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Article

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# Structure-Based Design of Potent Nicotinamide Phosphoribosyltransferase Inhibitors with Promising in Vitro and in Vivo Antitumor Activities

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## Abstract

Inhibition of nicotinamide phosphoribosyltransferase (NAMPT) has the potential to directly limit NAD production in cancer cells and is an effective strategy for cancer treatment. Using a structure-based strategy, we have designed a new class of potent small-molecule inhibitors of NAMPT. Several designed compounds showed promising antiproliferative activities *in vitro*. (*E*)-N-(5-((4-(((2-(1H-indol-3-yl)ethyl)(isopropyl)amino)methyl)phenyl)amino)pentyl)-3-(pyridin-3-yl)acrylamide, **30**, bearing an indole moiety has an $IC_{50}$ of 25.3 nM for binding to the NAMPT protein, and demonstrated promising inhibitory activities in the nanomolar range against several cancer cell lines (MCF-7 $GI_{50}$ = 0.13 nM; MDA-MB-231 $GI_{50}$ = 0.15 nM). Triple-negative breast cancer is the most malignant

subtype of breast cancer with no effective targeted treatments currently available. Significant antitumor efficacy of compound **30** was achieved (TGI was 73.8%) in an orthotopic MDA-MB-231 triple-negative breast cancer xenograft tumor model. This paper reports promising lead molecules for the inhibition of NAMPT which could serve as a basis for further investigation.

## Introduction

Nicotinamide phosphoribosyltransferase (NAMPT), also known as pre-B-cell colony-enhancing factor (PBEF) or visfatin, was originally found in peripheral lymphocytes and identified as a secreted growth factor for early B cells.[1] NAMPT is a rate limiting enzyme that converts nicotinamide (NAM) to nicotinamide mononucleotide (NMN) in the biosynthesis of nicotinamide adenine dinucleotide (NAD), which is a crucial cofactor or substrate for a wide range of metabolic enzymes.[2-4] This NAM-dependent process of NMN formation enables the efficient intracellular recycling of NAM, which is produced by NAD-consuming enzymes such as sirtuins and poly-ADP-ribose polymerases (PARPs).[5,6] Proper maintenance of NAD levels in cells is known to be crucial to sustaining the energy required for numerous physiologic processes related to cell metabolism.[7-10] Due to their uncontrolled growth and proliferation, cancer cells have increased energy demands for essential nutrients. They have a higher rate of NAD consumption than normal cells, which may make them more susceptible to NAMPT inhibition in comparison with non-cancerous cells. Thus, NAMPT is over-expressed in a number of cancer types and efficiently inhibition of NAMPT enzyme activity causes a direct inhibition of NAD production in cancer cells which leads to loss of ATP and ultimately cell death. [11,12] Normal cells on the other hand are protected from NAMPT inhibition as they have access to an alternative pathway, catalyzed by nicotinic acid phosphoribosyltransferase (NAPRT) for NAD synthesis from nicotinic acid (NA).[13,14] Many cancer cell lines however are deficient in NAPRT activity and are therefore

unable to convert NA to NAD.[15] Consequently, NAMPT inhibition has emerged as a promising and novel strategy for selective inhibition of cancer cell growth and is a promising target for cancer treatment.[16, 17]

Two small molecule inhibitors of NAMPT, (*E*)-N-(4-(1-benzoylpiperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide (**1**, APO-866, FK866)[18] and (*E*)-1-(6-(4-Chlorophenoxy)hexyl)-2-cyano-3-(pyridin-4-yl)- guanidine (**2**, CHS828)[19]are currently in phase II clinical trials (Figure 1). Although they potently inhibit NAMPT and/or NAD synthesis in cancer cells and thereby induce cell death, both compounds failed to demonstrate sustained efficacy in cancer patients due to dose-limiting toxicity, while thrombocytopenia and gastrointestinal toxicity are the most commonly encountered adverse events for them.[20,21] To date, a few other NAMPT inhibitors have been reported and shown potent *in vitro* antiproliferative activity in a spectrum of cancer cell lines (Figure 1).[17,22,23] Specific and potent NAMPT inhibitor with reduced side effects and improved pharmacokinetic properties is highly desirable. Here, we report the identification of a new series of NAMPT inhibitors using *in silico* rational design and structure activity analysis (SAR). The lead inhibitor demonstrated more potent NAMPT enzyme inhibitory effect in comparison to **1**, as well as promising *in vitro* anticancer activities against several cancer cell lines and good therapeutic effects in a human breast carcinoma (MDA-MB-231) xenograft model.

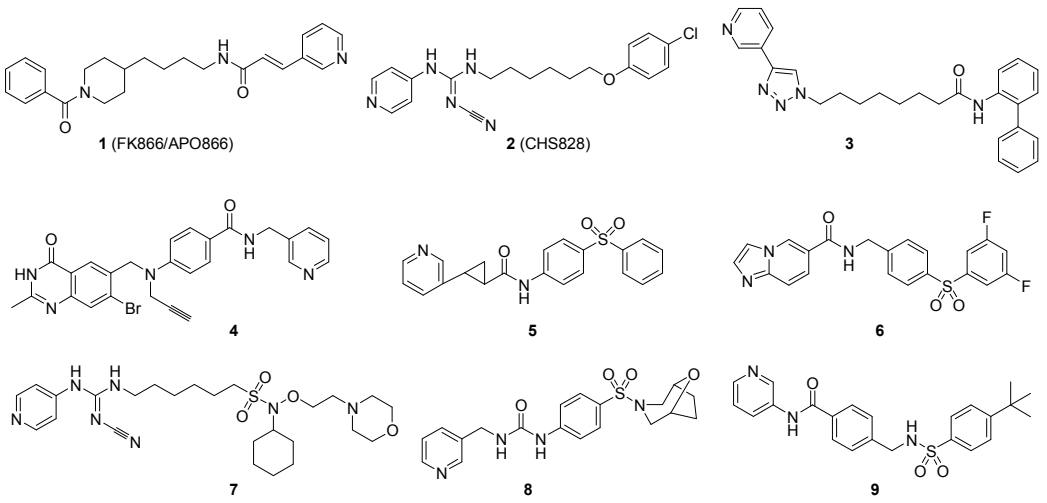



**Figure 1**. Reported NAMPT inhibitors.

## Results and Discussion

### A New Chemical Scaffold to Target NAMPT

The X-ray crystal structure of the enzyme NAMPT reveals that it functions as a homodimer with a head to tail interaction, and has two active sites accessible only through a long, narrow and predominantly hydrophobic tunnel (15 Å × 6 Å) at the interface between the monomers (Figure 2A). The crystal structure of NAMPT bound to **1** (PDB codes 2GVJ[1] and 4O1D[2]) shows that its pyridine moiety is sandwiched between the side chains of Phe193 and Tyr18, and the main binding interaction is a π-π offset stacking.[24-26] The oxygen atom and the nitrogen atom of the amide bond in **1** form two hydrogen bonds with the hydroxyl group of the Ser 275 side chain and a water molecule, respectively. This water molecule, as a hydrogen bond bridge, further forms three hydrogen bonds with Asp219 and Val242. Analysis of the co-crystal structure of **1** complexed with NAMPT showed that the tail group plays an important

role because of its ability to establish hydrophobic interactions with a hydrophobic cleft, which can accommodate a larger group than the benzoylpiperidine ring. Superimposition of two published co-crystal structures of **1** complexed with NAMPT (PDB codes: 2GVJ[1] and 4O1D[2]), reveals that **1** has two different conformations in the binding tunnel in these two crystal structures. The 3-(pyridin-3-yl)acrylamide moieties are almost identical, but the tail part of **1**, i.e., the benzoylpiperidine segment, has different orientations in the two structures (Figure 2B). This is also the case when **2** bound to the protein (Figure 2B): in the crystal structure of 4O12[3], different conformations of **2** can be observed: the chlorobenzene ring of **2** can interacts with the protein in two different ways (Figure 2B). Based on this structural information, we speculated that if we retain the 3-(pyridin-3-yl)acrylamide part of **1** and modify the linker and tail group and extend the length between the cap group and the tail group, they may occupy the two locations simultaneously as does the tail of **2**, and this may increase the binding affinity to NAMPT. Additionally, the Tyr188 residue in the rim of the binding tunnel of NAMPT is exposed to solvent, and inhibitors which have π-π stacking interaction with Tyr188 are also expected to show an increased binding affinity. Based on this strategy, we designed and synthesized compound **10** as a new template, in which the 3-(pyridin-3-yl)acrylamide part of **1** was retained, and the alkyl chain linker was replaced by a linker containing a more rigid aromatic ring. The benzoylpiperidine ring in the tail part in **1** was replaced by an indole moiety, which is connected with a flexible chain to the benzene ring. It was expected that this indole moiety in the designed NAMPT inhibitors could occupy the two locations of the rim

in the binding tube of NAMPT simultaneously, or at least, it could be located where it could have a π-π stacking interaction with Tyr188 of NAMPT. Accordingly, we designed and synthesized compound **10** as a template for further optimization (Figure 3). Compound **10** had an $IC_{50}$ value of 702 nM to NAMPT, and is 22 times less potent than **1** ($IC_{50}$ = 32.0 nM) (Table 1).

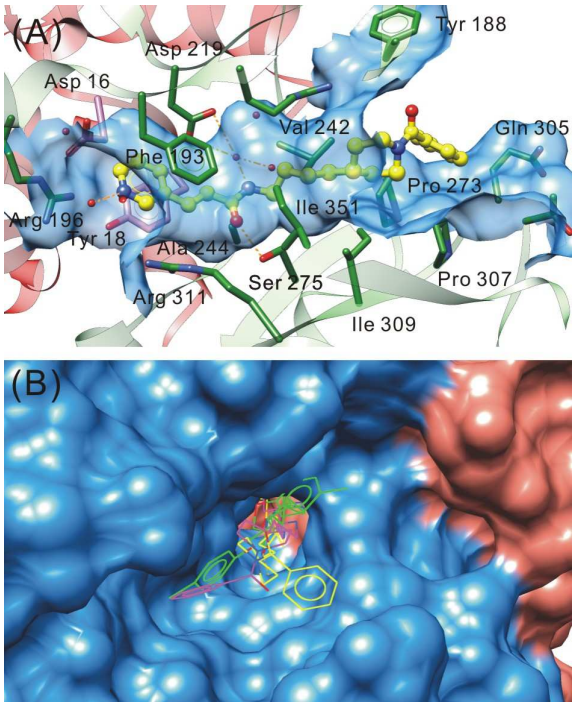
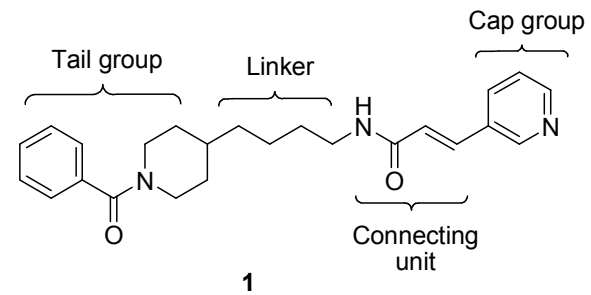


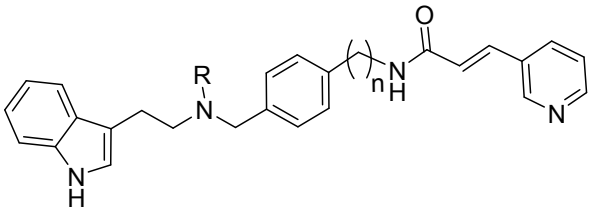

**Figure 2.** (A) The binding mode of **1** (PDB code: 4O1D). (B) The tail groups of NAMPT inhibitors may have different orientations in the active site. Yellow, **1** (PDB code: 4O1D); magenta, **1** (PDB code: 2GVJ); green, **2**(PDB code: 4O12).

The nitrogen substituent of compound **10** might have considerable impact on the efficacy against NAMPT of the related compounds. To assess the contribution of the isopropyl group to the efficacy, we first synthesized compound **11** in which this group

is replaced by a hydrogen atom. Compound **11** is half as potent as **10**, confirming the importance of the isopropyl group, which can easily be understood as the binding tube is hydrophobic. Therefore, introduction of increasingly hydrophobic groups into this nitrogen of **10** would improve the van der Waals interactions. Accordingly, we incorporated a series of hydrophobic groups such as methyl, ethyl, propyl, hydroxyethyl, phenyl or *t*-Boc groups at this position, as shown in Table 1. Among these compounds, **14** with hydroxyethyl at this position had an $IC_{50}$ value of 181 nM against NAMPT in the enzyme inhibition assay, and was therefore about 3.8 times more potent than the original compound **10**. Compared with the isopropyl group, the hydroxyethyl group in **14** is less hydrophobic, and its favorable binding affinity can be explained by its docking pose in which its two carbons have hydrophobic interactions with Val242 and Tyr188, while the hydroxyl group forms a hydrogen bond with Arg191. However, compound **14** was equally potent as compound **10** in cell inhibition assay (see Table 1). We assumed that compound **14** was very hydrophilic, which makes it difficult to across the cell membrane.




**1**



**10**: R = -CH(CH$_3$)$_2$, n = 3; **11**: R = -H; n = 3;
**12**: R = -CH$_2$CH$_3$, n = 3; **13**: R = -CH$_2$CH$_2$CH$_3$, n = 3;
**14**: R = -CH$_2$CH$_2$OH, n = 3; **15**: R = -CH$_2$Ph, n = 3;
**16**: R = -CH$_3$, n = 3; **17**: R = Boc, n = 3;
**18**: R = -CH(CH$_3$)$_2$, n = 1; **19**: R = -CH(CH$_3$)$_2$, n = 5;
**20**: R = -CH(CH$_3$)$_2$, n = 7.

**Figure 3**. Chemical structures of newly designed NAMPT inhibitors.

**Table 1.** *In Vitro* NAMPT Enzyme and MDA-MB-231 Inhibitory Activities of Compounds **1**, **10-20**.[a]

| Compd | NAMPT ($IC_{50}$ (nM)) | MDA-MB-231 ($GI_{50}$ (nM)) |
|---|---|---|
| **1** | 32.0±2.50 | 0.78±0.04 |
| **10** | 702±100.4 | 30.5±3.39 |

| | | |
|---|---|---|
| **11** | 1410±45.47 | 358±40.91 |
| **12** | 873±233.4 | 76.4±7.42 |
| **13** | 544±55.3 | 36.0±3.38 |
| **14** | 181±10.0 | 31.4±1.76 |
| **15** | 1000±59.42 | 269±26.15 |
| **16** | 780±69.86 | 84.7±17.08 |
| **17** | 715±67.56 | 123±14.85 |
| **18** | 213±17.77 | 134±14.59 |
| **19** | 66.9±7.0 | 0.89±0.00 |
| **20** | 52.7±1.2 | 0.84±0.16 |

[a]**1** was used as the positive control. Values are means of three experiments. The incubation time for MDA-MB-231 inhibitory activities is 72 h.

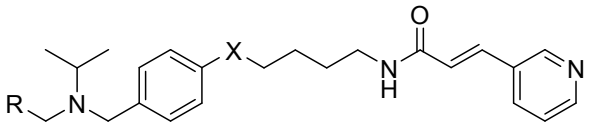
Having identified isopropyl as a suitable substituent group on the nitrogen for effective interaction with NAMPT, we sought to determine the optimal length (n) of the alkyl chain. We designed and synthesized compounds **18-20**, in which n = 1, 5,

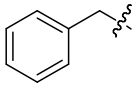
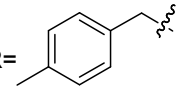
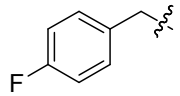
and 7 carbon atoms, respectively. Compound **18**, an analog with a single carbon atom in the side chain, was 3 times as potent against NAMPT as the compound **10**. When the alkyl chain was extended to n = 5, the resulting compound **19** had an $IC_{50}$ value of 66.9 nM, which was about 10 times as potent as the compound **10**. Compound **20** with seven methylene spacers, had $IC_{50}$ value of 52.7 nM and was approximately equivalent in activity to compound **19**. Molecular modeling indicated that the benzene ring in the linker of **20** was positioned in the external rim of the binding tube, indicating that in this binding tube a flexible alkyl chain is accommodated better than a chain with a bulkier benzene ring.

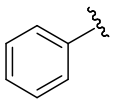
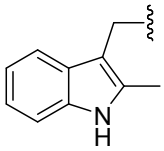
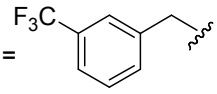
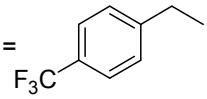
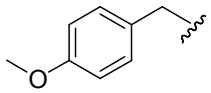
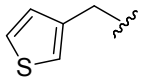
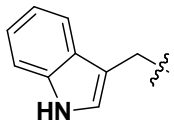
To further study the SAR of compound **19** and explore the effect of the tail group on the enzymatic inhibitory activities, we designed and synthesized compounds **21-29** (Table 2). Based on the binding mode of compound **19** to NAMPT, we assumed that replacement of the indole moiety of **19** with a phenyl ring would attenuate the affinity because the indole ring enjoys a hydrophobic interaction with the protein. Replacement of the indole ring by a phenyl had a significant impact on the effectiveness of the compounds against NAMPT; compound **21** was found to be almost 4 times less potent than compound **19**. It was observed that different electron withdrawing functional groups at the *meta*- or *para*-position of the phenyl ring decreased the inhibitory activity against NAMPT markedly (**26, 27**). For instance, when a trifluoromethyl group was introduced at the *meta*-position of compound **21**, the resulting compound (**26**) was 1.3 times less potent than **21**. Conversely, when electron donating functional groups such as methyl and methoxyl were introduced at

the *para*-position of compound **21** (**22** and **28** respectively), their inhibitory activities against NAMPT were significantly increased in comparison to that of compound **21**. Compound **28** with a methoxyl group at the *para*-position had an $IC_{50}$ value of 54.8 nM against NAMPT, and thus was about 5.2 times more potent than compound **21**. Interestingly, replacement of the phenyl ring with a thiophene group (**29**) increased the inhibitory activity, suggesting that tail group might be a feasible position for further optimization.

**Table 2.** *In Vitro* NAMPT Enzyme Inhibitory Activities of Compounds **21** – **30**.[a]




| Compd | NAMPT ($IC_{50}$, nM) |
|---|---|
| **1** | 32.0±2.50 |
| **21:** R =  X = $CH_2$ | 285±14.5 |
| **22:** R=  X = $CH_2$ | 99.6±11.6 |
| **23:** R =   X = $CH_2$ | 149±19.9 |

| | |
|---|---|
| **24:** R =  X = $CH_2$ | 82.5±12.0 |
| **25:** R =  X = $CH_2$ | 120±8.4 |
| **26:** R =  X = $CH_2$ | 381±18.6 |
| **27:** R =  X = $CH_2$ | 362±28.6 |
| **28:** R =  X = $CH_2$ | 54.8±6.20 |
| **29:** R =  X = $CH_2$ | 195±19.0 |
| **30:** R=  X = $NHCH_2$ | 25.3±2.10 |

[a]**1** was used as the positive control. Values are means of three experiments.

Based on the binding mode of compound **19** to NAMPT, we assumed that incorporation of a nitrogen atom into the linker could increase the affinity to the protein *via* hydrophobic interactions. When the nitrogen atom was inserted into the linker, the resulting compound **30** exhibited slightly better binding affinity for Nampt

than **1** (Table 2). A docking study of compound **30**, a very flexible compound, led to several possible docking poses. One pose (Figure 4) indicated that the isopropyl group can occupy the position of the phenyl ring of **1** as in the conformation of crystal structure of 4O1D, and the indole ring can occupy the position of the phenyl ring of **1** as in the conformation of crystal structure of 2GVJ. **1** at least has two different conformations when complexed with NAMPT and the tail of **1** can have two different hydrophobic interactions in the tunnel. Compound **30** may be able to have these two hydrophobic interactions similar to the two conformations of complexed **1** in NAMPT. Another docking pose showed that the indole ring has a π-π stacking interaction with Tyr188. Either of these two docking poses may explain why these two compounds have good binding affinity against NAMPT. In addition, we synthesized compound **S1** with a six methylene linker and showed that it was less potent than the compound **30** (supporting information), which demonstrated that NH-containing linker present in **30** was preferred.

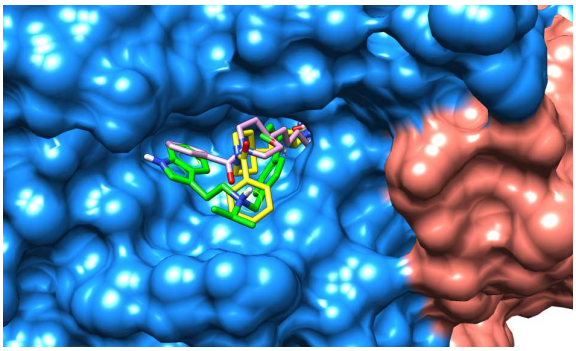


**Figure 4.** Green: one docking pose of compound **30**; Yellow: the binding pose of **1** (PDB code: 4O1D); Pink: the binding pose of **1** (PDB code: 2GVJ).

We further investigated the antiproliferative activities of these derivatives with Hela (human cervical cancer cell line), MCF7 (breast cancer cell line), H1975 (human non-small-cell lung cancer cell line), U937 (human histiocytic leukemia cell line) and MDA-MB-231（human breast adenocarcinoma cell line）with **1** and taxol as the positive controls. As shown in Table 3, compounds **19**, **20**, **25**, **27**, **28** and **30** have $GI_{50}$ values in the nanomolar range against human cancer cell lines. It was remarkable that compound **30**, especially in view of its higher NAMPT inhibitory activities, had anticancer activity against several cancer cell lines which was much better than that of **1**. In contrast, compounds **11**, **15**, **17** and **18**, weaker inhibitors of NAMPT, show much reduced activity against these cell lines. The results of the enzyme and cell-based assays thus supported for NAMPT a correlation between the *in vitro* inhibitory activity and cellular cytotoxicity. Compound **30** was therefore further examined in *in vivo* pharmacokinetic (PK) studies in rats and in a xenograft efficacy experiment to evaluate its anti-tumor effects.

**Table 3.** Antiproliferative Activities of Compounds **10-30** against Five Different Cancer Cell Lines.[a-c]

| Compd | $GI_{50}$ (nM) | | | | |
|---|---|---|---|---|---|
| | Hela | MCF7 | H1975 | U937 | MDA-MB-231 |
| **1** | 1.34±0.20 | 0.29±0.04 | 3.95±0.14 | 0.36±0.06 | 0.78±0.04 |
| **10** | 90.7±7.12 | 22.4±1.34 | 79.7±6.90 | 24.2±1.63 | 30.5±3.39 |
| **11** | 405±40.97 | 86.9±6.46 | 963±84.34 | 187±12.36 | 358±33.91 |

| | | | | | |
|---|---|---|---|---|---|
| **12** | 200±16.02 | 41.0±3.97 | 314±15.59 | 48.3±2.84 | 76.4±7.42 |
| **13** | 55.7±5.18 | 23.8±1.89 | 109±12.18 | 28.1±2.12 | 36.0±3.38 |
| **14** | 69.4±5.95 | 18.1±1.69 | 107±3.54 | 18.3±2.35 | 31.4±1.76 |
| **15** | 379±24.95 | 101±6.29 | 514±3.82 | 85.2±4.66 | 269±22.15 |
| **16** | 250±17.23 | 75.4±6.43 | 224±19.30 | 69.1±5.85 | 84.7±7.08 |
| **17** | 288±14.03 | 85.5±8.55 | 399±18.73 | 54.3±1.67 | 123±11.85 |
| **18** | 173±15.84 | 75.1±6.49 | 335±9.83 | 77.9±5.81 | 134±4.59 |
| **19** | 3.23±0.43 | 0.63±0.05 | 4.20±0.45 | 0.61±0.09 | 0.89±0.00 |
| **20** | 2.64±0.28 | 0.74±0.08 | 4.20±0.39 | 0.75±0.11 | 0.84±0.16 |
| **21** | 19.6±1.83 | 7.18±0.22 | 61.3±6.07 | 6.21±0.14 | 10.1±1.31 |
| **22** | 26.6±1.47 | 5.53±0.42 | 39.2±2.87 | 8.73±0.72 | 7.19±0.67 |
| **23** | 20.9±2.97 | 7.37±0.89 | 34.1±1.77 | 7.67±0.88 | 13.6±1.29 |
| **24** | 40.1±2.42 | 6.37±0.28 | 49.0±2.09 | 11.1±1.41 | 12.6±0.26 |
| **25** | 7.93±0.74 | 1.67±0.57 | 13.3±1.52 | 1.58±0.12 | 3.03±0.32 |
| **26** | 15.2±1.98 | 2.41±0.24 | 26.2±2.38 | 5.10±0.65 | 6.92±0.38 |
| **27** | 8.98±0.98 | 1.60±0.19 | 9.54±0.97 | 1.57±0.37 | 2.40±0.32 |
| **28** | 6.95±0.23 | 1.38±0.32 | 9.87±0.39 | 2.12±0.33 | 2.86±0.32 |

| | | | | | |
|---|---|---|---|---|---|
| **29** | 43.7±2.10 | 8.47±0.55 | 71.8±7.05 | 14.8±1.18 | 23.6±2.05 |
| **30** | 0.50±0.02 | 0.13±0.02 | 1.61±0.27 | 0.25±0.03 | 0.15±0.05 |
| Taxol | 2.34±0.24 | 4.38±0.40 | 5.77±0.98 | 2.12±0.35 | 8.67±0.84 |

[a]Taxol and **1** were used as the positive control. [b]Inhibition of cell growth by the listed compounds was determined by using CCK-8 assay.

To validate the target specificity at the cellular level, the compound **30** was evaluated for its ability in reducing the cellular NAD levels. After incubation with human MCF-7 cells for 24 hours, the compound **30** decreased the cellular NAD level significantly. The $IC_{50}$ for compound **30** reducing NAD level was 0.155 nM (Figure 5).

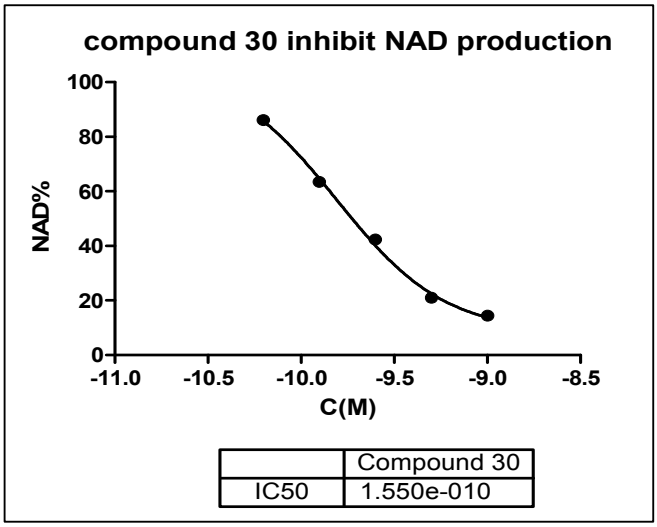



**Figure 5**. The NAD level response curve of the compound **30** on MCF-7 cells after 24 hours treatment.

As summarized in Table 4, compound **30** demonstrated good PK properties in rats.

After an IV administration of a dose of 5 mg/kg, it showed good plasma clearance and a high volume of distribution. The $C_{max}$ was about 500 times greater than the corresponding cell $IC_{50}$ value and compound **30** exhibited half-life of 1.55 h, longer than that of **1**, which is approximately 20 min[23].

**Table 4**. Pharmacokinetic Parameters of Compound **30** in Rats (n = 4)

| route | $C_{max}$ (ug/L) | $T_{max}$ (h) | $AUC_{0-\infty}$ (ug/L*h) | $T_{1/2}$ (h) | CL (L/h/kg) | F |
|---|---|---|---|---|---|---|
| IV (5 mg/kg) | 3790.6 | 0.033 | 1709.4 | 1.55 | 2.94 | |
| PO (25 mg/kg) | 107.6 | 2.0 | 373.9 | 2.37 | 68.13 | 4.4% |

[a]Values are the average of three runs. Vehicle: 2% DMSO, 4% EtOH, 4% castor oil and 90% doubly distilled $H_2O$. CL, clearance; $T_{1/2}$, half-life; $C_{max,}$ maximum concentration; $T_{max}$, time of maximum concentration; $AUC_{0-\infty}$, area under the plasma concentration time curve; F, oral bioavailability.

On the basis of the *in vivo* pharmacokinetic data, we next evaluated the antitumor efficacy of **30** in the MDA-MB-231 xenograft tumor model (Figure 6). The human breast adenocarcinoma cell line MDA-MB-231 has the triple-negative breast cancer phenotype, which is the most malignant type that poises worst prognosis in patients with no specific treatment. Our data showed that compound **30** at 15 mg/kg

effectively inhibits tumor growth, with a tumor growth inhibition (TGI) value of 73.8% ($p < 0.01$). During the treatment study, the mouse body weight was measured as shown in Figure S1 (Supporting Information). The result suggested the compound **30** at the current dose is well-tolerated, with no mortality or significant loss of body weight observed during treatment.

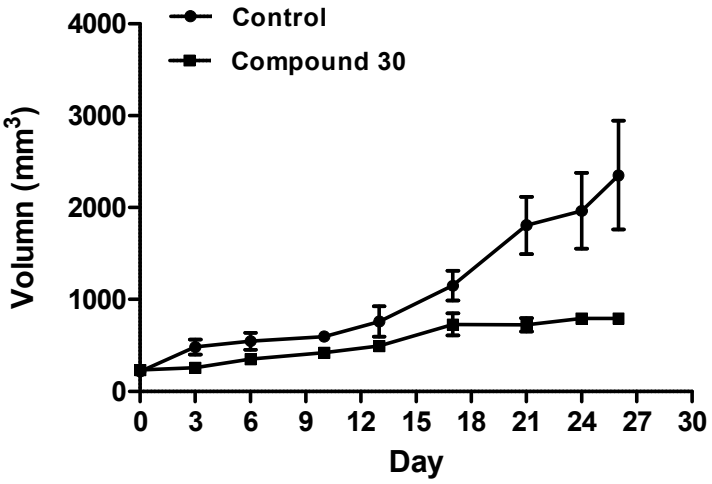
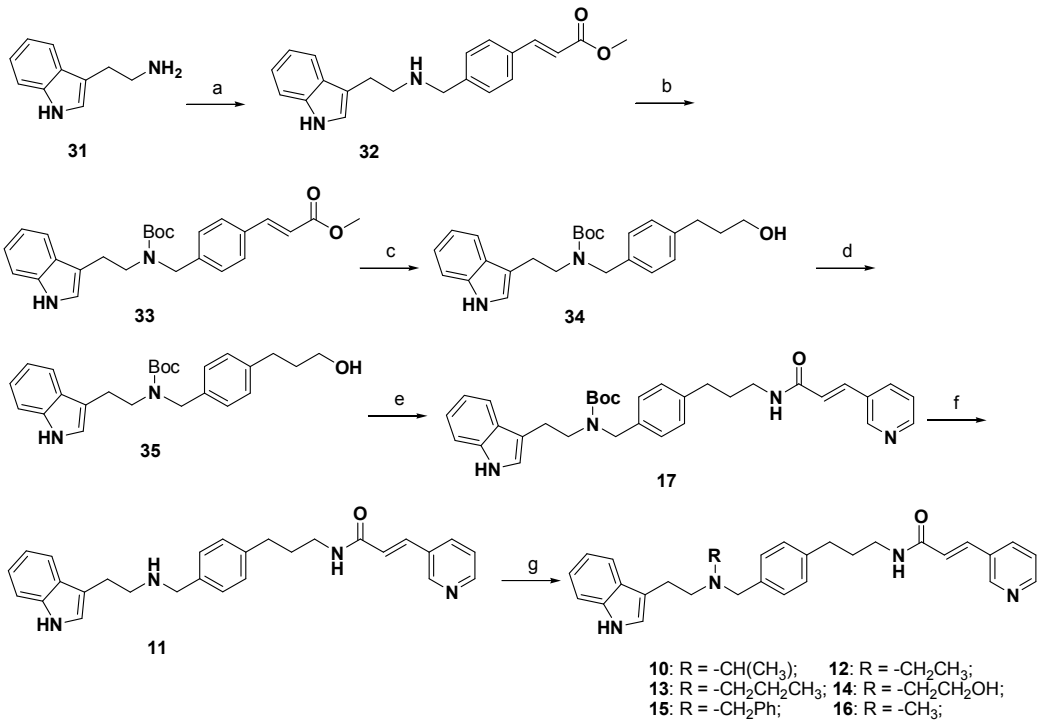



**Figure 6.** Growth curve of the MDA-MB-231 breast cancer xenograft in nude mice treated with i.v. injections of vehicle alone or compound **30** (15 mg/kg) 4 days a week. ($p < 0.01$). Data are expressed as the mean tumor volume ± SE of the animals in each treatment group.

## Chemistry

Compounds **10**-**17** were prepared according to the general procedures shown in Scheme 1. Tryptamine **31** was reacted with methyl 4-formylcinnamate **36** *via* reductive amination to generate substituted methyl 4-aminomethyl cinnamates (**32**). Compound **32** was protected with Boc group to give **33**, which was reacted with

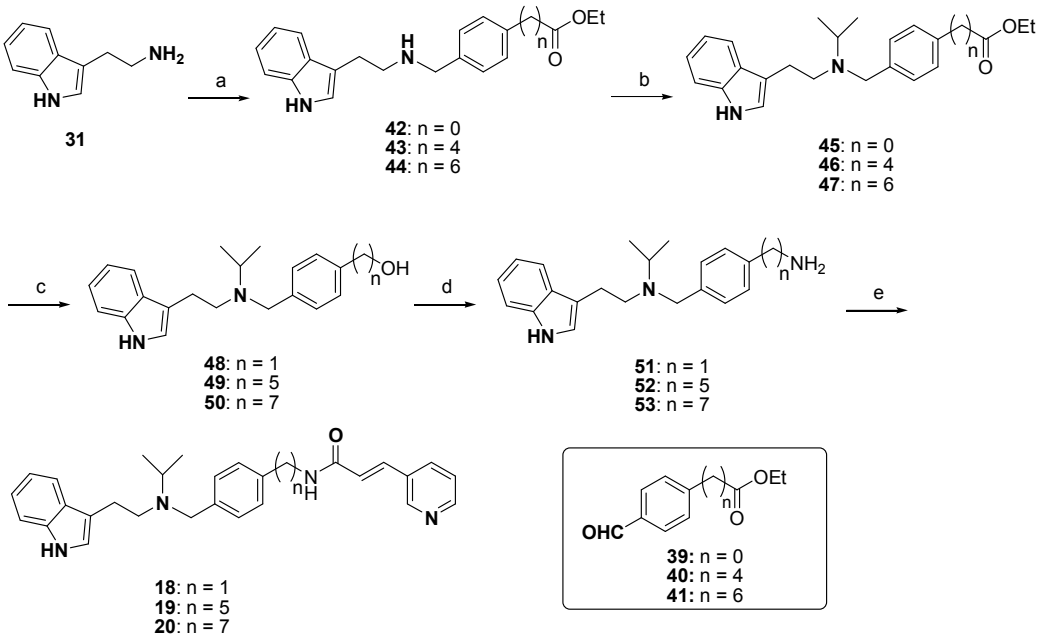
$LiAlH_4$ to furnish the alcohol **34** in 60% yield. Subsequent transformation of the alcohol **34** into the amine **35** involved a three-step sequence: (i) mesylation of alcohol **34**, (ii) formation of the azide, and (iii) reduction using $PPh_3$ (73% yield). Condensation of (*E*)-3-(pyridin-3-yl)acrylic acid **37** with amine **35** in the presence of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) gave compound **17**, which was further deprotected to give intermediate **11** in 85% yield. Subsequent coupling with alkyl halides gave **10** and **12-16** in 42-80% yields.




**Scheme 1.** General procedure for the synthesis of compounds **10-17**. Reagents and conditions: (a) methyl 4-formylcinnamate (**36**), $NaBH_3CN$, AcOH, MeOH, rt, 65%. (b) $(Boc)_2O$, triethylamine, dioxane, $H_2O$, rt, 85%. (c) $LiAlH_4$, THF, 0 °C - rt, 60%. (d) (i) MsCl, triethylamine, DCM, 0 °C. (ii) $NaN_3$, DMF, 80 °C. (iii) $PPh_3$, $H_2O$, THF, 60 °C, 73% in 3 steps. (e) (*E*)-3-(pyridin-3-yl)acrylic acid (**37**), EDCI, hydroxybenzotriazole (HOBt), triethylamine, DMF, rt, 86%. (f) 3M HCl, EtOAc, rt, 90%. (g) R-X, $K_2CO_3$, DMF, 80 °C, 42%-80%.

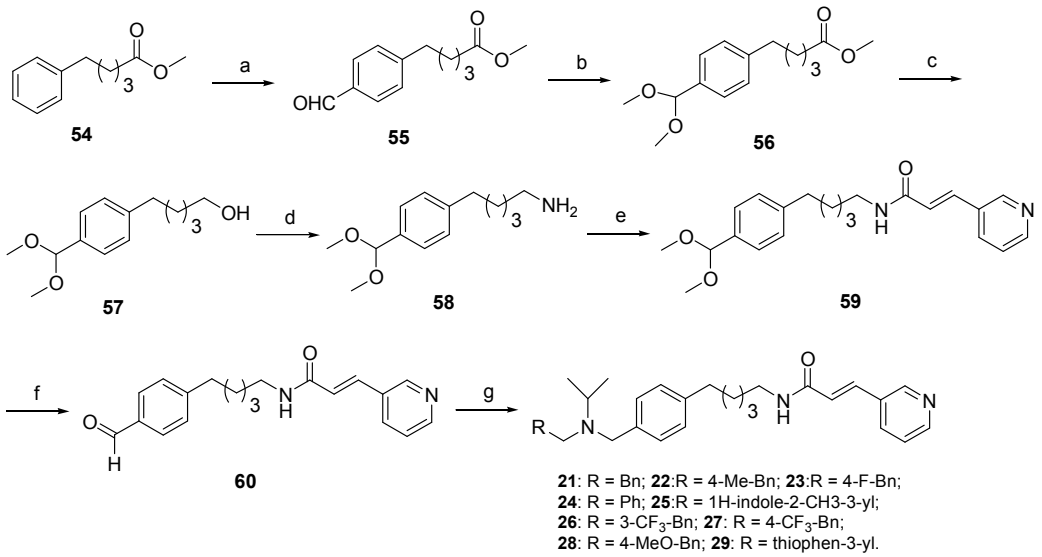
Compounds **18-20** were synthesized by using the procedure described in Scheme 2.

Tryptamine (**31**) was reacted with aldehydes **39-41** *via* reductive amination to afford the intermediates **42-44** in 42-60% yield. Coupling of which with 2-iodopropane afforded **45-47** was achieved in 50-70% yields, which were further reacted with $LiAlH_4$ to furnish the alcohols **48-50** in 63%-77% yields. Subsequently, transformation of alcohols **48-50** into amines **51-53** was achieved using similar methods, involving a three-step sequence: (i) mesylation of alcohol**s**, (ii) formation of the azide, and (iii) reduction using $PPh_3$ (49-70% yields). Condensation of (*E*)-3-(pyridin-3-yl)acrylic acid **37** with amines **51-53** in the presence of EDCI gave the final products **18-20** in 59-80% yields, respectively.



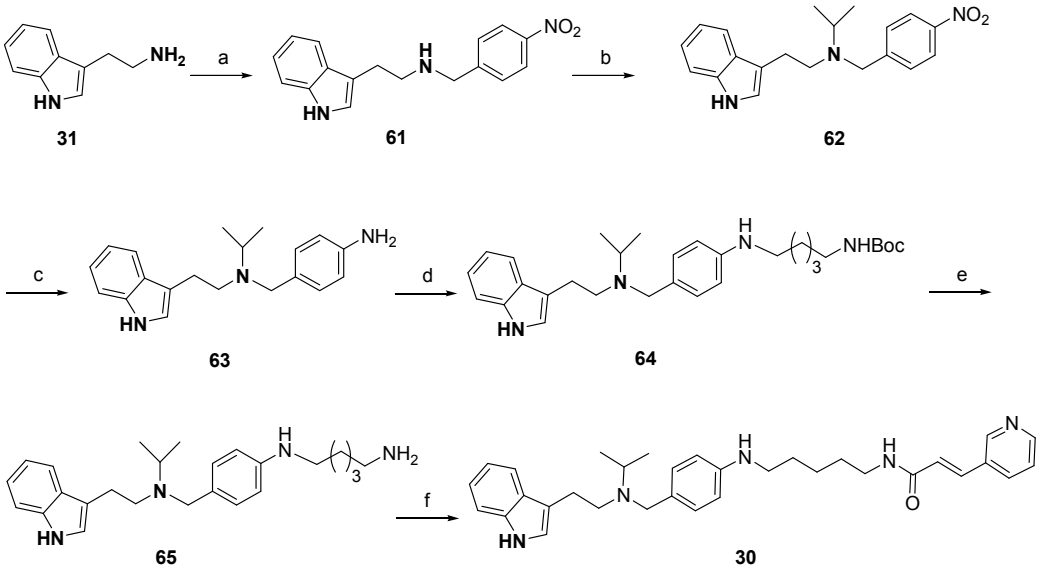

**Scheme 2.** Synthesis of compounds **18-20**. Reagents and conditions: (a) aldehyde **39-41**, $NaBH_3CN$, AcOH, MeOH, rt, 42%-60%. (b) 2-iodopropane, $K_2CO_3$, triethylamine, $CH_3CN$, 80 °C, 50-70%. (c) $LiAlH_4$, THF, 0 °C - rt, 63%-77%. (d) (i) MsCl, triethylamine, DCM, 0 °C. (ii) $NaN_3$, DMF, 80 °C. (iii) $PPh_3$, $H_2O$, THF, 60 °C, 49-70% in 3 steps. (e) (*E*)-3-(pyridin-3-yl)acrylic acid (**37**), EDCI, HOBt, triethylamine, DMF, rt, 59-80%.

To study the SAR of compound **19**, a new route was developed for synthesis of analogs **21-29**. The introduction of the aldehyde group selectively into the 4-position of methyl 5-phenylpentanoate (**54**) was achieved by a Duff reaction to give **55** in 32% yield, which was further allowed to react with methyl orthoformate affording compound **56** in 56% yield. Reduction of the ester group in the presence of $LiAlH_4$ provided compound **57** in high yields. Using similar methods, alcohol **57** was transformed into the amine (**58**) in 35% yield, and this was allowed to react with (*E*)-3-(pyridin-3-yl)acrylic acid (**37**) in the presence of EDCI giving **59** in 88% yield. Deprotection of **59**, reductive amination with different amines and finally, coupling with 2-iodopropane gave compounds **21-29** in 60-86% yield.




**Scheme 3.** Synthesis of compounds **21-29**. Reagents and conditions: (a) hexamethylenetetramine, TFA, reflux, 32%; (b) $(MeO)_3CH$, TsOH, MeOH, reflux, 56%; (c) LAH, THF, 0 $^oC$, 73%; (d) (i) MsCl, triethylamine, DCM, 0 °C. (ii) $NaN_3$, DMF, 80 °C. (iii) $PPh_3$, $H_2O$, THF, 60 °C, 35% in 3 steps. (e) (*E*)-3-(pyridin-3-yl)acrylic acid (**37**), EDCI, HOBt, TEA, DMF, 88% (f) 6M HCl, THF, rt, 90%; (g) (i) different amines, $NaBH_4$, MeOH, rt; (ii) 2-iodopropane, $K_2CO_3$, TEA, 60 $^oC$, 60-86% in two steps.

The synthesis of compound **30** is illustrated in scheme 4. Tryptamine (**31**) was reacted with 4-nitrobenzaldehyde *via* reductive amination to provide compound **61**, which was reacted with 2-iodopropane to generate compound **62**. The nitro group of compound (**62**) was reduced by $SnCl_2.2H_2O$ to obtain the amine **63** in 58% yield, which was coupled with *ter*t-butyl (5-iodopentyl) carbamate (**38**) to furnish the intermediate **64**. Deprotection of **64** and coupling with (*E*)-3-(pyridin-3-yl)acrylic acid (**37**) in the presence of EDCI gave **30** in 45% yield.




**Scheme 4.** Synthesis of compound **30**. Reagents and conditions: (a) 4-nitrobenzaldehyde, $NaBH_3CN$, AcOH, MeOH, rt, 70%. (b) 2-iodopropane, DIPEA, $CH_3CN$, 80 °C, 48 h, 93%. (c) $SnCl_2.2H_2O$, EtOH, rt, 58%. (d) *ter*t-butyl (5-iodopentyl) carbamate (**38**), $K_2CO_3$, MeCN, 50 °C, 43%. (e) 3M HCl, THF, rt, 82%. (f) (*E*)-3-(pyridin-3-yl)acrylic acid (**37**), EDCI, HOBt, triethylamine, DMF, rt, 45%.

## Conclusion

We have designed and synthesized a series of novel NAMPT inhibitors using a structure-based design strategy based upon the crystal structure of NAMPT complexed with **1**. Structure-activity relationships were thoroughly studied by

changing the length of the linker and the flexibility of the surface tail group. We identified a representative lead compound **30**, which inhibits NAMPT with an $IC_{50}$ value of 25.3 nM and shows promising *in vitro* anticancer activities against several cancer cell lines. It also exhibited significant antitumor efficacy in the MDA-MB-231 xenograft tumor model, which is an aggressive triple-negative breast cancer phenotype with no specific treatment so far. In addition, compound **30** showed an excellent half-life (1.55 h), much longer than that of **1** (20 min). These results make it clear that compound **30** is a promising NAMPT inhibitor well suited to further evaluations as a new anticancer agent.

## Experimental Section

**General.** Reagents and solvents were used as purchased without further purification. The progress of all reactions was monitored by TLC using EtOAc/n-hexane or DCM/MeOH as a solvent system, and spots were visualized by irradiation with ultraviolet light (254 nm). Flash chromatography was performed using silica gel (300−400 mesh). $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded on Bruker Avance ARX-400 (or Bruker Avance ARX-500). The low or high resolution of ESIMS was recorded on an Agilent 1200 HPLC-MSD mass spectrometer or Applied Biosystems Q-STAR Elite ESI-LC-MS/MS mass spectrometer, respectively. Anhydrous solvents were obtained as follows: THF by distillation from sodium and benzophenone; $CH_2Cl_2$, toluene and DMF from $CaH_2$. All other solvents were reagent grade. All moisture sensitive reactions were carried out in a flame dried flask under an argon

atmosphere. The purity of the final compounds was determined in an Agilent 1260 series HPLC system using the following conditions: C-18 column (DicKma, 4.6 mm × 250 mm) with the solvent system (elution conditions: mobile phase A consisting of MeOH; mobile phase B consisting of $H_2O$ containing 0.1% ammonia), with monitoring between 190 and 800 nm. A flow rate of 1.0 mL/min was used. The retention time was reported as $t_R$ (min). The purity of final compounds is >95%.

**(*E*)-N-(3-(4-(((2-(1H-indol-3-yl)ethyl)(isopropyl)amino)methyl)phenyl)propyl)-3-(pyridin-3-yl)acrylamide (10)**

To a stirred solution of compound **11** (22 mg, 0.05 mmol) in DMF (2 mL) was added potassium carbonate (14 mg, 0.1 mmol) and 2-iodopropane (10.2 mg, 0.06 mmol), the mixture was stirred for 8 h at room temperature. DMF was evaporated under reduced pressure. The residue was extracted with ethyl acetate (4 mL × 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The resulting mixture was purified by silica column chromatography and eluted with petroleum ether/EtOAc (2:1) to give **10** (12 mg, 52%) as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.72 (s, 1H), 8.56 (d, $J$ = 4.7 Hz, 1H), 8.16 - 8.01 (m, 1H), 7.75 (d, $J$ = 8.1 Hz, 1H), 7.59 (d, $J$ = 15.8 Hz, 1H), 7.45 (d, $J$ = 7.9 Hz, 1H), 7.31 (t, $J$ = 7.9 Hz, 4H), 7.19 - 7.00 (m, 4H), 6.92 (s, 1H), 6.37 (d, $J$ = 15.6 Hz, 1H), 5.63 (br, 1H), 3.63 (s, 2H), 3.42 (q, $J$ = 6.9 Hz, 2H), 3.11 - 3.00 (m, 1H), 2.89 - 2.79 (m, 2H), 2.78 - 2.61 (m, 4H), 1.92 (t, $J$ = 7.3 Hz, 2H), 1.05 (d, $J$ = 6.5 Hz, 6H) ppm. $^{13}$C NMR (125

MHz, $CDCl_3$): $\delta$ 165.1, 150.3, 149.1, 137.3, 136.2, 134.3, 130.6, 128.8, 128.1, 127.6, 126.4, 123.6, 122.7, 121.7, 121.5, 118.9, 118.8, 110.9, 53.9, 50.5, 39.5, 32.9, 31.1, 25.1, 18.2 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{31}H_{37}N_4O$, 481.2962; found, 481.2966. HPLC purity: 98.6%.

**(*E*)-N-(3-(4-((2-(1H-indol-3-yl)ethylamino)methyl)phenyl)propyl)-3-(pyridin-3-yl )acrylamide (11)**

To a stirred solution of compound **17** (38 mg, 0.07 mmol) in ethyl acetate (2 mL) was added 3 M HCl (2 mL), the mixture was stirred for 8 h at room temperature. The reaction mixture was quenched by 2 M NaOH (3 mL), extracted with ethyl acetate (6 mL × 3). Then the combined organic layers were washed with saturated $NaHCO_3$ solution and brine, dried over $Na_2SO_4$ (anhydrous), filtered and concentrated. The resulting mixture was purified by silica column chromatography and eluted with petroleum ether/EtOAc (1:1) to give **11** (28 mg, 90%) as a yellow solid. $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ 8.67 (s, 1H), 8.49 (d, $J$ = 4.9 Hz, 1H), 8.01 (dd, $J$ = 8.2, 1.9 Hz, 1H), 7.63 - 7.21 (m, 8H), 7.16 - 6.93 (m, 3H), 6.73 (d, $J$ = 15.9 Hz, 1H), 4.07 (s, 2H), 3.21 (t, $J$ = 7.6 Hz, 2H), 3.12 (t, $J$ = 7.5 Hz, 2H), 2.69 (t, $J$ = 7.7 Hz, 2H), 1.98 - 1.76 (m, 4H) ppm. $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ 167.7, 150.7, 149.7, 144.1, 138.3, 137.4, 136.2, 132.9, 131.6, 130.8, 130.2, 128.2, 125.5, 124.8, 124.0, 122.7, 119.9, 118.9, 112.50 , 110.8, 52.2, 40.1, 33.8, 32.0, 24.0, 23.7 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{28}H_{31}N_4O$, 439.2492; found, 439.2493. HPLC purity: 98.8%.

**(*E*)-N-(3-(4-(((2-(1H-indol-3-yl)ethyl)(ethyl)amino)methyl)phenyl)propyl)-3-(pyri**

**din-3-yl)acrylamide (12)**

The procedure was the same as described above for the synthesis of **10**. Compound **12** was obtained as a white solid (19 mg, 82%). $^{1}$H NMR (400 MHz, $CD_3OD$): $\delta$ 8.68 (s, 1H), 8.49 (d, $J$ = 3.2 Hz, 1H), 8.02 (d, $J$ = 8.0 Hz, 1H), 7.53 (d, $J$ = 15.8 Hz, 1H), 7.44 (dd, $J$ = 7.9, 5.2 Hz, 1H), 7.41 - 7.20 (m, 6H), 7.06 (d, $J$ = 6.1 Hz, 2H), 6.97 (t, $J$ = 7.5 Hz, 1H), 6.74 (d, $J$ = 15.8 Hz, 1H), 4.05 (s, 2H), 3.34 - 3.31 (m, 2H), 3.14 - 2.93 (m, 6H), 2.71 (t, $J$ = 7.7 Hz, 2H), 1.94 - 1.85 (m, 2H), 1.27 (t, $J$ = 7.2 Hz, 3H) ppm. $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ 167.7, 150.7, 149.7, 143.9, 138.2, 137.4, 136.2, 132.9, 131.6, 130.0, 128.3, 125.5, 124.8, 123.7, 122.6, 119.8, 119.0, 112.4, 111.6, 58.1, 53.8, 40.2, 33.9, 32.1, 22.1, 10.3 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{30}H_{35}N_4O$, 467.2805; found, 467.2806. HPLC purity: 97.3%.

**(*E*)-N-(3-(4-(((2-(1H-indol-3-yl)ethyl)(propyl)amino)methyl)phenyl)propyl)-3-(py ridin-3-yl)acrylamide (13)**

The procedure was the same as described above for the synthesis of **10**. Compound **13** was obtained as a white solid (12 mg, 50%). $^{1}$H NMR (400 MHz, $CD_3OD$): $\delta$ 8.70 (s, 1H), 8.50 (d, $J$ = 4.4 Hz, 1H), 8.02 (d, $J$ = 7.9 Hz, 1H), 7.54 (d, $J$ = 16.0 Hz, 1H), 7.45 (dd, $J$ = 7.9, 5.1 Hz, 1H), 7.33 (dd, $J$ = 21.4, 7.8 Hz, 4H), 7.22 (d, $J$ = 7.6 Hz, 2H), 7.10 - 6.90 (m, 3H), 6.72 (d, $J$ = 16.0 Hz, 1H), 3.81 (s, 2H), 3.35 - 3.31 (m, 2H), 3.01 - 2.93 (m, 2H), 2.89 - 2.85 (m, 2H), 2.72 - 2.64 (m, 4H), 1.93 - 1.86 (m, 2H), 1.67 - 1.59 (m, 2H), 0.92 (t, $J$ = 7.2 Hz, 3H) ppm. $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ 166.3, 149.2, 148.2, 141.1, 136.7, 135.9, 134.8, 131.5, 129.5, 129.4, 128.1, 127.1, 124.1, 123.4, 121.8, 120.8, 118.1, 117.7, 111.9, 110.8, 57.6, 55.3, 53.7, 38.8, 32.4, 30.7, 21.6,

18.9, 10.6 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{31}H_{37}N_4O$, 481.2962; found, 481.2963. HPLC purity: 96.4%.

**(*E*)-N-(3-(4-(((2-(1H-indol-3-yl)ethyl)(2-hydroxyethyl)amino)methyl)phenyl)propyl)-3-(pyridin-3-yl)acrylamide (14)**

The procedure was the same as described above for the synthesis of **10**. Compound **14** was obtained as a yellow solid (18 mg, 75%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.72 (s, 1H), 8.55 (d, $J$ = 4.4 Hz, 1H), 8.34 (s, 1H), 7.75 (d, $J$ = 7.6 Hz, 1H), 7.59 (d, $J$ = 15.6 Hz, 1H), 7.45 (d, $J$ = 8.0 Hz, 1H), 7.36 - 7.23 (m, 2H), 7.21 - 7.02 (m, 6H), 6.89 (s, 1H), 6.41 (d, $J$ = 15.6 Hz, 1H), 5.89 (br, 1H), 3.69 (s, 2H), 3.59 - 3.49 (m, 2H), 3.40 (q, $J$ = 6.0 Hz, 2H), 2.94 (t, $J$ = 7.0 Hz, 2H), 2.85 (t, $J$ = 7.0 Hz, 2H), 2.74 (t, $J$ = 5.4 Hz, 2H), 2.66 (t, $J$ = 7.2 Hz, 2H), 1.93 - 1.87 (m, 2H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.2, 150.2, 149.0, 140.2, 137.2, 136.3, 134.4, 130.7, 129.2, 128.4, 127.4, 123.7, 122.9, 122.8, 121.8, 121.6, 119.1, 118.6, 113.9, 111.2, 58.5, 58.2, 55.3, 54.0, 39.4, 32.9, 30.9, 23.1 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{30}H_{35}N_4O_2$, 483.2755; found, 483.2756. HPLC purity : 97.5% .

**(*E*)-N-(3-(4-(((2-(1H-indol-3-yl)ethyl)(benzyl)amino)methyl)phenyl)propyl)-3-(pyridin-3-yl)acrylamide (15)**

The procedure was the same as described above for the synthesis of **10**. Compound **15** was obtained as a yellow solid (22 mg, 85%). $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ 8.68 (s, 1H), 8.48 (d, $J$ = 4.4 Hz, 1H), 7.98 (d, $J$ = 8.0 Hz, 1H), 7.53 (d, $J$ = 15.6 Hz, 1H), 7.43 - 7.39 (m, 1H), 7.37 - 7.09 (m, 11H), 7.02 (t, $J$ = 7.6 Hz, 1H), 6.93 - 6.81 (m, 2H), 6.70 (d, $J$ = 15.6 Hz, 1H), 3.64 (s, 2H), 3.60 (s, 2H), 3.41 - 3.31 (m, 2H), 2.91 (t, $J$ =

7.6 Hz, 2H), 2.73 - 2.64 (m, 4H), 1.91 - 1.84 (m, 2H) ppm. $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ 167.7, 150.7, 149.6, 141.5, 140.9, 138.4, 138.1, 137.4, 136.2, 132.9, 130.2, 130.1, 129.3, 129.2, 128.8, 127.9, 125.5, 124.8, 123.0, 122.1, 119.4, 119.3, 114.4, 112.1, 59.4, 59.1, 55.2, 40.3, 33.9, 32.2, 23.9 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{35}H_{37}N_4O$, 529.2962; found, 529.2964. HPLC purity: 96.8%.

**(*E*)-N-(3-(4-(((2-(1H-indol-3-yl)ethyl)(methyl)amino)methyl)phenyl)propyl)-3-(pyridin-3-yl)acrylamide (16)**

The procedure was the same as described above for the synthesis of **10**. Compound **16** was obtained as a yellow solid (157 mg, 75%). $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ 8.69 (s, 1H), 8.49 (d, $J$ = 4.9 Hz, 1H), 8.00 (dd, $J$ = 8.1, 2.0 Hz, 1H), 7.53 (d, $J$ = 15.8 Hz, 1H), 7.48 - 7.37 (m, 2H), 7.34 - 7.14 (m, 5H), 7.12 - 6.86 (m, 3H), 6.71 (d, $J$ = 15.8 Hz, 1H), 3.60 (s, 2H), 3.42 - 3.31 (m, 2H), 2.96 (dd, $J$ = 10.3, 6.1 Hz, 2H), 2.74 - 2.66 (m, 4H), 2.33 (s, 3H), 1.92 - 1.85 (m, 2H) ppm. $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ 170.2, 153.2, 152.2, 144.8, 140.7, 139.9, 138.7, 138.6, 135.4, 133.5, 131.9, 131.2, 128.0, 127.3, 125.6, 124.8, 122.0, 121.8, 116.2, 114.7, 65.1, 61.3, 44.9, 42.8, 36.4, 34.7, 26.2 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{29}H_{33}N_4O$, 453.2649; found, 453.2649. HPLC purity: 96.3%.

**(*E*)-*tert*-butyl 2-(1H-indol-3-yl)ethyl(4-(3-(3-(pyridin-3-yl)acrylamido)propyl)benzyl)carbamate (17)**

To a mixture of (*E*)-3-(pyridin-3-yl)acrylic acid (18 mg, 0.12 mmol) in DMF (2 mL) was added EDCI (38 mg, 0.2 mmol), HOBt (20 mg, 0.15 mmol)，triethylamine (0.03 mL, 0.2 mmol) and compound **35** (41 mg, 0.1 mmol), the reaction mixture was stirred

for 5 h at room temperature. DMF was evaporated under reduced pressure. The residue was extracted with ethyl acetate (4 mL × 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution，saturated aqueous $NH_4Cl$ solution and brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The resulting mixture was purified by silica column chromatography and eluted with petroleum ether/EtOAc (5:1) to give **17** (46 mg, 86%) as a yellow solid.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.63 (s, 1H), 8.46 (d, $J$ = 4.9 Hz, 2H), 7.65 (d, $J$ = 7.9 Hz, 1H), 7.55 - 7.38 (m, 2H), 7.30 - 7.14 (m, 2H), 7.09 - 7.00 (m, 6H), 6.83 (d, $J$ = 19.7 Hz, 1H), 6.36 (d, $J$ = 15.7 Hz, 1H), 6.08 (br, 1H), 4.31 - 4.26 (m, 2H), 3.48 - 3.17 (m, 4H), 2.97 - 2.74 (m, 2H), 2.55 (d, $J$ = 7.7 Hz, 2H), 1.86 - 1.67 (m, 2H), 1.36 (s, 9H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 171.2, 165.2, 156.1, 150.2, 148.9, 140.2, 137.1, 136.3, 134.4, 130.7, 128.4, 127.9, 127.4, 123.7, 122.9, 121.9, 121.8, 119.1, 118.6, 113.1, 111.2, 79.6, 60.4, 39.4, 32.8, 31.1, 28.4, 21.0, 14.2 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{33}H_{39}N_4O_3$, 539.3017; found, 539.3016. HPLC purity : 99.5%.

**(*E*)-N-(4-(((2-(1H-indol-3-yl)ethyl)(isopropyl)amino)methyl)benzyl)-3-(pyridin-3-yl)acrylamide (18)**

The procedure was the same as described above as the synthesis of compound **10**. Compound **18** was obtained as yellow solid (10 mg, 42%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.68 (d, $J$ = 2.0 Hz, 1H), 8.50 (dd, $J$ = 4.8, 1.2 Hz, 1H), 8.21 (br, 1H), 7.71 - 7.69 (m, 1H), 7.61 (d, $J$ = 15.6 Hz,1H), 7.43 (d, $J$ = 7.6 Hz, 1H), 7.33 (d, $J$ = 8.0 Hz,

2H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.24 (dd, *J* = 4.8, 3.2 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 2H), 7.13 (t, *J* = 7.2 Hz,1H), 7.06 - 7.02 (m, 1H), 6.88 (d, *J* = 2.0 Hz, 1H), 6.45 (d, *J* = 15.6 Hz, 1H), 6.30 (t, *J* = 5.2 Hz, 1H), 4.52 (d, *J* = 5.6 Hz, 2H), 3.63 (s, 2H), 3.07 - 3.00 (m, 1H), 2.83 - 2.79 (m, 2H), 2.75 - 2.71 (m, 2H), 1.04 (d, *J* = 6.8 Hz, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.0, 150.2, 149.0, 141.1, 137.5, 136.2, 136.0, 134.4, 130.7, 128.9, 127.7, 127.6, 123.6, 122.8, 121.7, 121.5, 118.9, 118.8, 114.8, 111.0, 53.9, 50.6, 50.2, 43.8, 25.1, 18.1 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{29}H_{33}N_4O$, 453.2649; found, 453.2648. HPLC purity: 99.8%.

**(*E*)-N-(5-(4-(((2-(1H-indol-3-yl)ethyl)(isopropyl)amino)methyl)phenyl)pentyl)-3-(pyridin-3-yl)acrylamide (19)**

The procedure was the same as described above as the synthesis of compound **10**. Compound **19** was obtained as yellow solid (32 mg, 80%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.74 (s, 1H), 8.56 (d, *J* = 4.7 Hz, 1H), 7.87 (d, *J* = 8.2 Hz, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.60 (d, *J* = 15.4 Hz, 1H), 7.47 - 6.97 (m, 10H), 6.43 (d, *J* = 15.4 Hz, 1H), 5.73 (br, 1H), 3.60 (s, 2H), 3.38 (q, *J* = 6.7 Hz, 2H), 3.35 - 3.21 (m, 1H), 2.74 (s, 4H), 2.59 (q, *J* = 7.4 Hz, 2H), 1.79 - 1.48 (m, 4H), 1.46 - 1.26 (m, 2H), 1.04 (d, *J* = 6.5 Hz, 6H). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.2, 150.5, 149.3, 140.9, 138.6, 137.4, 135.4, 134.4, 134.0, 131.3, 130.9, 128.6, 128.3, 124.9, 123.8, 123.3, 123.0, 121.7, 119.9, 113.3, 54.2, 50.3, 49.4, 40.3, 39.9, 35.5, 31.2, 29.6, 26.6, 18.2 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{33}H_{41}N_4O$, 509.3275; found, 509.3278. HPLC purity: 99.3%.

**(*E*)-N-(7-(4-(((2-(1H-indol-3-yl)ethyl)(isopropyl)amino)methyl)phenyl)heptyl)-3-(pyridin-3-yl)acrylamide (20)**

The procedure was the same as described above as the synthesis of compound **10**. Compound **20** was obtained as yellow solid (32 mg, 59%). $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.73 (s, 1H), 8.55 (s, 1H), 8.32 (s, 1H), 7.76 (d, $J$ = 8.0 Hz, 1H), 7.59 (d, $J$ = 15.6 Hz, 1H), 7.48 - 7.23 (m, 5H), 7.21 - 7.00 (m, 4H), 6.93 (s, 1H), 6.44 (d, $J$ = 15.6 Hz, 1H), 5.88 (br, 1H), 3.78 (s, 2H), 3.34 (q, $J$ = 6.4 Hz, 2H), 3.25 - 3.18 (m, 1H), 2.87 - 2.83 (m, 4H), 2.59 (t, $J$ = 7.6 Hz, 2H), 1.61 (t, $J$ = 7.4 Hz, 2H), 1.51 (t, $J$ = 7.2 Hz, 2H), 1.31 - 1.24 (m, 6H), 1.14 (d, $J$ = 6.5 Hz, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.1, 150.0, 148.8, 141.8, 137.1, 136.2, 134.6, 130.9, 130.8, 130.7, 129.2, 128.4, 127.4, 123.7, 123.0, 121.8, 121.7, 119.1, 118.7, 111.2, 54.2, 51.3, 50.3, 39.8, 35.5, 31.2, 29.5, 29.1, 28.9, 26.8, 24.2, 17.9 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{35}H_{45}N_4O$, 537.3588; found, 537.3585. HPLC purity: 98.2% .

**(*E*)-N-(5-(4-((isopropyl(phenethyl)amino)methyl)phenyl)pentyl)-3-(pyridin-3-yl)acrylamide (21)**

A solution of compound **60** (200 mg, 0.62 mmol) and phenylmethanamine (82.3 mg, 0.68 mmol) in MeOH (10 mL) was stirred at room temperature for 1 hour, and then $NaBH_4$ (47 mg, 1.24 mmol) was added. The resulting solution was stirred for another hour. The solvent was evaporated to dryness, and the residue was portioned between ethyl acetate (10 mL) and saturated aqueous $NaHCO_3$ solution. The aqueous phase was extracted with ethyl acetate (5 ml x 3). Organic phases were combined and washed with brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was used for the next step without further purification.

The residue mentioned above was dissolved in DMF (2 mL), followed by adding

$K_2CO_3$ (115 mg, 0.84 mmol), TEA (85 mg, 0.84 mmol) and 2-iodopropane (355 mg, 2.09 mmol). The resulting mixture was warmed to 70 $^oC$ and stirred for 36 hours. The solvent was evaporated under reduced pressure. The residue was portioned between ethyl acetate (10 mL) and water (5 mL). The aqueous phase was extracted with ethyl acetate (5 ml x 3). Organic phases were combined and washed with brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica column chromatography and eluted with petroleum ether/EtOAc (2:1) to give **21** as brown solid (88 mg, 79%). $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 8.74 (d, $J$ = 1.2 Hz, 1H), 8.55 (d, $J$ = 3.6 Hz, 1H), 7.78 - 7.75 (m, 1H), 7.60 (d, $J$ = 15.6 Hz, 1H), 7.31 - 7.27 (m, 1H), 7.26 - 7.22 (m, 4H), 7.17 - 7.15 (m, 1H), 7.14 - 7.07 (m, 4H), 6.42 (d, $J$ = 15.6 Hz, 1H), 5.72 (s, 1H), 3.59 (s, 2H), 3.41 - 3.35 (m, 2H), 3.00 - 2.94 (m, 1H), 2.64 (d, $J$ = 4.8 Hz, 4H), 2.60 (t, $J$ = 7.6 Hz, 2H), 1.70 - 1.57 (m, 4H), 1.44 - 1.36 (m, 2H), 1.00 (d, $J$ = 6.8 Hz, 6H) ppm. $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 165.0, 150.3, 149.1, 141.0, 140.5, 138.6, 137.2, 134.3, 130.7, 128.8, 128.4, 128.1, 128.0, 125.7, 123.6, 122.9, 53.9, 51.7, 49.9, 39.8, 35.8, 35.4, 31.0, 29.5, 26.5, 18.0 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{31}H_{40}N_3O$, 470.3166; found, 470.3163. HPLC purity: 97.7% .

**(*E*)-N-(5-(4-((isopropyl(4-methylphenethyl)amino)methyl)phenyl)pentyl)-3-(pyridin-3-yl)acrylamide (22)**

The procedure was the same as described above as the synthesis of compound **21**. Compound **22** was obtained as brown oil (76 mg, 69%). $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 8.73 (d, $J$ = 1.6 Hz, 1H), 8.55 (dd, $J$ = 4.8, 1.6 Hz, 1H), 7.76 (d, $J$ = 8.0 Hz, 1H),

7.60 (d, $J$ = 15.6 Hz, 1H), 7.31 - 7.27 (m, 1H), 7.26 - 7.23 (m, 2H), 7.09 - 6.99 (m, 6H), 6.43 (d, $J$ = 15.6 Hz, 1H), 5.78 (s, 1H), 3.59 (s, 2H), 3.38 (t, $J$ = 6.8 Hz, 2H), 2.99 - 2.95 (m, 1H), 2.62 - 2.58 (m, 6H), 2.29 (s, 3H), 1.69 - 1.56 (m, 4H), 1.44 - 1.38 (m, 2H), 1.00 (d, $J$ = 6.8 Hz, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.0, 150.3, 149.1, 140.5, 138.6, 137.9, 137.2, 135.1, 134.3, 130.7, 128.8, 128.6, 128.4, 128.0, 123.6, 122.9, 53.9, 51.9, 49.9, 39.8, 35.4, 35.3, 31.0, 29.5, 26.5, 20.9, 18.0 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{32}H_{42}N_3O$, 484.3322; found, 484.3320. HPLC purity: 96.5%.

**(*E*)-N-(5-(4-(((4-fluorophenethyl)(isopropyl)amino)methyl)phenyl)pentyl)-3-(pyridin-3-yl)acrylamide (23)**

The procedure was the same as described above as the synthesis of compound **21**. Compound **23** was obtained as brown oil (39 mg, 81%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.74 (s, 1H), 8.55 (d, $J$ = 4.0 Hz, 1H), 7.77 (d, $J$ = 8.0 Hz, 1H), 7.60 (d, $J$ = 15.6 Hz, 1H), 7.30 (dd, $J$ = 7.6, 4.8 Hz, 1H), 7.19 (d, $J$ = 8.0 Hz, 2H), 7.08 - 7.02 (m, 4H), 6.91 (t, $J$ = 8.8 Hz, 2H), 6.44 (d, $J$ = 16.0 Hz, 1H), 5.77 (s, 1H), 3.57 (s, 2H), 3.41 - 3.36 (m, 2H), 2.98 - 2.94 (m, 1H), 2.61 - 2.57 (m, 6H), 1.68 - 1.57 (m, 4H), 1.45 - 1.39 (m, 2H), 0.98 (d, $J$ = 6.4 Hz, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.0, 150.3, 149.1, 140.6, 137.3, 136.7, 134.3, 130.7, 130.1, 130.0, 128.4, 128.1, 123.6, 122.9, 114.8, 114.7, 53.9, 51.6, 49.9, 39.8, 35.4, 34.8, 31.1, 29.5, 26.6, 17.9 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{31}H_{39}FN_3O$, 488.3072; found, 488.3076. HPLC purity: 96.8%.

**(*E*)-N-(5-(4-((benzyl(isopropyl)amino)methyl)phenyl)pentyl)-3-(pyridin-3-yl)acry**

**lamide (24)**

The procedure was the same as described above as the synthesis of compound **21**. Compound **24** was obtained as brown oil (41 mg, 66%). $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.73 (s, 1H), 8.55 (d, $J$ = 4.0 Hz, 1H), 7.74 (d, $J$ = 8.0 Hz, 1H), 7.59 (d, $J$ = 16.0 Hz, 1H), 7.37 (d, $J$ = 7.6 Hz, 2H), 7.29 - 7.25 (m, 5H), 7.18 (t, $J$ = 7.2 Hz, 1H), 7.07 (d, $J$ = 8.0 Hz, 2H), 6.45 (d, $J$ = 16.0 Hz, 1H), 5.95 (d, $J$ = 5.2 Hz, 1H), 3.54 (s, 2H), 3.51 (s, 2H), 3.39 - 3.34 (m, 2H), 2.95 - 2.88 (m, 1H), 2.57 (t, $J$ = 8.0 Hz, 2H), 1.67 - 1.55 (m, 4H), 1.42 - 1.34 (m, 2H), 1.04 (d, $J$ = 6.8 Hz, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.2, 150.4, 149.2, 141.2, 140.7, 138.4, 137.3, 134.5, 130.9, 128.6, 128.5, 128.2, 128.1, 126.6, 123.8, 123.1, 53.3, 53.0, 48.2, 39.9, 35.5, 31.2, 29.6, 26.7, 17.7 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{30}H_{38}N_3O$, 456.3009; found, 456.3008. HPLC purity: 98.7%.

**(*E*)-N-(5-(4-((isopropyl(2-(2-methyl-1H-indol-3-yl)ethyl)amino)methyl)phenyl)pentyl)-3-(pyridin-3-yl)acrylamide (25)**

The procedure was the same as described above as the synthesis of compound **21**. Compound **25** was obtained as a yellow solid (82 mg, 86%). $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.68 (d, $J$ = 1.6 Hz, 1H), 8.54 (dd, $J$ = 4.8, 1.6 Hz, 1H), 8.05 (s, 1H), 7.71 - 7.68 (m, 1H), 7.56 (d, $J$ = 15.6 Hz, 1H), 7.31 - 7.22 (m, 4H), 7.21 (d, $J$ = 8.0 Hz, 1H), 7.08 - 6.98 (m, 4H), 6.32 (d, $J$ = 15.6 Hz, 1H), 5.83 - 5.84 (m, 1H), 3.62 (s, 2H), 3.36 - 3.31 (m, 2H), 3.08 - 3.05 (m, 1H), 2.72 - 2.68 (m, 2H), 2.62 - 2.54 (m, 4H), 2.22 (s, 3H), 1.67 - 1.61 (m, 2H), 1.59 - 1.55 (m, 2H), 1.39 - 1.37 (m, 2H), 1.06 (d, $J$ = 6.4 Hz, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.1, 150.0, 148.9, 140.5, 138.6, 137.0,

136.9, 135.2, 134.4, 131.1, 128.8, 128.7, 128.1, 123.6, 123.0, 122.9, 120.6, 118.7, 117.9, 110.2, 54.3, 50.5, 50.4, 39.8, 35.3, 31.0, 29.4, 26.4, 24.5, 18.3, 11.4 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{34}H_{43}N_4O$, 523.3431; found, 523.3434. HPLC purity: 96.6%.

**(*E*)-N-(5-(4-((isopropyl(3-(trifluoromethyl)phenethyl)amino)methyl)phenyl)pentyl)-3-(pyridin-3-yl)acrylamide (26)**

The procedure was the same as described above as the synthesis of compound **21**. Compound **26** was obtained as a brown oil (30 mg, 64%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.73 (d, $J$ = 1.2 Hz, 1H), 8.55 - 8.54 (m, 1H), 7.76 (d, $J$ = 8.0 Hz, 1H), 7.60 (d, $J$ = 15.6 Hz, 1H), 7.34 (d, $J$ = 7.6 Hz, 1H), 7.34 - 7.31 (m, 2H), 7.31 - 7.25 (m, 2H), 7.16 (d, $J$ = 8.0 Hz, 2H), 7.05 (d, $J$ = 8.0 Hz, 2H), 6.46 (d, $J$ = 15.6 Hz, 1H), 5.92 - 5.91 (m, 1H), 3.58 (s, 2H), 3.38 (q, $J$ = 6.8 Hz, 2H), 3.00 - 2.93 (m, 1H), 2.71 - 2.63 (m, 4H), 2.58 (t, $J$ = 8.0 Hz, 2H), 1.68 - 1.57 (m, 4H), 1.44 - 1.36 (m, 2H), 0.98 (d, $J$ = 6.8 Hz, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.1, 150.2, 149.0, 141.9, 140.7, 137.9, 137.2, 137.1, 134.3, 132.2, 130.7, 129.0, 128.4, 128.1, 125.7, 125.6, 122.9, 122.5, 53.9, 51.0, 49.9, 39.8, 35.4, 35.2, 31.0, 29.5, 26.5, 17.8 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{32}H_{39}F_3N_3O$, 538.3040; found, 538.3037. HPLC purity: 96.6%.

**(*E*)-N-(5-(4-((isopropyl(4-(trifluoromethyl)phenethyl)amino)methyl)phenyl)pentyl)-3-(pyridin-3-yl)acrylamide(27)**

The procedure was the same as described above as the synthesis of compound **21**. Compound **27** was obtained as a brown oil (60 mg, 65%). $^1$H NMR (400 MHz,

$CDCl_3$): $\delta$ 8.73 (d, $J$ = 2.0 Hz, 1H), 8.55 (dd, $J$ = 4.4, 1.2 Hz, 1H), 7.78 - 7.74 (m, 1H), 7.60 (d, $J$ = 15.6 Hz, 1H), 7.46 (d, $J$ = 8.0 Hz, 2H), 7.29 (dd, J = 8.0, 4.8 Hz, 1H), 7.19 - 7.14 (m, 4H), 7.04 (d, $J$ = 8.0 Hz, 2H), 6.46 (d, $J$ = 15.6 Hz, 1H), 5.94 (s, 1H), 3.56 (s, 2H), 3.38 (q, $J$ = 6.8 Hz, 2H), 2.99 - 2.95 (m, 1H), 2.70 - 2.65 (m, 4H), 2.59 (t, $J$ = 7.6 Hz, 2H), 1.68 - 1.57 (m, 4H), 1.44 - 1.38 (m, 2H), 0.98 (d, $J$ = 6.4 Hz, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.1, 150.2, 149.0, 145.2, 140.6, 138.2, 137.1, 134.3, 130.7, 129.1, 128.3, 128.0, 124.9, 124.8, 123.6, 123.0, 122.9, 53.7, 51.1, 49.8, 39.8, 35.4, 35.3, 31.0, 29.5, 26.5, 17.9 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{32}H_{39}F_3N_3O$, 538.3040; found, 538.3036. HPLC purity: 97.8%.

**(*E*)-N-(5-(4-((isopropyl(4-methoxyphenethyl)amino)methyl)phenyl)pentyl)-3-(pyridin-3-yl)acrylamide (28)**

The procedure was the same as described above as the synthesis of compound **21**. Compound **28** was obtained as a brown oil (45 mg, 77%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.73 (d, $J$ = 2.0 Hz, 1H), 8.55 (dd, $J$ = 4.8, 1.2 Hz, 1H), 7.77 - 7.74 (m, 1H), 7.60 (d, $J$ = 15.6 Hz, 1H), 7.29 (dd, $J$ = 8.0, 4.8 Hz, 1H), 7.23 (d, $J$ = 8.0 Hz, 2H), 7.08 (d, $J$ = 8.0 Hz, 2H), 7.01 - 6.79 (m, 2H), 6.79 - 6.76 (m, 2H), 6.43 (d, $J$ = 15.6 Hz, 1H), 5.82 (br, 1H), 3.77 (s, 3H), 3.58 (s, 2H), 3.40 - 3.35 (m, 2H), 2.98 - 2.94 (m, 1H), 2.61 - 2.57 (m, 6H), 1.69 - 1.56 (m, 4H), 1.44 - 1.38 (m, 2H), 0.99 (d, $J$ = 6.8 Hz, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.1, 157.7, 150.2, 149.1, 140.5, 138.6, 137.2, 134.3, 133.1, 130.7, 129.6, 128.4, 128.1, 123.6, 122.9, 113.6, 55.2, 53.9, 51.9, 49.9, 39.8, 35.4, 34.8, 31.0, 29.5, 26.5, 18.0 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{32}H_{42}N_3O_2$, 500.3271; found, 500.3275. HPLC purity: 96.8%.

**(*E*)-N-(5-(4-((isopropyl(2-(thiophen-3-yl)ethyl)amino)methyl)phenyl)pentyl)-3-(pyridin-3-yl)acrylamide (29)**

The procedure was the same as described above as the synthesis of compound **21**. Compound **29** was obtained as a brown oil (50 mg, 60% ). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.74 (d, $J$ = 1.6 Hz , 1H), 8.56 (dd, $J$ = 4.4, 1.2 Hz, 1H), 7.78 - 7.75 (m, 1H), 7.60 (d, $J$ = 15.6 Hz, 1H), 7.31 - 7.24 (m, 3H), 7.10 - 7.08 (m, 3H), 6.89 - 6.87 (m, 1H), 6.74 - 6.72 (m, 1H), 6.43 (d, $J$ = 15.6 Hz, 1H), 5.71 (s, 1H), 3.59 (s, 2H), 3.41 - 3.35 (m, 2H), 2.98 - 2.94 (m, 1H), 2.88 - 2.84 (m, 2H), 2.72 - 2.68 (m, 2H), 2.60 (t, $J$ = 7.6 Hz, 2H), 1.68 - 1.56 (m, 4H), 1.44-1.38 (m, 2H), 1.01 (d, $J$ = 6.4 Hz, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.0, 150.3, 149.1, 143.4, 140.6, 138.5, 137.3, 134.3, 130.7, 128.4, 128.1, 126.5, 124.4, 123.6, 123.0, 122.9, 54.1, 51.8, 50.2, 39.8, 35.4, 31.0, 29.9, 29.5, 26.5, 18.0 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{29}H_{38}N_3OS$, 476.2730; found, 476.2731. HPLC purity: 99.1% .

**(*E*)-N-(5-((4-(((2-(1H-indol-3-yl)ethyl)(isopropyl)amino)methyl)phenyl)amino)pentyl)-3-(pyridin-3-yl)acrylamide (30)**

The procedure was the same as described above as the synthesis of compound **17**. the compound **30** was obtained as yellow solid (471 mg, 45%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.70 (d, $J$ = 1.6 Hz, 1H), 8.53 (dd, $J$ = 4.8, 1.2 Hz, 1H), 8.35 (br, 1H), 7.70 (d, $J$ = 8.0 Hz, 1H), 7.58 (d, $J$ = 15.6 Hz, 1H), 7.47 (d, $J$ = 7.6 Hz, 1H), 7.29 (d, $J$ = 8.0 Hz, 1H), 7.25 (t, $J$ = 6.4 Hz, 1H), 7.18 (d, $J$ = 8.4 Hz, 2H), 7.13 (t, $J$ = 7.6 Hz, 1H), 7.05 (t, $J$ = 7.6 Hz, 1H), 6.91 (d, $J$ = 1.6 Hz, 1H), 6.54 (d, $J$ = 8.4 Hz, 2H), 6.42 (d, $J$ = 15.6 Hz, 1H), 6.12 (t, $J$ = 5.6 Hz, 1H), 3.56 (s, 2H), 3.37 (q, $J$ = 6.8 Hz, 2H), 3.09 -

3.02 (m, 3H), 2.85 - 2.79 (m, 2H), 2.76 - 2.71 (m, 2H), 1.63 - 1.54 (m, 4H), 1.47 - 1.41 (m, 2H), 1.03 (d, $J$ = 6.8 Hz, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 165.2, 150.1, 148.9, 147.2, 137.1, 136.2, 134.4, 130.7, 129.7, 127.6, 123.6, 123.0, 121.6, 118.9, 114.8, 112.6, 111.0, 53.9, 50.2, 49.9, 43.9, 39.6, 29.3, 29.1, 25.0, 24.4, 18.1 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{33}H_{42}N_5O$, 524.3384; found, 524.3382. HPLC purity : 98.9% .

***tert*-butyl 2-(1H-indol-3-yl)ethyl(4-(3-hydroxypropyl)benzyl)carbamate (34)**

A solution of the compound **33** (870 mg, 2 mmol) in anhydrous THF (8 mL) was slowly added to a solution of $LiAlH_4$ (228 mg, 6 mmol) in anhydrous THF at 0 ℃ under argon atmosphere. Then the mixture was stirred for 2 h at room temperature, quenched with saturated aqueous $NH_4Cl$ (5 mL) solution and evaporated THF under reduced pressure. The residue was extracted with ethyl acetate (6 mL × 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The resulting mixture was purified by silica column chromatography and eluted with petroleum ether/EtOAc (3:1) to give **34** (490 mg, 60%) as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.14 (s, 1H), 7.53 (d, $J$ = 7.7 Hz, 1H), 7.34 (d, $J$ = 8.1 Hz, 1H), 7.21 - 6.93 (m, 6H), 6.94 (d, $J$ = 20.0 Hz, 1H), 4.41 - 4.35 (m, 2H), 3.66 (s, 2H), 3.51 - 3.43 (m, 2H), 3.09 - 2.82 (m, 2H), 2.69 (t, $J$ = 7.8 Hz, 2H), 1.91 - 1.84 (m, 2H), 1.47 (s, 9H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 139.1, 136.7, 136.2, 128.5, 128.1, 127.6, 127.4, 121.9, 121.8, 119.2, 118.7, 113.4, 111.1, 79.6, 69.1, 49.8, 47.3, 37.3, 31.1, 30.6, 28.4 ppm. MS (ESI, *m/z*): 409 ($M^+$ + 1).

**_tert_-butyl 2-(1H-indol-3-yl)ethyl(4-(3-aminopropyl)benzyl)carbamate (35)**

To a stirred solution of compound **34** (408 mg, 1 mmol) , triethylamine (0.4 mL, 3 mmol) in dichloromethane (2 mL) was slowly added methanesulfonyl chloride (0.12 mL, 1.5 mmol) at 0 ℃. After stirring at 0 ℃ for 1 h, the reaction mixture was extracted with dichloromethane (4 mL × 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was used in the next step without further purification.

The above intermediate was dissolved in DMF (2 mL), and then sodium azide (195 mg, 3 mmol) was added. After stirring at 80 ℃ for 2 h, the reaction mixture was cooled to room temperature. DMF was evaporated under reduced pressure. The residue was extracted with ethyl acetate (4 mL × 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was used in the next step without further purification.

The above intermediate was dissolved in THF (2 mL), then triphenylphosphine (393 mg, 1.5 mmol), $H_2O$ (0.1 mL, 6 mmol) was added. After stirring at 60 ℃ for 2 h, the reaction mixture was cooled to room temperature. THF was evaporated under reduced pressure. The residue was purified by silica column chromatography and eluted with petroleum ether/EtOAc (1:1) to give **35** (297 mg, 73% for 3 steps) as a yellow oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.22 (s, 1H), 7.53 (d, $J$ = 7.6 Hz, 1H), 7.33 (d, $J$ = 8.1 Hz, 1H), 7.23 - 7.04 (m, 6H), 6.94 (d, $J$ = 27.2 Hz, 1H), 4.41 - 4.34 (m, 2H), 3.50 - 3.42

(m, 2H), 2.97 - 2.92 (m, 2H), 2.72 (t, $J$ = 7.2 Hz, 2H), 2.63 (t, $J$ = 7.8 Hz, 2H), 1.79 - 1.72 (m, 2H), 1.46 (s, 9H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 140.9, 136.3, 136.1, 135.9, 128.4, 127.9, 127.4, 121.8, 121.7, 119.2, 118.7, 113.4, 111.1, 79.5, 49.8, 47.2, 41.6, 35.2, 32.8, 28.4, 24.3 ppm. MS (ESI, *m/z*): 408 ($M^+$ + 1).

**5-(4-(dimethoxymethyl)phenyl)pentan-1-ol (57)**

To a suspension of LAH (213 mg, 5.6 mmol) in THF (10 mL) cooled to 0 $^o$C, a solution of methyl 5-(4-(dimethoxymethyl)phenyl)pentanoate (**56**) (0.5 g, 1.9 mmol) in THF (3 mL) was added dropwise. Then the mixture was stirred for 0.5 h, quenched with saturated aqueous $NH_4Cl$ (1 mL) solution and evaporated THF under reduced pressure. The residue was extracted with ethyl acetate (10 mL × 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The resulting mixture was purified by silica column chromatography and eluted with petroleum ether/EtOAc (10:1) to give **57** (327 mg, 73%) as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.34 (d, $J$ = 7.6 Hz, 2H), 7.17 (d, $J$ = 7.6 Hz, 2H), 5.36 (s, 1H), 3.63 (t, $J$ = 6.0 Hz, 2H), 3.33 (s, 6H), 2.62 (t, $J$ = 7.6 Hz, 2H), 1.68 - 1.55 (m, 4H), 1.43-1.37 (m, 2H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 142.8, 135.4, 128.2, 126.6, 103.3, 62.9, 52.7, 35.6, 32.6, 31.1, 25.3 ppm.

**5-(4-(dimethoxymethyl)phenyl)pentan-1-amine (58)**

To a stirred solution of compound **57** (320 mg, 1.34 mmol)**,** triethylamine (0.37 mL, 2.68 mmol) in dichloromethane (7 mL) was slowly added methanesulfonyl chloride (0.12 mL, 1.61 mmol) at 0 °C. After stirring at 0 °C for 2 h, the reaction mixture was

extracted with dichloromethane (4 mL × 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was used in the next step without further purification.

The above intermediate was dissolved in DMF (5 mL), and then sodium azide (209 mg, 3.2 mmol) was added. After stirring at 80 °C for 2 h, the reaction mixture was cooled to room temperature. DMF was evaporated under reduced pressure. The residue was extracted with ethyl acetate (4 mL × 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was used in the next step without further purification.

The above intermediate was dissolved in THF (5 mL), then triphenylphosphine (393 mg, 1.0 mmol), $H_2O$ (0.1 mL, 4.1 mmol) was added. After stirring at 60 °C for 6 h, the reaction mixture was cooled to room temperature. THF was evaporated under reduced pressure. The residue was purified by silica column chromatography and eluted with petroleum ether/EtOAc (5:1) to give **58** (112 mg, 35% for 3 steps) as a colorless oil. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.34 (d, $J$ = 8.0 Hz, 2H), 7.17 (d, $J$ = 8.0 Hz, 2H), 5.36 (s, 1H), 3.32 (s, 6H), 2.67 (t, $J$ = 7.0 Hz, 2H), 2.61 (t, $J$ = 7.5 Hz, 2H), 1.66 - 1.59 (m, 2H), 1.49 - 1.43 (m, 2H), 1.36 - 1.33 (m, 2H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 142.9, 135.4, 128.2, 126.6, 103.3, 52.7, 42.1, 35.6, 33.6, 31.2, 26.5 ppm.

**(*E*)-N-(5-(4-(dimethoxymethyl)phenyl)pentyl)-3-(pyridin-3-yl)acrylamide (59)**

The procedure was the same as described above as the synthesis of compound **17**. Compound **59** was obtained as pale yellow solid (150 mg, 88%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.72 (s, 1H), 8.55 - 8.53 (m, 1H), 7.77 - 7.73 (m, 1H), 7.58 (d, $J$ = 16.0 Hz, 1H), 7.33 (d, $J$ = 8.0 Hz, 2H), 7.30 - 7.16 (m, 1H), 7.15 (d, $J$ = 8.0 Hz, 2H), 6.45 (d, $J$ = 16.0 Hz, 1H), 5.98 (s, 1H), 5.33 (s, 1H), 3.39 - 3.32 (m, 2H), 3.31 (s, 6H), 2.60 (t, $J$ = 8.0 Hz, 2H), 1.67 - 1.55 (m, 4H), 1.41-1.35 (m, 2H) ppm. MS (ESI, *m/z*): 369 ($M^+$ + 1).

**(*E*)-N-(5-(4-formylphenyl)pentyl)-3-(pyridin-3-yl)acrylamide (60)**

A solution of compound **59** (159 mg, 0.407 mmol) in 6M HCl (1 mL) and THF (2 mL) was stirred at room temperature overnight. The solvent was evaporated and the residue was extracted with ethyl acetate (3 x 4 mL). The combined organic phase was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica column chromatography and eluted with petroleum ether/EtOAc (5:1) to give **60** (138 mg, 90%) as a pale yellow solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.94 (s, 1H), 8.71 (d, $J$ = 2.0 Hz, 1H), 8.54 - 8.53 (m, 1H), 7.78 - 7.73 (m, 3H), 7.58 (d, $J$ = 15.6 Hz, 1H), 7.31 - 7.27 (m, 2H), 6.47 (d, $J$ = 15.6 Hz, 1H), 6.08 (s, 1H), 3.40 - 3.35 (m, 2H), 2.68 (t, $J$ = 8.0 Hz, 2H), 1.71 - 1.57 (m, 4H), 1.43 - 1.37 (m, 2H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 191.9, 165.1, 150.2, 149.9, 149.0, 137.1, 134.4, 134.3, 130.7, 129.9, 129.0, 123.7, 122.9, 39.6, 35.9, 30.5, 29.4, 26.4 ppm. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{20}H_{23}N_2O_2$, 323.1754; found, 323.1756.

***tert*-butyl (5-((4-(((2-(1H-indol-3-yl)ethyl)(isopropyl)amino)methyl)phenyl)amino)**

**pentyl)carbamate (64)**

To a solution of **63** (4.5 g, 14.6 mmol) in acetonitrile (60 mL), tert-butyl (5-iodopentyl)carbamate **38** (3.8 g, 12.1 mmol) and $K_2CO_3$ (3.4 g, 24.4 mmol) were added. The mixture was stirred at 50 $^oC$ for 48 h. Acetonitrile was evaporated under reduced pressure. The residue was extracted with ethyl acetate (50 mL × 3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The resulting mixture was purified by silica column chromatography and eluted with petroleum ether/EtOAc (5:1) to give **64** (3.13 g , 43%) as a brown oil. $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ 7.99 (br, 1H), 7.49 (d, $J$ = 8.0 Hz, 1H), 7.33 (d, $J$ = 8.0 Hz, 1H), 7.20 - 7.14 (m, 3H), 7.07 (t, $J$ = 7.0 Hz, 1H), 6.95 (d, $J$ = 1.5 Hz, 1H), 6.56 (d, $J$ = 8.5 Hz, 2H), 4.53 (br, 1H), 3.58 (s, 2H), 3.14 - 3.09 (m, 4H), 3.07 - 3.05 (m, 1H), 2.85 - 2.83 (m, 2H), 2.76 - 2.72 (m, 2H), 1.67 - 1.60 (m, 2H), 1.56 - 1.49 (m, 2H), 1.45 (s, 9H), 1.43 - 1.40 (m, 2H), 1.05 (d, $J$ = 6.0 Hz, 6H) ppm. $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 156.0, 147.2, 136.2, 129.7, 127.7, 121.7, 121.4, 119.0, 118.9, 115.2, 112.6, 110.9, 79.1, 53.9, 50.2, 44.1, 40.5, 29.9, 29.3, 28.4, 27.0, 25.2, 24.4, 18.1 ppm.

**N1-(4-(((2-(1H-indol-3-yl)ethyl)(isopropyl)amino)methyl)phenyl)pentane-1,5-diamine (65)**

To a solution of **64** (2.4 g, 4.8 mmol) in THF (15 mL), HCl solution (3.0 M, 10 mL ) was added. The solution was stirred for 4 hrs at room temperature. Solvent was evaporated under reduced pressure. The residue was extracted with ethyl acetate (50 mL × 4). The combined organic layers were washed with saturated aqueous $NaHCO_3$

solution and brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The resulting mixture was purified by silica column chromatography and eluted with petroleum ether/EtOAc (1:2) to give **65** (1.56 g, 82%) as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.19 (br, 1H), 7.48 (d, $J$ = 8.0 Hz, 1H), 7.28 (d, $J$ = 8.0 Hz, 1H), 7.18 (d, $J$ = 8.4 Hz, 2H), 7.14 (t, $J$ = 7.2 Hz, 1H), 7.07 - 7.03 (m, 1H), 6.91 (s, 1H), 6.56 (d, $J$ = 8.4 Hz, 2H), 3.56 (s, 2H), 3.10 (t, $J$ = 7.2 Hz, 2H), 3.07 - 3.00 (m, 1H), 2.85 - 2.81 (m, 2H), 2.74 - 2.68 (m, 4H), 1.66 - 1.58 (m, 2H), 1.50 - 1.41 (m, 4H), 1.03 (d, $J$ = 6.8 Hz, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 147.2, 136.2, 129.7, 129.6, 127.7, 121.6, 121.5, 118.9, 118.8, 115.0, 112.6, 111.0, 53.9, 50.3, 49.8, 44.2, 42.1, 33.5, 29.5, 25.2, 24.5, 18.2 ppm.

***In Vitro* NAMPT Inhibition Assay.** *In vitro* NAMPT enzyme inhibitory activity assays were performed using CycLex NAMPT Colorimetric Assay Kit (CycLex NAMPT Colorimetric Assay Kit, MBL International Corp., Woburn, MA) according to manufacturers' instructions. Before starting the assay to make assay buffer-1 and -2 as below, assay buffer-1contains: 10X NAMPT assay buffer 10 μL, 10X Nicotinamide 10 μL, 10X PRPP 10 μL, 10X ATP 10 μL, Recombinant NMNAT1 2 μL, $dH_2O$ 48 μL, total volume, 90 μL; assay buffer-2 contains: 50X WST-1 2 μL, 50X ADH 2 μL, 50X Diaphorase 2 μL, 10X EtOH 10 μL, distilled $H_2O$, 4 μL, total volume, 20 μL. The assay was performed by adding 2 μL of recombinant NAMPT and various concentrations of compounds or vehicle to each well of the microplate, then the reaction was initiated by adding 90 μL of assay buffer-1 to each well and mixing

thoroughly followed by incubation at 30 °C for 60 min, after this period, 20 μL of assay buffer-2 was added to each well of the microplate and mixed thoroughly. Then the absorbance at 450 nm was monitored for 30 min at 5 min intervals using a microtiter plate reader.

**Cell Culture and Cell Viability Assay.** Cell lines, Hela, MCF7, H1975, U937 and MDA-MB-231 were purchased from Shanghai Cell Bank, Chinese Academy of Sciences. Cells were routinely grown and maintained in mediums RPMI or DMEM with 10% FBS and with 1% penicillin/streptomycin. All cell lines were incubated in a Thermo/Forma Scientific $CO_2$ Water Jacketed Incubator with 5% $CO_2$ in air at 37 °C. Cell viability assay was determined by the CCK8 (DOjinDo, Japan) assay. Cells were seeded at a density of 400-800 cells/well in 384 well plates and treated with various concentration of compounds or solvent control. After 72 h incubation, CCk8 reagent was added, and absorbance was measured at 450 nm using Envision 2104 multi-label Reader (Perkin Elmer, USA). Dose response curves were plotted to determine the $IC_{50}$ values using Prism 5.0 (GraphPad Software Inc., USA).

**Docking Studies.** The compounds described in this article were docked into the active site of NAMPT (PDB code: 4O1D) by employing the program Glide 5.9 of the Schrödinger suite. The structure was downloaded from the protein data bank and prepared using the protein preparation wizard in the Schrödinger suite. The extra precision (XP) mode of Glide was employed. A post docking minimization was carried out for the best 25 poses for each ligand, and the 10 best poses were reported and analyzed.

**NAD measurement.** The effects of compounds on cellular level of NAD was measured by using a modified version of manufacturer instructions supplied with the NAD/NADH Glo Assay (Promega). Briefly, cells were seeded in 96-well plate and starved for over 12 h with serum-free medium, following by treatment with compounds or vehicle for 24 h. For extraction, cells were washed 3 times in ice cold PBS, extracted in 100 μL ice cold lysis buffer (1% Dodecyltrimethylammonium bromide (DTAB) in 0.2 N NaOH diluted 1:1 with PBS), and immediately frozen at -80 °C. To measure NAD, 20 μL of the samples was moved to PCR tubes containing 20 μL lysis buffer and 20 μL 0.4 N HCl and incubated at 60 °C for 15 min. Following incubations, samples were allowed to equilibrate to room temperature and then quenched by neutralizing with 20 μL 0.5 M Tris base. Manufacturer instructions were followed thereafter to measure NAD.

**Pharmacokinetic Analysis**. Male SD rats (180-220 g) were dosed with the test compound intravenously (i.v.) at 5 mg/kg or by oral administration (p.o.) at 25 mg/kg. Animals were randomly distributed into two experimental groups (n = 4 rats for each group). The compound was dissolved in a vehicle of 2% DMSO, 4% EtOH, 4% castor oil, and 90% $H_2O$. After a single administration, whole blood samples (100-200 μL) were obtained from the orbital venous plexus at the following time points: 5 min, 10 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 7 h, 11 h and 24 h (p.o.); 2 min, 10 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 7 h, 11 h and 24 h (i.v.). Whole blood samples were collected in heparinized tubes. The plasma fraction was immediately separated by centrifugation (8,000 rpm, 6 min, 4 °C) and stored at -20 °C until LC-MS analysis. Test sample

concentrations were determined by LC/MS using propranolol as an internal standard. Pharmacokinetic parameters were calculated using DAS 2.0 software. The rats were humanely euthanased without pain by carbon dioxide 24 h after the experiment.

***In Vivo* Antitumor Activity Assay.** All animal experiments were performed according to institutional ethical guidelines of animal care. Female SCID nude mice (6 weeks) were inoculated in the lower left mammary fat pad with MDA-MB-231 cells ($1 \times 10^6$/mouse in 200 μL). When the tumor volume reached 100−300 $mm^3$, the mice were randomly assigned into control and treatment groups (n = 5). Each group was dosed with either vehicle only or with compound **30** at 15 mg/kg intravenously (i.v.) 4 days a week. The length (L) and width (W) of the tumor mass were measured, and the tumor volume (TV) was calculated as: TV = (length × width2)/2. Tumor growth inhibition (TGI) was calculated using the following formula: TGI = [1 − (T − T0)/(C − C0)] × 100, where T and T0 are the mean tumor volumes on a specific experimental day and on the first day of treatment, respectively, for the experimental groups, and likewise where C and C0 are the mean tumor volumes for the control group. Beginning on day 0, tumor dimensions and body weight were measured daily during the experiments.

## ASSOCIATED CONTENT

### Supporting Information

Synthetic protocols, spectral data for all listed compounds and scanned NMR spectra

for compounds **10-65**. This material is available free of charge via the Internet at http://pubs.acs.org.

## PDB ID CODES

PDB code 4O1D was used for modeling docking in Nampt of compound **30**. Authors will release the Atomic Coordinates and experimental data upon article publication.

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### Notes

The authors declare no competing financial interest.

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## ABBREVIATIONS USED

NAMPT, Nicotinamide phosphoribosyltransferase; NAM, nicotinamide; NMN, nicotinamide mononucleotide; NAD, nicotinamide adenine dinucleotide; NAPRT, nicotinic acid phosphoribosyltransferase; DCM, dichloromethane; THF, tetrahydrofuran; DMF, N,N-dimethylformamide; DIPEA, *N,N'*-diisopropylethyl amine; $IC_{50}$, the half maximal inhibitory concentration; DMSO, dimethylsulfoxide; EDCI, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, hydroxybenzotriazole; $GI_{50}$, concentration at which cell growth is inhibited by 50%;

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