar−H), 8.16 (s, 1H, Ar-NH), 8.05−7.97 (m, 1H, Ar−H), 7.93 (dd, *J* = 8.0, 1.5, 1H, Ar−H), 7.70−7.64 (m, 1H, Ar−H), 7.64−7.58 (m, 1H, Ar−H), 7.57 (s, 1H, Ar-NH), 7.53 (d, *J* = 6.8, 1H, Ar−H), 7.26−7.23 (m, 1H, Ar−H), 6.77 (s, 1H, Ar−H), 4.94 (dd, *J* = 12.4, 5.4, 1H, COCHN), 4.54 (dt, *J* = 12.1, 6.1, 1H, OCH), 3.71−3.54 (m, 4H, OCH2), 3.49−3.43 (m, 2H, CH2), 3.29−3.18 (m, 2H, CH2), 3.04 (t, *J* = 5.9, 2H, CH2), 2.78 (dddd, *J* = 21.3, 18.4, 10.7, 5.0, 11H, CH2, CH), 2.53 (t, *J* = 6.2, 2H, CH2), 2.14 (s, 3H, Ar-CH3), 1.33 (dd, *J* = 11.8, 6.4, 12H, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.27, 171.65, 171.41, 168.53, 168.53, 168.26, 166.84, 157.45, 155.35, 144.80, 138.49, 137.33, 136.04, 134.64, 134.64, 131.48, 131.28, 127.94, 127.94, 127.12, 126.18, 124.89, 123.60, 123.13, 120.85, 118.47, 116.18, 111.07, 105.88, 71.79, 69.74, 55.48, 53.86, 49.25, 48.77, 44.77, 39.07, 37.43, 31.93, 31.45, 29.70, 29.36, 22.69, 22.24, 18.93, 15.37, 14.12. HRMS (DART-TOF) calculated for $C_{51}H_{64}ClN_{10}O_{10}S$ $[M + H]^+$ *m/z* 1043.4216, found 1043.4178.

*4.1.18. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(2-(2-(2-((3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3-oxopropyl) amino) ethoxy)ethoxy)ethyl)propenamide (**F2**)*

The title compound was obtained from *tert*-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate following similar synthesis procedure of **E1** (yellow powder, yield 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.51 (s, 1H, CONHCO), 8.82 (d, *J* = 8.0, 1H, Ar−H), 8.58 (d, *J* = 8.3, 1H, Ar−H), 8.17−8.13 (m, 1H, Ar−H), 8.00 (dd, *J* = 8.6, 4.2, 1H, Ar−H), 7.93 (dd, *J* = 8.0, 1.5, 1H, Ar−H), 7.68 (dd, *J* = 8.5, 7.3, 1H, Ar−H), 7.65−7.58 (m, 2H, Ar−H, Ar-NH), 7.53 (d, *J* = 6.7, 1H, Ar−H), 7.26−7.22 (m, 1H, Ar−H), 6.78 (d, *J* = 4.4, 1H, Ar−H), 5.01−4.88 (m, 1H, COCHN), 4.55 (dt, *J* = 12.2, 6.0, 1H, OCH), 3.79−3.36 (m, 12H, OCH2, NCH2), 3.04−2.98 (m, 1H, CH), 2.92−2.83 (m, 2H, CH2), 2.82−2.69 (m, 4H, CH2), 2.62−2.55 (m, 1H, CH), 2.46 (t, *J* = 6.5, 2H, CH), 2.25−2.11 (s, 3H, Ar-CH3), 1.76 (d, *J* = 17.4, 4H, CH2), 1.39−1.31 (m, 12H, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.05, 171.32, 168.47, 168.21, 166.85, 157.49, 155.35, 144.80, 138.51, 137.49, 137.31, 136.04, 134.64, 131.28, 127.73, 127.16, 127.07, 126.27, 124.88, 123.64, 123.10, 118.39, 116.15, 111.12, 71.68, 70.20, 70.13, 70.00, 55.47, 53.99, 48.74, 44.83, 38.95, 37.93, 37.30, 32.88, 32.47, 31.93, 29.70, 29.36, 22.69, 22.23, 18.94, 15.37. HRMS (DART-TOF) calculated for $C_{53}H_{68}ClN_{10}O_{11}S$ $[M + H]^+$ *m/z* 1087.4478, found 1087.4503.

*4.1.19. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)butyl)propanamide (**G1**)*

To a solution of 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyi soindoline-1,3-dione (411 mg, 0.15 mmol) in DMF (100 mL), *tert*-Butyl bromoacetate (293 mg, 0.15 mmol) and K_2CO_3 (621 mg, 4.5 mmol) were added. The resulting reaction mixture was stirred at room temperature for 15 h and monitored by TLC. Upon completion, the reaction mixture was then quenched with H_2O and extracted with EtOAc, then the organic layer was washed with water (100 mL) for

three times. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography, and the product was obtained as white powder (yield 70%). The product (78 mg, 0.2 mmol) was dissolved in trifluoroacetic acid (5 mL) and stirred at room temperature for 5 h. Upon completion, the reaction was then quenched with 10 mL DCM and the solution was removed by vacuum distillation to get white solid. The solid was dissolved in DMF (10 mL), to this solution *tert*-butyl (4-aminobutyl)carbamate (54 mg, 0.3 mmol), HATU (91 mg, 0.24 mmol) and DIPEA (38.7 mg, 0.3 mmol) were added and stirred at room temperature for 10 h and monitored by TLC. Upon completion, the reaction was then quenched with water and extracted with EtOAc. The organic layer was washed twice with water, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography, and the product was obtained as yellow powder (yield 56%). Then the product of this step was dissolved in trifluoroacetic acid (5 mL) and stirred at room temperature for 5 h. Upon completion, the reaction was then quenched with DCM (10 mL) and the solution was removed by vacuum distillation to get G1-M (brown oil). G1-M was applied in synthesis without further purification. Next, to a solution of G1-M (100 mg, 0.2 mmol) in DMF (10 mL), **K2** (63 mg, 0.1 mmol), HATU (45.6 mg, 0.12 mmol) and DIPEA (38.7 mg, 0.3 mmol) were added. The resulting reaction mixture was stirred at room temperature for 15 h and monitored by TLC. Upon completion, the reaction was then quenched with water and extracted with EtOAc. The organic layer was washed twice with water, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography to afford target molecule as yellow powder (yield 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.51 (s, 1H, CONHCO), 8.58 (d, J = 8.0, 1H, Ar−H), 8.16 (s, 1H, Ar-NH), 8.00 (s, 1H, Ar−H), 7.93 (dd, J = 8.0, 1.5, 1H, Ar−H), 7.73 (dd, J = 8.3, 7.4, 1H, Ar−H), 7.66−7.59 (m, 2H, Ar−H, Ar-NH), 7.55 (s, 2H, Ar−H), 7.27−7.23 (m, 1H, Ar−H), 7.19 (d, J = 8.3, 1H, Ar−H), 6.77 (s, 1H, Ar−H), 5.05−4.93 (m, 1H, COCHN), 4.60 (dt, J = 12.2, 10.2, 3H, OCH, COCH2), 2.98−2.64 (m, 6H, CH2), 2.50 (d, J = 6.2, 2H, CH2), 2.14 (s, 3H, Ar-CH3), 1.78 (d, J = 18.6, 4H, CH2), 1.70−1.53 (m, 4H, CH2), 1.42−1.29 (m, 12H, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.49, 168.82, 166.81, 166.59, 166.45, 157.48, 155.34, 154.76, 144.74, 138.50, 137.12, 134.65, 133.51, 131.27, 127.85, 127.10, 124.89, 123.63, 123.12, 120.79, 120.33, 118.41, 117.63, 111.03, 105.83, 71.80, 68.66, 60.39, 55.48, 53.92, 53.78, 49.44, 38.84, 38.75, 32.14, 31.53, 26.83, 26.62, 22.79, 22.24, 21.04, 18.92, 15.37, 14.20. HRMS (DART-TOF) calculated for $C_{50}H_{61}ClN_9O_{10}S$ $[M + H]^+$ m/z 1014.3951, found 1014.3975.

*4.1.20. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(6-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)hexyl)propenamide (**G2**)*

The title compound was obtained from *tert*-butyl (6-aminohexyl)carbamate following similar synthesis procedure of **G1** (yellow powder, yield 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.52 (s, 1H, CONHCO), 8.58 (d, J = 8.3, 1H, Ar−H), 8.16 (s, 1H, Ar-NH), 7.99 (s, 1H, Ar−H), 7.92 (dd, J = 8.0, 1.4, 1H, Ar−H), 7.77−7.69 (m, 1H, Ar−H), 7.66−7.59 (m, 1H, Ar−H), 7.58 (s, 1H, Ar-NH), 7.56−7.52 (m, 1H, Ar−H), 7.49 (t, J = 5.4, 1H, Ar−H), 7.25 (d, J = 8.0, 1H, Ar−H), 7.19 (d, J = 8.4, 1H, Ar−H), 6.75 (s, 1H, Ar−H), 5.07−4.92 (m, 1H, COCHN), 4.67−4.59 (m, 2H, COCH2O), 4.60−4.47 (m, 1H, OCH), 3.51−3.11 (m, 7H, NCH2, CH), 2.97−2.67 (m, 6H, CH2), 2.48 (t, J = 6.0, 2H, CH2), 2.28 (dd, J = 22.1, 11.6, 2H, CH2), 2.18 (d, J = 21.2, 4H, Ar-CH, NH), 1.90−1.67 (m, 4H, CH2), 1.55 (ddd, J = 26.9, 13.5, 6.7, 4H, CH2), 1.34 (dd, J = 15.3, 6.5, 12H, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.42, 171.67, 171.13, 168.61, 166.71, 166.67, 166.10, 157.47, 155.34, 155.29, 154.50, 144.80, 138.48, 137.01, 134.64, 133.59, 131.26, 127.84, 127.15, 124.86, 123.61, 123.12, 120.95, 119.65, 118.21, 117.35, 110.97, 105.82, 71.73, 68.10, 60.38, 55.48, 54.20, 53.82, 49.36, 39.11, 38.97, 37.67, 32.40, 32.15, 31.52, 29.54, 29.05, 26.69, 26.39, 22.71, 22.23, 21.04, 18.91, 15.36, 14.20. HRMS (DART-TOF) calculated for $C_{52}H_{65}ClN_9O_{10}S$ $[M + H]^+$ m/z 1042.4264, found 1042.4293.

*4.1.21. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino) pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(8-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)octyl)propenamide (**G3**)*

The title compound was obtained from *tert*-butyl (8-aminooctyl)carbamate following similar synthesis procedure of **G1** (yellow powder, yield 21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.52 (s, 1H, CONHCO), 8.58 (d, J = 8.2, 1H, Ar−H), 8.16 (s, 1H, Ar-NH), 7.99 (s, 1H, Ar−H), 7.93 (dd, J = 8.0, 1.5, 1H, Ar−H), 7.73 (dd, J = 10.1, 5.7, 1H, Ar−H), 7.62 (dd, J = 10.4, 3.2, 2H, Ar−H, Ar-NH), 7.55 (t, J = 6.0, 1H, Ar−H), 7.39 (t, J = 5.6, 1H, Ar−H), 7.26−7.23 (m, 1H, Ar−H), 7.19 (d, J = 8.4, 1H, Ar−H), 6.75 (s, 1H, Ar−H), 4.96 (dd, J = 12.3, 5.3, 1H, COCHN), 4.63 (t, J = 7.8, 2H, CONH2), 4.59−4.45 (m, 1H, OCH), 3.29 (dddd, J = 27.1, 23.9, 16.5, 8.6, 6H, NCH2), 2.98−2.67 (m, 6H, CH2), 2.49 (d, J = 5.7, 2H, CH2), 2.23 (d, J = 21.1, 2H, CH2), 2.15 (s, 3H, Ar-CH3), 1.89−1.69 (m, 4H, CH2), 1.62−1.49 (m, 4H, CH2), 1.44−1.29 (m, 18H, CH3, CH2). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.39, 168.35, 166.68, 166.61, 166.01, 157.46, 155.37, 155.27, 154.49, 144.86, 138.48, 137.03, 134.63, 133.61, 131.28, 124.91, 123.63, 123.13, 120.94, 119.49, 118.13, 117.33, 110.93, 105.87, 71.71, 68.00, 55.48, 53.85, 49.39, 39.09, 31.58, 29.70, 29.35, 29.12, 29.01, 26.78, 26.62, 22.59, 22.21, 18.93, 15.37. HRMS (DART-TOF) calculated for $C_{54}H_{69}ClN_9O_{10}S$ $[M + H]^+$ m/z 1070.4577, found 1070.4604.

*4.1.22. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethoxy)ethyl) propenamide (**H1**)*

The title compound was obtained from *tert*-butyl (2-(2-aminoethoxy)ethyl)carbamate following similar synthesis procedure of **G1** (yellow powder, yield 23%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.51 (s, 1H, CONHCO), 8.58 (d, J = 8.1, 1H, Ar−H), 8.15 (d, J = 4.4, 1H, Ar−H), 7.99 (s, 1H, Ar−H), 7.92 (dd, J = 8.0, 1.5, 1H, Ar−H), 7.76−7.71 (m, 1H, Ar−H), 7.67 (d, J = 4.9, 1H, Ar−H), 7.64−7.59 (m, 1H, Ar−H), 7.58−7.50 (m, 3H, Ar−H, Ar-NH, CONH), 7.25 (dd, J = 9.5, 2.3, 1H, Ar−H), 7.22−7.16 (m, 1H, Ar−H), 5.05−4.92 (m, 1H, COCHN), 4.63 (s, 2H, COCH2O), 4.54 (dt, J = 12.1, 6.0, 1H, OCH), 3.68−3.42 (m, 9H, OCH2, NCH2), 3.33−3.13 (m, 4H, CH2), 2.91−2.62 (m, 8H, CH2), 2.14 (s, 3H, Ar-CH3), 1.37−1.29 (m, 16H, CH2, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.67, 171.12, 168.96, 166.71, 166.67, 166.15, 157.48, 155.32, 154.28, 144.77, 138.49, 137.02, 134.65, 133.66, 131.25, 127.78, 127.08, 124.86, 123.63, 123.11, 120.82, 119.16, 117.97, 117.25, 111.07, 105.78, 71.75, 69.58, 69.37, 67.64, 60.38, 55.47, 54.09, 53.95, 53.84, 49.39, 39.37, 38.83, 37.73, 32.72, 32.05, 31.52, 29.68, 22.91, 22.24, 21.04, 18.92, 15.37, 14.20. HRMS (DART-TOF) calculated for $C_{50}H_{61}ClN_9O_{11}S$ $[M + H]^+$ m/z 1030.3900, found 1030.3875.

*4.1.23. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido) ethoxy)ethoxy)ethyl) propenamide (**H2**)*

The title compound was obtained from *tert*-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate following similar synthesis procedure of **G1** (yellow powder, yield 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.51 (s, 1H, CONHCO), 8.58 (d, J = 8.0, 1H, Ar−H), 8.21−8.09 (m, 1H, Ar−H), 7.98 (s, 1H, Ar−H), 7.92 (dd, J = 8.0, 1.5, 1H,

Ar−H), 7.73 (dd, J = 8.3, 7.5, 1H, Ar−H), 7.65−7.59 (m, 2H, Ar-NH), 7.58 (s, 1H, Ar−H), 7.53 (d, J = 7.2, 1H, Ar−H), 7.27 (d, J = 9.8, 1H, Ar−H), 7.19 (d, J = 8.4, 1H, Ar−H), 6.79 (s, 1H, Ar−H), 5.04−4.93 (m, 1H, COCHN), 4.66 (d, J = 14.3, 2H, COCH2O), 4.60−4.51 (m, 1H, OCH), 3.73−3.52 (m, 10H, OCH2, NCH2), 3.45 (dd, J = 10.7, 5.3, 2H, NCH2), 3.27 (dq, J = 13.7, 6.8, 1H, CH), 3.16 (d, J = 10.6, 2H, CH2), 2.92−2.63 (m, 6H, CH2), 2.18−2.12 (m, 4H, Ar-CH3, CH), 1.38−1.28 (m, 16H, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.63, 171.12, 168.67, 166.89, 166.66, 165.92, 157.49, 155.33, 155.29, 154.43, 144.81, 138.49, 136.98, 134.65, 133.68, 131.25, 127.72, 127.03, 124.84, 123.62, 123.10, 120.86, 119.38, 118.03, 117.29, 111.12, 105.76, 71.67, 70.33, 69.92, 69.53, 67.95, 60.38, 55.47, 54.13, 53.94, 49.35, 39.05, 39.00, 37.84, 32.75, 32.34, 31.52, 22.71, 22.23, 21.04, 18.93, 15.36, 14.20. HRMS (DART-TOF) calculated for $C_{52}H_{65}ClN_9O_{12}S$ $[M + H]^+$ m/z 1074.4162, found 1074.4153.

4.1.24. Synthesis of 3-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl) phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-N-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)propenamide (**H3**)

The title compound was obtained from *tert*-butyl (2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl)carbamate following similar synthesis procedure of **G1** (yellow powder, yield 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.44 (s, 1H, CONHCO), 8.51 (d, J = 8.3, 1H, Ar−H), 8.09 (s, 1H, Ar-NH), 7.91 (s, 1H, Ar-NH), 7.85 (dd, J = 7.9, 1.5, 1H, Ar−H), 7.72 (d, J = 4.9, 1H, Ar−H), 7.65 (dd, J = 10.7, 5.0, 1H, Ar−H), 7.59 (s, 1H, Ar−H), 7.57−7.53 (m, 1H, Ar−H), 7.52 (s, 1H, Ar−H), 7.45 (dd, J = 7.3, 2.0, 1H, Ar-NH), 7.20−7.15 (m, 1H, Ar−H), 7.15−7.08 (m, 1H, Ar−H), 6.76−6.66 (m, 1H, Ar−H), 4.90 (dd, J = 11.9, 5.4, 1H, COCHN), 4.57 (s, 2H, COCH2O), 4.47 (dt, J = 12.1, 6.1, 1H, OCH), 3.78−3.44 (m, 16H, OCH2, NCH2), 3.09 (d, J = 10.3, 2H, CH2), 2.90−2.54 (m, 7H, CH2, CH), 1.39−1.25 (m, 16H, CH3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.37, 171.76, 168.78, 166.92, 166.64, 165.91, 157.46, 155.31, 155.28, 154.43, 144.78, 138.45, 137.03, 134.69, 133.62, 131.25, 127.66, 127.04, 124.78, 123.61, 123.13, 120.86, 119.42, 117.99, 117.27, 111.02, 105.73, 71.60, 70.32, 70.20, 69.93, 69.43, 67.89, 60.40, 55.45, 54.29, 53.94, 49.28, 39.06, 38.95, 37.76, 32.62, 32.32, 31.45, 29.69, 22.73, 22.22, 21.07, 18.96. HRMS (DART-TOF) calculated for $C_{54}H_{69}ClN_9O_{13}S$ $[M + H]^+$ m/z 1118.4424, found 1118.4403.

4.2. ALK inhibition assays

The ALK activity assay was conducted by Sundia MediTech Company, Ltd. (China). Briefly, the compound, ALK enzyme (Carna, 08−518), Kinase substrate22 and ATP (Km, Sigma) were diluted in kinase buffer to the indicated concentrations. The assay plate was covered and incubated at room temperature. The data were collected on Caliper EZ Reader and the curves were fitted by Graphpad Prism 5.0.

4.3. Cell viability assay

The human cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in RPMI 1640 or DMEM supplemented with 10% (v/v) fetal bovine serum (Gibco) and 1% (v/v) penicillin− streptomycin (HyClone) at 37 °C with 5% CO_2. Cells were seeded in a 96-well plate for 24 h and then an equal volume of medium containing various concentrations of compounds were added to each well. After 72 h, MTT was added, and the cells were incubated for an additional 1−4 h. The absorbance values (OD) of the 96-well plate were measured at 450 nm using a Spectra MAX M5 microplate spectrophotometer (Molecular Devices). The IC_{50} values were the means of at least three independent experiments and calculated by GraphPad Prism5 software.

4.4. Western blot assay

H3122 cells were treated with **B3** at different concentrations at 37 °C, then the cells were washed with PBS before being lysed with RIPA buffer, protease inhibitors, phosphatase cocktails A and B, and PMSF (1 mM). Protein in lysate was determined by the BCA Protein Assay Kit (Beyotime#p0012s). The samples were subjected to SDS−PAGE and then transferred onto PVDF membranes (Millpore). The membranes were incubated overnight at 4 °C with the primary antibody in 5% BSA/TBST buffer with gentle shaking, then washed with 1 × TBS/T 3 times, followed by incubation for 1 h with a 1/5000 dilution of secondary HRP antibody in 5% nonfat milk/TBST. Primary antibodies to anti ALK (no. 3633T), pALK (no. 3341s), IGF1R (no. 9750T), INSR (no.23413T) and GAPDH (no. 2118s) were from Cell Signaling Technology, primary antibodies to STAT3 (no. 10253-2-ap) were from Proteintech Group and primary antibodies to p-STAT3 (Af3293) were from Affinity. The target blots were detected with chemiluminescence system.

4.5. Pharmacokinetics determination

The pharmacokinetic properties were determined at XPiscoric lnc., China. We carried out pharmacokinetics determinations using adult SD rats (180−250 g, 6−7 weeks, N = 3 per group). **B3** was formulated in DMA/Solutol HS 15/saline (5:10:85, v/v/v) and administered intravenously at a dose of 1 mg/kg. Plasma samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, and 12 h after treatment. Plasma was obtained from the blood samples by centrifugation (8000 rpm for 6 min at 2−8 °C) and stored at −80 °C. All samples of the compound were determined by LC−MS/MS (Shimadzu; API 4000).

4.6. In vivo xenograft studies

The female BALB/c nude mice were purchased from Beijing HFK Bioscience Co. ltd., (Beijing, China). H3122 cells (5×10^6) suspended in 0.1 mL of serum were transplanted to the mice at 6−7 weeks old and weighed 18−22 g. The mice were divided randomly (6 mice for each group) when the size of tumors reached 60−100 mm^3. The mice were dosed orally with **B3** (25, 50 mg/kg/d, dissolved in 10% NMP/90% PEG300), vehicle (10% NMP/90% PEG300), and LDK378 (positive control, 25 mg/kg/d, dissolved in 10% NMP/90% PEG300). The body weight and tumor volume were measured every 3 days. The tumor volume was determined with Vernier calipers and calculated as follows: tumor volume = a × $b^2/2$ (a, long diameter; b, short diameter). All animal experiments have been approved by Institutional Animal Care and Treatment Committee of Sichuan University in China.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2020.113150.

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