Development of selective mono or dual PROTAC degrader probe of CDK isoforms

Fei Zhou, Luyu Chen, Chaoguo Cao, Jiang Yu, Xiaojiao Luo, Peiting Zhou, Lifeng Zhao, Wu Du, Jijun Cheng, Yongmei Xie, Yuanwei Chen

PII: S0223-5234(19)31104-3

DOI: https://doi.org/10.1016/j.ejmech.2019.111952

Reference: EJMECH 111952

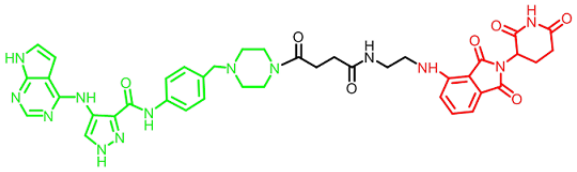
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 9 October 2019

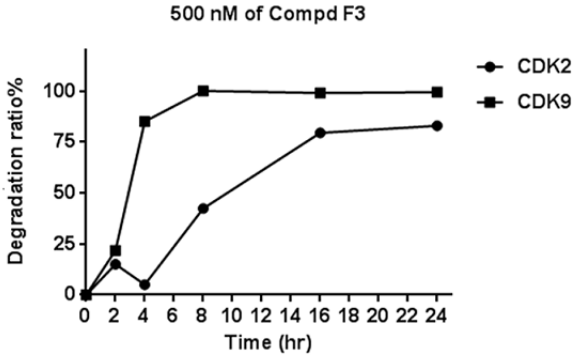
Revised Date: 27 November 2019

Accepted Date: 3 December 2019

Please cite this article as: F. Zhou, L. Chen, C. Cao, J. Yu, X. Luo, P. Zhou, L. Zhao, W. Du, J. Cheng, Y. Xie, Y. Chen, Development of selective mono or dual PROTAC degrader probe of CDK isoforms, *European Journal of Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.ejmech.2019.111952.



Dual CDK2/9 degrader (compound F3)
CDK2(DC_{50}): 62 nM
CDK9(DC_{50}): 33 nM
PC-3(IC_{50}): 0.12 μM



Development of selective mono or dual PROTAC degrader probe of CDK isoforms

Fei Zhou[a], Luyu Chen[a], Chaoguo Cao[a], Jiang Yu[a], Xiaojiao Luo[a], Peiting Zhou[a], Lifeng Zhao[b], Wu Du[c], Jijun Cheng[c], Yongmei Xie[a], Yuanwei Chen[a, c, *]

[a] State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University and Collaborative Innovation Center of Biotherapy, Chengdu 610041, China

[b] Sichuan Industrial Institute of Antibiotics, Chengdu University, Chengdu 610052, China

[c] Hinova Pharmaceuticals Inc, 4th Floor, Building RongYao A, No. 5, Keyuan South Road, Chengdu, 610041, China

* Corresponding author.

E-mail address: ywchen@scu.edu.cn

Highlights

1. We designed and synthesized two series PROTAC compounds by tethering CDK inhibitors with CRBN ligands.
2. We identified compounds differentially induced dual CDK2/9 degradation, or selective to CDK2 or to CDK9.
3. Compound **F3** is a potent dual degrader for CDK2 (DC_{50}: 62 nM) and CDK9 (DC_{50}: 33 nM).
4. Compound **F3** suppresses prostate cancer PC-3 cell proliferation by interfering with cell cycle progression.

Abstract

Cyclin-dependent kinase (CDK) family members are promising molecular targets in discovering potent inhibitors in disease settings, they function differentially. CDK2, CDK4 and CDK6, directly regulate the cell cycle, while CDK9 primarily modulates the transcription regulation. In discovering inhibitors of these CDKs, toxicity associated with off-target effect on other CDK homologs often posts as a clinical issue and hinders their further therapeutic development. To improve efficacy and reduce toxicity, here, using the Proteolysis Targeted Chimeras (PROTACs) approach, we design and further optimize small molecule degraders targeting multiple CDKs. We showed that heterobifunctional compound **A9** selectively degraded CDK2. We also identified a dual-degrader, compound **F3**, which potently induced degradation of both CDK2 (DC_{50}: 62 nM) and CDK9 (DC_{50}: 33 nM). In human prostate cancer PC-3 cells, compound **F3** potently inhibits cell proliferation by effectively blocking the cell cycle in S and G2/M phases. Our preliminary data suggests that PROTAC-oriented CDK2/9 degradation is potentially an effective therapeutic approach.

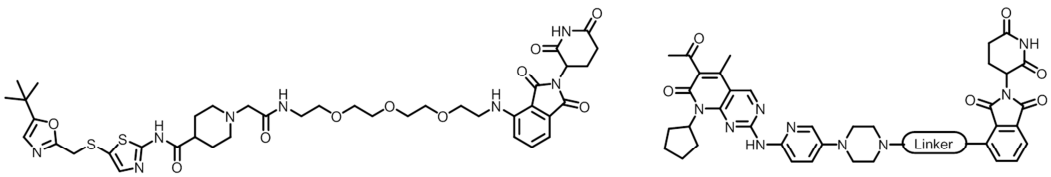
Key words: PROTAC, Cell cycle, CDK2, CDK9, Prostate cancer.

1. Introduction

Cyclin dependent kinases (CDKs) are serine/threonine protein kinases encoded by 21 genes in human [1, 2]. One class of CDKs, CDKs 1, 2, 4 and 6, play extensive roles in eukaryotic cell cycle checkpoint regulation. A second class of the sub-branch of the family, CDKs 7, 8, 9, 12 and 13, were identified as transcriptional regulators [3].

Of particular interest is CDK4/6, which, in the cell cycle forms an active complex with cyclin D that which phosphorylates the retinoblastoma protein (pRb) to reduce the inhibition of transcription factor activity of the E2F family. Later during late G1 phase, CDK2 takes turn, upon binding to cyclin E, to further phosphorylate the Rb protein, potently relieving the E2F suppression to allow cell cycle entry into S phase. During the entire S phase, CDK2 in complex with cyclin A controls the progression of DNA synthesis [4, 5]. To further understand the activation mechanism of CDKs, the crystal structure of CDK2 was first identified within the CDK family [6,7], followed by structure resolution of the cyclinA-CDK2 complex, providing a computational model to understand the mechanism of CDKs activation [8]. In cancers where cell proliferation is deregulated, overexpression of CDKs is often the driver of cancer pathogenesis, and thus, targeting CDKs has become of much interest to combat cancer. [9].

Small molecule CDK inhibitors have been used in clinical studies to treat various cancers, including, but not limited to, acute myeloid leukemia (AML), breast cancer (BC), non-small cell lung cancer (NSCLC), and prostate cancer (PC) [10-13]. However, poor therapeutic efficacy and serious toxicity response have hindered their clinical development [14]. Emerging Proteolysis Targeted Chimeras (PROTACs) technique, which tethers a small molecule to a ligand for the E3 ubiquitin ligase, converts target inhibitors into advantageous target degraders [15, 16]. Determination of crystal structure of the DDB1–CRBN bound to the drug thalidomide, and studies of phthalimide binding to the CRL4CRBN E3 complex for ubiquitination and subsequent proteasome-mediated degradation, have recently helped to extend the capacities of the PROTAC technique [17-20]. The PROTAC technique has been shown to selectively degrade specific homologous proteins, including members of the bromodomain-containing proteins, as well as CDKs [21-23]. Out of the few reported CDK9 degraders, THAL-SNS-032 stand out as a highly efficient degrader for CDK9 [23], and showed differential pharmacological effects between inhibitors and degraders. Recently, degraders designed based on three FDA approved CDK4/6 inhibitors palbociclib, ribociclib, and abemaciclib, also achieved selective degradation of CDK4 and CDK6 or dual CDK4/6 [24] (**Figure 1**).



THAL-SNS-032 palbociclib-based CDK4/6 degraders

Figure 1. Representative CDK PROTACs

To date, PROTAC technique has been applied to a number of kinase targets [25-27]. CDK2 has not been identified as a degradable target. The ubiquitination of CDK2 could suppress tumor cell growth effectively and safely [28], and the degradation of CDK2 could eliminate the block of tumor cell differentiation [29]. Development of degrader probe targeting CDK2 may help to understand the CDK2 function as well as to explore potential therapeutic intervention to cancer. In this study,

for the first time we report the design and synthesis of novel CDK small molecule PROTAC degraders which degrade CDK2 solely or CDK2/9 dually. Some of them strongly inhibit proliferation of prostate cancer PC-3 cells through mechanism of down-regulating the CDK2/9 signaling pathways including proto-oncogene products, suggesting potential therapeutic usage.

2. Results and discussion

2.1. Binding-mode analysis

Two pan CDK inhibitors which designed based on CDK2 crystal structure are proposed to conjugate to cereblon (CRBN) ligands [30, 31]. To identify suitable attachment points, we modeled the structure of CDK2 complexed with AT-7519 and FN-1501 respectively. The best poses were visualized with DS3.5 (**Figure 2A**). As expected, hydrophobic end of the inhibitor should be attached with linkers. Linker length and the attachment points of CRBN ligands are uncertain factors [32]. Thus, we designed series compounds and predicted that the chemical structures would not affect the ability of the degraders to bind the target proteins (**Figure 2B**).

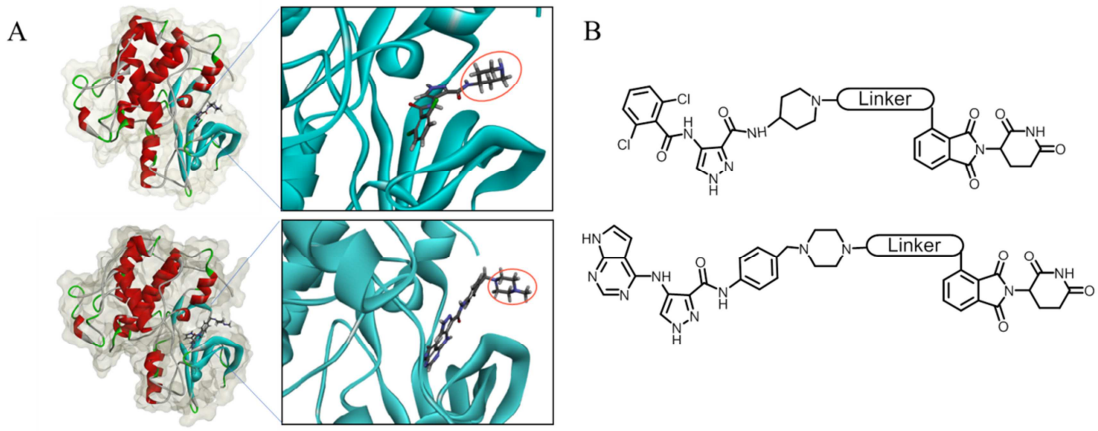
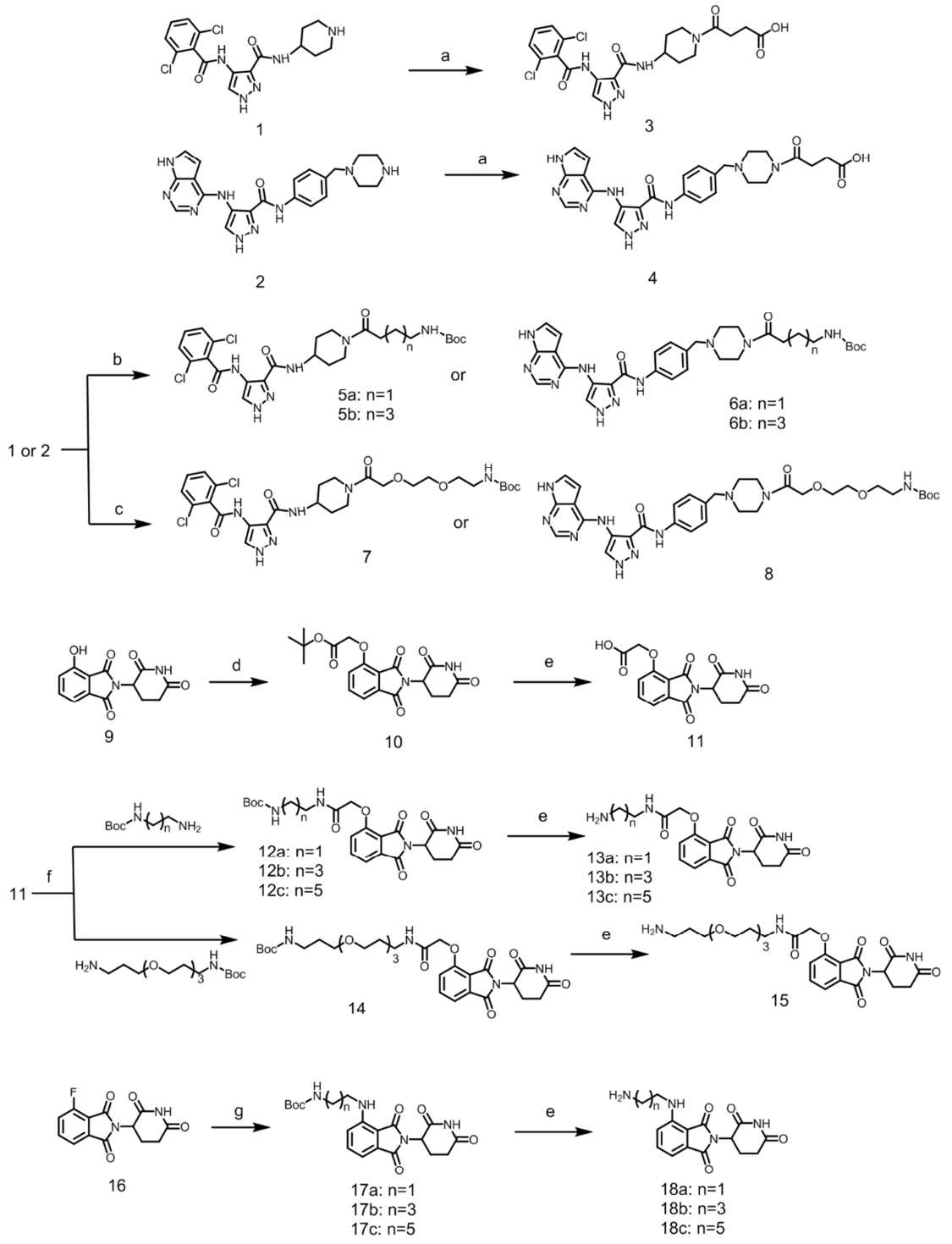


Figure 2. Cocrystal structures of CDK2 complexed with AT-7519 and FN-1501 (PDB code: 2VTH). (A) The best pose of AT-7519 (top panel) and FN-1501 (bottom panel) in CDK2 crystal structure. (B) Chemical structures of designing PROTAC molecules based on AT-7519 and FN-1501.

2.2. Chemistry

AT-7519-based (**A1**-**A10**) and FN-1501-based (**F1**-**F10**) compound series share the same flexible chains connecting CDK inhibitors with pomalidomide or 3-hydroxy thalidomide. In addition to alkyl linkers with different lengths, we employed two hydrophilic alkyl ether chains (**Figure 3**). CDKs ligands **1** and **2** and CRBN ligands **9** and **16** were prepared as previously described [24, 30, 31]. Intermediates **3** and **4** were prepared from **1** and **2** with succinic anhydride. Compounds **5**-**8** were prepared from compounds **1** and **2** by acylation with various linkers. Compounds **12** and **14** were obtained from **9** through three steps synthesis. A mixture of compound **16** and Mono-Boc protected alkyl diamines in 1-methyl-2-pyrrolidinone (NMP) and DIPEA was stirred at 90 °C for 12 hours to give compound **17**. Finally, target compounds were prepared by connecting the intermediates after Boc-deprotection (**Scheme 1**).



Scheme 1. Reagents and conditions: (a) Succinic anhydride, dichloromethane (DCM), Et_3N, rt, 24 h; (b) N-Boc-γ-aminobutyric acid or N-Boc-aminohexanoic acid, HATU, DIPEA, DMF, rt, 12 h; (c) 8-tert-butyloxycarbonylamino-3,6-dioxaoctanoic acid, HATU, DIPEA, DMF, rt, 12 h; (d)

Tert-butyl bromoacetate, KI, $KHCO_3$, DMF, 60 °C, 12 h; (e) CF_3COOH, DCM, rt, 2 h; (f) HATU, DIPEA, DMF, rt, 12 h; (g) NMP, DIPEA, 90 °C, 12 h.

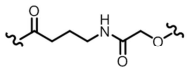
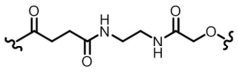
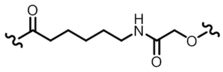
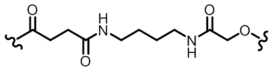
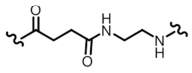
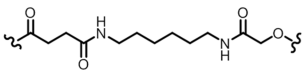
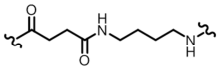
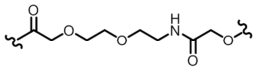
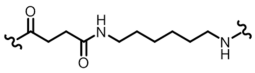
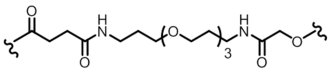
Code	Linker	Code	Linker
A1/F1		A6/F6	
A2/F2		A7/F7	
A3/F3		A8/F8	
A4/F4		A9/F9	
A5/F5		A10/F10	

Figure 3. List of linkers used in designed degraders.

2.3. Western blotting and cellular activity analysis

We first evaluated the ability of 20 target compounds to induce degradation of the primary CDK targets of AT-7519 in AR-negative human prostate cancer PC-3 cells by Western blotting. Cells were treated with **A1**-**A10** and **F1**-**F10** respectively for 12 hours. Western blotting showed that compounds **A2** and **A9** induced selective CDK2 degradation at 1 μM effectively while sparing CDK5 and CDK9 (**Figure 4A**). Compound **A2** showed degradation activity against both CDK2 and CDK9 at concentration of 5 μM. Compound **A9** induced a modest reduction of CDK9 similar to inhibition with AT-7519 treatment at concentration of 5 μM (**Figure 4B**). Compounds **F1**-**F3** achieved dual CDK2/9 degradation at 1 μM; compounds **F5**, **F6**, and **F9** have longer linker connecting the same CDK and CRBN ligands as **F1**-**F3**, achieving selective CDK9 degradation (**Figure 4C**). This selectivity of CDK is due to the differential distance between different CDK subunits and CRBN and the redundant chains affect ternary complex formation. Interestingly, different from low concentration, **F4** could not induce CDK2 degradation at 1 μM and 30μM, demonstrating a 'hook effect'. On the other hand, we did not find any degraders that lost the ability to degrade CDK9 at 30 μM (**Figure 4C and Figure 4E**). Thus, it is feasible to degrade CDK9 by **F** series PROTAC molecules with linkers containing 8-12 atoms. The chain length of 10 atoms is the maximum linker length for achieving degradation of CDK2 (**Figure 4C and Figure 4D**). Compound **F6** has a linker length between those of compounds **F4** and **F5**, but its degradation ability is weaker than both of them, indicating that pomalidomide recruits E3 ubiquitin ligase better than 4-hydroxy thalidomide (**Figure 4D**). To find the most effective degrader, compounds **F2**, **F3**, **F4**, **F5** and **F9** were evaluated at 50 nM concentration (**Figure 4F**), and we found that **F3** is the best whereas all other compounds have little degradation. To further investigate the correlation between CDKs degradation and anti-proliferative, all of target compounds were evaluated against PC-3 cells using the cck-8 assay (**Table 1**). The CDK2 degrader **A9** has an IC_{50} value of 0.84 μM, showing comparable potency to that of AT-7519. The most potent CDK2/9 degrader compound **F3** achieves

an IC_{50} of 0.12 μM and is 4-20 times more potent than other CDK2/9 degraders **F1**, **F2** and **F4**. Compounds **F5**，**F6** and **F9** with selective CDK9 degradation activity showed weaker cell activity than CDK2/9 degraders **F1**-**F4** generally. In contrast, compounds **F7**, **F8**, and **F10** failed to decrease the level of CDK2/9 and thus displayed decreased inhibitory activities, with IC_{50} values ranging from 7.30 to 18.34 μM. In general, the cell activity result correlated well with CDKs degradation activity. The data obtained are summarized in **Table 1**.

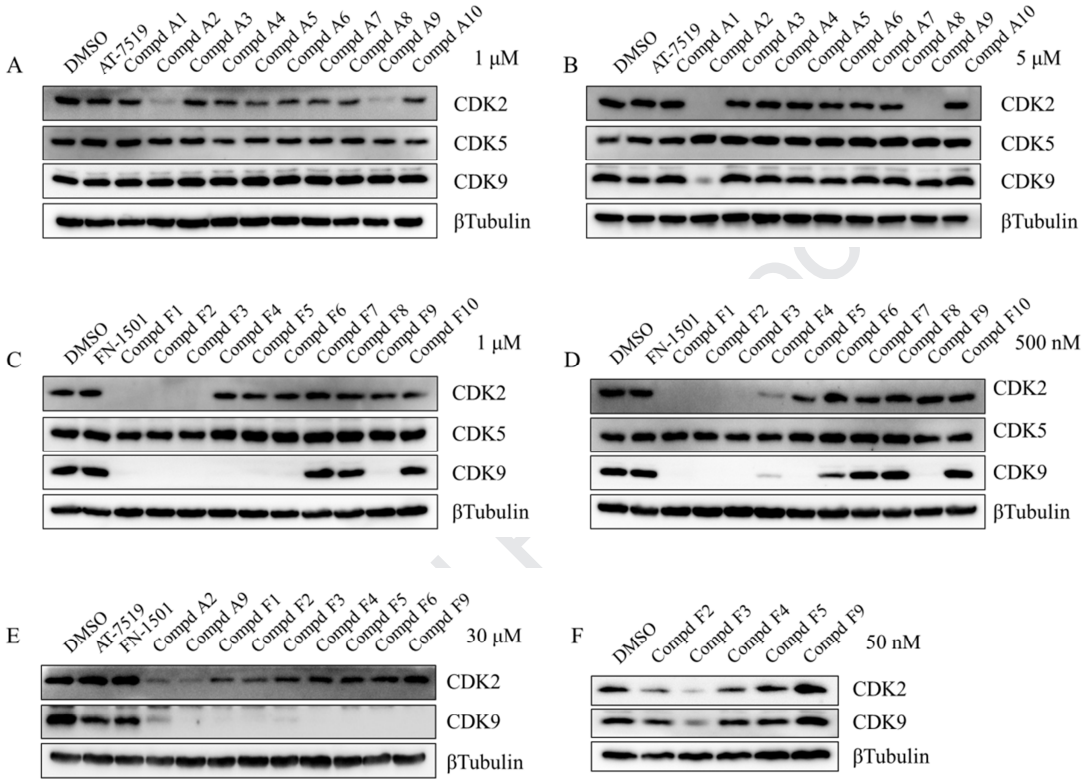


Figure 4. Degradation of CDK2, CDK5 and CDK9 by degraders. PC-3 cells were treated with (A) Compounds **A1**-**A10** at 1 μM, (B) Compounds **A1**-**A10** at 5 μM, (C) Compounds **F1**-**F10** at 1 μM, (D) Compounds **F1**-**F10** at 500 nM, (E) Compounds AT-7519, FN-1501, **A2**, **A9**, **F1**-**F4**, **F5**, **F6 and F9** at 30 μM, (F) Compounds **F2**-**F5** and **F9** at 50 nM, for 12 hours, followed by Western blotting analysis of CDK2, CDK5, CDK9, and loading control β-tubulin.

Table 1 Activity of compounds *in vitro*

Compd	PC-3 IC_{50}±SD (μM)[a]	Degradation	
		CDK2	CDK9
AT-7519	0.71±0.13	−	−
A1	NT	−	−
A2	NT	+	+
A3	5.95±1.16	−	−
A4	23.32±3.17	−	−
A5	14.56±1.63	−	−
A6	NT	−	−
A7	NT	−	−
A8	1.31±0.02	−	−

A9	0.84±0.13	+	−
A10	2.02±0.55	−	−
FN-1501	0.69±0.02	−	−
F1	2.21±0.36	+	+
F2	0.54±0.02	+	+
F3	0.12±0.02	+	+
F4	1.38±0.15	+	+
F5	3.60±0.78	−	+
F6	8.03±1.95	−	+
F7	13.39±1.56	−	−
F8	7.30±1.14	−	−
F9	3.54±0.71	−	+
F10	18.34±0.69	−	−

[a] IC_{50} values were the average of three separate determinations (Mean ± SD). NT, indicates not tested. (-) indicates not degradation detected, (+) indicates degradation detected

2.4. PROTACs Mechanistic studies

To further assess the mechanism of action of these PROTACs beyond CDK degradation, we first performed dose-response and time-course studies with compound **F3**. As shown in **Figure 5A**, compound **F3** showed clearly concentration-dependent CDK2 and CDK9 degradation activity, achieving DC_{50} values as 62 nM and 33 nM, respectively. To determine whether compound **F3** degrades CDK2 and CDK9 synchronously, we evaluated their degradation kinetics in PC-3 cells by treatment with 500 nM compound **F3** in a time course. As shown in **Figure 5B**, degradation ratio of CDK9 increased dramatically by >80% in only 4 hours while CDK2 degradation is minimal. This suggests **F3** preferentially degrade CDK9 over CDK2. Meanwhile, we did not find significant CDK4/6 downregulation, which indicates selective CDK2/9 isoforms degradation. Transcription factor c-Myc stimulates the cell cycle progression and the cellular proliferation. Uncontrolled expression of c-Myc confers cells immortalization. Given c-Myc is historically undruggable, depletion of CDKs may actually be an effective strategy for down-regulating its expression [33]. While studying the kinetics of CDK2/9 degradation, we investigated changes in the protein level of c-Myc (**Figure 5B**). Our data suggests that our PROTAC caused a significant down-regulation of c-Myc in response to CDK2/9 downregulation. Similarly, compound **F3** also down-regulated the Mcl-1 protein level in PC-3 cells (**Figure 5B**). Next, we further explored the mechanism of CDK2/9 degradation induced by compound **F3**. CDK2/9 degradation was inhibited by pretreatment with Pomalidomide and FN-1501, indicating that degradation required engagement of both CRBN and CDK2/9. Furthermore, the degradation was blocked by pretreatment with proteasome inhibitor MG-132, which is consistent with that PROTAC-induced protein degradation is proteasome dependent (**Figure 5C**). Compound **A9** was investigated under similar conditions. We found that it's degradation of CDK2 is also CDK2, CRBN, and proteasome dependent. (**Figure 5D**).

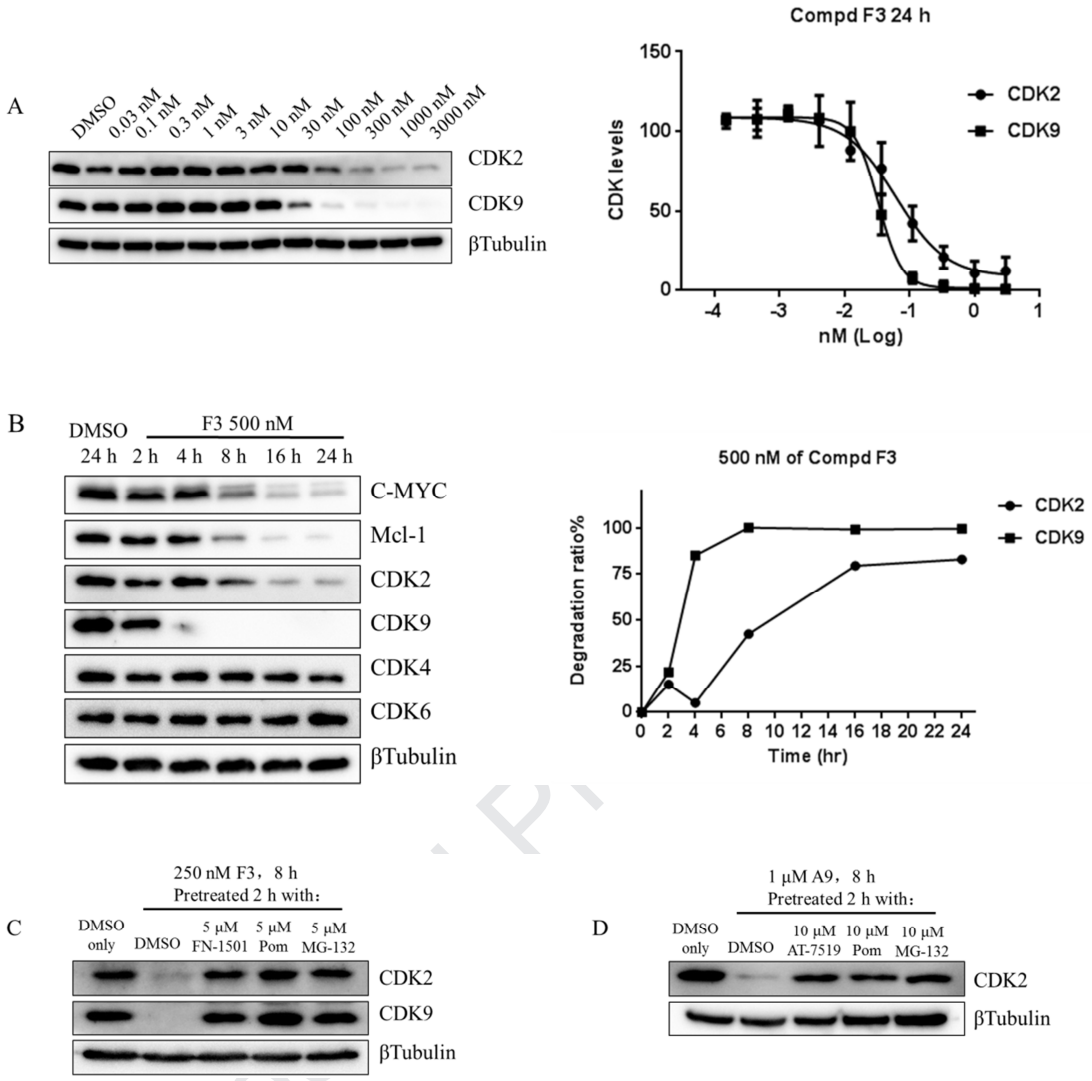
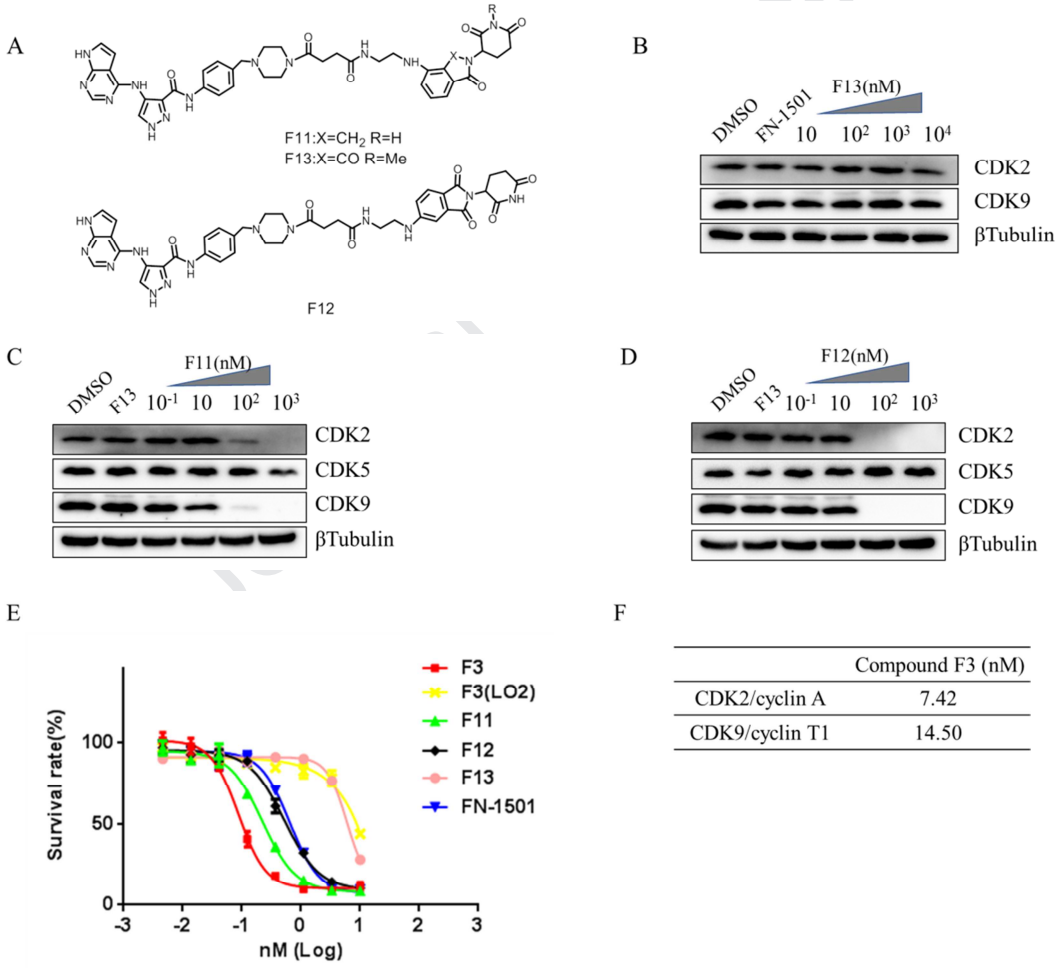


Figure 5. Kinetics and mechanisms of CDK2/9 degradation by compound **F3**. (A) CDK2 and CDK9 degradation dose-response for compound **F3**. PC-3 Cells were treated with compound **F3** at different doses as indicated for 24 hours, followed by Western botting of CDK2, CDK9 and loading control β-tubulin; DC_{50} data represent the mean of 2 determinations. (B) Time-dependent degradation of CDK2, CDK4, CDK6, CDK9, c-Myc and Mcl-1. PC-3 cells were treated with 500 nM of compound **F3** for times as indicated, followed by Western botting analysis. (C) CDK2/9, CRBN, and proteasome-dependent CDK2/9 degradation by compound **F3**. PC-3 cells were pretreated 2 h with 5 μM DMSO, FN-1501, Pomalidomide and MG-132, respectively, followed by 8 h treatment with compound **F3** at 250 nM, followed by Western botting of CDK2, CDK9 and loading control β-tubulin. (D) CDK2, CRBN, and proteasome-dependent CDK2 degradation by compound **A9.** PC-3 cells were pretreated 2 h with 10 μM DMSO, AT-7519, Pomalidomide and MG-132, respectively, followed by 8 h treatment with compound **A9** at 1 μM, followed by Western botting of CDK2 and loading control β-tubulin.

2.5. Development of analogues of compound F3

To further study the structure–degradation activity relationship, we synthesized additional analogs compounds **F11** and **F12** by replacing pomalidomide of compound **F3** with lenalidomide and 4-amino thalidomide, and prepared compound **F13** as a

control analog which lack of ability to bind CRBN (**Figure 6A**) (**Scheme S1**). Compounds **F11** and **F12** significantly reduced CDK2/9 levels, similar to that of compound **F3**, whereas CDK levels were unaffected in the presence of compound **F13** (**Figure 6B**, **Figure 6C and Figure 6D**). Compared to FN-1501 and its analogs, compound **F3** showed the best anti-cancer activities against PC-3 cells with IC_{50} values of 0.12 μM, and weak activity against non-tumor LO2 cells (IC_{50}: 7.99 μM), indicating good selectivity (**Figure 6E**). Interestingly, the anti-proliferation activity of **F13** decreased strikingly, indicating that compound **F3** inhibits cell growth by acting as a CDK degrader but not as a CDK inhibitor (**Figure 6E**). These data suggests that CDK2/9 elimination was critical to inhibit cancer cell proliferation. The cell-free kinase assay confirmed that **F3** inhibits both CDK2 and CDK9, with IC_{50} as 7.42 nM and 14.50 nM, respectively (**Figure 6F)**. Compound **F3** was also able to effectively degrade CDK2/9 in cell lines MCF-7, HCT-116 and 22Rv1, which all have high CDK2/9 expression. (**Figure 6G**).



F

	Compound F3 (nM)
CDK2/cyclin A	7.42
CDK9/cyclin T1	14.50

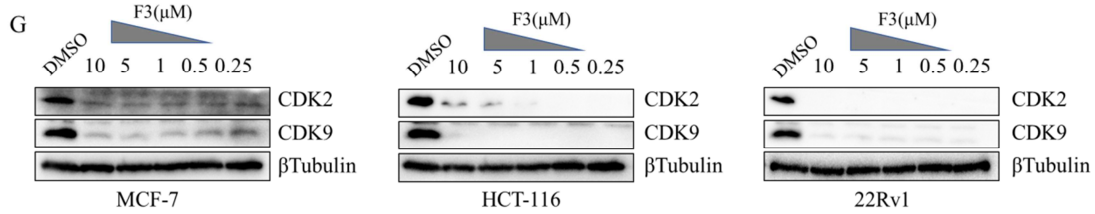
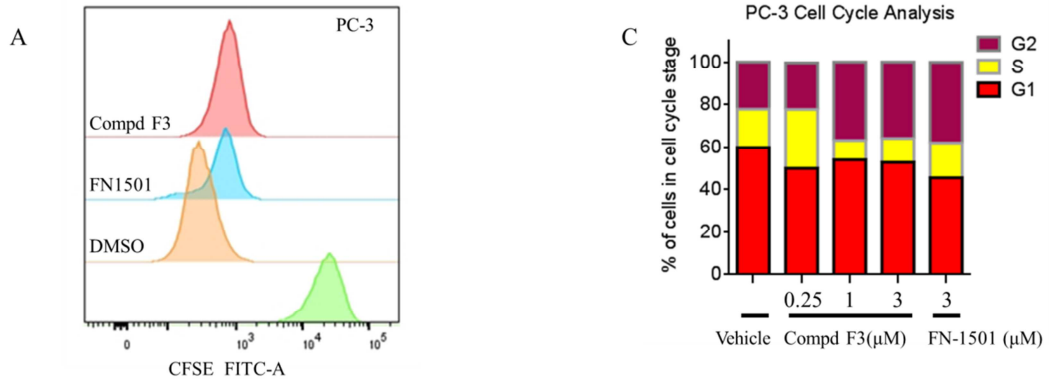


Figure 6. Further characterization of compound **F3**. (A) Chemical structures of compounds **F11-F13**. (B) Dose-dependent CDK2/5/9 degradation studies by compound **F13**, (C) compound **F11** and (D) compound **F12**; PC-3 cells were treated with compounds **F11**, **F12**and **F13** at indicated concentrations for 12 hours, followed by Western blotting analysis of CDK2, CDK5, CDK9 and β-tubulin. Compound **F13** and FN-1501 at 1 μM used as a control. (E) Growth inhibitory curves of indicated compounds in PC-3 cells and LO2 cells. IC_{50} values were the average of three separate determinations. (F) Inhibitory activities of compound **F3** toward CDK2 and CDK9 kinase in vitro. (G) Degradation of CDK2 and CDK9 by **F3** in different cell lines as indicated. The cells were treated with compound **F3** at indicated concentrations for 12 hours, followed by Western botting of CDK2, CDK9 and loading control β-tubulin.

2.6. F3 induced cell cycle arrest

To investigate mechanisms of **F3** anti-proliferation activity, we utilized the CFSE-labeling. After PC-3 cells were treated with 2 μM compound **F3** for 24 hours, the fluorescence signal significantly different from that of the DMSO treated control group, demonstrating that compound **F3** severely affect the proliferation of PC-3 cells (**Figure 7A**). To further investigate whether the anti-proliferation activities were due to cell cycle arrest, we performed cell cycle arrest assay by flow cytometry (**Figure 7B**). Compound **F3** obviously prolonged the S phase by 9.8% at 250 nM concentration, and, when the concentration increases, induced cell cycle blockage at the G2/M phase.



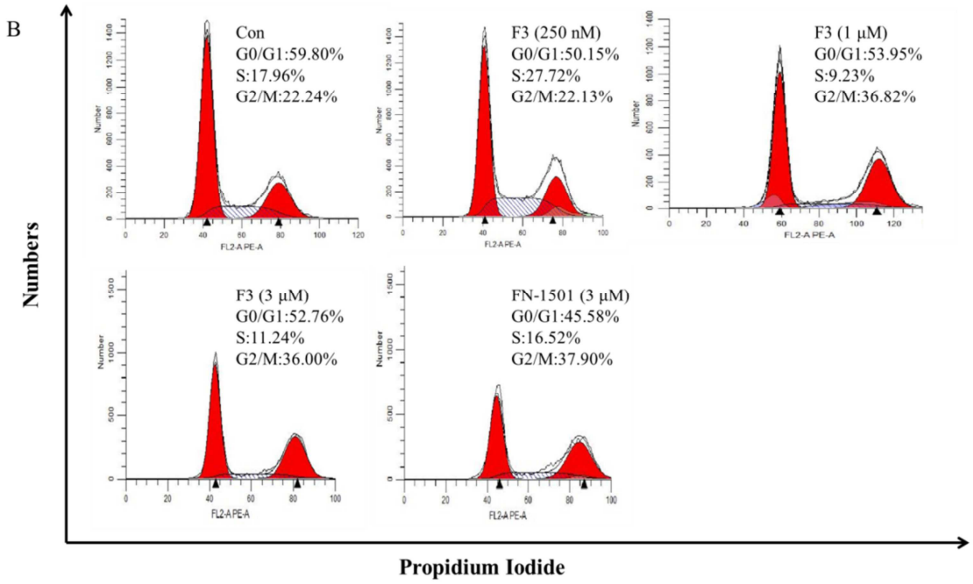


Figure 7. Compound **F3** inhibits PC-3 cell proliferation and arrest cell cycle in G2/M phases. (A) Flow cytometry analysis of CFSE-labeled PC-3 cells; PC-3 cells were treated with compound **F3** at 2 μM for 24 hours. CFSE was added to the single cell suspension to achieve a final concentration of 5 μM, and then incubated for 15 min at room temperature in the dark, followed by flow cytometry analysis (B-C) Effects of **F3** on PC-3 cell cycle progress. PC-3 cells were incubated with compound **F3** or FN-1501 at the indicated concentrations for 48 hours, and further incubated with 50 mg/mL propidium iodide for 20 minutes in the dark, followed by flow cytometry analysis.

3. Conclusion

In this study, we designed and synthesized two series of compounds, and evaluated their degradation activity against CDKs by Western blotting. In one series, we identified a CDK2 selective degrader **A9**, which are valuable as starting prototypes in designing more potent CDK2 degraders. Most compounds in FN-1501-based series achieved either dual CDK2/9 degradation or selective CDK9 degradation. Structure–degradation relationship studies led to compound **F3**, which showed potent and rapid degradation of CDK2 and CDK9. Cell cycle analysis revealed that compound **F3** suppressed PC-3 proliferation by delaying/arresting the cell cycle in S/G2/M phases. Degradation of CDK2/9 activities of compound **F3** in three different cancer cell lines suggests that it has potential to treat multiple cancer types. In summary, targeted degradation of CDK2/9 in cancers is a promising therapeutic method, and compound **F3** and its analogs worth further investigations.

4. Experimental section

4.1. Chemistry

All commercially obtained reagents and solvents were used as received without further purification. Flash chromatography was performed using Biotage Isolera One apparatus with Agela normal-phase silica cartridges. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AMX 400 spectrometer. The spectra were referenced against the deuterated solvents with tetramethylsilane (TMS). In the spectral data reported, the format (δ) chemical shift (multiplicity, *J* values in Hz,

integration) was used with the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. HRMS spectra were measured on Q-Tofmicro Premier mass spectrometer (Micromass, Manchester, UK) with an electrospray ionization (ESI) source.

4.1.1. *4-(4-(4-(2,6-dichlorobenzamido)-1H-pyrazole-3-carboxamido)piperidin-1-yl)-4-oxobutanoic acid (**3**)*

Et_3N (150 μL, 1.1mmol, 3 eq) was added to a solution of 4-(2,6-dichlorobenzamido)-N-(piperidin-4-yl)-1H-pyrazole-3-carboxamide(**1**) (180 mg, 0.37 mmol, 1.0 eq) and succinic anhydride (44 mg, 0.44 mmol, 1.2 eq) in DCM. The solution was stirred at room temperature for 24 h. After concentration, the residue was purified by flash column chromatography to afford the title compound (**3**) as a white solid (140 mg, 80% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.34 (s, 1H), 7.61 – 7.52 (m, 3H), 4.34 (d, *J* = 12.8 Hz, 1H), 3.97 – 3.89 (m, 2H), 3.04 (t, *J* = 12.4 Hz, 1H), 2.63 – 2.50 (m, 3H), 2.42 – 2.93 (m, 2H), 1.80 – 1.71 (m, 2H), 1.58 – 1.39 (m, 2H). HRMS (ESI$^+$): calcd for $C_{20}H_{21}Cl_2N_5O_5Na$ $[M + Na]^+$ 504.0817, found 504.0812.

4.1.2. *4-(4-(4-(4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-1H-pyrazole-3-carboxamido)benzyl)piperazin-1-yl)-4-oxobutanoic acid (**4**)*

Compound **4** (927 mg, 90% yield) was obtained according to similar synthetic procedures for **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.42 (s, 1H), 11.88 (s, 1H), 10.25 (s, 1H), 9.53 (s, 1H), 8.58 (s, 1H), 8.39 (s, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.34 – 7.26 (m, 3H), 6.50 (dd, *J* = 3.2, 2.0 Hz, 1H), 3.47 – 3.45 (m, 6H), 3.18 (s, 2H), 2.46 – 2.27 (m, 6H). HRMS (ESI$^+$): calcd for $C_{25}H_{28}N_9O_4$ $[M + H]^+$ 518.2264, found 518.2263.

4.1.3. *tert-butyl(4-(4-(4-(2,6-dichlorobenzamido)-1H-pyrazole-3-carboxamido)piperidin-1-yl)-4-oxobutyl)carbamate(**5a**)*

DIPEA (46 mg, 0.36 mmol, 2.0 eq) and HATU (102 mg, 0.27 mmol, 1.5 eq) were added to a solution of **1** (70 mg, 0.18 mmol, 1.0 eq) and N-Boc-γ-aminobutyric acid (47 mg, 0.23 mmol, 1.3 eq) in DMF (5 mL). The reaction mixture was quenched with H_2O and extracted with EtOAc. The organic layer was separated, washed with brine, dried, and evaporated. The residue was purified by flash column chromatography to afford **5a** (60 mg, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (s, 1H), 8.48 (d, *J* = 2.8 Hz, 1H), 7.35 – 7.27 (m, 3H), 4.91 – 4.80 (m, 1H), 4.63 – 4.53 (m, 1H), 4.25 – 4.07 (m, 1H), 3.94 – 3.81 (m, 1H), 3.23 – 3.08 (m, 2H), 2.80 – 2.66 (m, 1H), 2.39 (t, *J* = 7.2 Hz, 2H), 2.11 – 1.78 (m, 4H), 1.65 – 1.48 (m, 2H), 1.43 (s, 9H). HRMS (ESI$^+$): calcd for $C_{25}H_{32}Cl_2N_6O_5Na$ $[M + Na]^+$ 589.1709, found 589.1703.

4.1.4. *tert-butyl(6-(4-(4-(2,6-dichlorobenzamido)-1H-pyrazole-3-carboxamido)piperidin-1-yl)-6-oxohexyl)carbamate(**5b**)*

Compound **5b** (70 mg, 38% yield) was obtained according to the synthetic procedures for **5a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.40 (d, *J* = 8.4 Hz, 1H), 8.35 (s, 1H), 7.60 – 7.50 (m, 3H), 6.75 (t, *J* = 5.2 Hz, 1H), 4.38 (d, *J* = 12.8Hz, 1H), 4.01 – 3.92 (m, 1H), 3.87 (d, *J* = 13.2 Hz, 1H), 3.10 – 2.97 (m, 1H), 2.90 (q, *J* = 6.8 Hz, 2H), 2.65 – 2.53 (m, 1H), 2.28 (t, *J* = 7.2 Hz, 2H), 1.85 – 1.68 (m,

2H), 1.60 – 1.41 (m, 4H), 1.41 – 1.34 (m, 11H), 1.28 – 1.21(m, 2H). HRMS (ESI^+): calcd for $C_{27}H_{36}Cl_2N_6O_5Na$ $[M + Na]^+$ 617.2022, found 617.2026.

4.1.5. *tert-butyl(4-(4-(4-(4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-1H-pyrazole-3-carboxamido)benzyl)piperazin-1-yl)-4-oxobutyl)carbamate(**6a**)*

Compound **6a** (58 mg, 38% yield) was obtained according to the synthetic procedures for 5a. ^1H NMR (400 MHz, DMSO-d_6) δ 13.46 (s, 1H), 11.90 (s, 1H), 10.27 (s, 1H), 9.54 (s, 1H), 8.59 (s, 1H), 8.40 (s, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.35 – 7.25 (m, 3H), 6.79 (t, *J* = 5.2 Hz, 1H), 6.50 (d, *J* = 3.2 Hz, 1H), 3.51 – 3.39 (m, 6H), 2.94 (q, *J* = 6.8 Hz, 2H), 2.40 – 2.24 (m, 6H), 1.60 (p, *J* = 6.8 Hz, 2H), 1.38 (s, 9H). HRMS (ESI^+): calcd for $C_{30}H_{38}N_{10}O_4Na$ $[M + Na]^+$ 625.2975, found 625.2971.

4.1.6. *tert-butyl(6-(4-(4-(4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-1H-pyrazole-3-carboxamido)benzyl)piperazin-1-yl)-6-oxohexyl)carbamate(**6b**)*

Compound **6b** (100 mg, 52% yield) was obtained according to the synthetic procedures for **5a**. ^1H NMR (400 MHz, DMSO-d_6) δ 13.42 (s, 1H), 11.87 (s, 1H), 10.25 (s, 1H), 9.51 (s, 1H), 8.57 (s, 1H), 8.38 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.35 – 7.24 (m, 3H), 6.74 (t, *J* = 5.6 Hz, 1H), 6.48 (dd, *J* = 3.2, 1.6 Hz, 1H), 3.51 – 3.40 (m, 6H), 2.88 (q, *J* = 6.8 Hz, 2H), 2.40 – 2.22 (m, 6H), 1.50 – 1.42 (p, *J* = 6.8 Hz, 2H), 1.39 – 1.32 (m, 11H), 1.28 – 1.22 (m, 2H). HRMS (ESI^+): calcd for $C_{32}H_{43}N_{10}O_4$ $[M + H]^+$ 631.3469, found 631.3463.

4.1.7.*tert-butyl(2-(2-(2-(4-(4-(2,6-dichlorobenzamido)-1H-pyrazole-3-carboxamido)piperidin-1-yl)-2-oxoethoxy)ethoxy)ethyl)carbamate(**7**)*

Compound **7** (80 mg, 37% yield) was obtained according to the synthetic procedure for 5a. ^1H NMR (400 MHz, $CDCl_3$) δ 9.87 (s, 1H), 8.46 (s, 1H), 7.35 – 7.28 (m, 3H), 7.20 – 7.12 (m, 1H), 5.17 (s, 1H), 4.52 (d, *J* = 12.8 Hz, 1H), 4.33 – 4.08 (m, 2H), 3.87 (d, *J* = 12.0 Hz, 1H), 3.78 – 3.64 (m, 4H), 3.54 (t, *J* = 5.2 Hz, 2H), 3.36 – 3.25 (m, 2H), 3.16 – 3.10 (m, 1H), 2.83 – 2.77 (m, 1H), 2.11 – 1.95 (m, 2H), 1.63 – 1.47 (m, 2H), 1.42 (s, 9H). HRMS (ESI^+): calcd for $C_{27}H_{36}Cl_2N_6O_7Na$ $[M + Na]^+$ 649.1920, found 649.1919.

4.1.8. *tert-butyl(2-(2-(2-(4-(4-(4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-1H-pyrazole-3-carboxamido)benzyl)piperazin-1-yl)-2-oxoethoxy)ethoxy)ethyl)carbamate(**8**)*

Compound **8** (66 mg, 50% yield) was obtained according to the synthetic procedures for 5a. ^1H NMR (400 MHz, MeOD-d_4) δ 8.62 (s, 1H), 8.38 (s, 1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 3.6 Hz, 1H), 6.61 (d, *J* = 3.6 Hz, 1H), 4.22 (s, 2H), 3.67 – 3.56 (m, 6H), 3.54 – 3.45 (m, 6H), 3.22 (t, *J* = 5.6 Hz, 2H), 2.47 – 2.41 (m, 4H), 1.43 (s, 9H). HRMS (ESI^+): calcd for $C_{32}H_{42}N_{10}O_6Na$ $[M + Na]^+$ 685.3186, found 685.3167.

4.1.9. *tert-butyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate (**10**)*

2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (**9**) (274 mg, 1 mmol, 1.0 eq) was dissolved in DMF (10 mL) at room temperature. Potassium bicarbonate (150 mg, 1.5 mmol, 1.5 eq) and tert-butyl bromoacetate (175 μL, 1.2 mmol, 1.2 eq) were then added. The resulting mixture was stirred at 60 °C for 12 h. The reaction mixture was quenched with H_2O and extracted with EtOAc. The organic layer was separated, washed with brine, dried, and evaporated. The residue was purified by flash

column chromatography to afford **10** (260 mg, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.67 (t, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 7.2 Hz, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 5.02 – 4.92 (m, 1H), 4.79 (s, 2H), 2.94 – 2.68 (m, 3H), 2.18 – 2.08 (m, 1H), 1.48 (s, 9H). HRMS (ESI$^+$): calcd for $C_{19}H_{20}N_2O_7Na$ $[M + Na]^+$ 411.1168, found 411.1170.

4.1.10. *tert-butyl(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acet amido)ethyl)carbamate(**12a**)*

Compound **12a** (93 mg, 72% yield) was given according to the synthetic procedures for **5a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 7.80 – 7.66 (m, 2H), 7.54 (d, *J* = 7.2 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 5.16 (t, *J* = 5.6 Hz, 1H), 5.00 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.67 (s, 2H), 3.56 – 3.40 (m, 2H), 3.38 – 3.23 (m, 2H), 2.92 – 2.74 (m, 3H), 2.22 – 2.10 (m, 1H), 1.40 (s, 9H). HRMS (ESI$^+$): calcd for $C_{22}H_{26}N_4O_8Na$ $[M + Na]^+$ 497.1648, found 497.1644.

4.1.11. *tert-butyl(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acet amido)butyl)carbamate(**12b**)*

Compound **12b** (130 mg, 94% yield) was obtained according to the synthetic procedures for **5a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.22 (s, 1H), 7.74 (t, *J* = 7.6 Hz, 1H), 7.63 (s, 1H), 7.55 (d, *J* = 7.2 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 1H), 5.10 – 4.59 (m, 4H), 3.65 – 3.07 (m, 4H), 2.94 – 2.69 (m, 3H), 2.23 – 2.07 (m, 1H), 1.66 – 1.50 (m, 4H), 1.44 (s, 9H). HRMS (ESI$^+$): calcd for $C_{24}H_{30}N_4O_8Na$ $[M + Na]^+$ 525.1961, found 525.1962.

4.1.12. *tert-butyl(6-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acet amido)hexyl)carbamate(**12c**)*

Compound **12c** (113 mg, 80% yield) was obtained according to the synthetic procedures for **5a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (s, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 7.2 Hz, 1H), 7.45 (t, *J* = 5.6 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 5.00 (m, 1H), 4.72 (s, 1H), 4.64 (s, 2H), 3.46 – 3.27 (m, 2H), 3.20 – 3.00 (m, 2H), 2.95 – 2.74 (m, 3H), 2.25 – 2.10 (m, 1H), 1.60 (p, *J* = 6.8 Hz, 2H), 1.54 – 1.32 (m, 15H). HRMS (ESI$^+$): calcd for $C_{26}H_{34}N_4O_8Na$ $[M + Na]^+$ 553.2274, found 553.2279.

4.1.13. *tert-butyl(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2-oxo -7,10,13-trioxa-3-azahexadecan-16-yl)carbamate(**14**)*

Compound **14** (146 mg, 77% yield) was obtained according to the synthetic procedures for **5a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1H), 7.74 (t, *J* = 8.0 Hz, 1H), 7.60 – 7.51 (m, 2H), 7.22 (d, *J* = 8.4 Hz, 1H), 5.05 (t, *J* = 5.6 Hz, 1H), 5.02 – 4.95 (m, 1H), 4.65 (s, 2H), 3.72 – 3.43 (m, 14H), 3.29 – 3.13 (m, 2H), 2.93 – 2.74 (m, 3H), 2.28 (s, 1H), 2.21 – 2.11 (m, 1H), 1.88 (p, *J* = 6.4 Hz, 2H), 1.74 (p, *J* = 6.4 Hz, 2H), 1.43 (s, 9H). HRMS (ESI$^+$): calcd for $C_{30}H_{42}N_4O_{11}Na$ $[M + Na]^+$ 657.2748, found 657.2748.

4.1.14. *tert-butyl(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethy l)carbamate(**17a**)*

DIPEA (199 μL, 1.2 mmol, 3 eq) was added to a solution of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione **16** (110 mg, 0.4 mmol, 1 eq) and N-Boc-Ethylenediamine (70 mg, 0.44 mmol, 1.1 eq) in NMP (5 mL). The mixture was heated to 90 °C for 12 h. Then, the mixture was diluted with EtOAc and washed

with 10% citric acid (aq), saturated sodium bicarbonate, water and three times with brine. The residue was purified by flash column chromatography to afford **17a** (137 mg, 82% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.08 (s, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 7.03 (d, *J* = 7.2 Hz, 1H), 6.99 (t, *J* = 5.6 Hz, 1H), 6.70 (t, *J* = 6.4 Hz, 1H), 5.05 (dd, *J* = 12.8, 5.6 Hz, 1H), 3.43 – 3.37 (m, 2H), 3.13 (q, *J* = 6.0 Hz, 2H), 2.90 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.68 – 2.52 (m, 2H), 2.08 – 1.98 (m, 1H), 1.37 (s, 9H). HRMS (ESI$^+$): calcd for $C_{20}H_{24}N_4O_6Na$ [M + Na]$^+$ 439.1594, found 439.1592.

4.1.15. *tert-butyl(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butyl)carbamate (**17b**)*

Compound **17b** (120 mg, 81% yield) was obtained according to the synthetic procedures for **17a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.08 (s, 1H), 7.57 (t, *J* = 7.6 Hz, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 7.02 (d, *J* = 7.2 Hz, 1H), 6.82 (t, *J* = 5.6 Hz, 1H), 6.54 (t, *J* = 6.4 Hz, 1H), 5.05 (dd, *J* = 12.8, 5.6 Hz, 1H), 3.32 – 3.25 (q, *J* = 6.8 Hz, 2H), 2.95 (q, *J* = 6.0 Hz, 2H), 2.92 – 2.83 (m, 1H), 2.66 – 2.51 (m, 2H), 2.11 – 1.97 (m, 1H), 1.55 (p, *J* = 7.2 Hz, 2H), 1.46 (p, *J* = 7.2 Hz, 2H), 1.37 (s, 9H). HRMS (ESI$^+$): calcd for $C_{22}H_{28}N_4O_6$ Na [M + Na]$^+$ 467.1907, found 467.1912.

4.1.16. *tert-butyl(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)carbamate (**17c**)*

Compound **17c** (110 mg, 68% yield) was obtained according to the synthetic procedures for **17a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.08 (d, *J* = 7.2 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.24 (t, *J* = 5.6 Hz, 1H), 4.92 (dd, *J* = 12.0, 5.6 Hz,, 1H), 3.25 (q, *J* = 6.8 Hz, 2H), 3.11 (q, *J* = 6.8 Hz, 2H), 2.93 – 2.68 (m, 3H), 2.18 – 2.06 (m, 1H), 1.65 (p, *J* = 7.2 Hz, 2H), 1.53 – 1.33 (m, 15H). HRMS (ESI$^+$): calcd for $C_{24}H_{32}N_4O_6Na$ [M + Na]$^+$ 495.2220, found 495.2216.

General procedure for synthesis of compounds **A1**-**A10** and **F1**-**F13**. A solution of compound **5a** (119 mg, 0.21 mmol, 1.0 eq) in 1:1 TFA/DCM was stirred at rt for 2 h. The solvents were evaporated under reduced pressure to give the corresponding de-protected intermediate, which was added to a solution of DIPEA (74 μL, 0.42 mmol, 2.0 eq), HATU (120 mg, 0.31 mmol, 1.5 eq), and compound **11** (70 mg, 0.21 mmol, 1.0 eq) in DMF (5 mL). The resulting mixture was stirred at rt for 12 h. The reaction mixture was quenched with H_2O and extracted with EtOAc. The organic layer was separated, washed with brine, dried, and evaporated. The residue was purified by flash column chromatography to afford **A1** (127 mg, 77% yield) as white solid. Compounds **A1**-**A10** and **F1**-**F13** were obtained using similar procedures for **A1**.

4.1.17. *4-(2,6-dichlorobenzamido)-N-(1-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)butanoyl)piperidin-4-yl)-1H-pyrazole-3-carboxamide (**A1**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (s, 1H), 11.10 (s, 1H), 10.16 (s, 1H), 8.38 (s, 1H), 8.35 (s, 1H), 7.99 (t, *J* = 5.6 Hz, 1H), 7.81 (t, *J* = 7.6 Hz, 1H), 7.62 – 7.47 (m, 4H), 7.40 (d, *J* = 8.4 Hz, 1H), 5.12 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.78 (s, 2H), 4.36 (d, *J* = 12.8 Hz, 1H), 4.01 – 3.89 (m, 1H), 3.81 (d, *J* = 13.2 Hz, 1H), 3.17 (q, *J* = 6.4 Hz, 2H), 3.00 (t, *J* = 12.4 Hz, 1H), 2.90 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.63 – 2.53 (m,

3H), 2.31 (t, *J* = 7.0 Hz, 2H), 2.08 – 1.97 (m, 1H), 1.81 –1.62 (m, 4H), 1.59 – 1.37 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.19 , 170.39 , 170.31 , 167.20 , 167.19 , 165.94 , 163.09 , 160.78 , 155.54 , 137.37 , 135.82 , 133.51 , 133.33 , 132.33 , 131.73 (2C) , 128.88 (2C) , 121.97 , 121.19 , 120.82 , 117.30 , 116.49 , 68.14 , 49.28 (2C) , 46.46 , 44.41 , 40.73 , 38.61 , 32.15 , 31.41 , 30.08 , 25.25 , 22.48 . HRMS (ESI$^+$): calcd for $C_{35}H_{34}Cl_2N_8O_9Na$ $[M + Na]^+$ 803.1723, found 803.1724.

4.1.18. *4-(2,6-dichlorobenzamido)-N-(1-(6-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)hexanoyl)piperidin-4-yl)-1H-pyrazole-3-carboxamide (**A2**)*

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.99 (s, 1H), 10.46 (s, 1H), 9.90 (s, 1H), 8.46 (s, 1H), 7.72 (t, *J* = 8.0 Hz, 1H), 7.62 – 7.47 (m, 2H), 7.34 – 7.24 (m, 4H), 7.19 (dd, *J* = 8.4, 3.2 Hz, 1H), 5.00 (p, *J* = 5.6 Hz, 1H), 4.67 (s , 2H), 4.52 (d, *J* = 13.2 Hz, 1H), 4.20 – 4.03 (m, 1H), 3.83 (d, *J* = 12.0 Hz, 1H), 3.43 –3.30 (m, 2H), 3.10 (t, *J* = 12.4 Hz, 1H), 2.90 – 2.68 (m, 5H), 2.39 – 2.26 (m, 2H), 2.15 (m, 1H), 2.06 – 1.96 (m, 1H), 1.89 (m, 1H), 1.68 – 1.44 (m, 6H), 1.42 – 1.33 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.27 , 171.81 , 169.20 , 167.14 , 166.67 , 166.15 , 163.02 , 161.55 , 154.66 , 137.12 , 135.37 , 133.45 , 132.75 , 132.47 (2C) , 131.00 , 128.13 (2C) , 122.67 , 121.60 , 120.08 , 118.12 , 117.44 , 68.46 , 49.20 , 46.34 , 44.73 , 40.87 , 39.09 , 32.92 , 32.51 , 31.32 , 29.68 , 28.88 , 26.61 , 25.13 , 22.76 . HRMS (ESI$^+$): calcd for $C_{37}H_{38}Cl_2N_8O_9Na$ $[M + Na]^+$ 831.2036, found 831.2039.

4.1.19. *4-(2,6-dichlorobenzamido)-N-(1-(4-((2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)amino)-4-oxobutanoyl)piperidin-4-yl)-1H-pyrazole-3-carboxamide (**A3**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.45 (s, 1H), 11.09 (s, 1H), 10.17 (s, 1H), 8.39(d, *J* = 8.0 Hz, 1H), 8.35 (s, 1H), 8.08 (t, *J* = 5.6 Hz, 1H), 7.63 – 7.52 (m, 4H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.03 (d, *J* = 6.8 Hz, 1H), 6.73 (t, *J* = 6.2 Hz, 1H), 5.06 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.35 (d, *J* = 12.8 Hz, 1H),4.03 – 3.92 (m, 1H), 3.88 (d, *J* = 13.2 Hz, 1H), 3.42 – 3.37 (m, 2H), 3.25 (q, *J* = 6.0 Hz, 2H), 3.03 (t, *J* = 12.8 Hz, 1H), 2.90 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.66 –2.53 (m, 5H), 2.31 (t, *J* = 7.2 Hz, 2H), 2.09 – 1.08 (m, 1H), 1.83 –1.68 (m, 2H), 1.64 – 1.37 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.25 , 172.54 , 170.54 , 170.00 , 169.18 , 167.76 , 163.10 , 160.81 , 146.85 , 136.64 , 135.83 , 132.67 (2C) , 132.33 , 131.72 (2C) , 128.88 (2C) , 121.99 (2C) , 117.66 , 111.00 , 109.71, 49.00 (2C) , 46.48 , 44.28 , 41.87 , 40.80 , 38.62 , 32.09 , 31.45 , 30.99 , 28.33 , 22.65 . HRMS (ESI$^+$): calcd for $C_{35}H_{36}Cl_2N_9O_8$ $[M + H]^+$ 780.2064, found 780.2067.

4.1.20. *4-(2,6-dichlorobenzamido)-N-(1-(4-((4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butyl)amino)-4-oxobutanoyl)piperidin-4-yl)-1H-pyrazole-3-carboxamide (**A4**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.43 (s, 1H), 11.08 (s, 1H), 10.17 (s, 1H), 8.39(d, *J* = 8.4 Hz, 1H), 8.35 (s, 1H) , 7.82 (t, *J* = 5.6 Hz, 1H), 7.61 – 7.52 (m, 4H), 7.10 (d, *J* = 8.4 Hz, 1H), 7.02 (d, *J* = 7.2 Hz, 1H), 6.54 (t, *J* = 6.0 Hz, 1H), 5.05 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.34 (d, *J* = 13.2 Hz, 1H), 4.01 – 3.92 (m, 1H), 3.88 (d, *J* = 13.6 Hz, 1H), 3.31 – 3.26 (m, 2H), 3.07 – 3.01 (m, 3H), 2.90 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.61 – 2.52 (m, 5H), 2.30 (t, *J* = 7.2 Hz, 2H), 2.02 – 1.98 (m, 1H), 1.83 – 1.68 (m, 2H),

1.60 – 1.39 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.25 , 171.73 , 170.54 , 170.08 , 169.40 , 167.76 , 163.13 , 160.78 , 146.87 , 136.72 , 135.83, 132.66 (2C) , 132.34 , 131.72 (2C) , 128.89 (2C) , 121.97 (2C) , 117.70 , 110.85 , 109.54, 49.01 (2C) , 46.49 , 44.29 , 42.03 , 40.78 , 38.59 , 32.10 , 31.44 , 30.98 , 28.35 , 27.01 , 26.65 , 22.63 . HRMS (ESI$^+$): calcd for $C_{37}H_{39}Cl_2N_9O_8Na$ [M + Na]$^+$ 830.2196, found 830.2192.

4.1.21. *4-(2,6-dichlorobenzamido)-N-(1-(4-((6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)amino)-4-oxobutanoyl)piperidin-4-yl)-1H-pyrazole-3-carboxamide(**A5**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.41 (d, *J* = 8.0 Hz, 1H), 8.35 (s, 1H), 7.77 (t, *J* = 5.6 Hz, 1H), 7.62 – 7.52 (m, 4H), 7.09 (d, *J* = 8.4 Hz, 1H), 7.02 (d, *J* = 7.2 Hz, 1H), 6.53 (t, *J* = 6.0 Hz, 1H), 5.06 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.34 (d, *J* = 12.8 Hz, 1H), 4.00 – 3.93 (m, 1H), 3.88 (d, *J* = 13.6 Hz, 1H), 3.29 (q, *J* = 6.4 Hz, 2H), 3.09 – 2.95 (m, 3H), 2.89 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.65 – 2.52 (m, 5H), 2.30 (t, *J* = 7.2 Hz, 2H), 2.11 – 1.97 (m, 1H), 1.85 – 1.66 (m, 2H), 1.63 – 1.26 (m, 10H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.25 , 171.63 , 170.54 , 170.10, 169.41 , 167.76 , 162.93 , 160.81 , 146.89 , 136.72 , 135.85 , 132.65 (2C) , 132.31 , 131.72 (2C) , 128.87 (2C) , 122.02 , 117.62 (2C) , 110.83 , 109.49 , 49.02 (2C) , 46.48 , 44.28 , 42.26 , 40.78 , 38.92 , 32.10 , 31.45 , 30.98 , 29.56 , 29.11 , 28.37 , 26.59 , 26.52 , 22.63 . HRMS (ESI$^+$): calcd for $C_{39}H_{43}Cl_2N_9O_8Na$ [M + Na]$^+$ 858.2509, found 858.2515.

4.1.22. *4-(2,6-dichlorobenzamido)-N-(1-(4-((2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethyl)amino)-4-oxobutanoyl)piperidin-4-yl)-1H-pyrazole-3-carboxamide (**A6**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.44 (s, 1H), 11.11 (s, 1H), 10.17 (s, 1H), 8.41 (d, *J* = 8.4 Hz, 1H), 8.35 (s, 1H), 8.01 (t, *J* = 5.6 Hz, 1H), 7.89 (t, *J* = 5.6 Hz, 1H), 7.81 (t, *J* = 7.6 Hz, 1H), 7.61 – 7.47 (m, 4H), 7.41 (d, *J* = 8.4 Hz, 1H), 5.13 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.78 (s, 2H), 4.33 (d, *J* = 12.8Hz, 1H), 4.04 –3.90 (m, 1H), 3.87 (d, *J* = 13.6 Hz, 1H), 3.22 (q, *J* = 5.6 Hz, 2H), 3.14 (q, *J* = 5.6 Hz, 2H), 3.02 (t, *J* = 12.8 Hz, 1H), 2.90 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.65 – 2.53 (m, 5H), 2.30 (t, *J* = 7.2 Hz, 2H), 2.10 – 1.99 (m, 1H), 1.82 – 1.68 (m, 2H), 1.61 – 1.38 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.22 , 172.25 , 170.32 , 170.11 , 167.51 , 167.20 , 165.91 , 163.07 , 160.81 , 155.55 , 137.40 , 135.83 , 133.49 (2C) , 132.33 , 131.73 (2C) , 128.88 (2C) , 121.98 (2C) , 120.93 , 117.27 , 116.50 , 68.07 , 49.28 (2C) , 46.47 , 44.27 , 40.79 , 38.73 , 38.66 , 32.08 , 31.42 , 31.00 , 28.29 , 22.48 . HRMS (ESI$^+$): calcd for $C_{37}H_{37}Cl_2N_9O_{10}Na$ [M + Na]$^+$ 860.1938, found 860.1937.

4.1.23. *4-(2,6-dichlorobenzamido)-N-(1-(4-((4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)butyl)amino)-4-oxobutanoyl)piperidin-4-yl)-1H-pyrazole-3-carboxamide (**A7**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.40 (d, *J* = 8.0 Hz, 1H), 8.34 (s, 1H), 7.97 (t, *J* = 5.6 Hz, 1H), 7.85 – 7.77 (m, 2H), 7.61 – 7.47 (m, 4H), 7.40 (d, *J* = 8.4 Hz, 1H), 5.13 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.78 (s, 2H), 4.34 (d, *J* = 12.4 Hz, 1H), 4.02 – 3.92(m, 1H), 3.89 (d, *J* = 13.2 Hz, 1H), 3.15 (q, *J* = 6.4 Hz, 2H), 3.07 – 2.98 (m, 3H), 2.90 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.65 – 2.51 (m, 5H), 2.30 (t, *J* = 7.2 Hz, 2H),

2.10 – 2.00 (m, 1H), 1.83 – 1.70 (m, 2H), 1.57 – 1.37 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.22 , 171.70 , 170.33 , 170.10 , 167.19 , 167.14 , 165.97 , 162.93 , 160.81 , 155.53 , 137.40 , 135.83 , 133.50 (2C) , 132.32 , 131.73 (2C) , 128.87 (2C) , 122.00 , 120.88 (2C) , 117.29 , 116.51 , 68.14 , 49.29 (2C) , 46.48 , 44.29 , 40.79 , 38.63 , 38.58 , 32.10 , 31.42 , 30.98 , 28.35 , 26.99 , 26.97 , 22.47 . HRMS (ESI$^+$): calcd for $C_{39}H_{41}Cl_2N_9O_{10}Na$ [M + Na]$^+$ 888.2251, found 888.2248.

4.1.24. *4-(2,6-dichlorobenzamido)-N-(1-(4-((6-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)hexyl)amino)-4-oxobutanoyl)piperidin-4-yl)-1H-pyrazole-3-carboxamide(**A8**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (s, 1H), 11.12 (s, 1H), 10.17 (s, 1H), 8.39 (d, *J* = 8.4 Hz, 1H), 8.36 (s, 1H), 7.93 (t, *J* = 5.6 Hz, 1H), 7.81 (t, *J* = 8.0 Hz, 1H), 7.76 (t, *J* = 5.6 Hz, 1H), 7.62 – 7.47 (m, 4H), 7.40 (d, *J* = 8.4 Hz, 1H), 5.13 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.78 (s, 2H), 4.36 (d, *J* = 12.8 Hz, 1H), 4.04 – 3.94 (m, 1H), 3.89 (d, *J* = 13.2 Hz, 1H), 3.16 (q, *J* = 6.4 Hz, 2H), 3.05 – 2.98 (m, 3H), 2.91 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.64 – 2.54 (m, 5H), 2.30 (t, *J* = 7.2 Hz, 2H), 2.09 – 2.01 (m, 1H), 1.84 – 1.68 (m, 2H), 1.59 – 1.33 (m, 6H), 1.33 – 1.25 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.22 , 171.65 , 170.32 , 170.10 , 167.18 , 167.08 , 165.98 , 163.12 , 160.75 , 155.48 , 137.37 , 135.82 , 133.49 , 133.36 , 132.31 , 131.73 (2C) , 128.87 (2C) , 121.98 , 121.17 , 120.84 , 117.31 , 116.51 , 68.14 , 49.29 (2C) , 46.49 , 44.31 , 40.81 , 38.89 , 38.74 , 32.10 , 31.42 , 30.98 , 29.57 , 29.42 , 28.37 , 26.53 , 26.46 , 22.48 . HRMS (ESI$^+$): calcd for $C_{41}H_{45}Cl_2N_9O_{10}Na$ [M + Na]$^+$ 916.2564, found 916.2536.

4.1.25. *4-(2,6-dichlorobenzamido)-N-(1-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethoxy)ethoxy)acetyl)piperidin-4-yl)-1H-pyrazole-3-carboxamide (**A9**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.39 (s, 1H), 11.10 (s, 1H), 10.15 (s, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.35 (s, 1H), 8.01 (t, *J* = 5.6 Hz, 1H), 7.80 (t, *J* = 8.0 Hz, 1H), 7.60 – 7.46 (m, 4H), 7.39 (d, *J* = 8.4 Hz, 1H), 5.11 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.79 (s, 2H), 4.29 (d, *J* = 12.8 Hz, 1H), 4.22 – 4.05 (m, 2H), 4.03 – 3.92 (m, 1H), 3.79 (d, *J* = 13.2 Hz, 1H), 3.61 – 3.52 (m, 4H), 3.48 (t, *J* = 5.6 Hz, 2H), 3.38 – 3.33 (m, 2H), 3.05 – 2.83 (m, 2H), 2.64 – 2.53 (m, 3H), 2.08 – 1.99 (m, 1H), 1.78 – 1.68 (m, 2H), 1.60 – 1.40 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.21 , 170.31 , 167.52 , 167.36 , 167.18 , 165.90 , 163.09 , 160.75 , 155.44 , 137.38 , 135.82 , 133.50 , 133.35 , 132.34 , 131.72 (2C) , 128.89 (2C) , 121.97 , 121.17 , 120.82 , 117.24 , 116.49 , 70.21 , 69.91 (2C) , 69.28 , 68.01 , 49.29 (2C) , 46.33 , 43.84 , 40.85, 38.92 , 32.08 , 31.42 , 22.48 . HRMS (ESI$^+$): calcd for $C_{37}H_{38}Cl_2N_8O_{11}Na$ [M + Na]$^+$ 863.1935, found 863.1934.

4.1.26. *4-(2,6-dichlorobenzamido)-N-(1-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2,18-dioxo-7,10,13-trioxa-3,17-diazahenicosan-21-oyl)piperidin-4-yl)-1H-pyrazole-3-carboxamide(**A10**)*

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.16 (s, 1H), 10.15 (s, 1H), 9.90 (s, 1H), 8.45 (s, 1H), 7.71 (t, *J* = 8.0 Hz, 1H), 7.67 – 7.60 (m, 1H), 7.52 (d, *J* = 7.2 , 1H), 7.35 –7.27 (m, 4H), 7.21 (d, *J* = 8.4 Hz, 1H), 6.86 – 6.78 (m, 1H), 5.01 (dd, *J* = 11.6 Hz, 6.0 Hz 1H), 4.67 (s, 2H), 4.49 (d, *J* = 12.8 Hz, 1H), 4.16 – 4.03 (m, 1H), 3.87 (d, *J* = 13.6 Hz, 1H), 3.66 – 3.54 (m, 10H), 3.51 (t, *J* = 6.0 Hz, 2H), 3.46 (q, *J* = 6.4 Hz, 2H), 3.31 (q, *J* =

6.4 Hz, 2H), 3.10 (t, *J* = 12.4 Hz, 1H), 2.85 – 2.49 (m, 8H), 2.18 – 2.10 (m, 1H), 2.06 – 1.89 (m, 2H), 1.85 (p, *J* = 6.4 Hz, 2H), 1.74 (p, *J* = 6.4 Hz, 2H), 1.58 – 1.44 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.60 , 172.02 , 170.46 , 168.99 , 167.09 , 166.69 , 166.07 , 163.10 , 161.49 , 154.62 , 137.05 , 135.41 , 133.49 , 132.70 , 132.47 (2C) , 130.96 , 128.12 (2C) , 122.56 , 121.62 , 119.93 , 118.01 , 117.35 , 70.38 , 70.35 , 70.10 , 69.99 , 69.46 , 68.75 , 68.28 , 49.28 (2C) , 46.32 , 44.34 , 41.00 , 37.54 , 36.63 , 32.20 , 31.38 , 29.67 , 29.24 , 28.92 , 28.56 , 22.70 . HRMS (ESI^+): calcd for $C_{45}H_{53}Cl_2N_9O_{13}Na$ $[M + Na]^+$ 1020.3038, found 1020.3038.

4.1.27. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)butanoyl)piperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide (**F1**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.52 (s, 1H), 11.89 (s, 1H), 11.12 (s, 1H), 10.29 (s, 1H), 9.52 (s, 1H), 8.56 (s, 1H), 8.38 (s, 1H), 8.01 (t, *J* = 5.6 Hz, 1H), 7.84 – 7.76 (m, 3H), 7.50 (d, *J* = 7.2 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.33 – 7.24 (m, 3H), 6.48 (d, *J* = 3.2 Hz, 1H), 5.12 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.78 (s, 2H), 3.50 – 3.38 (m, 6H), 3.17 (q, *J* = 6.8 Hz, 2H), 2.90 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.65 – 2.52 (m, 2H), 2.39 – 2.19 (m, 6H), 2.10 – 1.99 (m, 1H), 1.67 (p, *J* = 7.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.21 , 170.51 , 170.31 , 167.21 , 167.19 , 165.94 , 163.28 , 155.54 , 152.16 , 151.65 , 151.03 , 137.60 , 137.37 , 133.63 , 133.50 , 132.76 , 129.60 (2C) , 125.14 , 123.50 , 121.09 (2C) , 120.81 , 120.57 , 117.29 , 116.48 , 103.56 , 97.19 , 68.13 , 61.93 , 53.22 , 52.70 , 49.29 , 45.29 , 41.51 , 38.57 , 31.42 , 29.98 , 25.13 , 22.48 . HRMS (ESI^+): calcd for $C_{40}H_{41}N_{12}O_8$ $[M + H]^+$ 817.3170, found 817.3165.

4.1.28. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(6-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)hexanoyl)piperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide (**F2**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.42 (s, 1H), 11.87 (s, 1H), 11.11 (s, 1H), 10.24 (s, 1H), 9.52 (s, 1H), 8.58 (s, 1H), 8.39 (s, 1H), 7.93 (t, *J* = 5.6 Hz, 1H), 7.86 – 7.78 (m, 3H), 7.50 (d, *J* = 7.2 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.34 – 7.25 (m, 3H), 6.49 (dd, *J* = 3.2, 1.6 Hz, 1H), 5.13 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.77 (s, 2H), 3.50 – 3.37 (m, 6H), 3.15 (q, *J* = 6.4 Hz, 2H), 2.91 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.65 – 2.54 (m, 2H), 2.38 – 2.23 (m, 6H), 2.10 – 2.01 (m, 1H), 1.55 – 1.39 (m, 4H), 1.34 – 1.25 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.20 , 170.90 , 170.31 , 167.18 , 167.08 , 165.98 , 163.25 , 155.52 , 152.12 , 151.65 , 151.04 , 137.60 , 137.39 , 133.66 , 133.49 , 132.76 , 129.58 (2C) , 125.08 , 123.49 , 121.12 (2C) , 120.91 , 120.57 , 117.32 , 116.52 , 103.56 , 97.17 , 68.19 , 61.94 , 53.32 , 52.80 , 49.30 , 45.41 , 41.46 , 38.70 , 32.66 , 31.43 , 29.32 , 26.52 , 24.95 , 22.48 . HRMS (ESI^+): calcd for $C_{42}H_{44}N_{12}O_8Na$ $[M + Na]^+$ 867.3303, found 867.3307.

4.1.29. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(4-((2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)amino)-4-oxobutanoyl)piperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide (**F3**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (s, 1H), 11.87 (s, 1H), 11.08 (s, 1H), 10.24 (s, 1H), 9.51 (s, 1H), 8.57 (s, 1H), 8.38 (s, 1H), 8.05 (t, *J* = 5.6 Hz, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.34 – 7.25 (m, 3H), 7.18 (d, *J* = 8.8 Hz, 1H), 7.03 (d, *J* = 7.2 Hz, 1H), 6.73 (t, *J* = 6.0 Hz, 1H), 6.48 (dd, *J* = 3.2, 1.6 Hz, 1H), 5.05 (dd, *J*

= 12.8, 5.6 Hz, 1H), 3.48 – 3.39 (m, 6H), 3.36 (q, *J* = 6.0 Hz, 2H), 3.31 (s, 2H), 3.23 (q, *J* = 6.0 Hz, 2H), 2.89 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.64 – 2.53 (m, 2H), 2.40 – 2.25 (m, 6H), 2.07 – 1.97 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.24 , 172.50 , 170.53 , 170.14 , 169.17 , 167.76 , 163.25 , 152.12 , 151.66 , 151.04 , 146.85 , 137.60 , 136.65 , 133.65 , 132.77 , 132.68 , 129.59 (2C) , 125.08 , 123.50 , 121.14 (2C) , 120.58 , 117.65 , 110.99 , 109.73 , 103.56 , 97.18 , 61.95 , 53.17 , 52.73 , 49.01 , 45.20 , 41.88 , 41.61 , 38.61 , 31.46 , 30.93 , 28.31 , 22.65 . HRMS (ESI$^+$): calcd for $C_{40}H_{42}N_{13}O_7$ $[M + H]^+$ 816.3330, found 816.3326.

4.1.30. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(4-((4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butyl)amino)-4-oxobutanoyl)piperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide* ***(F4)***

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.42 (s, 1H), 11.87 (s, 1H), 11.09 (s, 1H), 10.24 (s, 1H), 9.52 (s, 1H), 8.58 (s, 1H), 8.39 (s, 1H), 7.85 – 7.75 (m, 3H), 7.57 (t, *J* = 7.6 Hz, 1H), 7.35 – 7.24 (m, 3H), 7.10 (d, *J* = 8.4 Hz, 1H), 7.02 (d, *J* = 7.2 Hz, 1H), 6.57 – 6.46 (m, 2H), 5.05 (dd, *J* = 12.8, 5.6 Hz, 1H), 3.52 – 3.40 (m, 6H), 3.33 – 3.23 (m, 4H), 3.07 (q, *J* = 6.4 Hz, 2H), 2.89 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.65 – 2.53 (m, 2H), 2.40 – 2.25 (m, 6H), 2.08 – 1.98 (m, 1H), 1.64 – 1.53 (m, 2H), 1.52 – 1.41 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.25 , 171.71 , 170.54 , 170.23 , 169.40 , 167.76 , 163.24 , 152.13 , 151.65 , 151.04 , 146.87 , 137.59 , 136.71 , 133.65 , 132.74 , 132.65 , 129.58 (2C) , 125.09 , 123.50 , 121.12 (2C) , 120.52 , 117.69 , 110.85 , 109.48 , 103.56 , 97.18 , 61.95 , 53.16 , 52.74 , 49.02 , 45.21 , 42.04 , 41.60 , 38.58 , 31.46 , 30.94 , 28.33 , 27.01 , 26.65 , 22.64 . HRMS (ESI$^+$): calcd for $C_{42}H_{46}N_{13}O_7$ $[M + H]^+$ 844.3643, found 844.3639.

4.1.31. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(4-((6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)amino)-4-oxobutanoyl)piperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide* ***(F5)***

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (s, 1H), 11.87 (s, 1H), 11.07 (s, 1H), 10.24 (s, 1H), 9.51 (s, 1H), 8.57 (s, 1H), 8.38 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.75 (t, *J* = 5.6 Hz, 1H), 7.57 (t, *J* = 7.6 Hz, 1H), 7.34 – 7.24 (m, 3H), 7.08 (d, *J* = 8.8 Hz, 1H), 7.02 (d, *J* = 6.8 Hz, 1H), 6.52 (t, *J* = 6.0 Hz, 1H), 6.48 (dd, *J* = 3.2, 1.6 Hz, 1H), 5.05 (dd, *J* = 12.8, 5.6 Hz, 1H), 3.51 – 3.39 (m, 6H), 3.33 – 3.25 (m, 4H), 3.01 (q, *J* = 6.4 Hz, 2H), 2.88 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.65 – 2.52 (m, 2H), 2.41 – 2.25 (m, 6H), 2.07 – 1.97 (m, 1H), 1.62 – 1.50 (m, 2H), 1.44 – 1.29 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.26 , 171.65 , 170.54 , 170.27 , 169.41 , 167.77 , 163.25 , 152.13 , 151.65 , 151.02 , 146.89 , 137.57 , 136.74 , 133.64 , 132.78, 132.64 , 129.60 (2C) , 125.07 , 123.50 , 121.12 (2C) , 120.60 , 117.63 , 110.84 , 109.48 , 103.56 , 97.20 , 61.94 , 53.15 , 52.73 , 49.02 , 45.21 , 42.24 , 41.59 , 38.90 , 31.44 , 30.94 , 29.54 , 29.10 , 28.33 , 26.55 , 26.50 , 22.63 ..HRMS (ESI$^+$): calcd for $C_{44}H_{50}N_{14}O_7$ $[M + H]^+$ 872.3956, found 872.3953.

4.1.32. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(4-((2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethyl)amino)-4-oxobutanoyl)piperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide* ***(F6)***

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.43 (s, 1H), 11.88 (s, 1H), 11.11 (s, 1H), 10.25 (s, 1H), 9.52 (s, 1H), 8.58 (s, 1H), 8.39 (s, 1H), 7.99 (t, *J* = 5.6 Hz, 1H), 7.88 (t, *J* = 5.6

Hz, 1H), 7.85 – 7.76 (m, 3H), 7.50 (d, *J* = 7.2 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 7.34 – 7.24 (m, 3H), 6.49 (dd, *J* = 3.2, 1.6 Hz, 1H), 5.13 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.77 (s, 2H), 3.59 – 3.40 (m, 6H), 3.31 (s, 2H), 3.22 (q, *J* = 6.0 Hz, 2H), 3.15 (q, *J* = 6.0 Hz, 2H), 2.91 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.67 – 2.53 (m, 2H), 2.40 – 2.24 (m, 6H), 2.10 – 2.00 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.22 , 172.25 , 170.31 , 170.26 , 167.51 , 167.20 , 165.91 , 163.25 , 155.55 , 152.14 , 151.65 , 151.03 , 137.60 , 137.40 , 133.63 , 133.48 , 132.76 , 129.60 (2C) , 125.09 , 123.50 , 121.13 (2C) , 120.96 , 120.57 , 117.28 , 116.51 , 103.56 , 97.20 , 68.09 , 61.93 , 53.13 , 52.71 , 49.29 , 45.18 , 41.59 , 38.74 , 38.64 , 31.43 , 30.94 , 28.28 , 22.49 . HRMS (ESI$^+$): calcd for $C_{42}H_{43}N_{13}O_9Na$ $[M + Na]^+$ 896.3204, found 896.3204.

4.1.33. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(4-((4-(2-((2-(2,6-dioxo piperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)butyl)amino)-4-oxobutanoyl)p iperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide(**F7**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (s, 1H), 11.87 (s, 1H), 11.10 (s, 1H), 10.24 (s, 1H), 9.51 (s, 1H), 8.57 (s, 1H), 8.38 (s, 1H), 7.94 (t, *J* = 5.6 Hz, 1H), 7.87 – 7.73 (m, 4H), 7.49 (d, *J* = 7.2 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.33 – 7.24 (m, 3H), 6.48 (dd, *J* = 3.2, 1.6 Hz, 1H), 5.12 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.77 (s, 2H), 3.50 – 3.37 (m, 6H), 3.31 (s, 2H), 3.15 (q, *J* = 6.0 Hz, 2H), 3.02 (q, *J* = 6.0 Hz, 2H), 2.90 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.69 – 2.55 (m, 2H), 2.43 – 2.26 (m, 6H), 2.09 – 1.98 (m, 1H), 1.48 – 1.35(m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.22 , 171.69 , 170.32 , 170.26 , 167.19 , 167.14 , 165.96 , 163.24 , 155.53 , 152.14 , 151.65 , 151.03 , 137.59 , 137.41 , 133.63 , 133.49 , 132.76 , 129.61 (2C) , 125.08 , 123.50 , 121.13 (2C) , 120.89 , 120.63 , 117.29 , 116.52 , 103.56 , 97.19 , 68.15 , 61.94 , 53.17 , 52.73 , 49.29 , 45.21 , 41.60 , 38.62 , 38.57 , 31.42 , 30.93 , 28.32 , 26.98 , 26.95 , 22.47 . HRMS (ESI$^+$): calcd for $C_{44}H_{47}N_{13}O_9Na$ $[M + Na]^+$ 924.3517, found 924.3518.

4.1.34. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(4-((6-(2-((2-(2,6-dioxo piperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)hexyl)amino)-4-oxobutanoyl) piperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide (**F8**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.42 (s, 1H), 11.88 (s, 1H), 11.12 (s, 1H), 10.25 (s, 1H), 9.53 (s, 1H), 8.59 (s, 1H), 8.39 (s, 1H), 7.92 (t, *J* = 5.6 Hz, 1H), 7.84 – 7.78 (m, 3H), 7.75 (t, *J* = 5.6 Hz, 1H), 7.50 (d, *J* = 7.2 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.34 – 7.25 (m, 3H), 6.49 (dd, *J* = 3.2, 1.6 Hz, 1H), 5.13 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.77 (s, 2H), 3.52 – 3.40 (m, 6H), 3.31 (s, 2H), 3.15 (q, *J* = 6.0 Hz, 2H), 3.01 (q, *J* = 6.0 Hz, 2H), 2.91 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.66 – 2.53 (m, 2H), 2.39 – 2.25 (m, 6H), 2.09 – 2.00 (m, 1H), 1.48 – 1.24 (m, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.22 , 171.63 , 170.32 , 170.25 , 167.19 , 167.07 , 165.97 , 163.25 , 155.48 , 152.12 , 151.65 , 151.03 , 137.59 , 137.37 , 133.65 , 133.48 , 132.76 , 129.58 (2C) , 125.08 , 123.49 , 121.13 (2C) , 120.84 , 120.57 , 117.30 , 116.51 , 103.56 , 97.19 , 68.14 , 61.95 , 53.18 , 52.75 , 49.29 , 45.21 , 41.60 , 38.88 , 38.74 , 31.42 , 30.93 , 29.57 , 29.42 , 28.34 , 26.52 , 26.46 , 22.48 . HRMS (ESI$^+$): calcd for $C_{46}H_{52}N_{13}O_9$ $[M + H]^+$ 930.4011, found 930.4005.

4.1.35. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(2-(2-(2-(2-((2-(2,6-dio xopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethoxy)ethoxy)acetyl)piper azin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide(**F9**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (s, 1H), 11.87 (s, 1H), 11.11 (s, 1H), 10.24 (s, 1H), 9.51 (s, 1H), 8.58 (s, 1H), 8.38 (s, 1H), 8.00 (t, *J* = 5.6 Hz, 1H), 7.84 – 7.76 (m, 3H), 7.49 (d, *J* = 7.2 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.32 – 7.27 (m, 3H), 6.48 (dd, *J* = 3.2, 1.6 Hz, 1H), 5.12 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.79 (s, 2H), 4.13 (s, 2H), 3.55 (br, 4H), 3.48 (t, *J* = 5.6 Hz, 2H), 3.46 – 3.35 (m, 8H), 2.90 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.66 – 2.52 (m, 2H), 2.39 – 2.27 (m, 4H), 2.10 – 2.01 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.21 , 170.32 , 167.63 , 167.34 , 167.18 , 165.90 , 163.24 , 155.42 , 152.12 , 151.65 , 151.04 , 137.60 , 137.39 , 133.58 , 133.50 , 132.74 , 129.59 (2C) , 125.08 , 123.50 , 121.12 (2C) , 120.83 , 120.57 , 117.24 , 116.51 , 103.55 , 97.19 , 70.23 , 69.93 , 69.81 , 69.26 , 68.01 , 61.92 , 53.14 , 52.63 , 49.29 , 44.74 , 41.58 , 38.93 , 31.42 , 22.48 . HRMS (ESI$^+$): calcd for $C_{42}H_{44}N_{12}O_{10}Na$ [M + Na]$^+$ 899.3201, found 899.3209.

4.1.36. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2,18-dioxo-7,10,13-trioxa-3,17-diazahenicosan-21-oyl)piperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide* ***(F10)***

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (s, 1H), 11.87 (s, 1H), 11.10 (s, 1H), 10.24 (s, 1H), 9.51 (s, 1H), 8.57 (s, 1H), 8.38 (s, 1H), 7.94 (t, *J* = 5.6 Hz, 1H), 7.83 – 7.77(m, 3H), 7.75 (t, *J* = 5.6 Hz, 1H), 7.49 (d, *J* = 7.2 Hz, 1H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.34 – 7.25 (m, 3H), 6.48 (d, *J* = 3.6 Hz, 1H), 5.12 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.77 (s, 2H), 3.53 – 3.36 (m, 18H), 3.30 (s, 2H), 3.25 – 3.17 (m, 2H), 3.11 – 3.02 (m, 2H), 2.90 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.66 – 2.53 (m, 2H), 2.43 – 2.25 (m, 6H), 2.10 – 2.00 (m, 1H), 1.67 (p, *J* = 6.4 Hz, 2H), 1.59 (q, *J* = 6.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.22 , 171.75 , 170.31 , 170.26 , 167.19 (2C) , 165.95 , 163.243 , 155.49 , 152.12 , 151.65 , 151.02 , 137.40 (2C) , 133.49 (2C) , 132.69 , 129.64 (2C) , 125.06 , 123.50 , 121.13 (2C) , 120.87 , 120.58 , 117.29 , 116.54 , 103.55 , 97.20 , 70.21 (2C) , 70.03 , 69.96 , 68.53 , 68.40 , 68.14 , 61.87 , 53.13 , 52.70 , 49.29 , 45.18 , 41.53 , 39.66 , 36.26 , 31.41 , 30.90 , 29.82 , 29.68 , 28.30 , 22.47 . HRMS (ESI$^+$): calcd for $C_{50}H_{59}N_{13}O_{12}Na$ [M + Na]$^+$ 1056.4304, found 1056.4299.

4.1.37. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(4-((2-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)ethyl)amino)-4-oxobutanoyl)piperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide****(F11)***

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (s, 1H), 11.87 (s, 1H), 11.00 (s, 1H), 10.24 (s, 1H), 9.51 (s, 1H), 8.57 (s, 1H), 8.38 (s, 1H), 7.97 (d, *J* = 5.2 Hz, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.35 – 7.25 (m, 4H), 6.95 (d, *J* = 7.2 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.48 (dd, *J* = 3.2, 1.6 Hz, 1H), 5.64 (t, *J* = 4.8 Hz, 1H), 5.11 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.28 – 4.10 (m, 2H), 3.49 – 3.41 (m, 6H), 3.32 (s, 2H), 3.27 – 3.19 (m, 4H), 2.93 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.69 – 2.53 (m, 2H), 2.39 – 2.27 (m, 6H), 2.14 – 1.94 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.34 , 172.41 , 171.66 , 170.24 , 169.32 , 163.25 , 152.13 , 151.66 , 151.03 , 143.92 , 137.59 , 133.63 , 132.77 , 132.57 , 129.72 , 129.61 (2C) , 127.04 , 125.37, 123.51 , 121.14 (2C) , 120.60 , 112.21 , 110.69 , 103.56 , 97.19 , 61.94 , 53.15 , 52.73 , 52.01 , 46.11 , 45.21 , 42.75 , 41.61 , 38.37 , 31.71 , 30.97 , 28.30 , 23.29 . HRMS (ESI$^+$): calcd for $C_{40}H_{44}N_{13}O_6$ [M + H]$^+$ 802.3532, found 802.3539.

4.1.38. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(4-((3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)propyl)amino)-4-oxobutanoyl)piperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide(**F12**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (s, 1H), 11.87 (s, 1H), 11.05 (s, 1H), 10.25 (s, 1H), 9.51 (s, 1H), 8.57 (s, 1H), 8.38 (s, 1H), 7.98 (t, *J* = 5.6 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.35 – 7.25 (m, 3H), 7.08 (t, *J* = 5.2 Hz, 1H), 6.99 (d, *J* = 2.0 Hz, 1H), 6.88 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.48 (dd, *J* = 3.2, 1.6 Hz, 1H), 5.03 (dd, *J* = 12.8, 5.2 Hz, 1H), 3.55 – 3.39 (br, 6H), 3.29 (s, 2H), 3.27 – 3.20 (br, 4H), 2.88 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.64 – 2.54 (m, 2H), 2.43 – 2.23 (m, 6H), 2.09 – 1.89 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.27 , 172.43 , 170.60 , 170.25 , 168.11 , 167.59 , 163.25 , 154.82 , 152.14 , 151.66 , 151.03 , 137.59 , 134.66 , 133.64 , 132.73 , 129.62 (2C) , 125.57 , 125.09 , 123.51 , 121.13 (2C) , 120.60 , 116.73 , 116.05 , 105.95 , 103.56 , 97.21 , 61.93 , 53.15 , 52.73 , 49.12 , 45.20 , 42.41 , 41.63 , 38.17 , 31.46 , 30.97 , 28.31 , 22.71 . HRMS (ESI$^+$): calcd for $C_{40}H_{42}N_{13}O_7$ $[M + H]^+$ 816.3330, found 816.3325.

4.1.39. *4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-(4-((2-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)amino)-4-oxobutanoyl)piperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide(F13)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (s, 1H), 11.87 (s, 1H), 10.24 (s, 1H), 9.52 (s, 1H), 8.58 (s, 1H), 8.39 (s, 1H), 8.06 (t, *J* = 5.6 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.59 (t, *J* = 7.2 Hz, 1H), 7.33 – 7.26 (m, 3H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.03 (d, *J* = 7.2 Hz, 1H), 6.73 (t, *J* = 6.4 Hz, 1H), 6.49 (dd, *J* = 3.2, 1.6 Hz, 1H), 5.12 (dd, *J* = 12.8, 5.2 Hz, 1H), 3.52 – 3.41 (m, 6H), 3.41 – 3.34 (m, 2H), 3.24 (d, *J* = 6.4 Hz, 2H), 3.02 (s, 3H), 2.95 (ddd, *J* = 17.6, 14.0, 5.2 Hz, 1H), 2.81 – 2.71 (m, 1H), 2.60 – 2.51 (m, 2H), 2.42 – 2.25 (m, 6H), 2.10 – 1.99 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.50 , 172.25 , 170.27 , 170.15 , 169.15 , 167.73 , 163.25 , 152.12 , 151.65 , 151.03 , 146.89 , 137.62 , 136.68 , 133.59 , 132.76 , 132.66 , 129.61 (2C) , 125.08 , 123.50 , 121.13 (2C) , 120.56 , 117.69 , 111.02 , 109.69 , 104.49 , 97.17 , 61.93 , 53.15 , 52.73 , 49.56 , 45.19 , 41.89 , 41.60 , 38.60 , 31.59 , 30.95 , 28.32 , 27.07 , 21.87 . HRMS (ESI$^+$): calcd for $C_{41}H_{43}N_{13}O_7$ $[M + H]^+$ 816.3325, found 830.3486.

4.2. Molecular modeling

Molecular docking studies were performed using DS 3.5 (Discovery studio 3.5). The crystal structure of CDK2 complex (PDB code: 2VTH) was obtained from the RCSB Protein Data Bank. With the water deleted, ligand removed and force field applied, the receptor was used for the subsequent docking studies. Ligand structure was protonated and conformation searched with DS 3.5. The other parameters were as default. The best output poses of the ligands generated according to CDOCKER ENERGY.

4.3. Cell culture

PC-3(SCSP-532) and MCF-7(SCSP-531) cells were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells were cultured in a 37 °C incubator with 5% CO_2. PC-3 were maintained in DMEM and F12 (1:1) nutrient medium supplemented with 10% (v/v) fetal bovine serum (Gibco) and 1% (v/v) penicillin/streptomycin (HyClone, SV30010). LO2, MCF-7, HCT-116 and

22Rv1 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin.

4.4. Western Blot Analysis.

Cells (5×10^5 cells/well) were plated in 6-well plates and treated with compounds under the indicated conditions. After 24 hours, the cells were washed with ice-cold PBS, lysed and collected with RIPA buffer and protease inhibitors. The lysate was centrifuged at 12000 rpm for 15 min at 4 °C, the concentration of the supernatants was determined using the BCA Protein Assay Kit (beyotime#p0012s). Total proteins (50 μg) were subjected to 10% SDS−PAGE and were then transferred onto PVDF membranes (Millpore). Membranes were blocked for 30 min, then incubated with primary antibodies overnight at 4 °C, then washed with 1 × TBST 3 times, then incubated with secondary HRP antibody for 2 h at room temperature, then washed with 1 × TBST 3 times, followed by washing and detection with chemiluminescence system (Clinx Science Instruments, Chemiscope 5300). The band intensities were quantified using GelQuant.NET software. Skimmed (5%) milk was dissolved in 0.1% TBS-T (10 mM Tris-HCl and Tween 20) and was then used for blocking membranes and diluting antibodies. Primary antibodies used were anti-CDK2 (1/1000 dilution, CST#78B2), anti-CDK5 (1/1000 dilution, CST#D1F7M), anti-CDK9 (1/1000 dilution, CST#C12F7), anti-CDK4 (1/1000 dilution, CST#D9G3E), anti-CDK6 (1/1000 dilution, CST#D4S8S), anti-βTubulin (1/1000 dilution, CST#2148), anti-c-Myc (1/1000 dilution, CST#D84C12), anti-Mcl-1 (1/1000 dilution, D2W9E).

4.5. CCK-8 assay

Cells (80 μL) were seeded in 96-well plates at a density of 3×10^3 per well for 24 h. Compound was tested in 10-dose IC_{50} mode with a 3-fold serial dilution starting at 10 μM. After 5 days, 10 μL CCK-8 reagents (SAB, CP002) was added to each well and the plates were incubated at 37 °C for another 4 h. The experiment was repeated at least twice independently with duplicate measurements. The luminescence was measured at 450 nm via spectrophotometry (Thermo Multiskan MK3). Data was analyzed using GraphPad Prism 5 software.

4.6. Kinase IC_{50} tests.

The *in vitro* kinase enzymatic inhibition assays were carried out by Reaction Biology Corporation (USA). HEK293 cells were seeded into the wells of 384-well plates. The cells were pretreated with the NanoBRET^{TM} Tracer K-10 and then treated with compound for 1 hour. Reactions were carried out at 10 μM ATP. The BRET signal was measured on an Envision 2104 Multilabel Reader. IC_{50} value was calculated and IC_{50} curve was plotted using the GraphPad Prism 4 program based on a sigmoidal dose response equation. Compound was tested in 10-dose IC_{50} mode with a 3-fold serial dilution starting at 1 μM. Control compound (staurosporine) was tested in 10-dose IC_{50} mode with 4-fold serial dilution starting at 20 μM. (http://www.reactionbiology.com)

4.7. Flow cytometry assay

CFSE assay was performed by BD Horizon™ CFSE (565082). Cell cycle distribution was determined by Cell Cycle Kit (KeyGEN#KGA512). The experimental conditions are described in the notes and the experimental methods follow the protocol of the instruction. Data analysis was performed using FlowJo and Modfit software.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81672951).

References

[1] M. Malumbres, M. Barbacid, Mammalian cyclin-dependent kinases, Trends in Biochemical Sciences, 30 (2005) 630-641.

[2] M. Malumbres, E. Harlow, T. Hunt, T. Hunter, J.M. Lahti, G. Manning, D.O. Morgan, L.-H. Tsai, D.J. Wolgemuth, Cyclin-dependent kinases: a family portrait, Nature Cell Biology, 11 (2009) 1275 -1276.

[3] Y.A. Sonawane, M.A. Taylor, J.V. Napoleon, S. Rana, J.I. Contreras, A. Natarajan, Cyclin Dependent Kinase 9 Inhibitors for Cancer Therapy, Journal of Medicinal Chemistry, 59 (2016) 8667-8684.

[4] D.J. Wood, J.A. Endicott, Structural insights into the functional diversity of the CDK-cyclin family, Open Biol, 8 (2018) pii: 180112.

[5] I. Sánchez, B.D. Dynlacht, New insights into cyclins, CDKs, and cell cycle control, Seminars in Cell & Developmental Biology, 16 (2005) 311-321.

[6] H.L. De Bondt, J. Rosenblatt, J. Jancarik, H.D. Jones, D.O. Morgant, S.-H. Kim, Crystal structure of cyclin-dependent kinase 2, Nature, 363 (1993) 595-602.

[7] W. Cheng, Z. Yang, S. Wang, Y. Li, H. Wei, X. Tian, Q. Kan, Recent development of CDK inhibitors: An overview of CDK/inhibitor co-crystal structures, European Journal of Medicinal Chemistry, 164 (2019) 615-639.

[8] P.D. Jeffrey, A.A. Russo, K. Polyak, E. Gibbs, J. Hurwitz, J. Massagué, N.P. Pavletich, Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex, Nature, 376 (1995) 313-320.

[9] M. Johansson, J.L. Persson, Cancer Therapy: Targeting Cell Cycle Regulators, Anti-Cancer Agents in Medicinal Chemistry- Anti-Cancer Agents), 8 (2008) 723-731.

[10] M. Malumbres, P. Pevarello, M. Barbacid, J.R. Bischoff, CDK inhibitors in cancer therapy: what is next?, Trends in Pharmacological Sciences, 29 (2008) 16-21.

[11] J. Cicenas, K. Kalyan, A. Sorokinas, A. Jatulyte, D. Valiunas, A. Kaupinis, M. Valius, Highlights of the Latest Advances in Research on CDK Inhibitors, Cancers, 6 (2014) 2224-2242.

[12] A.B. Heptinstall, I. Adiyasa, C. Cano, I.R. Hardcastle, Recent advances in CDK inhibitors for cancer therapy, Future Medicinal Chemistry, 10 (2018) 1369-1388.

[13] M.H. Rahaman, M. Kumarasiri, L.B. Mekonnen, M. Yu, S. Diab, H. Albrecht, R.W. Milne, S. Wang, Targeting CDK9: a promising therapeutic opportunity in prostate cancer, Endocr Relat Cancer, 23 (2016) T211-T226.

[14] U. Asghar, A.K. Witkiewicz, N.C. Turner, E.S. Knudsen, The history and future of targeting cyclin-dependent kinases in cancer therapy, Nat Rev Drug Discov, 14 (2015) 130-146.
[15] D.P. Bondeson, A. Mares, I.E.D. Smith, E. Ko, S. Campos, A.H. Miah, K.E. Mulholland, N. Routly, D.L. Buckley, J.L. Gustafson, N. Zinn, P. Grandi, S. Shimamura, G. Bergamini, M. Faelth-Savitski, M. Bantscheff, C. Cox, D.A. Gordon, R.R. Willard, J.J. Flanagan, L.N. Casillas, B.J. Votta, W. den Besten, K. Famm, L. Kruidenier, P.S. Carter, J.D. Harling, I. Churcher, C.M. Crews, Catalytic in vivo protein knockdown by small-molecule PROTACs, Nature Chemical Biology, 11 (2015) 611-617.
[16] K.G. Coleman, C.M. Crews, Proteolysis-Targeting Chimeras: Harnessing the Ubiquitin-Proteasome System to Induce Degradation of Specific Target Proteins, Annual Review of Cancer Biology, 2 (2018) 41-58.
[17] J. Lu, Y. Qian, M. Altieri, H. Dong, J. Wang, K. Raina, J. Hines, James D. Winkler, Andrew P. Crew, K. Coleman, Craig M. Crews, Hijacking the E3 Ubiquitin Ligase Cereblon to Efficiently Target BRD4, Chemistry & Biology, 22 (2015) 755-763.
[18] D.P. Bondeson, C.M. Crews, Targeted Protein Degradation by Small Molecules, Annu Rev Pharmacol Toxicol, 57 (2017) 107-123.
[19] P.M. Cromm, C.M. Crews, Targeted Protein Degradation: from Chemical Biology to Drug Discovery, Cell Chemical Biology, 24 (2017) 1181-1190.
[20] E.S. Fischer, K. Böhm, J.R. Lydeard, H. Yang, M.B. Stadler, S. Cavadini, J. Nagel, F. Serluca, V. Acker, G.M. Lingaraju, R.B. Tichkule, M. Schebesta, W.C. Forrester, M. Schirle, U. Hassiepen, J. Ottl, M. Hild, R.E.J. Beckwith, J.W. Harper, J.L. Jenkins, N.H. Thomä, Structure of the DDB1–CRBN E3 ubiquitin ligase in complex with thalidomide, Nature, 512 (2014) 49-53.
[21] M. Zengerle, K.H. Chan, A. Ciulli, Selective Small Molecule Induced Degradation of the BET Bromodomain Protein BRD4, ACS Chem Biol, 10 (2015) 1770-1777.
[22] C.M. Robb, J.I. Contreras, S. Kour, M.A. Taylor, M. Abid, Y.A. Sonawane, M. Zahid, D.J. Murry, A. Natarajan, S. Rana, Chemically induced degradation of CDK9 by a proteolysis targeting chimera (PROTAC), Chem Commun (Camb), 53 (2017) 7577-7580.
[23] C.M. Olson, B. Jiang, M.A. Erb, Y. Liang, Z.M. Doctor, Z. Zhang, T. Zhang, N. Kwiatkowski, M. Boukhali, J.L. Green, W. Haas, T. Nomanbhoy, E.S. Fischer, R.A. Young, J.E. Bradner, G.E. Winter, N.S. Gray, Pharmacological perturbation of CDK9 using selective CDK9 inhibition or degradation, Nature Chemical Biology, 14 (2017) 163-170.
[24] B. Jiang, E.S. Wang, K.A. Donovan, Y. Liang, E.S. Fischer, T. Zhang, N.S. Gray, Development of Dual and Selective Degraders of Cyclin-Dependent Kinases 4 and 6, Angew Chem Int Ed Engl, 58 (2019) 6321-6326.
[25] L. Tan, N.S. Gray, When Kinases Meet PROTACs, Chinese Journal of Chemistry, 36 (2018) 971-977.

[26] W. Li, C. Gao, L. Zhao, Z. Yuan, Y. Chen, Y. Jiang, Phthalimide conjugations for the degradation of oncogenic PI3K, Eur J Med Chem, 151 (2018) 237-247.
[27] C.E. Powell, Y. Gao, L. Tan, K.A. Donovan, R.P. Nowak, A. Loehr, M. Bahcall, E.S. Fischer, P.A. Janne, R.E. George, N.S. Gray, Chemically Induced Degradation of Anaplastic Lymphoma Kinase (ALK), J Med Chem, 61 (2018) 4249-4255.
[28] W. Chen, J. Lee, S.Y. Cho, H.A. Fine, Proteasome-Mediated Destruction of the Cyclin A/Cyclin-Dependent Kinase 2 Complex Suppresses Tumor Cell Growth in Vitro and in Vivo, Cancer Research, 64 (2004) 3949-3957.
[29] M. Ying, X. Shao, H. Jing, Y. Liu, X. Qi, J. Cao, Y. Chen, S. Xiang, H. Song, R. Hu, G. Wei, B. Yang, Q. He, Ubiquitin-dependent degradation of CDK2 drives the therapeutic differentiation of AML by targeting PRDX2, Blood, 131 (2018) 2698-2711.
[30] P.G. Wyatt, A.J. Woodhead, V. Berdini, J.A. Boulstridge, M.G. Carr, D.M. Cross, D.J. Davis, L.A. Devine, T.R. Early, R.E. Feltell, E.J. Lewis, R.L. McMenamin, E.F. Navarro, M.A. O'Brien, M. O'Reilly, M. Reule, G. Saxty, L.C.A. Seavers, D.-M. Smith, M.S. Squires, G. Trewartha, M.T. Walker, A.J.A. Woolford, Identification of N-(4-Piperidinyl)-4-(2,6-dichlorobenzoylamino)-1H-pyrazole-3-carboxamide (AT7519), a Novel Cyclin Dependent Kinase Inhibitor Using Fragment-Based X-Ray Crystallography and Structure Based Drug Design, Journal of Medicinal Chemistry, 51 (2008) 4986-4999.
[31] Y. Wang, Y. Zhi, Q. Jin, S. Lu, G. Lin, H. Yuan, T. Yang, Z. Wang, C. Yao, J. Ling, H. Guo, T. Li, J. Jin, B. Li, L. Zhang, Y. Chen, T. Lu, Discovery of 4-((7H-Pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide (FN-1501), an FLT3- and CDK-Kinase Inhibitor with Potentially High Efficiency against Acute Myelocytic Leukemia, J Med Chem, 61 (2018) 1499-1518.
[32] K. Cyrus, M. Wehenkel, E.-Y. Choi, H.-J. Han, H. Lee, H. Swanson, K.-B. Kim, Impact of linker length on the activity of PROTACs, Molecular BioSystems, 7 (2011) 359-364.
[33] G. Bretones, M.D. Delgado, J. Leon, Myc and cell cycle control, Biochim Biophys Acta, 1849 (2015) 506-516.

Highlights

1. We designed and synthesized two series PROTAC compounds by tethering CDK inhibitors with CRBN ligands.
2. We identified compounds differentially induced dual CDK2/9 degradation, or selective to CDK2 or to CDK9.
3. Compound **F3** is a potent dual degrader for CDK2 (DC_{50}: 62 nM) and CDK9 (DC_{50}: 33 nM).
4. Compound **F3** suppresses prostate cancer PC-3 cell proliferation by interfering with cell cycle progression.

Declaration of Interest Statement

The authors declare no conflicts of interest.