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Discovery of novel pyrimidine molecules containing boronic acid as VCP/p97 Inhibitors

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ABSTRACT

Valine-containing protein (VCP) is a member of the adenosine triphosphate family involved in a variety of cellular activities. VCP/p97 is capable of maintaining protein homeostasis and mediating the degradation of misfolded polypeptides by the ubiquitin–proteasome system (UPS). In this manuscript, a series of novel p97 inhibitors with pyrimidine as core structure were designed, synthesized and biologically evaluated. Based on the enzymatic results, a detailed structure–activity relationship discussion of the synthesized compounds was carried out. Furthermore, cellular activities of the compounds with enzymatic potency of less than 200 nM were investigated by using A549 and RPMI8226 cell lines. Among the screened inhibitors, compound **17** (IC_{50}, 54.7 nM) showed good enzymatic activity. Investigation of cellular activities with non-small cell lung cancer A549 and multiple myeloma (MM) RPMI8226 further confirmed the potency of **17** with the IC_{50} values of 2.80 μM and 0.86 μM, respectively. Compound 17 is now being developed as a candidate. Finally, docking studies were carried out to explore the possible binding mode between the active inhibitor **17** and p97.

1. Introduction

Valine-containing protein (VCP) is a member of the adenosine triphosphate family involved in a variety of cellular activities1, which is rich in cells and exhibits many biological effects.[2,3] For instance, it can activate the ubiquitin dependence of transcription factors, endoplasmic reticulum-associated degradation (ERAD), control the nuclear membrane fusion, and promote the degradation of polymerized proteins by autophagy.[4–11] Furthermore, p97 can sustain the protein homeostasis and is critical to the degradation pathway of ubiquitin–proteasome system (UPS).[12] Studies showed that siRNA knockdown of p97 led to ER stress and activated unfolded protein response (UPR), and led to apoptosis through UPS inhibition and caspase activation. In addition, inhibiting the ATPase function of p97 can inhibit UPS, activate UPR and induce apoptosis.[13] P97 is a cyclic structure composed of six subunits, each of which has a molecular weight of 92kD and contains an *N*-terminal domain, as well as D1 and D2 domains.[14,15] Some results showed that the activity of the D2 enzyme will also be affected due to the D1 and D2 synergy between the domains when the enzyme in the D1 domain is mutated. This function is essential for cell growth. The D2 domain provides the main mechanical force for a wide range of cellular processes.[16–18]

P97 plays an important role in regulating a variety of physiological reactions, and its function is related to many pathological conditions.[19–21] Studies showed that elevated levels of p97 in cancer patients were associated with cancer occurrence and poor clinical outcomes. This is true in many types of cancer, such as colorectal cancer, pancreatic cancer, thyroid cancer, breast cancer, squamous cell carcinoma, gastric cancer, osteosarcoma and lung cancer, etc. Inhibition of p97 can preferentially kill cancer cells with high protein synthesis load.[22–29] Therefore, in recent years, p97 has attracted much attention as a potential drug target for the development of small molecules for cancer treatment, several inhibitors were reported so far (Fig. 1). Bursavich et al.[30] used the high-throughput screening method to obtain the first p97 inhibitor 2-anilino-4-aryl-1,3-thiazole (Fig. 1). Through optimization, a series of 2-anilinothiazole analogs with submicromolar p97

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inhibitory activity have been discovered. However, it is reported that this series of compounds were not specific and also potent against other enzymes. Subsequently, Polucci et al.[31] designed a methylsulfonyl-substituted aryl-1,2,4-triazole compound NMS-873 (Fig. 1), which was a more effective allosteric inhibitor of p97, which can induce the accumulation of polyubiquitinated proteins, leading to endoplasmic reticulum stress and inhibiting autophagosome maturation. However, the compound had poor metabolic stability and was difficult to be used in clinic. In 2011, Chou et al.[32] used the high-throughput method to screen the first reversible ATP-competitive p97 inhibitor DBeQ from the NIH compound library (Fig. 1). It can reversibly inhibit p97 in a competitive manner. Further studies have shown that the quinazoline ring of DBeQ is a known protein kinase inhibitor. Through the activity screening of 170 kinases, it was not found that the compound had a significant inhibitory effect on one of the kinases. In order to develop more effective p97 inhibitors, the researchers further optimized the structure of DBeQ and obtained two analogues ML240 and ML241. Their inhibitory effect on p97 increased nearly 10 times. Both ML240 and ML241 are ATP-competitive p97 inhibitors and have similar in vitro ATPase activities. Studies have shown that ML240 and ML241 have different mechanisms of action.[33] Among these p97 inhibitors, as the first-generation selective inhibitor CB-5083, has a highly selective inhibitory effect and also shows significant anti-tumor activity.[34] However, due to the fact that binding of CB-5083 with PDE6 led to off-target effects and toxicity,[35] the clinical trial on CB-5083 discontinued, and so it is necessary to discover new p97 inhibitor to meet the unmet clinical need. In this manuscript, the design, synthesis and structure–activity relationship discussion of a series of novel p97 inhibitors were introduced. Detailed structure activity relationship (SAR) was discussed based on the enzymatic results and the cellular activities of the potent compounds were furthermore investigated. From both enzymatic and cellular results, compound **17** were screened with good enzyme inhibitory activity and potency againstnon-small cell lung cancer A549 cells and multiple myeloma RPMI8226 tumor cells and could be developed as a candidate.

2. Results and discussion

2.1. Chemistry

The synthesis of key intermediates **4**, **9a** and **9b** was outlined in scheme 1. 3-Nitroaniline **1** condensed with acetone under strongly basic conditions to give 2-methyl-4-nitro indole **2** (yield 54%). The nitro group of intermediates **2** was reduced to amino group to give amine **3** (yield 94%). Then the Boc protecting group was introduced to obtain intermediate **4** (yield 84%). Compound **5** reacted with urea and then refluxed in aqueous sodium hydroxide to give **6** (yield 69%). Compound **6** reacted with $POCl_3$ to produce key intermediate **9a** (yield 43%). Starting material **7** reacted with urea and MeONa to give 2,4-diketopyrimidine **8** (yield 83%). Compound **8** reacted with $POCl_3$ to produce key intermediate **9b** (yield 45%).

[a]Reagents and conditions: (a) Acetone, *t*-BuOK, DMSO, rt; (b) Pd$(OH)_2$, H_2, EtOH, rt; (c) $(Boc)_2O$, DCM, rt; (d) Urea, HCl, EtOH, NaOH; (e) $POCl_3$, DMA, 120 °C; (f) Urea, MeONa, EtOH, 80 °C;

The synthesis of target molecules **17**–**38** was summarized in scheme 2. Key intermediates **9a-9e** reacted with 3-(aminomethyl) phenol to produce critical intermediates **11a-11e** (yields 35%-45%), which were coupled with compound **4** respectively in the presence of $Pd_2(dba)_3$ as the catalyst and Cs_2CO_3 as base to give compounds **12a-12e** (yields 42%-60%). Finally, **12a-12e** reacted with $PhNTf_2$ to form compounds **13a-13e** (yields 50%-73%). Intermediates **13a-13e** reacted with $(BPin)_2$ in the presence of $Pd(dppf)_2Cl_2$ as the catalyst to give boronic esters **14a-14e** (yields 65%-80%). Boronic esters **14a-14e** reacted with $NaIO_4$ to give boric acids **15a-15e** and then Boc groups were deprotected to yield **16a-16e** (yields 35%-55%). Intermediates **16a-16e** reacted with different carboxylic acid or acid chloride to produce target molecules **17**–**38** (yields 62%-78%).

[a]Reagents and conditions: (a) IPA, TEA, 80 °C. (b) Cs_2CO_3, $Pd_2(dba)_3$, X-Phos, dioxane,105 °C; (c) $PhNTf_2$, TEA, THF, 50 °C; (d) $(BPin)_2$, $Pd(dppf)_2Cl_2$, KOAc, dioxane, 110 °C; (e) $NaIO_4$, NH_4OAc, THF, H_2O, rt; (f) TFA, DCM, rt; (g) carboxylic acid, N,N′-dicyclohexylcarbodiimide (DCC), DCM; or carboxylic acid, *N*-ethyl-N′-(3-dimethylaminopropyl)carbodiimide (EDC), 1-hydroxybenzotriazole (HOBt), DIEA, DMF; or acid chloride, TEA, DCM.

2.2. Discussion

Enzymatic. It was reported that introduction of a substitution in the meta-position of phenyl group could avoid the off-target toxicity of **CB-5083**,[35] so boronic acid group was employed in the target compounds in this manuscript. We first investigated the structure–activity relationship (SAR) of different groups on indole ring while a hydrophobic cyclopentane ring was used to replace the hydrophilic pyran one of **CB-5083**. Table 1 showed the enzymatic activities of compounds **17**–**27** and **CB-5083** was used as the standard. The 4-position formamide of indole structure group was then changed to methanesulfonamide (**17**, 54.7

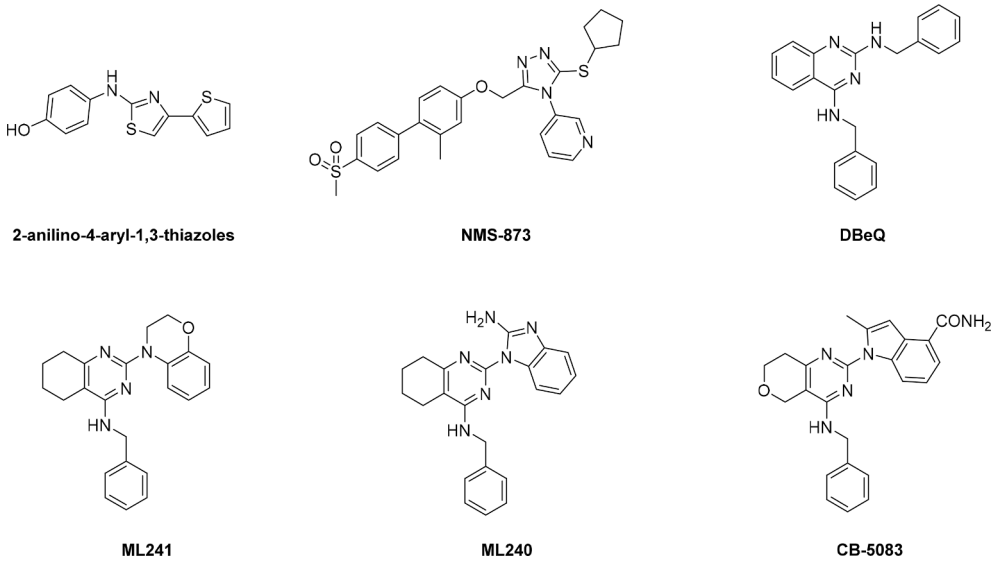
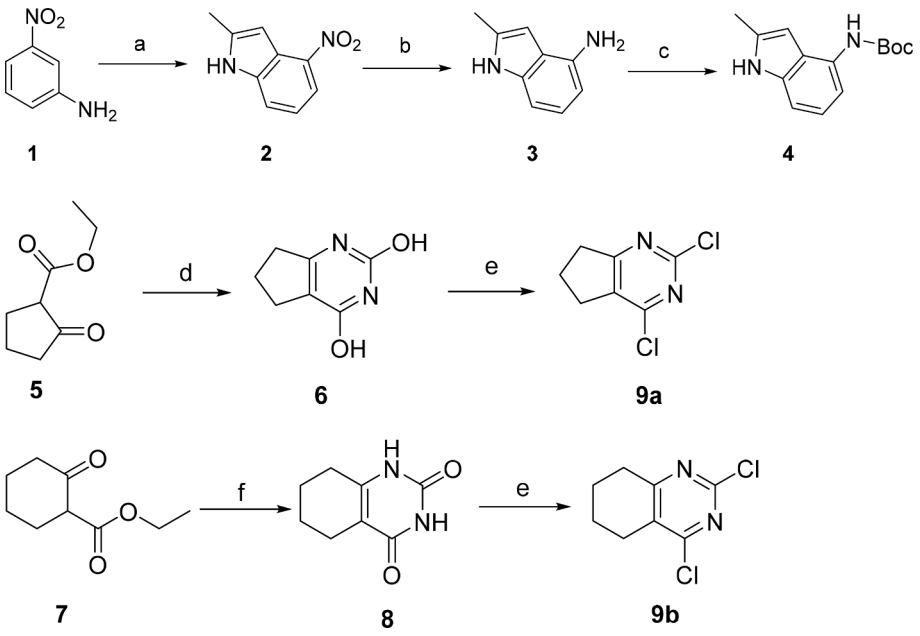


Fig. 1. The p97 inhibitors currently reported.



Scheme 1. Synthesis of intermediates **4, 9a** and **9b**.[a]

nM), and the resulting activity was close to that of **CB-5083** (44.0 nM). While the 4-position formamide of indole group was substituted by acrylamide (**18**, 192.4 nM) and crotonamide (**19**, 166.2 nM), separately, the activities nearly dropped about three times, which showed that α,β-unsaturated ketone was not beneficial to the activity. However, the introduction of alkyne groups greatly improved the activities, for example, propynamide (**21**, 91.4 nM) and butyne (**20**, 58.6 nM). Subsequently, the introduction of alkyl groups with electron withdrawing groups at the terminal, such as cyano (**22**, 692.3 nM), chloro (**23**, 258.4 nM) and chloromethylene (**24**, 436.2 nM) greatly reduced the activities. Finally, the effects of heterocycles at the same position on the activity were also investigated. It showed that thiazole (**25**, 468.3 nM) and oxazole (**27**, 241.4 nM) greatly reduced the activities, while the activity of furan (**26**, 57.3 nM) was maintained and was similar to that of **CB-5083**.

The SAR in Table 1 showed that methanesulfonyl, alkynyl and furan groups were beneficial to the activities, so these groups were still employed in the next optimization. Compounds **28**–**38** were designed based on the modification of the core structure. First, the cyclopentane was changed to cyclohexane (**28**, 45.6 nM), pyran (**31**, 117.2 nM) and benzene ring (**34**, 88.9 nM), respectively with the methanesulfonamide at the R position of indole ring. The results revealed that the cyclohexane ring compound was more potent than other two compounds. Next, cyclohexane ring (**29**, 89.8 nM), pyran ring (**32**, 117 nM), phenyl ring (**35**, 113.7 nM) and 5-methoxy group (**37**, 32.0 nM) were used to as the core structures without changing the butyne at the R position of indole. Compound **37** was found to be the best active and the aromatic and unsaturated heterocyclic ring were not beneficial to the activities. Finally, the furan ring at the R position of indole was kept, and the core structure was substituted by cyclohexane ring (**30**, 93.4 nM), pyran ring (**33**, 91.0 nM), benzene ring (**36**, 162.1 nM) and 5-methoxy group (**38**, 97.4 nM). The resulting compounds had similar activities except for the compound substituted by the phenyl ring **36**.

Cellular. Compounds with enzymatic activities below 200 nM were further evaluated for their potential effects of inhibiting the proliferation of non-small cell lung cancer (NSCLC) A549 and multiple myeloma (MM) RPMI8226 tumor cells. The biological results were shown in Table 2, which indicated that most compounds were notably potent against two types of cells and their IC_{50} values were below 10 μM. The cellular activities of most compounds were consistent with the enzymatic ones. Among these compounds, the inhibitory effects of compounds **17**, **21**, **28** and **29** on the two kinds of cells even reached less than 3 μM. Therefore, these inhibitors could be developed as candidates to evaluate the drug-availability.

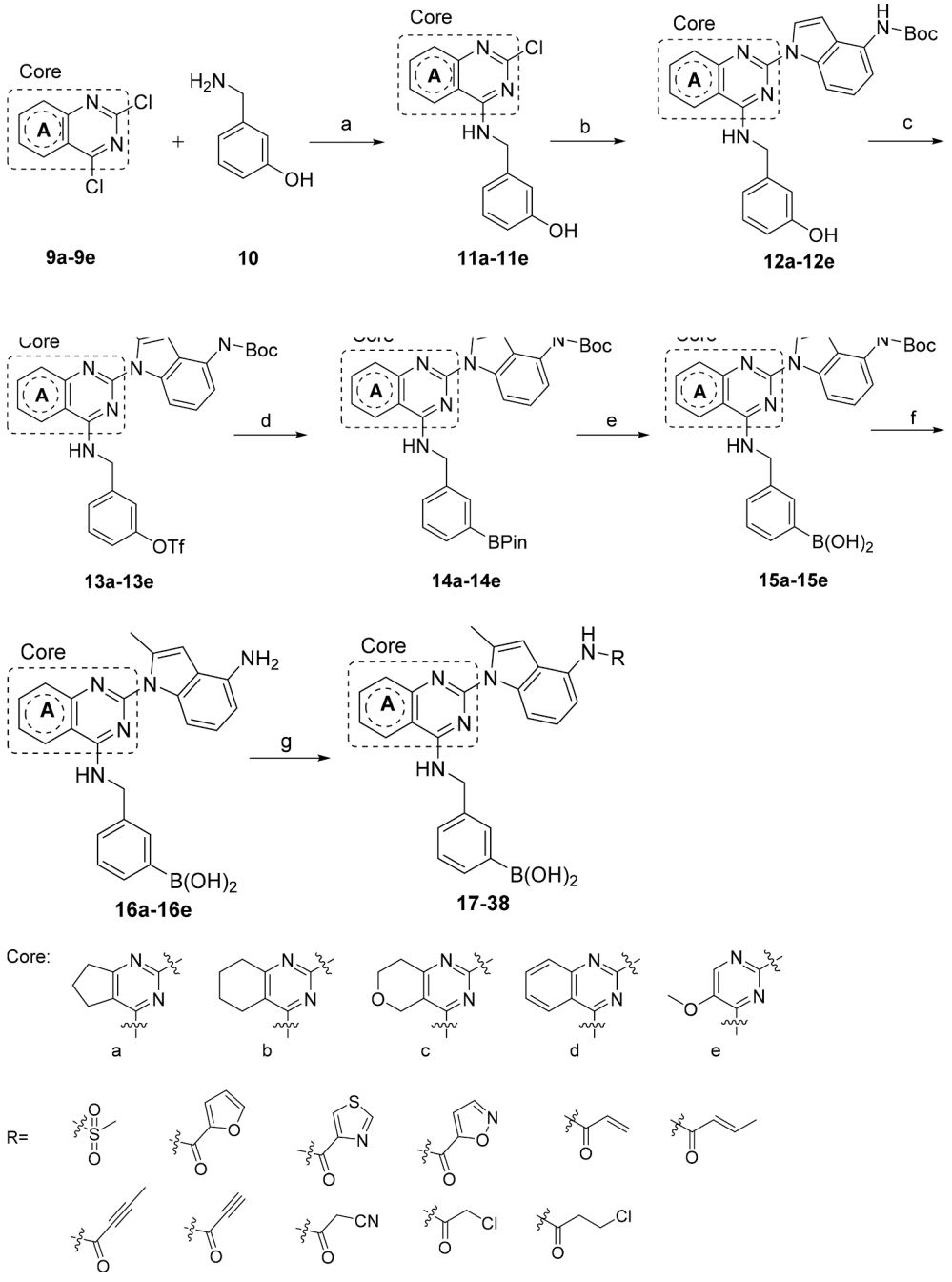
2.3. Molecular docking research

To investigate the interactions between the designed compounds and the p97 critical residues, molecular docking of the selected compounds was performed using Glide software. P97 structures co-crystalized with **CB-5083** (PDB ID: 6MCK) were first extracted from the RCSB protein database, and then hydrogenated, dehydrated and energy-minimized in the force field of OPLS-2005 using the protein preparation module of Schrödinger's software prior to molecular docking. Afterwards, the acceptor grid was generated in Glide's "Acceptor Grid Generation" panel and docking sites of 20 × 20 × 20 Å were set. The ligand was then bound to the receptor using the Precision Docking (XP) mode. Finally, the ligand was scored based on the docking score, the hydrogen bond score and the number of hydrogen bonds formed.

The docking results (Fig. 2 and Table 3) suggested that docking score of compound **CB-5083** with the highest activity was −9.220 kcal/mol, and the oxygen atom of the ligand molecule indolecarboxamide formed a 2.00 Å-long hydrogen bond with key amino acid Thr-688, and the NH on the benzylamine forms a 2.48 Å-long hydrogen bond with Asp478. Compound **17** achieved the second highest docking score of −6.600 kcal/mol, and the N atom in the pyrimidine structure of the ligand formed a 2.13 Å-strong hydrogen bond with key amino acid Thr-688, in the structural formula, the two oxygen atoms on the methanesulfonamide and the amino acid Arg662 on the p97 protein form a hydrogen bond of 1.83 Å-long and 1.99 Å-long respectively. Compared with active compound **17**, much inactive inhibitor **31** had the lowest docking score of −1.837 kcal/mol, the NH on the indole structure amide of this compound only forms a 1.96 Å-long hydrogen bond with Pro520 on the p97 protein.

3. Conclusion

In this manuscript, 22 new p97 inhibitors were designed by varying the core structure and the substitutions on the indole ring. After synthesis and enzymatic activity evaluation, the SAR was discussed in



Scheme 2. Synthesis of the target molecules **17**–**38**.[a]

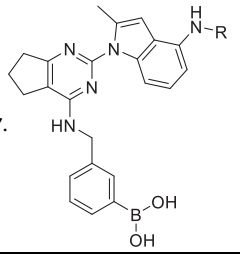
detail. The SAR indicated that methanesulfonic, alkyne and furan groups on the R position of indole ring were beneficial to the activities and the IC_{50} values were less than 100 nM, but the α,β-unsaturated ketone and alkane groups with electron withdrawing groups at the terminal reduced the activities. As to the variation of core structures, it showed that different combination of core structures with R groups led to active or inactive results. The cellular results of A549 and RPMI8226 showed that IC_{50} values of compounds **17**, **21**, **28** and **29** on the two kinds of cells reached less than 3 μM, which suggested those compounds were worthy to be developed. So our future work will focus on the in vivo evaluation of these inhibitors to determine their drug-availability.

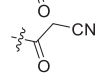
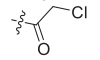
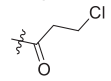
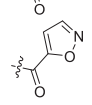
4. Experimental section

4.1. General methods

Unless otherwise indicated, chemicals, solvents and reagents were purchased from commercial suppliers and they were used without any purification. Absolutely anhydrous solvents (CH_2Cl_2, THF, DMF, etc.) were purchased from Energy packaged under nitrogen in Sure/Seal bottles. All reactions involving air or moisture-sensitive reagents were performed under an argon atmosphere. All reactions were detected by thin layer chromatography on silica gel 60 plate coated with 0.25 mm layer and spotted with UV light or iodine. All final products were purified to > 95% purity. The purity of the final products was determined by HPLC (Thermo) on an Agilent Poroshell 120 EC-C18 column (50 mm × 4.6 mm, 2.7 μm) with 0.1% FA/ACN (gradient eluted program: 0–5 min

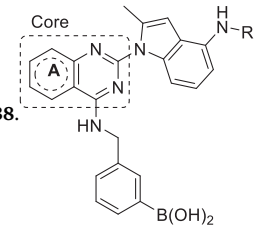
Table 1
Inhibitory activities of **CB-5083** and compounds **17–27**.

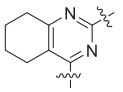
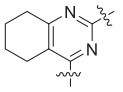
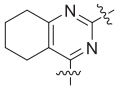
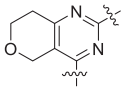
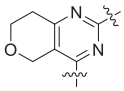
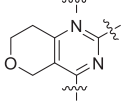
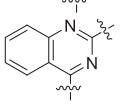
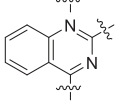
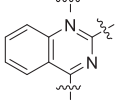
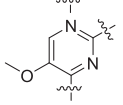
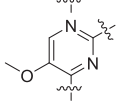


Compd.	R	IC_{50} (nM)[a]
17		54.7
18		192.4
19		166.2
20		58.6
21		91.4
22		692.3
23		258.4
24		436.2
25		468.3
26		57.3
27		241.4
CB-5083		44.0

[a] All experiments were repeated three times.

Table 2
Inhibitory activities of **CB-5083** and compounds **28–38**.



Compd.	Core	R	IC_{50} (nM)[a]
28			45.6
29			89.8
30			93.4
31			117.2
32			117.0
33			91.0
34			88.9
35			113.7
36			162.1
37			32.0
38			97.4
CB-5083			44.0

[a] All experiments were repeated three times.

90/10–5/95 v/v; 5–11.9 min 5/95 v/v; 11.9–12.1 min 5/95–90/10 v/v; 12.1–15 min 90/10 v/v) at 0.3 mL/min flow rate and 254 nm detector wavelength. 1H and 13C spectra were acquired in $CDCl_3$, DMSO-d_6 or CD_3OD at room temperature on a Bruker Avance 400 spectrometer with chemical shift (δ, ppm) reported relative to TMS as an internal standard. High-resolution mass spectra (HRMS) were recorded on a ZAB-HS instrument using an electrospray source (ESI).

4.2. Chemistry

4.2.1. tert-butyl (2-methyl-1H-indol-4-yl)carbamate (4)

At room temperature, to a solution of **1** (10 g, 74 mmol) in DMSO (100 mL) was added acetone (7.5 mL, 100 mmol). The mixture was stirred for 5 min, and *t*-BuOK (19 g, 169 mmol) was then added. The reaction mixture was stirred at room temperature for 24 h and then was added water (200 mL) and the pH was adjusted to 4 and extracted with DCM (100 mL × 3), the combined organic layers were washed with water (100 mL) and brine (100 mL), dried over Na_2SO_4 ,filtered and evaporated in vacuo. The residue was purified by column chromatography (petroleum ether/EtOAc = 8:1) to give **2** (7 g, yield 54%, purity 98%) as a yellow solid. MS (ESI) *m/z* 177.4 $[M+H]^+$.

At room temperature, to a solution of **2** (7 g, 39.7 mmol) in ethanol (200 mL) was added $Pd(OH)_2$ (2 g, 14.2 mmol). The mixture was degassed and refilled with hydrogen three times, Then the mixture was stirred at room temperature for 3 h under a hydrogen atmosphere. The resulting solution was filtered and concentrated in vacuo to give **3** as yellow solid (5.5 g, yield 94%, purity 97%). MS (ESI) *m/z* 147.6 $[M+H]^+$.

At room temperature, to a solution of **3** (5.5 g, 37.6 mmol) in DCM (150 mL) were added $(Boc)_2O$ (11 mL, 48.88 mmol). Then the mixture was stirred at room temperature for 24 h. The reaction was washed with water (100 mL) and brine (50 mL), dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (petroleum ether/EtOAc = 10:1) to give **4** (7.8 g, yield 84%, purity 98%) as a yellow solid. MS (ESI) *m/z* 247.3 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H, —NH—), 8.85 (s, 1H, —NH—), 7.25 (s, 1H, Ph), 6.96 (d, J = 8.0 Hz, 1H, Ph), 6.88 (s, 1H, Ph), 6.37 (s, 1H, 3-H of indole), 2.35 (s, 3H, Me), 1.49 (s, 9H, Boc).

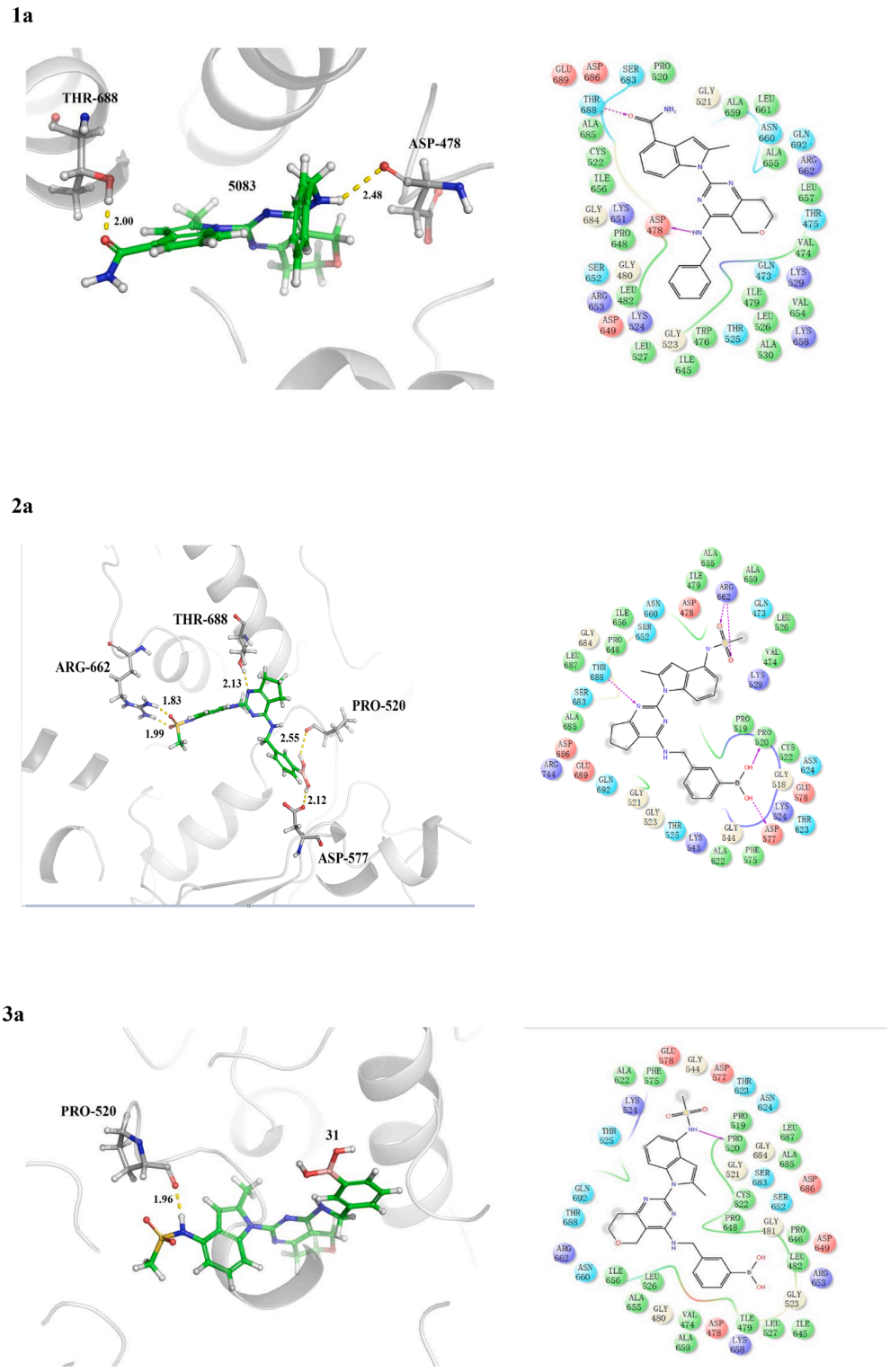


Fig. 2. (1a) The compound **CB-5083** of 3D and 2D ligand-protein interaction diagrams of the ligand molecule. (2a) The compound **17** of 3D and 2D ligand-protein interaction diagrams of the ligand molecule. (3a) The compound **31** of 3D and 2D ligand-protein interaction diagrams of the ligand molecule.

*4.2.2. 2,4-dichloro-6,7-dihydro-5H-cyclopenta[d]pyrimidine **(9a)***

A solution of **5** (2 g, 12.8 mmol), urea (1.15 g, 19.2 mmol) andhydrochloric acid (0.2 mL) in ethanol (10 mL) was refluxed under an atmosphere of argon for 4 h. The resulting mixture was then cooled down to room temperature and concentrated in vacuo. the residue was diluted with aqueous sodium hydroxide solution (10 mL) and the resulting mixture was refluxed for 30 min, then it was cooled down and the precipitated solids were collected and dried to afford **6** (1.3 g, yield 69%, purity 98%) as a colorless solid. MS (ESI) *m/z* 153.1 $[M+H]^+$.

A solution of **6** (1.3 g, 8.5 mmol) in $POCl_3$ (10 mL) and then added to the N,*N*-dimethylaniline (2.06 g, 17 mmol)the reaction mixture was refluxed and stirred for 3 h. After being cooled to room temperature, the mixture was concentrated in vacuo. The residue was diluted with water (100 mL) and extracted with hexane (50 mL × 4). The combined organic

Table 3
Cell viabilities of representative compounds against A549 cancer cell lines and RPMI8226 cell lines.

Compd.	RPMI8226 (μM)[a]	A549 (μM)[a]
17	0.86	2.80
18	2.94	4.75
19	NA[b]	4.45
20	1.73	3.15
21	1.27	2.10
26	2.30	3.55
28	1.55	2.42
29	1.50	2.15
30	2.33	3.12
31	5.10	>10
32	2.90	4.10
33	2.70	4.38
34	3.60	5.60
35	2.20	5.60
36	3.20	5.80
37	2.40	6.10
38	1.90	3.77
CB-5083	0.80	1.70

[a] All experiments were repeated three times.
[b] NA: No activity.

layers were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to give **9a** (0.72 g, yield 43%, purity 98%) as a yellow solid. MS (ESI) *m/z* 190.1 $[M+H]^+$.

4.2.3. 2,4-dichloro-5,6,7,8-tetrahydroquinazoline (9b)

The solution of **7** (5 g, 29.4 mmol), urea (2.29 g, 38.2 mmol) and MeONa (10 mL, 5.4 mol/L in ethanol, 58.8 mmol) in ethanol (50 mL) were stirred at 80 ℃ for 4 h. The mixture was then cooled down to room temperature and concentrated in vacuo, and diluted with aqueous sodium hydroxide solution (15 mL) and the resulting mixture was refluxed for 30 min, then it was cooled down and the precipitated solids were collected and dried to afford **8** (4.05 g, yield 83%, purity 98%) as a colorless solid. MS (ESI) *m/z* 167.6 $[M+H]^+$.

A solution of **8** (4.05 g, 24.4 mmol) was dissolved in $POCl_3$ (15 mL) and then added to the N,N-dimethylaniline (5.9 g, 48.8 mmol). The reaction mixture was refluxed and stirred for 3 h. After being cooled to room temperature, the mixture was concentrated in vacuo. The residue was diluted with water (100 mL) and extracted with hexane (50 mL × 4). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to give intermediate **9b** (2.21 g, yield 45%, purity 98%) as a colorless solid. MS (ESI) *m/z* 204.7 $[M+H]^+$.

4.2.4. 3-(((2-(4-amino-2-methyl-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d] pyrimidin-4-yl)amino) methyl)phenol (12a)

TEA (1 g, 10.6 mmol) was added to a solution of **9a** (1 g, 5.3 mmol) and **10** (0.8 g, 6.8 mmol) in IPA (10 mL) and the mixture was stirred at 80 ℃ for 12 h under N_2 atmosphere. Then cooled to room temperature and concentrated .The residue was purified by chromatography (DCM/MeOH = 100:1) to yield **11a** as a colorless solid (0.57 g, yield 40%, purity 97%). MS (ESI) *m/z* 276.2 $[M+H]^+$.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H, —OH), 7.88 (t, J = 6.1 Hz, 1H, —NH—), 7.11 (t, J = 7.8 Hz, 1H, Ph), 6.74–6.67 (m, 2H, Ph), 6.63 (m, 1H, Ph), 4.48 (d, J = 6.1 Hz, 2H, $\underline{CH_2}$Ph), 2.76–2.70 (t, J = 7.6 Hz, 2H, CH_2), 2.65 (t, J = 7.4 Hz, 2H, CH_2), 2.03 (m, J = 7.7 Hz, 2H, CH_2).

At room temperature, to the solution of **11a** (1.3 g, 4.7 mmol) and **4** (1.1 g, 4.7 mmol) in 1,4-dioxane (50 mL) was added cesium carbonate (2.3 g, 7.05 mmol), $Pd_2(dba)_3$ (0.64 g, 0.7 mmol) and X-Phos (0.33 g, 0.7 mmol). The mixture was degassed and refilled with nitrogen three times. The resulting mixture was stirred at 105 °C for 12 h and cooled to room temperature. The volatiles were evaporated in vacuo, and the resulting residue was dissolved in DCM (50 mL), washed with water (50 mL) and brine (30 mL), dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was purified by column chromatography (DCM/MeOH = 100:1) to give intermediate **12a** as a colorless solid (1.6 g, yield 52%, purity 97%). MS (ESI) *m/z* 486.3 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.32 (s, 1H, —OH), 8.96 (s, 1H, —NH—), 7.80 (t, J = 6.1 Hz, 1H, Ph), 7.50 (d, J = 8.3 Hz, 1H, Ph), 7.31 (d, J = 7.8 Hz, 1H, Ph), 7.12 (t, J = 7.7 Hz, 1H, Ph), 6.86 (t, J = 8.1 Hz, 1H, Ph), 6.74 (s, 1H, Ph), 6.72 (d, J = 2.6 Hz, 1H, 3-H of indole), 6.66–6.61 (m, 1H, Ph), 6.59 (s, 1H, —NH—), , 4.56 (d, J = 6.0 Hz, 2H, $\underline{CH_2}$Ph), 2.85 (t, J = 7.7 Hz, 2H, CH_2), 2.80 (s, 2H, CH_2), 2.48–2.42 (m, 3H, Me), 2.11 (m, J = 7.6 Hz, 2H, CH_2), 1.50 (s, 9H, Boc).

4.2.5. (3-(((2-(4-amino-2-methyl-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d] pyrimidin-4-yl)amino)methyl)phenyl)boronic acid (16a)

To a solution of **12a** (2.3 g, 4.74 mmol) and TEA (2.8 g, 28.44 mmol) in THF (200 mL) was added $PhNTf_2$ (5.08 g, 14.22 mmol). The mixture was stirred under an Ar atmosphere for 12 h at 50 °C. The volatiles were evaporated in vacuo. The residue was purified by chromatography (petroleum ether/EtOAc = 5:1) to give **13a** (1.68 g, yield 58%, purity 97%) as a yellow solid. MS (ESI) *m/z* 618.4 $[M+H]^+$.

Bis(pinacolato)diboron (1.5 g, 6.01 mmol), $Pd(dppf)_2Cl_2$ (0.59 g, 0.8 mmol) and KOAc (0.78 g, 8.02 mmol) were added to a solution of **13a** (2.48 g, 4.01 mmol) in dioxane (80 mL) under Ar. The solution was stirred at 110 °C for 6 h. Then cooled to room temperature and concentrate, the residue was purified by chromatography ((petroleum ether/EtOAc = 5:1) to give **14a** (1.72 g, yield 74%, purity 98%) as a yellow solid. MS (ESI) *m/z* 469.7 $[M+H]^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.82 (d, J = 1.7 Hz, 1H, —NH—), 7.80–7.72 (m, 1H, Ph), 7.55 (d, J = 9.2 Hz, 1H, Ph), 7.47 (m, J = 7.7, 1.6 Hz, 1H, Ph), 7.38 (t, J = 7.5 Hz, 1H, Ph), 7.33–7.17 (m, 2H, Ph), 7.08 (t, J = 8.1 Hz, 1H, Ph), 6.67 (s, 1H, Ph), 6.31 (s, 1H, 3-H of indole), 4.91 (s, 1H, —NH—), 4.76 (d, J = 5.3 Hz, 2H, NH$\underline{CH_2}$), 3.00 (t, J = 7.7 Hz, 2H, CH_2), 2.72 (t, J = 7.4 Hz, 2H, CH_2), 2.20 (m, J = 7.6 Hz, 2H, CH_2), 1.37 (s, 9H, Boc), 1.25 (s, 12H, CH_3).

A mixture of **14a** (1.72 g, 2.96 mmol), $NaIO_4$ (1.26 g, 5.92 mmol), NH_4OAc (0.45 g, 5.92 mmol) was dissolved in THF/H_2O (20 mL/1:1) , and stirred at room temperature for 12 h . The mixture was poured into a water (50 mL) and extracted with EtOAc (40 mL × 3) The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (DCM/MeOH = 100:1) to yield product **15a** as a yellow solid (0.9 g, yield 60%, purity 99%) as a yellow solid. MS (ESI) *m/z* 514.2 $[M+H]^+$. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.81–7.53 (m, 2H, Ph), 7.47 (d, J = 7.8 Hz, 1H, Ph), 7.43–7.34 (m, 2H, Ph), 7.21 (d, J = 8.3 Hz, 1H, Ph), 7.12 (t, J = 8.0 Hz, 1H, Ph), 6.67 (s, 1H, 3-H of indole), 4.85 (s, 2H, $\underline{CH_2}$Ph), 3.16 (t, J = 7.7 Hz, 2H, CH_2), 2.96 (t, J = 7.4 Hz, 2H, CH_2), 2.44 (s, 3H, Me), 2.42–2.35 (m, 2H, CH_2), 1.57 (s, 9H, Boc).

To a 0 °C solution of **15a** (1.2 g, 2.3 mmol) in DCM (20 mL) was added trifluoroacetic acid (5 mL, 30.6 mmol). Then the reaction was stirred at room temperature for 3 h and evaporated in vacuo and then added hexane and methyl tertiary ether = 1:5, stirred for 30 min and filter to give **16a** as a yellow solid (0.4 g, yield 44%, purity 98%). MS (ESI) *m/z* 414.2 $[M+H]^+$.

4.2.6. (3-(((2-(2-methyl-4-(methylsulfonamido)-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)methyl)phenyl)boronic acid (17)

To a 0 °C solution of **16a** (0.1 g, 0.22 mmol) and TEA (67 mg, 0.66 mmol) in DCM (5 mL) were added methanesulfonyl chloride (27 mg, 0.24 mmol). Then the solution was stirred at room temp for 3 h, and concentrated under reduced pressure. The residue was purified by chromatography (DCM/MeOH = 70:1) to give **17** (80 mg, yield 75%, purity 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (s, 1H, —NH—), 8.04 (s, 2H, —OH), 7.85 (t, J = 6.1 Hz, 1H, —NH—), 7.76 (s, 1H, Ph), 7.69 (d, J = 7.0 Hz, 1H, Ph), 7.58 (d, J = 8.2 Hz, 1H, Ph), 7.30–7.20 (m, 2H, Ph), 6.98 (d, J = 7.6 Hz, 1H, Ph), 6.89 (t, J = 8.0 Hz, 1H, Ph), 6.61 (s, 1H, 3-H of indole), 4.64 (d, J = 6.0 Hz, 2H, C$\underline{H_2}$Ph), 2.90 (s, 3H, Me), 2.86 (t, J =

7.7 Hz, 2H, CH_2), 2.80 (t, $J = 7.4$ Hz, 2H, CH_2), 2.45 (s, 3H, Me), 2.12 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.44, 159.81, 156.38, 139.05, 137.70, 137.27, 133.02, 128.86, 128.57, 127.83, 123.69, 122.08, 115.65, 113.59, 111.55, 102.48, 49.07, 43.98, 34.16, 27.19, 21.67, 16.18. HRMS calcd for $C_{24}H_{26}BN_5O_4S$ $[M+Na]^+$ 514.1697, found 514.1687.

4.2.7. (3-(((2-(4-acrylamido-2-methyl-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)methyl)phenyl)boronic acid **(18)**

Compound **18** was synthesized from **16a** and acryloyl chloride according to the procedure for preparing **17**. 71% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (s, 1H, —CONH—), 8.04 (s, 2H, —OH), 7.84 (t, $J = 6.0$ Hz, 1H, —NH—), 7.78 (s, 1H, Ph), 7.68 (dd, $J_1 = 7.5$ Hz, $J_2 = 11.3$ Hz, 2H, Ph), 7.53 (d, $J = 8.3$ Hz, 1H, Ph), 7.32 (dt, $J_1 = 7.4$ Hz, $J_2 = 14.7$ Hz, 2H, Ph), 6.88 (t, $J = 8.1$ Hz, 1H, Ph), 6.69 (dd, $J_1 = 10.2$ Hz, $J_2 = 17.0$ Hz, 1H, —CHCH$_2$), 6.61 (s, 1H, 3-H of indole), 6.27 (dd, $J = 2.2$ Hz, $J_2 = 16.9$ Hz, 1H, —CHCH$_2$), 5.76 (d, $J = 9.6$ Hz, 1H, —CHCH$_2$), 4.65 (d, $J = 5.8$ Hz, 1H, CH$_2$Ph), 2.86 (t, $J = 7.6$ Hz, 2H, CH_2), 2.80 (t, $J = 7.2$ Hz, 2H, CH_2), 2.48 (s, 3H, Me), 2.12 (m, $J = 7.7$ Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.43, 163.66, 159.81, 156.45, 139.07, 137.46, 136.59, 133.08, 133.01, 132.49, 131.22, 129.62, 128.91, 127.82, 126.94, 121.89, 121.11, 113.50, 113.32, 110.50, 102.32, 49.07, 44.01, 34.16, 27.19, 21.68, 16.15. HRMS calcd for $C_{26}H_{26}BN_5O_3$ $[M+Na]^+$ 490.2027, found 490.2018.

4.2.8. (E)-(3-(((2-(4-(but-2-enamido)-2-methyl-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)methyl)phenyl)boronic acid **(19)**

Compound **19** was synthesized from **16a** and (E)-2-butenoyl chloride according to the procedure for preparing **17**. 64% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (d, $J = 18.5$ Hz, 1H, —CONH—), 8.04 (s, 2H, —OH), 7.84 (t, $J = 6.2$ Hz, 1H, —NH—), 7.76 (d, $J = 8.2$ Hz, 1H, Ph), 7.69 (d, $J = 7.0$ Hz, 2H, Ph), 7.51 (d, $J = 8.2$ Hz, 1H, Ph), 7.32 (dt, $J_1 = 7.2$ Hz, $J_2 = 13.9$ Hz, 2H, Ph), 6.86 (t, $J = 8.2$ Hz, 1H, Ph), 6.82–6.75 (m, 1H, CH), 6.60 (t, $J = 15.3$ Hz, 1H, 3-H of indole), 6.38 (d, $J = 15.2$ Hz, 1H, CH), 4.65 (d, $J = 6.0$ Hz, 2H, CH$_2$Ph), 2.85 (t, $J = 7.4$ Hz, 2H, CH_2), 2.8 (s, 2H, CH_2), 2.47 (s, 3H, Me), 2.11 (m, 2H, CH_2), 1.88 (d, $J = 6.9$ Hz, 3H, Me). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.43, 159.80, 156.47, 139.80, 139.08, 137.45, 136.59–136.27 (m), 133.09, 133.01, 129.94, 128.99, 128.92, 127.83, 126.66, 121.89, 113.46, 102.37, 44.00, 34.16, 27.19, 21.68, 18.04, 16.18. HRMS calcd for $C_{27}H_{28}BN_5O_3$ $[M+Na]^+$ 504.2183, found 504.2175.

4.2.9. (3-(((2-(4-(but-2-ynamido)-2-methyl-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)methyl)phenyl)boronic acid **(20)**

To a solution of **16a** (0.1 g, 0.22 mmol) and 2-butynoic acid (18 mg, 0.22 mmol) in DMF (1 mL) were added DIPEA (56 mg, 0.44 mmol), EDC hydrochloride (84 mg, 0.44 mmol) and HOBt (29 mg, 0.22 mmol), and the mixture was stirred at rt overnight. The reaction mixture was then diluted with water (10 mL) and extracted with CH_2Cl_2 (10 mL × 3). The combined organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography using DCM/MeOH = 50:1 to afford compound **20** (0.072 g, yield 62%, purity 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H, —NH—), 9.32 (s, 1H, —NH—), 7.82 (t, $J = 6.1$ Hz, 1H, Ph), 7.57 (d, $J = 8.3$ Hz, 1H, Ph), 7.39 (d, $J = 7.8$ Hz, 1H, Ph), 7.12 (t, $J = 7.8$ Hz, 1H, Ph), 6.88 (t, $J = 8.1$ Hz, 1H, Ph), 6.78–6.69 (m, 2H, —OH), 6.66–6.60 (m, 1H, Ph), 6.56 (s, 1H, Ph), 5.77 (s, 1H, 3-H of indole), 4.56 (d, $J = 6.0$ Hz, 2H, NHCH$_2$),2.85 (t, $J = 7.7$ Hz, 2H, CH_2), 2.78 (t, $J = 7.3$ Hz, 2H, CH_2), 2.46 (s, 3H, Me), 2.12 (m, $J = 7.5$ Hz, 2H, CH_2), 2.06 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO-d_4) δ 161.76, 158.92, 141.04, 138.95, 138.73, 138.62, 137.87, 130.81, 129.71, 127.50, 125.19, 123.86, 119.06, 118.49, 117.13, 114.83, 112.16, 104.93, 104.45, 86.21, 75.84, 45.84, 33.16, 27.93, 22.69, 14.46, 3.01. HRMS calcd for $C_{27}H_{26}BN_5O_3$ $[M+Na]^+$ 502.2025, found 502.2019.

4.2.10. (3-(((2-(2-methyl-4-propiolamido-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)methyl)phenyl)boronic acid **(21)**

Compound **21** was synthesized from **16a** and propiolic acid according to the procedure for preparing **20**. 77% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (s, 1H, —CONH—), 7.90 (s, 1H, —NH—), 7.76 (s, 1H, Ph), 7.69 (d, $J = 7.0$ Hz, 1H, Ph), 7.55 (d, $J = 8.3$ Hz, 1H, Ph), 7.44–7.32 (m, 3H, Ph), 6.87 (t, $J = 8.3$ Hz, 1H, Ph), 6.54 (s, 1H, 3-H of indole), 4.64 (d, $J = 5.9$ Hz, 1H, CH$_2$Ph), 4.40 (s, 1H, CH), 2.83 (s, 2H, CH_2), 2.79 (d, $J = 6.8$ Hz, 2H, CH_2), 2.45 (s, 3H, Me), 2.12 (m, 1H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.81, 150.44, 139.02, 137.52, 136.93, 133.04, 128.89, 128.37, 127.84, 121.78 (d, $J = 7.4$ Hz), 114.72, 113.64, 111.33, 102.74, 79.06, 77.66, 44.01, 34.11, 27.20, 21.68, 16.09. HRMS calcd for $C_{26}H_{24}BN_5O_3$ $[M+Na]^+$ 488.1868, found 488.1857.

4.2.11. (3-(((2-(4-(2-cyanoacetamido)-2-methyl-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)methyl)phenyl)boronic acid **(22)**

Compound **22** was synthesized from **16a** and cyanoacetic acid according to the procedure for preparing **20**. 66% yield. ^{1}H NMR (400 MHz, Methanol-d_6) δ 7.14–6.38 (m, 6H, Ph), 6.14 (m, $J_1 = 7.9$ Hz, $J_2 = 9.8$ Hz,1H, Ph), 5.65 (d, $J = 12.1$ Hz, 1H, 3-H of indole), 3.90 (d, $J = 12.0$ Hz, 2H, NHCH$_2$), 2.68–2.56 (m, 3H, Me), 2.11 (m, $J = 7.9, 7.0$ Hz, 2H, CH_2), 2.02 (t, $J = 7.4$ Hz, 2H, CH_2), 1.61 (s, 2H, CH_2), 1.42 (m, $J_1 = 8.9$, $J_2 = 15.1, 7.5$ Hz, 2H, CH_2). ^{13}C NMR (100 MHz, Methanol-d_4) δ 161.76, 158.92, 141.04, 138.95, 138.73, 138.62, 137.87, 130.81, 129.71, 127.50, 125.19, 123.86, 123.62, 119.06, 118.49, 117.13, 115.23, 114.83, 112.16, 110.66, 104.93, 104.45, 86.21, 75.84, 45.84, 33.16, 27.93, 22.69, 14.46, 3.01. HRMS calcd for $C_{26}H_{25}BN_6O_3$ $[M+Na]^+$ 503.1977, found 503.2212.

4.2.12. (3-(((2-(4-(2-chloroacetamido)-2-methyl-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)methyl)phenyl)boronic acid **(23)**

Compound **23** was synthesized from **16a** and chloroacetyl chloride according to the procedure for preparing **17**. 65% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H, —CONH—), 8.05 (s, 2H, —OH), 7.86 (t, $J = 6.0$ Hz, 1H, —NH—), 7.77 (s, 1H, Ph), 7.69 (d, $J = 7.2$ Hz, 1H, Ph), 7.53 (dd, $J_1 = 4.6$ Hz, $J_2 = 8.1$ Hz, 2H, Ph), 7.31 (m, 2H, Ph), 6.89 (t, $J = 8.1$ Hz, 1H, Ph), 6.55 (s, 1H, 3-H of indole), 4.65 (d, $J = 6.0$ Hz, 2H, CH$_2$Ph), 4.38 (s, 2H, COCH$_2$), 2.86 (t, $J = 7.7$ Hz, 2H, CH_2), 2.79 (d, $J = 7.3$ Hz, 2H, CH_2), 2.48 (s, 3H, Me), 2.11 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.43, 165.18, 163.59, 159.81, 156.40, 139.06, 137.47, 136.82, 133.07, 133.01, 129.04, 128.90, 127.83, 121.89, 121.27, 113.56, 113.39, 110.85, 102.09, 49.07, 43.95, 34.16, 27.19, 21.68, 16.14. HRMS calcd for $C_{25}H_{25}BClN_5O_3$ $[M+Na]^+$ 512.1635, found 512.1651.

4.2.13. (3-(((2-(4-(3-chloropropanamido)-2-methyl-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)methyl)phenyl)boronic acid **(24)**

Compound **24** was synthesized from **16a** and 3-chloropropionyl chloride according to the procedure for preparing **17**. 72% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.71 (s, 1H, —CONH—), 8.11 (s, 1H, —OH), 7.88–7.80 (m, 1H, —NH—), 7.77 (s, 1H, Ph), 7.69 (d, $J = 7.2$ Hz, 1H, Ph), 7.53 (t, $J = 7.9$ Hz, 2H, Ph), 7.36–7.29 (m, 1H, Ph), 7.29–7.24 (m, 1H, Ph), 6.87 (t, $J = 8.1$ Hz, 1H, Ph), 6.58 (s, 1H, 3-H of indole), 4.65 (d, $J = 5.7$ Hz, 2H, CH$_2$Ph), 3.92 (t, $J = 6.2$ Hz, 2H, COCH$_2$CH$_2$), 2.94 (t, $J = 6.2$ Hz, 2H, COCH$_2$CH$_2$), 2.86 (t, $J = 7.7$ Hz, 2H, CH_2), 2.80 (t, $J = 7.2$ Hz, 2H, CH_2), 2.47 (s, 3H, Me), 2.09 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.42, 168.47, 159.79, 156.46, 139.07, 137.48, 136.54, 136.48, 133.08, 133.01, 129.61, 128.91, 127.82, 121.85, 121.21, 113.47, 113.36, 110.42, 102.42, 49.07, 44.00, 41.58, 34.16, 27.19, 21.68, 16.17. HRMS calcd for $C_{26}H_{27}BClN_5O_3$ $[M+Na]^+$ 526.1792, found 526.1823.

4.2.14. (3-(((2-(2-methyl-4-(thiazole-4-carboxamido)-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)methyl)phenyl) boronic acid (25)

Compound **25** was synthesized from **16a** and thiazole-4-carboxylic acid according to the procedure for preparing **20**. 78% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H, —CONH—), 9.30 (d, J = 2.0 Hz, 1H, thiazole), 8.53 (d, J = 2.0 Hz, 1H, thiazole), 8.04 (s, 2H, —OH), 7.86 (t, J = 6.1 Hz, 1H, —NH—), 7.78 (d, J = 1.8 Hz, 1H, Ph), 7.70 (dt, J_1 = 1.4 Hz, J_2 = 7.1 Hz, 1H, Ph), 7.57 (dd, J_1 = 7.9 Hz, J_2 = 11.3 Hz, 2H, Ph), 7.38–7.28 (m, 2H, Ph), 6.94 (t, J = 8.1 Hz, 1H, Ph), 6.39–6.35 (m, 1H, 3-H of indole), 4.66 (d, J = 6.0 Hz, 2H, C$\underline{H_2}$Ph), 2.87 (t, J = 7.7 Hz, 2H, CH_2), 2.80 (t, J = 7.4 Hz, 2H, CH_2), 2.48 (d, J = 1.0 Hz, 3H, Me), 2.12 (m, J = 7.8 Hz, 2H, CH_2).^{13}C NMR (100 MHz, DMSO-d_6) δ 171.45, 159.82, 159.10, 156.42, 155.76, 151.10, 139.07, 137.45, 137.28, 133.08, 133.01, 128.91, 128.74, 127.83, 125.62, 121.93, 121.84, 113.99, 113.59, 111.11, 101.69, 44.03, 34.17, 27.20, 21.69, 16.07. HRMS calcd for $C_{27}H_{25}BN_6O_3S$ $[M+Na]^+$ 547.1698, found 547.1686.

4.2.15. (3-(((2-(4-(furan-2-carboxamido)-2-methyl-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)methyl)phenyl)boronic acid (26)

Compound **26** was synthesized from **16a** and furan-2-carboxylic acid according to the procedure for preparing **20**. 82% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H, —CONH—), 8.04 (s, 2H, —OH), 7.94 (d, J = 1.7 Hz, 1H, furan), 7.85 (t, J = 6.1 Hz, 1H, —NH—), 7.78 (s, 1H, Ph), 7.70 (d, J = 7.3 Hz, 1H, Ph), 7.61 (d, J = 8.3 Hz, 1H, Ph), 7.36 (dd, J_1 = 5.0 Hz, J_2 = 8.2 Hz, 2H, Ph), 7.31 (t, J = 7.4 Hz, 1H, furan), 7.23 (d, J = 7.6 Hz, 1H, Ph), 6.91 (t, J = 8.0 Hz, 1H, Ph), 6.71 (dd, J_1 = 1.8 Hz, J_2 = 3.5 Hz, 1H, furan), 6.38 (s, 1H, 3-H of indole), 4.66 (d, J = 5.8 Hz, 2H, C$\underline{H_2}$Ph), 2.87 (t, J = 7.8 Hz, 2H, CH_2), 2.80 (t, J = 7.3 Hz, 2H, CH_2), 2.47 (s, 3H, Me), 2.11 (m, 2H, CH_2).^{13}C NMR (100 MHz, DMSO-d_6) δ 171.44, 159.81, 156.71, 156.48, 148.19, 146.00, 139.08, 137.59, 136.89, 133.08, 133.01, 128.92, 128.54, 127.83, 123.39, 121.67, 116.11, 114.84, 113.49, 112.49, 111.42, 103.13, 44.03, 34.18, 27.20, 21.69, 16.11. HRMS calcd for $C_{28}H_{26}BN_5O_4$ $[M+Na]^+$ 530.1974, found 530.1964.

4.2.16. (3-(((2-(4-(isoxazole-5-carboxamido)-2-methyl-1H-indol-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)methyl)phenyl) boronic acid (27)

Compound **27** was synthesized from **16a** and isoxazole-5-carboxylic acid according to the procedure for preparing **20**. 79% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (d, J = 1.9 Hz, 1H, isoxazole), 6.96 (s, 1H, Ph), 6.89–6.76 (m, 1H, Ph), 6.72 (t, J = 9.0 Hz, 1H, Ph), 6.62–6.46 (m, 3H, Ph), 6.34 (d, J = 1.9 Hz, 1H, Ph), 6.17 (t, J = 7.6 Hz, 1H, 3-H of indole), 5.58 (s, 1H, isoxazole), 3.92 (s, 2H, C$\underline{H_2}$Ph), 2.54 (s, 2H, CH_2), 2.13 (t, J = 7.7 Hz, 2H, CH_2), 2.05 (t, J = 7.4 Hz, 3H, Me), 1.43 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.14, 162.25, 159.23, 155.19, 154.29, 150.25, 137.89, 137.06, 136.44, 131.01, 127.72, 127.26, 126.70, 125.85, 122.55, 120.43, 114.96, 113.05, 110.22, 105.46, 100.53, 42.99, 32.52, 25.53, 20.51, 13.08. HRMS calcd for $C_{27}H_{25}BN_6O_4$ $[M+Na]^+$ 531.1927, found 531.1919.

4.2.17. (3-(((2-(2-methyl-4-(methylsulfonamido)-1H-indol-1-yl)-5,6,7,8-tetrahydroquinazolin-4-yl)amino)methyl)phenyl)boronic acid (28)

Compound **28** was synthesized from **17b** and methanesulfonyl chloride according to the procedure for preparing **17**. 75% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H, —CONH—), 8.04 (s, 2H, —OH), 7.74 (s, 1H, Ph), 7.69–7.60 (m, 1H, Ph), 7.51 (d, J = 8.3 Hz, 1H, Ph), 7.33–7.24 (m, 2H, Ph), 6.95 (dd, J_1 = 0.9 Hz, J_2 = 7.8 Hz, 1H, Ph), 6.84 (t, J = 8.0 Hz, 1H, Ph), 6.58 (d, J = 1.3 Hz, 1H, 3-H of indole), 4.64 (d, J = 5.9 Hz, 2H, C$\underline{H_2}$Ph), 2.89 (s, 3H, Me), 2.66 (s, 2H, CH_2), 2.45–2.37 (s, 3H, Me), 1.83 (d, J = 6.8 Hz, 4H, CH_2).^{13}C NMR (100 MHz, DMSO-d_6) δ 162.10, 161.84, 154.31, 139.21, 137.63, 137.22, 132.91, 132.80, 128.64, 128.52, 127.79, 123.66, 122.03, 115.61, 111.51, 109.80, 102.36, 44.28, 31.95, 29.47, 22.25, 16.11. HRMS calcd for $C_{25}H_{28}BN_5O_4S$ $[M+Na]^+$ 528.1851, found 528.1843.

4.2.18. (3-(((2-(4-(but-2-ynamido)-2-methyl-1H-indol-1-yl)-5,6,7,8-tetrahydroquinazolin-4-yl)amino)methyl)phenyl)boronic acid (29)

Compound **29** was synthesized from **17b** and 2-butynoic acid according to the procedure for preparing **20**. 68% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H, —CONH—), 8.02 (d, J = 5.5 Hz, 2H, —OH), 7.75 (s, 1H, —NH—), 7.68 (d, J = 6.6 Hz, 1H, Ph), 7.65–7.61 (m, 1H, Ph), 7.48 (d, J = 8.3 Hz, 1H, Ph), 7.37 (d, J = 8.0 Hz, 1H, Ph), 7.34–7.26 (m, 2H, Ph), 6.82 (t, J = 8.0 Hz, 1H, Ph), 6.54 (s, 1H, 3-H of indole), 4.65 (d, J = 6.2 Hz, 2H, C$\underline{H_2}$Ph), 2.68 (d, J = 4.6 Hz, 2H, CH_2), 2.43 (s, 3H, Me), 2.06 (s, 3H, Me), 1.84 (m, J = 7.2 Hz, 4H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.09, 161.83, 154.36, 151.27, 139.22, 137.45, 136.66, 132.90, 132.85, 128.81, 128.67, 127.77, 121.65, 121.56, 114.26, 110.95, 109.72, 102.61, 84.54, 76.57, 44.30, 29.48, 22.50, 22.27, 16.10, 3.81. HRMS calcd for $C_{28}H_{28}BN_5O_3$ $[M+Na]^+$ 516.2182, found 516.2175.

4.2.19. (3-(((2-(4-(furan-2-carboxamido)-2-methyl-1H-indol-1-yl)-5,6,7,8-tetrahydroquinazolin-4-yl)amino)methyl)phenyl)boronic acid (30)

Compound **30** was synthesized from **17b** and furan-2-carboxylic acid according to the procedure for preparing **20**. 67% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H, —CONH—), 8.05 (s, 2H, —OH), 7.94 (d, J = 1.7 Hz, 1H, furan), 7.78 (s, 1H, —NH—), 7.70 (d, J = 7.0 Hz, 1H, Ph), 7.64 (s, 1H, Ph), 7.56 (d, J = 8.3 Hz, 1H, Ph), 7.38 (d, J = 3.5 Hz, 1H, furan), 7.33 (d, J = 4.8 Hz, 1H, Ph), 7.29 (d, J = 7.5 Hz, 1H, Ph), 7.23 (d, J = 7.7 Hz, 1H, Ph), 6.88 (t, J = 8.0 Hz, 1H, Ph), 6.71 (dd, J_1 = 1.8 Hz, J_2 = 3.5 Hz, 1H, furan), 6.37 (s, 1H, 3-H of indole), 4.67 (d, J = 5.9 Hz, 2H, C$\underline{H_2}$Ph), 2.68 (t, J = 5.9 Hz, 2H, CH_2), 2.45 (s, 3H, Me), 1.93–1.77 (m, 4H, CH_2).^{13}C NMR (100 MHz, DMSO-d_6) δ 164.31, 164.06, 158.92, 156.63, 150.42, 148.19, 141.47, 139.76, 139.06, 136.72, 135.13, 135.09, 130.92, 130.72, 129.99, 125.56, 123.84, 118.24, 117.04, 114.69, 113.59, 111.91, 105.21, 46.55, 34.19, 27.81, 24.73, 24.50, 18.24. HRMS calcd for $C_{29}H_{28}BN_5O_4$ $[M+Na]^+$ 544.2131, found 544.2121.

4.2.20. (3-(((2-(2-methyl-4-(methylsulfonamido)-1H-indol-1-yl)-7,8-dihydro-5H-pyrano[4,3-d]pyrimidin-4-yl)amino)methyl)phenyl)boronic acid (31)

Compound **31** was synthesized from **16c** and methanesulfonyl chloride according to the procedure for preparing **17**. 64% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H, —NH—), 7.96 (s, 2H, —OH), 7.85 (d, J = 1.7 Hz, 1H, Ph), 7.69 (s, 1H, Ph), 7.61 (d, J = 7.0 Hz, 1H, Ph), 7.55 (s, 1H, Ph), 7.47 (d, J = 8.3 Hz, 1H, Ph), 7.29 (d, J = 3.5 Hz, 1H, Ph), 7.24 (dd, J_1 = 2.4 Hz, J_2 = 4.2 Hz, 1H, Ph), 6.28 (s, 1H, 3-H of indole), 4.59 (d, J = 5.9 Hz, 2H, NHC$\underline{H_2}$), 3.52 (dd, J_1 = 5.5 Hz, J_2 = 8.4 Hz, 1H, —NH—), 3.30 (s, 3H, Me) 2.60 (t, J = 5.9 Hz, 2H, CH_2), 2.36 (s, 3H, Me), 1.82–1.68 (m, 4H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.65, 159.23, 155.17, 138.83, 137.61, 137.57, 137.24, 133.01, 132.80, 128.71, 128.59, 127.84, 122.19, 115.76, 111.60, 108.64, 105.97, 102.76, 64.40, 63.16, 44.42, 40.48

HRMS calcd for $C_{24}H_{26}BN_5O_5S$ $[M+Na]^+$ 530.1644, found 530.1678.

4.2.21. (3-(((2-(4-(but-2-ynamido)-2-methyl-1H-indol-1-yl)-7,8-dihydro-5H-pyrano[4,3-d]pyrimidin-4-yl)amino)methyl)phenyl)boronic acid (32)

Compound **32** was synthesized from **16c** and 2-butynoic acid according to the procedure for preparing **20**. 67% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H, —CONH—), 8.23 (s, 2H, —OH), 7.75 (s, 1H, Ph), 7.72–7.60 (m, 1H, Ph), 7.54 (d, J = 8.3 Hz, 1H, Ph), 7.38 (d, J = 7.8 Hz, 1H, Ph), 7.34–7.24 (m, 1H, Ph), 6.85 (t, J = 8.1 Hz, 1H, Ph), 6.56 (s, 1H, 3-H of indole), 4.64 (d, J = 5.8 Hz, 1H, C$\underline{H_2}$Ph), 4.60 (s, 2H, OC$\underline{H_2}$), 3.99 (t, J = 5.6 Hz, 2H, OC$\underline{H_2}$CH$_2$), 2.75 (t, J = 5.8 Hz, 2H, OCH$_2$C$\underline{H_2}$), 2.44 (s, 3H, Me), 2.06 (s, 3H, Me).^{13}C NMR (100 MHz, DMSO-d_6) δ 166.59, 159.36, 159.21, 155.23, 151.29, 138.83, 133.02, 132.85, 128.72, 127.82, 121.83, 121.64, 114.46, 111.07, 108.57,

103.01, 76.55, 64.41, 63.20, 44.18, 31.13, 16.23, 3.81. HRMS calcd for $C_{27}H_{26}BN_5O_4$ $[M+Na]^+$ 518.1974, found 518.1938.

4.2.22. (3-(((2-(4-(furan-2-carboxamido)-2-methyl-1H-indol-1-yl)-7,8-dihydro-5H-pyrano[4,3-d]pyrimidin-4-yl)amino)methyl)phenyl)boronic acid **(33)**

Compound **33** was synthesized from **16c** and furan-2-carboxylic acid according to the procedure for preparing **20**. 60% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H, —CONH—), 8.06 (s, 2H, —OH), 7.94 (dd, J_1 = 0.8 Hz, J_2 = 1.8 Hz, 1H, furan), 7.77 (s, 1H, —NH—), 7.70 (dt, J_1 = 1.7 Hz, J_2 = 7.0 Hz, 1H, Ph), 7.66 (t, J = 6.0 Hz, 1H, Ph), 7.60 (d, J = 8.3 Hz, 1H, Ph), 7.37 (dd, J_1 = 0.8 Hz, J_2 = 3.4 Hz, 1H, furan), 7.34 (dd, J_1 = 2.6 Hz, J_2 = 4.3 Hz, 1H, Ph), 7.30 (d, J = 7.5 Hz, 1H, Ph), 7.23 (d, J = 7.6 Hz, 1H, Ph), 6.90 (t, J = 8.0 Hz, 1H, Ph), 6.71 (dd, J_1 = 1.8 Hz, J_2 = 3.5 Hz, 1H, furan), 6.38 (t, J = 0.9 Hz, 1H, 3-H of indole), 4.66 (d, J = 5.8 Hz, 2H, C$\underline{H}_2$Ph), 4.61 (s, 2H, OC$\underline{H}_2$), 3.99 (t, J = 5.6 Hz, 2H, OC$\underline{H}_2$CH$_2$), 2.77 (t, J = 5.6 Hz, 2H, OCH$_2$C$\underline{H}_2$), 2.46 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.36, 159.21, 156.72, 155.28, 148.17, 146.02, 138.89, 137.51, 136.87, 133.03, 132.86, 128.78, 128.55, 127.85, 123.45, 121.79, 116.25, 114.86, 112.50, 111.49, 108.55, 103.42, 64.42, 63.21, 44.20, 31.14, 16.16. HRMS calcd for $C_{28}H_{26}BN_5O_5$ $[M+Na]^+$ 546.1924, found 546.1921.

4.2.23. (3-(((2-(2-methyl-4-(methylsulfonamido)-1H-indol-1-yl) quinazolin-4-yl)amino)methyl)phenyl)boronic acid **(34)**

Compound **34** was synthesized from **16d** and methanesulfonyl chloride according to the procedure for preparing **17**. 80% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.46 (s, 1H, —CONH—), 9.24 (t, J = 6.0 Hz, 1H, —NH—), 8.44–8.38 (m, 1H, Ph), 8.11 (s, 2H, —OH), 7.83 (m, 2H, Ph), 7.79–7.72 (m, 2H, Ph), 7.71 (m, 1H, Ph), 7.55 (m, 1H, Ph), 7.43–7.37 (m, 1H, Ph), 7.32 (t, J = 7.5 Hz, 1H, Ph), 7.01 (d, J = 7.5 Hz, 1H, Ph), 6.92 (t, J = 8.0 Hz, 1H, Ph), 6.66 (d, J = 6.2 Hz, 1H, 3-H of indole), 4.86 (d, J = 5.8 Hz, 2H, C$\underline{H}_2$Ph), 2.92 (s, 3H, Me), 2.57 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.39, 154.49, 150.52, 138.35, 137.87, 137.59, 133.82, 133.17, 132.92, 128.88, 128.62, 127.94, 127.58, 125.69, 123.89, 123.54, 122.26, 115.88, 113.56, 111.99, 103.06, 44.54, 40.80, 16.61. HRMS calcd for $C_{25}H_{24}BN_5O_4S$ $[M+H]^+$ 502.1719, found 502.1750.

4.2.24. (3-(((2-(4-(but-2-ynamido)-2-methyl-1H-indol-1-yl)quinazolin-4-yl)amino)methyl)phenyl)boronic acid **(35)**

Compound **35** was synthesized from **16d** and 2-butynoic acid according to the procedure for preparing **20**. 72% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H, —CONH—), 9.25 (t, J = 5.9 Hz, 1H, —NH—), 8.42 (d, J = 8.0 Hz, 1H, Ph), 8.16 (s, 2H, —OH), 7.82 (d, J = 7.3 Hz, 2H, Ph), 7.76–7.68 (m, 3H, Ph), 7.54 (t, J = 7.4 Hz, 1H, Ph), 7.41 (t, J = 7.1 Hz, 2H, Ph), 7.31 (t, J = 7.4 Hz, 1H, Ph), 6.88 (t, J = 8.0 Hz, 1H, Ph), 6.62 (s, 1H, 3-H of indole), 4.85 (d, J = 5.6 Hz, 2H, C$\underline{H}_2$Ph), 2.56 (s, 3H, Me), 2.06 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.60, 161.36, 154.53, 151.29, 150.54, 138.38, 137.67, 137.04, 133.79, 133.16, 132.97, 128.92, 128.90, 127.92, 127.56, 125.64, 123.57, 121.89, 121.77, 114.55, 113.54, 111.39, 103.30, 84.59, 76.56, 44.54, 16.60, 3.83. HRMS calcd for $C_{28}H_{24}BN_5O_3$ $[M+Na]^+$ 512.1869, found 512.1904.

4.2.25. (3-(((2-(4-(furan-2-carboxamido)-2-methyl-1H-indol-1-yl) quinazolin-4-yl)amino)methyl)phenyl)boronic acid **(36)**

Compound **36** was synthesized from **16d** and furan-2-carboxylic acid according to the procedure for preparing **20**. 64% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.00 (s, 1H, —NH—), 8.47–8.38 (m, 1H, Ph), 8.09 (s, 2H, —OH), 7.94 (d, J = 1.7 Hz, 1H, —CH), 7.87–7.69 (m, 5H, Ph), 7.55 (m, J_1 = 6.8 Hz, J_2 = 8.2 Hz, 1H, Ph), 7.45–7.37 (m, 2H, Ph), 7.32 (t, J = 7.5 Hz, 1H, —CH), 7.27 (d, J = 7.7 Hz, 1H, Ph), 6.94 (t, J = 8.0 Hz, 1H, Ph), 6.72 (dd, J_1 = 1.8 Hz, J_2 = 3.5 Hz, 1H, 3-H of indole), 6.44 (s, 1H, CH), 5.77 (s, 1H, —NH—), 4.87 (d, J = 5.8 Hz, 2H, —NH$\underline{CH}_2$), 2.59 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.38, 156.74, 154.60, 150.58, 148.20, 146.02, 138.39, 137.77, 137.21, 133.84, 133.18, 133.00, 129.44, 128.95, 128.60, 127.93, 127.58, 125.63, 123.59, 123.55 121.85, 116.34, 114.86, 113.55, 112.49, 111.87, 103.71, 44.59, 16.53. HRMS calcd for $C_{29}H_{24}BN_5O_4$ $[M+Na]^+$ 518.1999, found 518.1978.

4.2.26. (3-(((2-(4-(but-2-ynamido)-2-methyl-1H-indol-1-yl)-5-methoxypyrimidin-4-yl)amino)methyl)phenyl)boronic acid **(37)**

Compound **37** was synthesized from **16e** and 2-butynoic acid according to the procedure for preparing **20**. 66% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H, —CONH—), 8.03 (s, 2H, —OH), 7.94 (s, 1H, —NH—), 7.73 (s, 1H, Ph), 7.68 (d, J = 6.7 Hz, 1H, Ph), 7.36 (t, J = 7.6 Hz, 2H, Ph), 7.32–7.24 (m, 2H, Ph), 6.82 (t, J = 8.0 Hz, 1H, Ph), 6.53 (s, 1H, 3-H of indole), 4.60 (d, J = 6.3 Hz, 2H, C$\underline{H}_2$Ph), 3.96 (s, 3H, Me), 2.40 (s, 3H, Me), 2.06 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.80, 151.27, 150.03, 138.82, 138.56, 137.44, 136.52, 133.06, 132.95, 128.83, 127.77, 121.60, 121.40, 114.13, 110.41, 102.14, 84.56, 56.60, 43.89, 15.72, 3.81. HRMS calcd for $C_{25}H_{24}BN_5O_4$ $[M+Na]^+$ 492.1817, found 492.1811.

4.2.27. (3-(((2-(4-(furan-2-carboxamido)-2-methyl-1H-indol-1-yl)-5-methoxypyrimidin-4-yl)amino)methyl)phenyl)boronic acid **(38)**

Compound **38** was synthesized from **16e** and furan-2-carboxylic acid according to the procedure for preparing **20**. 62% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H, —CONH—), 8.04 (s, 2H, —OH), 7.96 (s, 1H, —NH—), 7.94 (dd, J_1 = 0.8 Hz, J_2 = 1.8 Hz, 1H, furan), 7.75 (s, 1H, Ph), 7.69 (d, J = 6.8 Hz, 1H, Ph), 7.42 (d, J = 8.3 Hz, 1H, Ph), 7.37 (d, J = 3.4 Hz, 1H, furan), 7.35–7.30 (m, 1H, Ph), 7.28 (d, J = 7.5 Hz, 1H, Ph), 7.21 (d, J = 7.6 Hz, 1H, Ph), 6.87 (t, J = 8.0 Hz, 1H, Ph), 6.71 (dd, J_1 = 1.8 Hz, J_2 = 2.5 Hz, 1H, furan), 6.35 (d, J = 1.1 Hz, 1H, 3-H of indole), 4.62 (d, J = 6.1 Hz, 2H, C$\underline{H}_2$Ph), 3.97 (s, 3H, Me), 2.45–2.37 (m, 3H, Me). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.70, 154.81, 150.11, 148.18, 146.01, 138.84, 138.55, 137.53, 136.70, 133.09, 132.97, 128.86, 128.55, 127.79, 123.19, 121.56, 115.91, 114.84, 112.50, 110.84, 102.55, 56.61, 43.91, 15.65. HRMS calcd for $C_{26}H_{24}BN_5O_5$ $[M+Na]^+$ 520.1767, found 520.1760.

4.3. Biological assay

4.3.1. P97 ATPase assay

The ATPase assay was performed according to the following protocol: compounds were diluted in DMSO with a 3-fold 10-point serial dilution starting at 10 μM. The tenth concentration point was solvent control group (no drug). The assay was done in a 384-well plate with each row as a single dilution series with duplicate of each compound concentration point. In 4 μL total volume, 60 μg/mL p97 hexamer enzyme and 100 μM ATP were added to start the reaction. The plate was sealed and incubated at 25 °C for 60 min after mixing thoroughly in an orbital shaker. ADP Glo reagents 1 and 2 were added according to the manufacturer's protocol (Promega, Madison, WI). The luminescence was measured by CLARIO Star Plate Reader as the end point of the reaction. The program Graphpad Prism 6 was used to fit nonlinear curve and calculate IC_{50} of each compounds.

4.3.2. Cell culture and inhibition of cell proliferation

Cell lines were cultured according to ATCC guidelines. Cells were plated (2000 cells/well) in a volume of 90 μL/well of complete media in 96-well cell culture plates and cultured at 37 °C with 5% CO_2 for 24 h. Inhibitors were dissolved in DMSO (less than 0.1%) and tested in duplicate utilizing 3-fold serial dilutions with the highest concentration at 10 μM. Inhibitors were incubated with cells at 37 °C with 5% CO_2. After 72 h treatment, Cell Counting Kit-8 (CCK-8) was added to the plates to measure cell viabilities. Absorbance at 450 nm was measured and using GraphPad software to fit sigmoidal curve to determine IC_{50} value.

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 material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2021.116114.

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