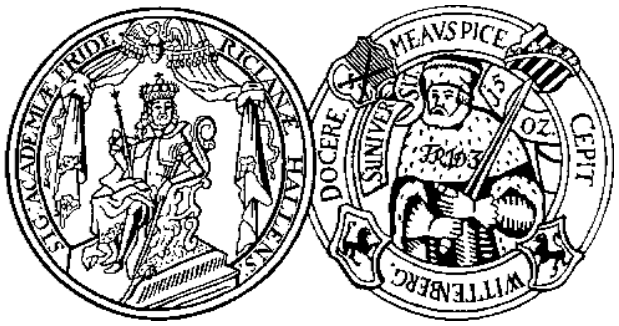

Class I HDAC Selective Modulators - Design, Synthesis, and Biological Characterization

Dissertation

zur Erlangung des

Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

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Halle-Wittenberg

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Halle (Saale), Datum der Disputation: 15.07.2022

That person is like a tree planted by streams of water, which yields its fruit in season and whose leaf does not wither— whatever they do prospers.

Psalm 1:3

Für meine Familie

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Abbreviations

$(Boc)_2O$	Di-tert-butyl dicarbonate
µM	Micromolar
^{13}C-NMR	Carbon-13 nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance
AcCl	Acetyl chloride
acety-CoA	Acetyl coenzyme A
ACN/CH_3CN	Acetonitrile
AcOH	Acetic acid
APCI	Atmospheric-pressure chemical ionization
aq	Aqueous
Ar	Aryl-residue
BF_3-EtO_2	Boron trifluoride etherate
Boc	tert-Butyloxycarbonyl
Boc-Gly-OH	*N*-(tert-Butoxycarbonyl)-glycine
bp	Base pairs
Br_2	Bromine
cAMP	Cyclic adenosine monophosphate
Cbz	benzyloxycarbonyl group
CbzCl	Benzyl chloroformate
CCl4	Carbon tetrachloride
CDI	Carbonyldiimidazole
CH_3I	Iodomethane
CoREST	Co-repressor of repressor element-1 silencing transcription factor
CRBN	Cereblon
Cs_2CO_3	Caesium carbonate
Cu_2O	Copper(I) oxide
CuI	Copper(I) iodide
$CuSO_4 \cdot 5H_2O$	Copper(II) sulfate pentahydrate
DCM	Dichloromethane
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMEM	Gibco dulbecco's modified eagle medium
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E2	E2 ubiquitin-conjugating enzyme
E3	E3 ubiquitin ligase
EC_{50}	Half maximal effective concentration
EDCI	1-Ethyl-3-(3′-dimethylaminopropyl)carbodiimide·HCl

eq	Equivalent
ESI	Electrospray-ionization
EtOH	Ethanol
FRS2a	Fibroblast growth factor receptor substrate 2a
h	Hour
H3K27	27^{th} amino acid in Histone H3
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
HCl	Hydrochloric acid
Hda1	Histone Deacetylase Hda1 in yeast
hHDAC	Human Histone deacetylase
HIV	Human immunodeficiency virus
HOBT	Hydroxybenzotriazole
Hos3	Histone deacetylase HOS_3 in yeast
HPβCD	(2-Hydroxypropyl)-beta-cyclodextrin
HPLC	High pressure/performance liquid chromatography
HRMS	High-resolution mass spectrometry
Hsp90	heat shock protein 90
IAPs	Inhibitors of apoptosis proteins
IC_{50}	The half maximal inhibitory concentration
K_2CO_3	Potassium carbonate
Ki	Inhibitory constant
K_m	Michaelis constant
KOAc	Potassium acetate
KOH	Potassium hydroxide
KOtBu	Potassium tert-butoxide
LC-MS	Liquid chromatography-mass spectrometry
LiOH	Lithium hydroxide
LSD1	lysine-specific demethylase 1A
MeOH	Methanol
min	Minute
mL	Milliliter
MS	Mass spectrometry
MSCl	Methanesulfonyl chloride
$N_2H_4 \cdot H_2O$	Hydrazine monohydrate
Na_2CO_3	Sodium carbonate
Na_2SO_4	Sodium sulfate
$NaB(OAC)_3H$	Sodium triacetoxyborohydride
$NaBH_4$	Sodium borohydride
NaH	Sodium hydride
$NaHCO_3$	Sodium Hydrogen Carbonate
NaN_3	Sodium azide
NaOH	Sodium hydroxide
NaOMe	Sodium methoxide

NBS	*N*-Bromosuccinimide
NCoR	Nuclear receptor co-repressor
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
$NH_2OH{\cdot}HCl$	Hydroxylammonium chloride
$NiCl_2{\cdot}6H_2O$	Nickel chloride hexahydrate
nM	Nanomolar
NMI	1-Methylimidazole
NuRD	Nucleosome remodeling and deacetylase
P27	Cyclin-dependent kinase inhibitor 1B
p53	Tumor protein P53
PCC	Pyridinium chlorochromate
$Pd(dba)_2$	Tris(dibenzylideneacetone)dipalladium(0)
$Pd(OAc)_2$	Palladium(II) acetate
$Pd(PPh_3)_2Cl_2$	Bis(triphenylphosphine)palladium chloride
$Pd(PPh_3)_4$	Tetrakis(triphenylphosphine)palladium(0)
PDB	Protein data bank
PD-L1	Programmed death-ligand 1
PhMe	Toluene
PPh_3	Triphenylphosphine
pTSA	p-Toluenesulfonic acid
r.t.	Room temperature
REST 1-3	Repressor element-1silencing transcription factor 1-3
Rpd3	Histone deacetylase RPD3 in yeast
Sir2	NAD-dependent protein deacetylase sirtuin-2 in yeast
SMC3	Structural maintenance of chromosomes protein 3
SMRT/N-CoR	Silencing mediator for retinoid and thyroid receptor (SMRT) corepressor and nuclear receptor corepressor (NCoR)
$SnCl_2$	Tin(II) chloride
$SOCl_2$	Thionyl chloride
STAT3	Signal transducer and activator of transcription 3
SUMO	Small ubiquitin-related modifier
t-BuOH	Tert-Butyl alcohol
TCFH	Chlor-*N*,*N*,*N*',*N*'-tetramethylformamidinium-hexafluorophosphat
TEA	Triethylamine
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofurane
TLC	Thin layer chromatography
TosCl	4-Toluenesulfonyl chloride
Trityl	Triphenyl methyl
TrtCl	Tritylchlorid

Ts	Toluenesulfonyl group,
UDP-glucuronosyltransferase	Uridine 5'-diphospho-glucuronosyltransferase
VHL	von Hippel-Lindau E3 ubiquitin protein ligase

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Part 1 Introduction and aim of the thesis

1.1 Epigenetics and histone modifications

Epigenetics, the biological conception principally refers to the organism phenotype changes which affect genome functions without alterations of the original nucleic acids sequence. It can be also inheritable to next generations. Both behaviors and environmental factors impact the organism's genome expression as well as organism susceptibilities to diseases epigenetic mechanisms. Moreover, the epigenetic machinary plays a crucial role in cellular differentiation and multiple physiological processes throughout the whole life. Epigenetic modifications are mediated by covalenty working erasers and writers which work simultaneously and determine the outcome collectively. Theses mechanisms control different states of DNA itself as well as the histone proteins which are embraced by DNA. Additionally, the epigenetic enzymes also affect non-histone proteins. Based on molecular level, epigenetic modifications can be grouped into three main types: i) DNA methylation which occurs exclusively in cytosine-phosphate guanine (CpG) islands, is the most widely explored epigenetic modification, and is linked to gene silencing; ii) nucleosome positioning which control the accessebilty of transcription factors to their target nucleic acids sequence; and iii) histone modifications which generally take place in histone tails and include acetylation by histone acetytransferases, methylation by histone lysine methyltransferases, phosphorylation by kinases, ubiquitination, SUMOylation, ADP-ribosylation, and other modifications. Histone modifications influence the process of DNA repair, replication and splicing, and have an important role in chromosome condensation and recruitment of epigenetic binding partners to regulate gene transcription (Figure 1).[1-6]

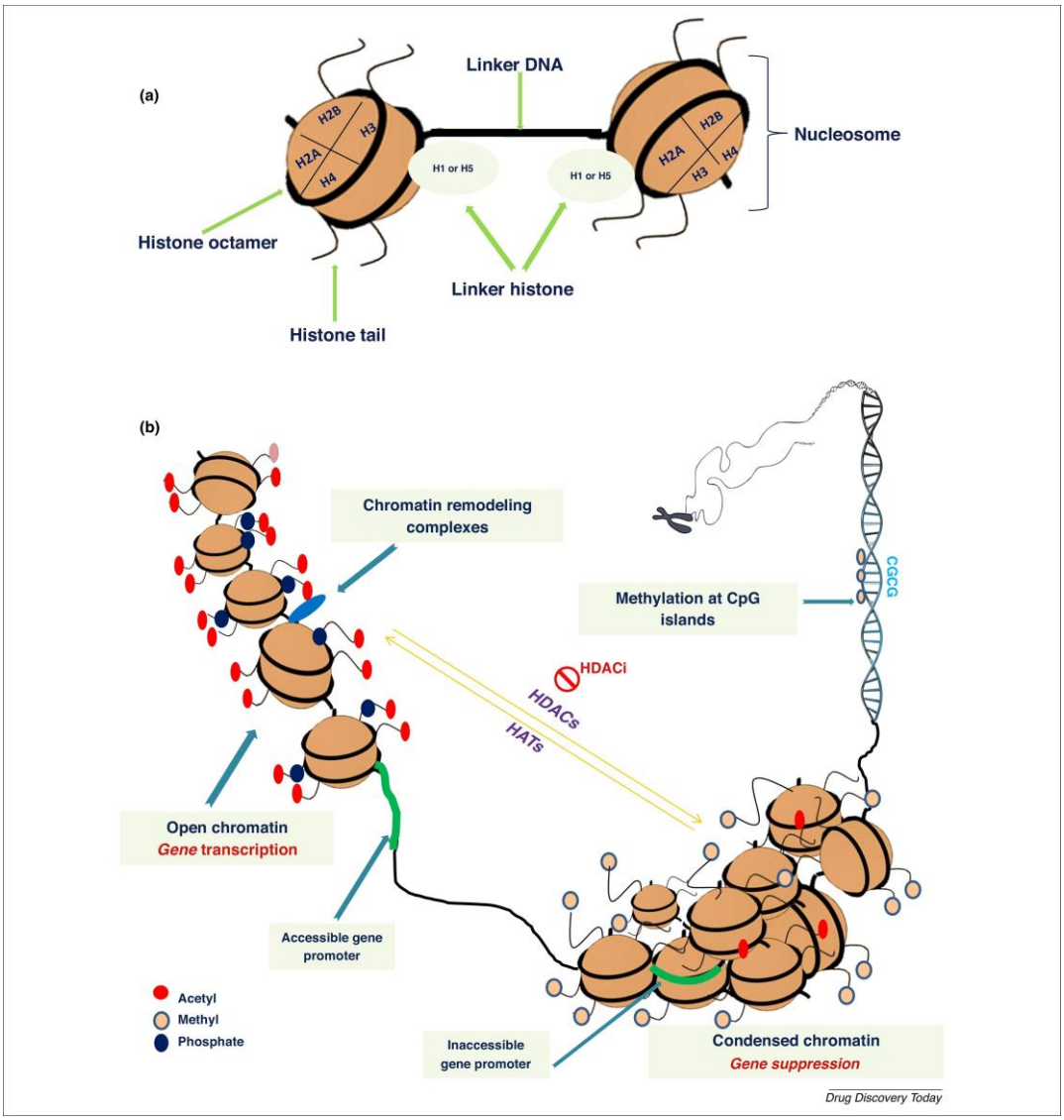


Figure 1. Epigenetic mechanisms and nucleosome structure (adapted from Ediriweera, M. K. et.al. [7])

To date, there are five different subtypes of histone proteins known as linker histone H1 and core histones namely H2A, H2B, H3, H4 present in the chromatin of eukaryotic cells (Figure 1).[8] Two copies of each of the 4 core histone proteins generate a histone octamer which binds and wraps around by 147 bp DNA. The packaging is called nucleosome which represents the basic chromatin unit. The highly ordered structures make them tightly condensed and possible to fit inside the nucleus. The flexible N-terminal tail region of histones contains a high portion of lysine and arginine residues that interact with the acidic phosphate-sugar backbone of the DNA. Each of the nucleosome is joined by the linker DNA.[8-11]

Epigenetic modifications remodel the chromatin structure resulting in alteration of gene expression. In the state of highly condensed hetero chromatin, a gene is repressed and cannot be transcribed. Conversely, in the opened and active state of the nucleosome (called euchromatin) a gene is able to be transcribed. Post-transcriptional modifying enzymes can control the process by changing the packaging of histones. On the basis of histone modifications, histone modifying proteins are classified into main three categories.

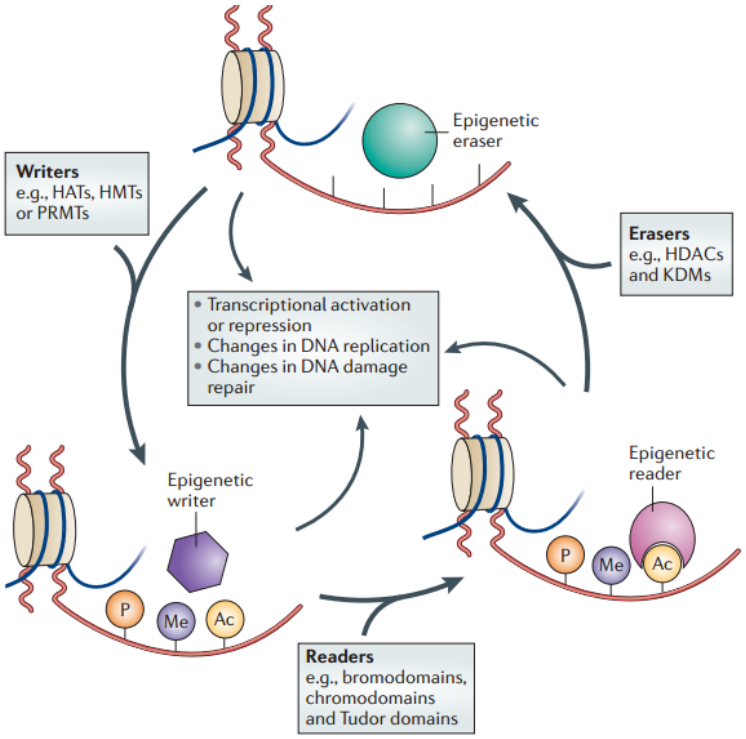


Figure 2. Histone modifying proteins can be grouped into 3 subgroups (adapted from Falkenberg, K. J. et.al. [12])

In the dynamic processes (Figure 2), epigenetic "writers" such as histone acetyltransferases (HATs), histone methyltransferases (HMTs), protein arginine methyltransferases (PRMTs) place respective marks on histone tails, subsequently epigenetic "reader" like bromodomains, chromodomains or tudor domains recognize these marks and interact with them, at the same time epigenetic "erasers" catalyze the removal of these marks for example histone deacetylases (HDACs), lysine demethylases (KDMs) etc.[7, 12]

1.2 Histone deacetylases and their actions

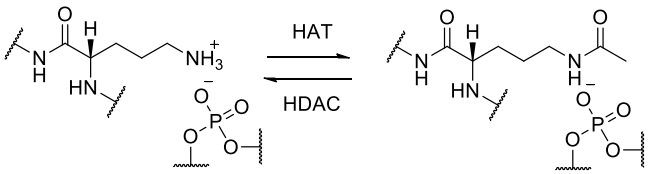


Figure 3. The balance of acetylation and deacetylation in histones

Histone acetyltransferases (HATs) form a family of enzymes that use acety-CoA to acetylate ε-amino group of lysine in histone tails. Besides acetylated lysine also acylated lysines have been reported. The acetylated or acylated lysine, which lost the positive charge of the lysine, leads to weakening the electrostatic interactions of histone tails and the negatively charged phosphate backbone of the DNA. Reversely, histone deacetylases (HDACs) - the natural antagonists of HATs - catalyze the removal of acyl groups from acylated lysine residue of histone proteins or non-histone proteins to regulate lysine acetylation status and consequently suppress gene transcription (Figure 3). In addition to histones, HDACs also erase acetyl/acyl marks from non-histone proteins including transcription factors such as NF-κB and E2F, structural proteins like α-tubulin or tumor suppressors like p53 and chaperone like protein Hsp90.[6, 12-15]

1.3 HDAC family

All the human 18 histone deacetylases can be divided into 4 classes based on their original homology to yeast orthologues Rpd3, Hda1, Hos3 and Sir2, namely class I (HDAC1, HDAC2, HDAC3, HDAC8), class II (HDAC4, HDAC5, HDAC7, HDAC9, HDAC 6 and HDAC10), class III (Sirt1-7) and the most recently identified class IV (HDAC11). The three classic histone deacetylases classes (class I, II, IV) are zinc-depended enzymes and the term of HDAC predominantly refers to these HDACs, whereas class III isoforms sirtuins need nicotinamide adenine dinucleotide (NAD^+) to

initiate their functions. HDAC substrates usually are challenging to define due to the complex cross regulatory functions of HDACs and the different substrate preferences within diverse multi-protein HDAC complexes.

Class I HDACs are closely related to yeast Rpd3 and share a highly conserved catalytic domain, these HDACs are ubiquitously expressed and mainly located in the nucleus. With the exception of HDAC8 that can function by itself,[16] class I HDACs generally form large complexes with other HDACs or their co-repressors to carry out repression of associated genes or participate in other cellular processes. For instance, HDAC1 and HDAC2 combine with corepressor of REST1-3 proteins and LSD1 protein to generate the CoREST complex. The NuRD complex contains HDAC1/2 units and six other protein units, while HDAC3 needs to bind to SMRT/N-CoR to exhibit its enzymatical activities. Studies also showed that class I HDACs harbor non-histone substrates like AMP-activated protein kinase (AMPK), SMC3 and P53.[17-18] As tissue specific expressed isotypes, class II HDACs are similar with yeast Hda1. This class could be further subdivided into class IIa (HDAC4, HDAC5, HDAC7, HDAC9) which possesses only one catalytic domain and shuttle between nucleus and cytoplasm as response to some physiological signals, while class IIb (HDAC6 and HDAC10) own two catalyzed domains and is usually discovered in cytoplasm. The functions of HDAC10 is still not completely clear but it is involved in cellular autophagy survival process,[19-21] while HDAC6 impacts the deacetylation process of α-tubulin, cortactin, chaperones, giving its important role in cell migration and cell-cell interactions. HDAC11, the exclusive class IV HDAC is homologous to Hos3 of yeast and shares similar sequences with both class I and class II HDACs. HDAC11 has so far not been well profiled but several studies display that it is tissue specific expressed and linked to certain cellular immune functions.[22-26]

1.4 Class I HDAC structural features and their catalytic mechanism

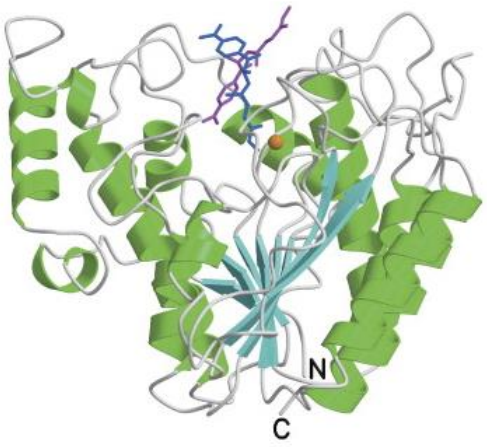


Figure 4. Structure of HDAC8 with bound trichostatin A (TSA), the two bound molecules of TSA are shown in blue and pink, the zinc ion is in orange (adapted from Somoza, J. R. et.al[27]). Only one TSA molecule is interacting with the zinc ion whereas the second bound TSA might be a crystallization artefact.

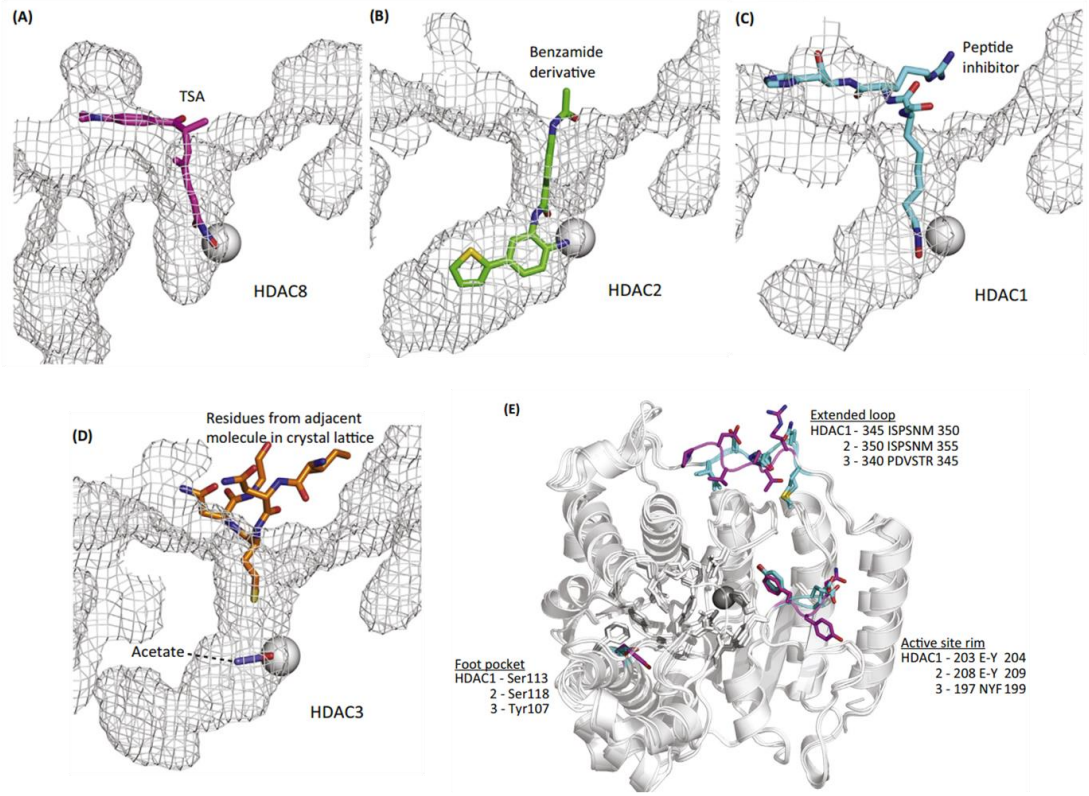


Figure 5. Structural comparison of HDAC1-HDAC3 and HDAC8 with their ligands (adapted from Millard et.al.[28])

HDAC8, despite being a class I member, is structurally distinct and show less

sequence similarity to HDAC1-3. This has been demonstrated by numerous solved X-ray structures over the years. The first disclosed human HDAC crystal structure is HDAC8 with certain hydroxamic acid inhibitors complexes (Figure 4). After that many HDACs crystal structures have been published which give many details to get insight into HDACs structures.[27, 29-30] HDAC8 possesses a sandwich-like structure which contains a single α/β domain with an 8-stranded β-sheet buried between helices and other loops.[27, 29] A long, narrow hydrophobic binding tunnel and a coordinated zinc ion at the bottom constitute the active site, on the rim of the tunnel there is another conserved Asp101 residue which play a vital role for substrate interaction as observed in the crystal structures.[31]

However, there are also many structural differences within class I HDAC isoforms (Figure 5), for example HDAC8 has a shorter C-terminal domain, a shorter loop1 but wider substrate binding pocket and a larger surface opening compared with HDAC1-3.[27] Moreover, the region of class I HDACs common 14-Å “foot pocket” (also called acetate release channel) is narrower in HDAC8 than in HDAC1-3, whereas Ser113/Ser118 in HDAC1/2 is replaced by tyrosine in HDAC3 leading to the difficulty for inhibitors with bulky groups to access to the footpocket.[28, 32-33] In addition, structural differences are also discovered in the unique solvent exposed site in HDAC3, the diverse extended loops in HDAC 1/2/3 as well as an essential inositol tetraphosphate molecule that firmly associates within the HDAC3 complex.[28, 33-34] All above specific features can be exploited for the discovery of specific HDAC inhibitors.

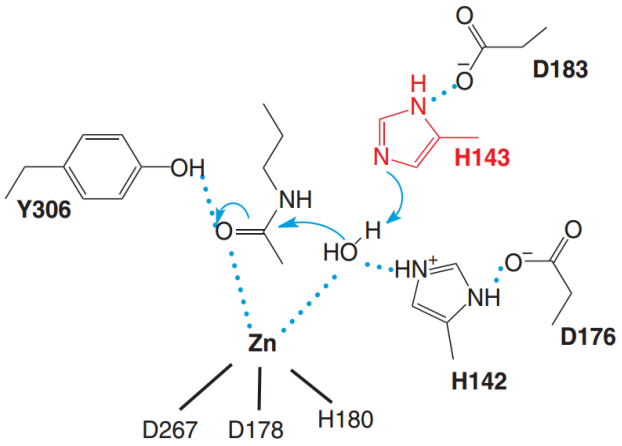


Figure 6. HDAC catalytic mechanism (adapted from Seto, E. et.al[34])

In the catalytic site (Figure 6) of HDAC6 the zinc ion is coordinated with two aspartic acids residues (D267, D178) and one histidine (H180), the carbonyl oxygen of the substrate and one water molecule to form a tetrahedral intermediate. The water molecule, which is polarized the Zn ion and a Asp-His charge relay system (H142, H143, D176, D183), nucleophilically attacks the electrophilic carbonyl of the substrate. Consequently, HDAC inhibitors are designed to mimic the residues of substrates to block HDAC activities and regulate diseases processes.[34]

1.5 Class I HDACs as drug targets

Class I HDAC hypoacetylations are highly linked to a large variety of cancers for example prostate, gastric, colon, lung, and pancreatic cancer (specially associated with HDAC1-3), in addition to neuroblastoma, hepatocellular carcinoma and other solid tumours or haematological malignancies.[35-45] HDAC inhibitors can lead to hyperacetylation of histone or non-histone proteins hence mediating an antitumour activity by influencing various cellular pathways involved in the process of development, differentiation, apoptosis and anti-angiogenesis or by inducing tumour suppressors and immune response modulation[39, 45-48]. HDAC inhibitors thus constitute important approaches in cancer therapy. Class I HDACs have also been identified as promising targets for neurodegenerative disease like Alzheimer's disease, immune

defficiency disease such as HIV infection, metaboilic disorders, inflammatory diseases etc.[35, 41, 49-61]

In the current work we will focus on class I HDACs and their potential role as valuable therapeutic targets for drug discovery.

It has already been shown that HDAC8 expression is highly associated with advanced-stage and metastasis outcomes in neuroblastoma which is one of the common childhood cancer forms. Knockdown studies of HDAC8 as well well as studies using selective HDAC8 inhibitors showed neuroblastoma cells proliferation regression, clone formation inhibition and induction of cellular differentiation without side effects. Furthermore, HDAC8 selective inhibitors also showed anti-poliferative activity and no significant toxic effects were found *in vivo*. Beneficial synergistic effects were also observed when retinoic acid was combined with a HDAC8 inhibitor. [41, 62-63]

Very recently, a study reported that in hepatocellular carcinoma (HCC), which is a type of non-T cell inflamed cancer, down-regulation of HDAC8 can result in hyperacetylation of H3K27 and reactivation of the production of T cell-trafficking chemokines to relieve T cell exclusion in mouse models. Subsequently, using selective HDAC8 inhibitors enhanced tumor-infiltrating $CD8^+$ T cells and facilitated outcome of anti-PD-L1 therapy in established hepatomas without obvious adverse effect. After the combination therapy treatment, mice obtained a tumor-free life longer than 15 months due to introduction of memory T cells.[64]

Many studies displayed that high expression of HDAC1-3 are significantly observed in pancreatic cancers and related to negative tumor differentiation[65-66]. Meanwhile blocked HDAC1-2 or 3 can bring promising therapeutic outcome for pancreatic cancers by complicated and comprehensive mechanisms for example reducing

leukocyte infiltration[67], enhancing sensitization to cells apoptosis [68-69], regulation of p53 and p27 [70-71] and immune modulation.[72-73]

Since the first HDAC inhibitor trichostatin A (TSA) has been discovered more than 30 years ago,[74] nowadays the United States Food and Drug Administration (FDA), the China Food and Drug Administration (CFDA) and/or the European Medicines Agency (EMA) have approved 5 pan-HDAC inhibitors (vorinostat - hydroxamic acid derivative, romidepsin - cyclic peptide derivative, belinostat - hydroxamic acid derivative, chidamide - benzamide derivative, panobinostat - hydroxamic acid derivative),[75-79] for the treatment of blood cancers such as T-cell lymphoma and multiple myeloma, and many more of HDAC inhibitors are in different phases of clinic trials. Several limitations have appeared from the use of these approved drugs: for example a reduced therapeutic window and drug resistance, which is perhaps due to the lack of isoform specificity profiles. Improvement of target selectivity might be a promising approach to decrease off-target side effects, but we should still keep in mind that it must be demonstrated in clinic whether the advantages of selective HDAC inhibitors could be transformed into clinical benefits.[44, 80]

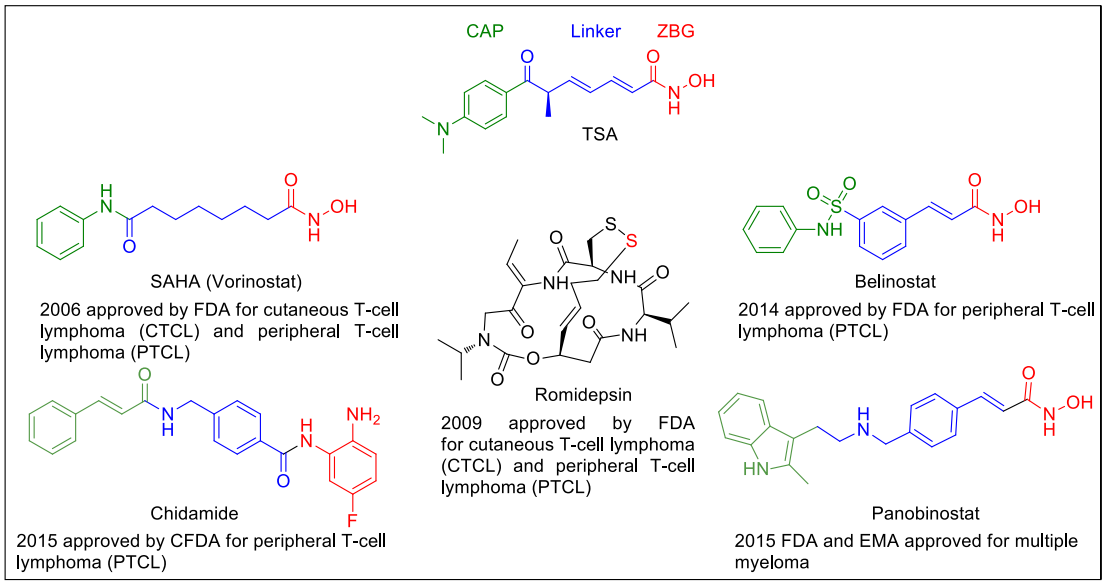


Figure 7. Classical pharmacophore of HDAC inhibitors and approved HDAC inhibitors. The different pharmacophoric parts are colored as following: zinc-binding group in red, linker in blue and capping group in green. Romidepsin is a prodrug (disulfide) that is cleaved *in vivo* into a thiol.

HDAC inhibitors have a well described pharmacophore (Figure 7). Taking the most frequently reported hydroxamic acids derived HDAC inhibitors as example the pharmacophore can be described as follows: a zinc binding group (ZBG) to form a tightly bidentate chelatation with the catalytic zinc ion (hydroxamic acid group), a cap group binding to the rim of the protein channel, a linker group placed into the catalytic tunnel. Although a high sequence identity can be observed between class I HDACs, especially HDAC1-3 (85% between HDAC1 and HDAC2, 64% between HDAC1 and HDAC3), the previously described structural differences still could be used for designing selective HDAC inhibitors development, through modifying the different parts of the classical HDAC pharmacophore.[53, 56, 80-83]

1.6 The technology of proteolysis-targeting chimera (PROTAC) and hydrophobic tagging

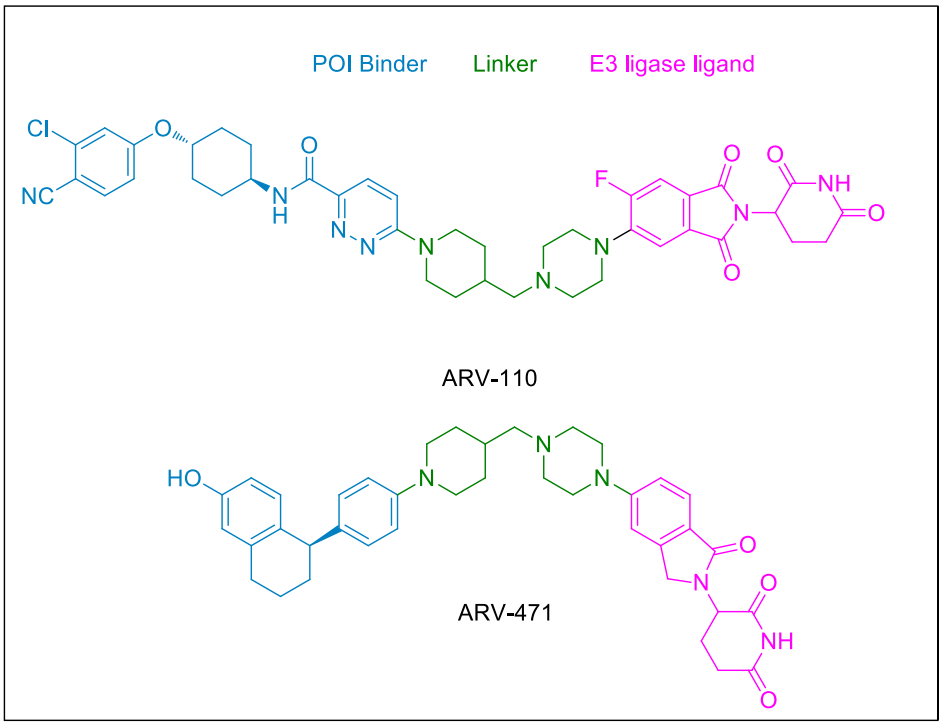


Figure 8. Examples of PROTACs in clinical trials. The ubiquitin ligase binding part is colored magenta and the ligand part that binds to the protein to be degraded is colored blue, linker is colored green.

20 years ago Crews and Deshaies et al. reported the first example of chimeric molecules that target proteins and induce degradation of the protein of interest (POI)

utilizing the endogenous 26S proteasome of the ubiquitin proteasome system (UPS). UPS plays a crucial role in quality control of protein homeostasic regulation.[84-85] Targeting protein degradation (TPD) has emerged as a charming territory and expanded its landscape from proteolysis-targeting chimera (PROTAC) to hydrophobic tagging (HyT),[86] molecular glues (using small molecular to degrade unligandable proteins by directly orchestrating target-ligase interactions),[87] lysosome-targeting chimeras (LYTACs, a conjugate of glycopolypeptide-POI binder that can bind to POI then be transported into the lysosome and subsequently degraded)[88] and autophagy-targeting chimeras (AUTACs, consisting of a guanine tag and POI binder that bind to POI to then be transported to the autophagosome and eliminated)[89] as well as others[90-92]. PROTACs are at the forefront among all these PTD approaches due to their tremendous experience principles for rational design, moreover the orally bioavailable PTOTACs of ARV-110 and ARV 470 (Figure 8) from Arvinas[93-94] as well as KT474[95] from Kymera have already demonstrated the enormous potential of PROTACs as therapeutic approaches in clinical applications and many more of them are expected to enter into clinical test in the near future.[96]

1.6.1 Mechanism of PROTACs

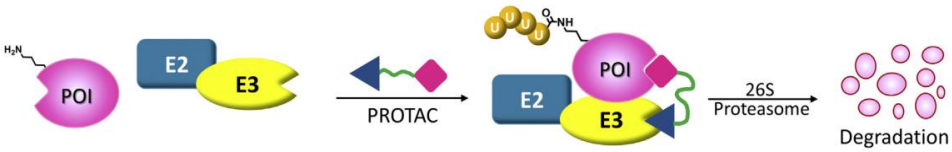


Figure 9. Schematic representation of the molecular mechanism of PROTACs (adapted from Wang, Y. et. al [97])

In general, PROTACs are heterobifunctional systems which consist of three parts: a POI ligand, an E3 ligase ligand and a linker connecting both. When a PROTAC binds to the POI and E3 ligase, a stable ternary complex is formed and then the E3 ligase coordinates the transfer of ubiquitin from the E2 ubiquitin-conjugating enzyme to the surface lysine residues of POI. The ubiquitinated proteins are subsequently

recognized by proteasomes for degradation. Finally the degrader is released from previous ternary complex and may participate in the next cycle of target recruitment (Figure 9).[85, 98]

1.6.2 Mechanism of Hydrophobic Tag (HyT)

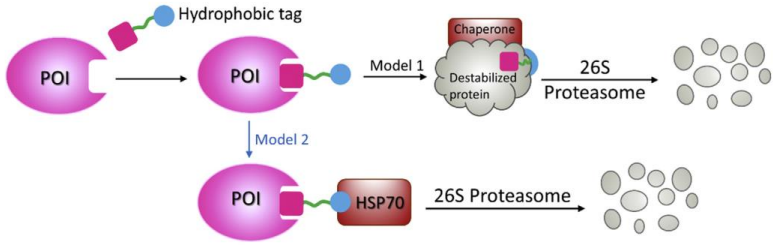


Figure 10. Proposed molecular mechanism of HyT (adapted from Wang, Y. et. al.[97])

With a similar composition of PROTAC, a heterobifunctional molecule of HyT also contains a POI ligand part linked with a bulky hydrophobic tag (usually a lipophilic small molecular fragment such as adamantane derivatives or tert-butyl carbamate-protected arginine (Boc_3-Arg) derivatives) by a linker. Although its exact mechanism is still indistinct, it has been suggested to employ molecular chaperones mediated degradation system: when a molecule of HyT binds to the POI, the hydrophobic fragment bound to the POI can mimic an unfolded protein and hence be recognized by protein folding machinery triggering cell quality control system which detect the protein folding status. Molecular chaperones like Hsp90, Hsp70 and Hsp40 are thus recruited to the POI and mediate its ubiquitination resulting in the POI degradation by 26S proteasomes (Figure 10).[99-102]

1.6.3 PROTAC/HyT versus inhibitors advantages and challenges

Considering the above catalyst-like degradation mechanism, PROTACs and

hydrophobic tag containing degraders could be regarded as event-driven pharmacology compared with the conventional small molecule inhibitors/occupancy-driven pharmacology which usually need a high target binding affinity to compete in the active site with the protein substrate. Hence, high drug doses are usually required whereas the risk of undesired side effects will be simultaneously increased due to off-target binding under higher drug concentrations. On the contrary, the chemical knockdown approach usually can function under low doses due to its catalyst-like character which could be employed iteratively and can degrade proteins entirely to affect their non-enzymatic function or down-regulate over-expressed proteins to normal levels under proper doses.[103] In addition, the essential procedure of PROTACs to recruit proteasomes is based on stable POI-PROTAC-E3 ligase ternary complex formation and week binary affinity to POI can still induce effective degradation outcome, this is exemplified by the work of Joshua P. Smalley and co-workers, where they used a class I selective inhibitor (CI-994) as POI binder and VHL ligase ligand to induce HDAC1-3 degradation. PROTAC4 displayed significant HDAC3 degradation, despite showing the worst inhibition of the enzyme (IC_{50} = 16.8 μM to HDAC3) compared with the stronger HDAC3 inhibiting PROTAC1 and 3 which showed almost no degradation.[104] Thus the reported results are not satisfying in total and further development of more effective HDAC degraders is necessary. It is also assumed that PROTACs own the capacity to degrade “undruggable” proteins with shallow or without proper binding pockets[105] like scaffolding proteins and transcription factors. Several studies have already demonstrated that this is indeed possible for example peptide-based phosphoPROTACs as FRS2a degraders [106] and STAT3-targeting PROTACs as STAT3 degragers were discovered.[107] Drug resistance is another serious problem that small molecule inhibitors have to face in clinical treatment, however PROTACs can still evade these by disease-causing proteins degradation.[108] Besides, many PROTACs exhibit improved selectivity and specificity among homologous targets over their parent inhibitors or protein ligands perhaps due to additive effects of PROTACs components and the spatial conformations of ternary complex.[109]

As with small molecule inhibitors, PROTACs associated off-target effects are possible. One reason is that the nature of the E3 ligase substrate is not completely understood, for example several studies disclosed zinc-finger CRBN neo-substrates as off-targets of immunomodulatory drugs (IMiD)-based PROTAC.[110-111] Furthermore, till now only a few of the 600 studied human E3 ligases have known small molecular ligands[112] and the most popularly employed E3 ligase ligands belong to CRBN, VHL and IAPs which have also limited the applied scope of PROTACs.[113] Another vital problem limiting PROTACs development is how to establish more systematic, more rational and more efficient screening system, since finding a potential PROTAC is still a time-consuming and empirical performance. The composition of the PROTAC structure as well as the biological validation processes are much more complicated and different from small molecule development, for example linker length, composition and attachement position can influence the PROTAC's structural conformations, hydrophobicity, solubility and possibility for ternary complex formation, subsequent degradation and biological activities.[114] Last but not least, PROTACs structural features always violate the Lipinski's "rule-of-five" principle and the traditional pharmacokinetics (PK)/pharmacodynamics (PD) evaluation strategy maybe not suitable for PROTACs. Nevertheless, the thus far obtained results from PROTACs are still highly encouraging.[103, 109, 115-116]

1.7 Aim of the work

Most of the so far reported HDAC isoform selective inhibitors use hydroxamic acids as zinc binding groups (ZBG). However, the hydroxamic acid feature as ZBG is considered to be responsible for several crucial disadvantages such as significant off-target effects as well as pharmacokinetic and metabolic problems.[117-121] Thus, the design of other metal-chelating non-hydroxamic acids may overcome these limitations.

Another intractable challenge for selective class I HDAC inhibitors discovery results from the highly conserved catalytic tunnel of HDAC enzymes.[80, 83, 122-123] On the basis of the previously described structural differences between HDAC isoforms, we set to develop class I HDAC (HDAC1, 2, 3 and 8) isoform specific modulators comprising inhibitors and degraders.

1.7.1 Development of HDAC8 selective inhibitors using a novel chemotype

For HDAC8 selective inhibitors, our recent studies determined that an HDAC8 specific L shaped bindning pocket is formed between the catalytic tunnel and the protein periphery which could be a exploited for next generation selective HDAC8 inhibitor design.[124] Additionally, Novartis group described two amino acid (**AA1** and **AA2**) derivatives which exhibited pronounced HDAC selective inhibitory profiles and showed a novel binding mode, where the ligand partly binds into the deeper acetate-release channel namely the foot pocket (FP).[125] Based on this finding, another research group explored structure-activity relationship of this very original type of amino acid-based HDAC inhibitors and identified a promising lead (**AA3**) with a two fold improved inhibitory activity against HDAC8 compared to the original lead (**AA2**) (Figure 11). However, no detailed selectivity profiles or further cellular activity were disclosed.[126]

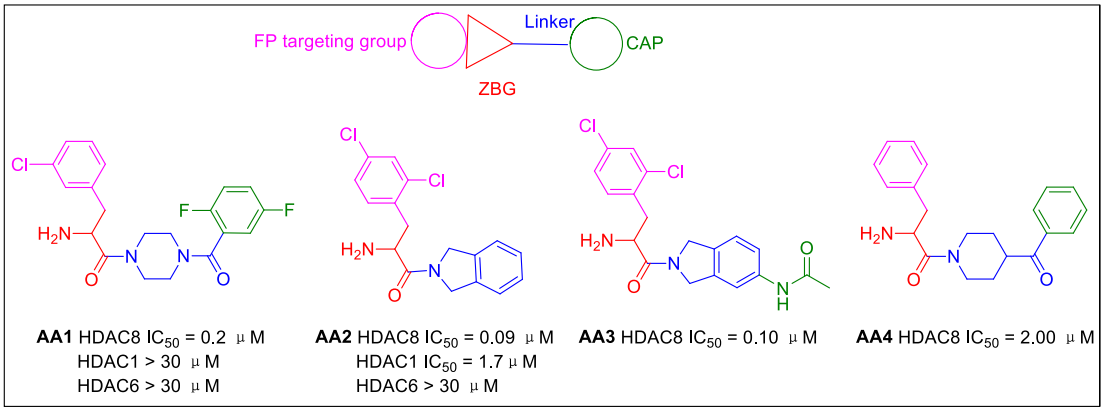


Figure 11. Amino acid (AA) derivatives: **AA1** and **AA2** were reported by a Novartis group, **AA3** was discovered by a UK group (the IC_{50} of **AA2** is 0.21 µM in their study), **AA4** is identified by our inhouse virtual screening. Foot-pocket targeting group is colored magenta, zinc-binding group is colored red, linker is blue and capping group is colored green.

We started with a virtual screening campaign from several compound libraries against the available crystal structures of wild type HDAC8. This screening and subsequent *in vitro* tresting also yielded about 50 active compounds. Besides several hydroxamic acids, the most promising amino acid hit (**AA4**) showed an IC_{50} value of 2.0 µM (Figure 11) as well as a very good selectivity profiles (against HDAC1with 16.9 % inhibition at 10 µM and against HDAC6 with 34.7 % inhibition at 10 µM) similar with the compound **AA1**. On the basis of our previous HDAC8 selective inhibitors discovery practice[63, 83] and the initial results obtained from the virtual screening, we were interested in exploring the structure-activity relationship (SAR) of HDAC8 inhibitors bearing an *R*-amino acid unit as Zinc binding group. These derivatives will be designed based on the pharmacophore (Figure 11) of previously found amino acid derived HDAC8 selective inhibitors. In the present work we will use chemical optimization guided by docking studies to alter the foot-pocket moiety, which is adjacent to the amino acid unit, as well as linker and cap groups to improve the HDAC8 inhibitory potency and selectivity against other HDAC isoforms. In addition, the developed compounds will be tested in cancer cell lines with upregulated HDAC8 such as neuroblastoma cell line and human T lymphocytes cell lines to confirm the antiproliferative potential of these derivatives.

1.7.2 Development of alkylhydrazides as novel HDAC inhibitors

HDAC1-3 subtypes have already been established as targets for numerous diseases. To date however, only a few selective inhibitors have been reported for the individual subtypes of class I. Rational inhibitor design for HDACi is still challenging, due to the high structural similarity of the individual HDAC isoforms.

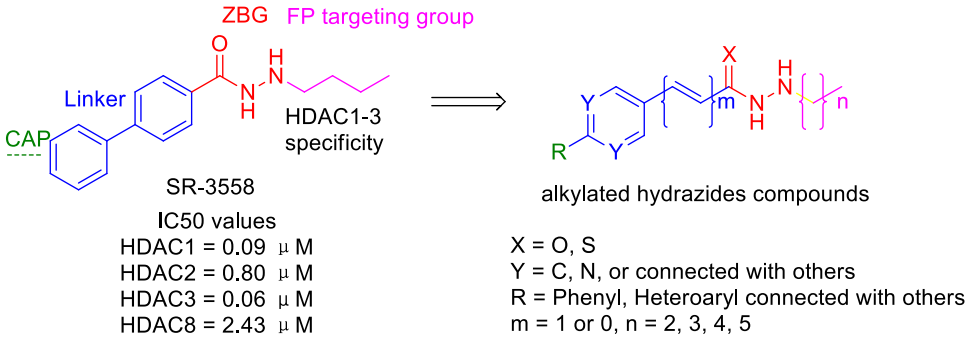


Figure 12. Left: reported novel class I selective inhibitor, right: planned modifications for its optimization.

Recently, a series of alkylated hydrazides that displayed encouraging HDAC3 selectivity against other HDAC isoforms were reported.[127-130] It was suggested that these novel alkylated hydrazide-derived inhibitors can use the hydrazide moiety as ZBG, the alkane tail can occupy the foot pocket of class I HDAC at the same time. However, until now there is no clear experimental proof that the hydrazide group is acting as ZBG.

The chemical structures of this type of inhibitors are more stable *in vitro*/*in vivo* than hydroxamic acid and aminobenzamide ZBG for UDP-glucuronosyltransferase mediated metabolism. Moreover they were confirmed as much safer than hydroxamic acid *in vitro*, and several of them exhibited excellent pharmacokinetic profile and oral bioavailability.[56, 128-132] Therefore, in order to obtain novel HDAC1-3 inhibitors with improved selectivity profiles and pharamcokinetic properties that can conquer the

limitations of hydroxamic acid, we utilized the reported compound SR-3558 (Figure 12) as a starting point to employ structure-based design as well as chemical optimization and explore the SAR of novel generated alkylated hydrazides against class I HDACs (HDAC1-3). The optimization campaign includes systematic modifications of the alkanyl tail, linker part, cap group and ZBG moiety. The synthesized compounds will be subsjected to enzymatic assays to determine their inhibitory potency and selectivity profiles over HDAC subtypes. Further target related phenotypic activity and biomarkers alterations in T lymphocytes cell line, human colon cell line and human pancreatic cell line will be characterized for the assessment of the most promising compounds.

1.7.3 Development of class I HDAC degraders

As we described in the previous part, TPD is a powerful and novel technology for drug discovery. In this work, we are going to employ this technology including PROTAC and hydrophobic tags to find more specific modulators for class I HDAC (Figure 13). Although several PROTACs targeting class I HDACs have been published very recently, it is still necessary to develop further degarders due to the suboptimal specificity, degradation activity and phenotypic activity limitations of the reported compounds.[104, 130, 133-135]

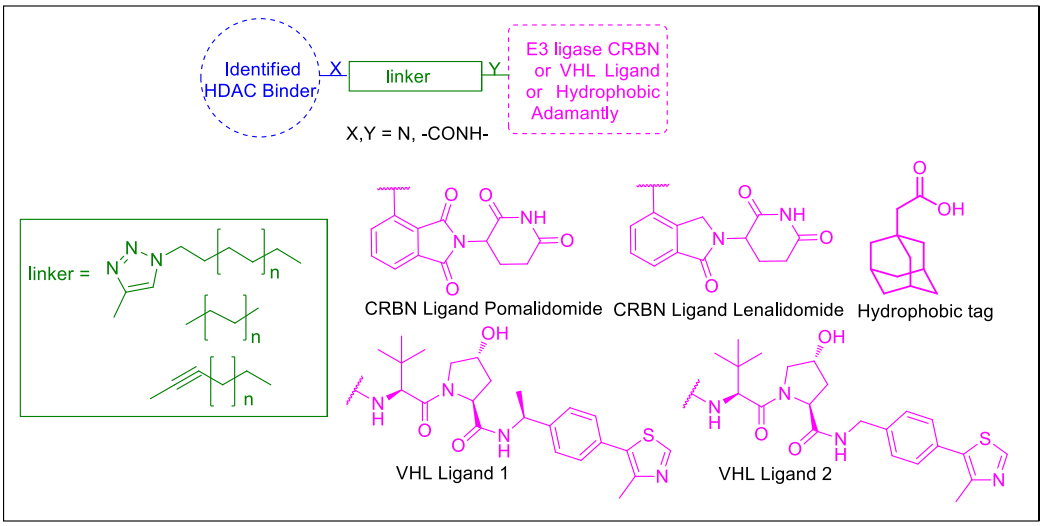


Figure 13. Degraders tool box for this class I HDAC degraders work.

In the past years, 2-aminobenzamide derivatives have been characterized as potent and selective HDAC1-3 inhibitors including CI-994, MS-275[136] (in clinical trials) (Figure 14), the CFDA-approved drug chidamide as well as other reported derivatives in preclinal studies.[137-142] Generally, veritable specific class I (HDAC1/2 or HDAC3) isotype profiles could be obtained by modification of the 2-aminobenzamide phenyl ring with aryl or fluoro substitutions.[143-148]

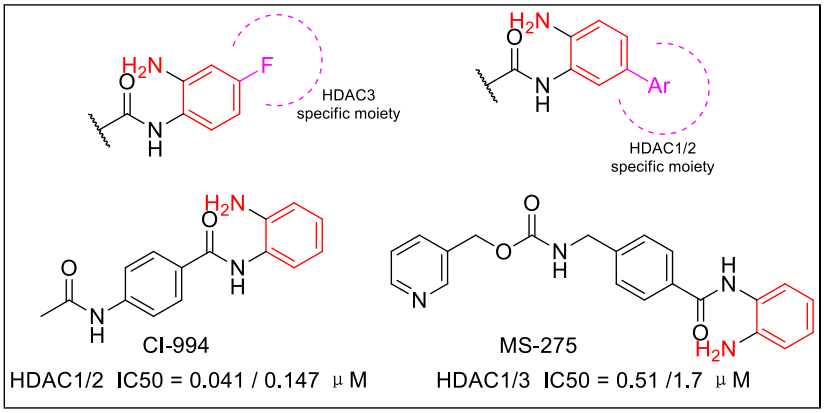


Figure 14. Examples of 2-aminobenzamide-based class I HDAC inhibitors and its modification to obtain class I HDAC subtypes specificity.

Consequently, new class I selective HDAC inhibitors will be developed and used together with published classical HDAC inhibitors as POI binders for the development of new class I HDAC degarders. The conventionally used E3 ligase ligands like pomalidomide, lenalidomide and VHL ligands will be included to recruit the E3 ligase. Additionally, the adamantyl group will be employed as hydrophobic tag (Figure 13). The developed class I HDAC degraders will be evaluated in enzymatic assay and on cellular level in human colon and pancreatic cell lines.

Part 2 Amino acid derivatives as HDAC8 selective inhibitors: synthesis, characterization and *in vitro* biological activity

2.1 Chemistry

2.1.1 Synthesis of amino acid building blocks

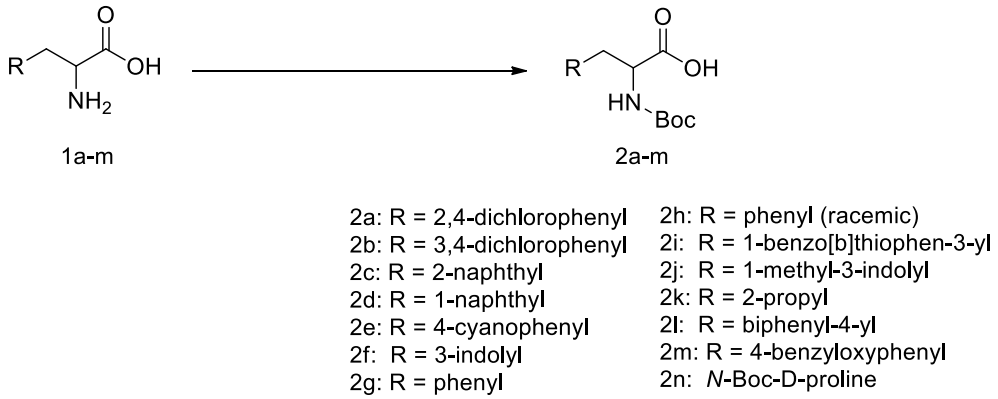


Figure 15. Reagents and conditions: $(Boc)_2O$, NaOH (10%) aq, THF/H_2O, ice bath to rt, overnight.

Firstly, we prepared the Boc-protected amino acid analogues **2a-m** in an almost quantitative yield using reported reaction conditions.[149] Whereas compound **2n** was directly commercially available and the racemic **2h** was a mixture of D and L configuration of Boc-phenylalanine generated from D and L configuration of Phenylalanine using the discribed method in Figure 15, for the other copounds D-configured starting materials (**1a-g**, and **1i-m**) were purchased and used (Figure 15).

2.1.2 Synthesis of products with phenyl(piperidin-4-yl)methanone linker

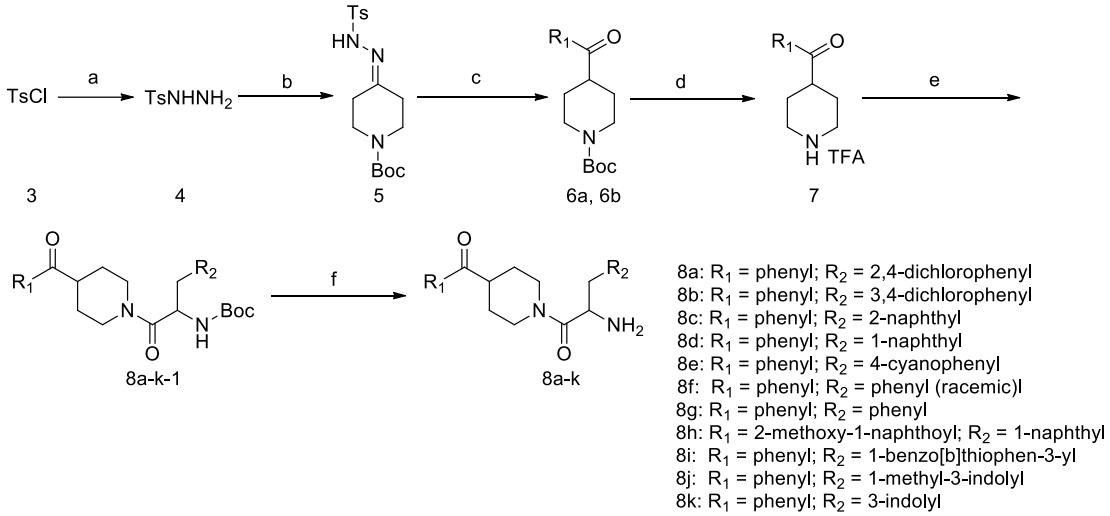


Figure 16. Reagents and conditions: (a) $N_2H_4 \cdot H_2O$, THF, 0-5 °C to rt, overnight; (b) 1-Boc-4-piperidone, MeOH, rt; (c) R_1CHO, Cs_2CO_3, 1,4-dioxane, 110 °C, microwave, 2.5 h; (d) TFA, DCM, rt; (e) Corresponding **2a-2j**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (f) TFA, DCM, ice bath.

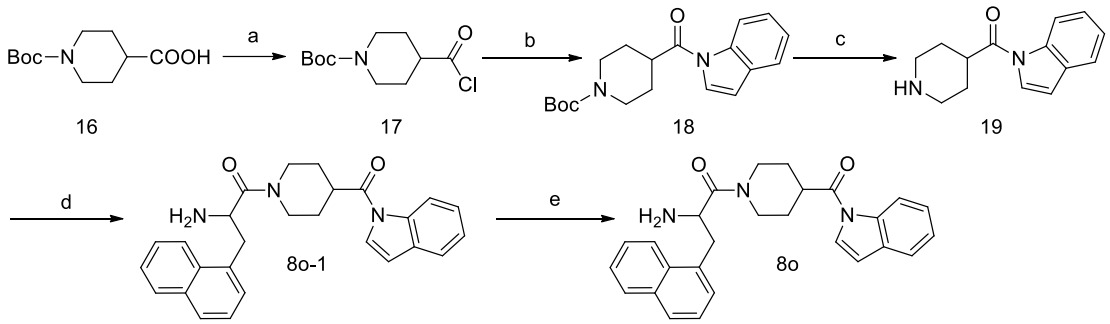


Figure 17. Reagents and conditions: (a) $SOCl_2$, TEA, DCM, rt; (b) Indole, ECF, TEA, THF, ice bath to rt, 4 days; (c) TFA, DCM, rt; (d) **2d**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (e) TFA, DCM, ice bath.

AA4 derivatives, which include phenyl(piperidin-4-yl)methanone unit as linker group, were investigated as the first batch for the optimization studies. In the synthetic procedure, we used 4-toluenesulfonyl chloride as starting material which was reacted with hydrazine monohydrate to yield the hydrazide **4**, which was coupled with 1-Boc-4-piperidone and the respective aldehyde to afford **6a-6b**. The latter step involves a diazonium alkoxide transition state and a formyl C-H bond insertion reaction as reported in the literature (Figure 16, step c, compound **5** to **6**).[150] Microwave under 110 °C was employed in this procedure to promote the reaction, which finished within 2.5 h. It's worth mentioning that the reaction mixture become chaotic when temperature increased up to 120 °C within 30 min, perhaps due to the

sensitivity to temperature of Boc group in compound **5**.

Subsequently, the Boc group was cleaved using TFA, The final products **8a-8k** were generated from the intermediate **7** by reaction with the above mentioned Boc-protected amino acid building blocks (**2a-2n**) followed by another Boc-deprotection procedure. The product **8o** was obtained via the condensation of acylchloride **17** and indole to yield **18**, which was subsequently Boc-deprotected and coupled with the Boc-protected amino acid **2d** (Figure 17).

2.1.3 Synthesis of products with rigid linker

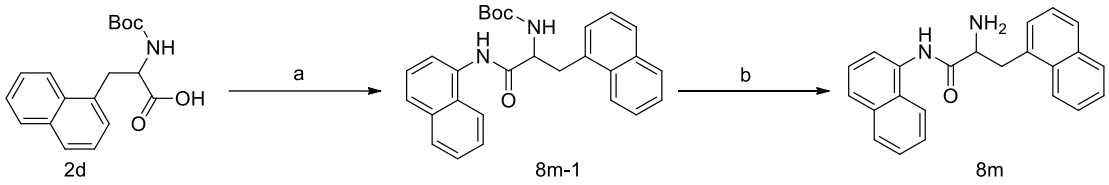


Figure 18. Reagents and conditions: (a) 1-Naphthylamine, ECF, TEA, DMF, ice bath to rt; (b) TFA, DCM, ice bath, 3 h.

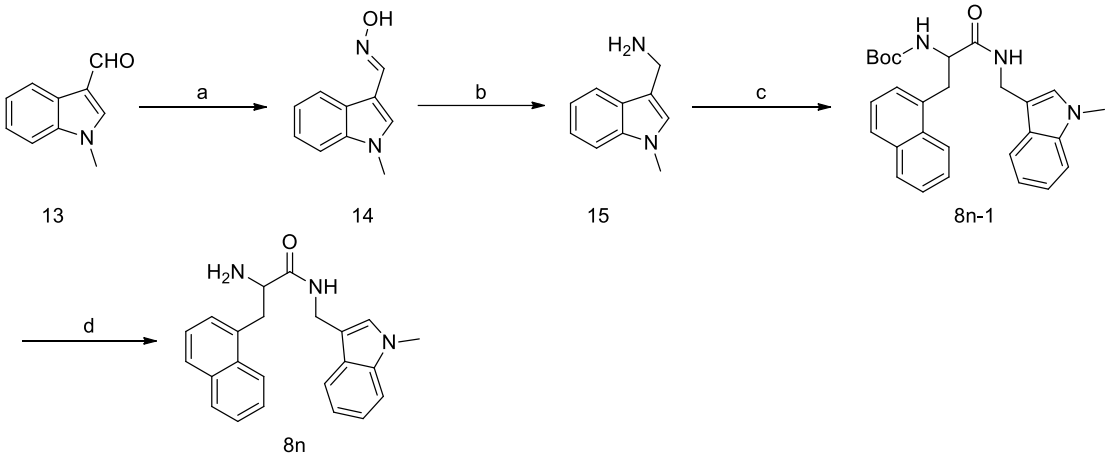


Figure 19. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, NaOAc, MeOH/H_2O (2 : 1), rt, overnight; (b) $NaBH_4$, $NiCl_2{\cdot}6H_2O$, MeOH, ice bath, 10 min; (c) **2d**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (d) TFA, DCM, ice bath.

For **8n**, the Schiff base **14** was first reduced to the amine **15** under the combined compounds of sodium borohydride and $NiCl_2{\cdot}6H_2O$ (which was used as lewis acid

here) in the second procedure within 10 min (Figure 19, step b).

To get the rigid **8p-8r**, general cyclodehydration for the oxadiazole derivaties (Figure 20, **8p** and **8q**) and click reactions for the triazole derivatives were implemented (Figure 21, **8r**).

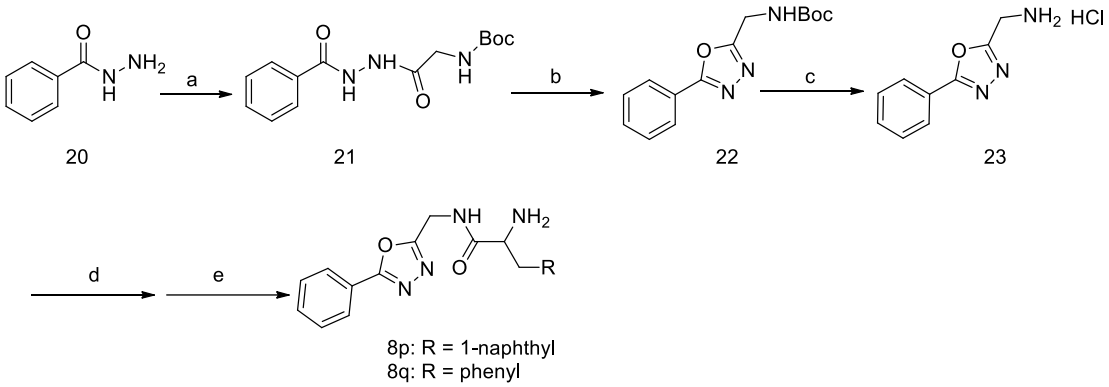


Figure 20. Reagents and conditions: (a) Boc-Gly-OH, EDCI, DIPEA, DCM, rt; (b) TosCl, TEA, DCM, rt; (C) TFA, DCM, rt; (d) **2d** or **2g**, EDCI, HOBt, DIPEA, DMF, ice bath to rt; (e) TFA, DCM, ice bath.

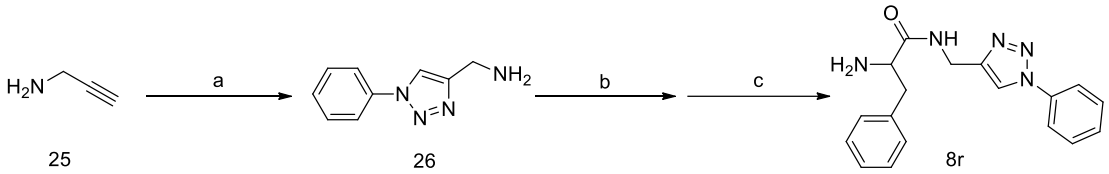


Figure 21. Reagents and conditions: (a) Phenyl azide, $CuSO_4 \cdot 5H_2O$, L-ascorbatic, H_2O/t-BuOH (1 : 1); (b) **2g**, EDCI, HOBt, DIPEA, DMF, ice bath to rt; (c) TFA, DCM, ice bath.

2.1.4 Synthesis of products with isoindoline linker and (R)-2-amino-3-(1-methyl-indol-3-yl)propanoic acid moiety

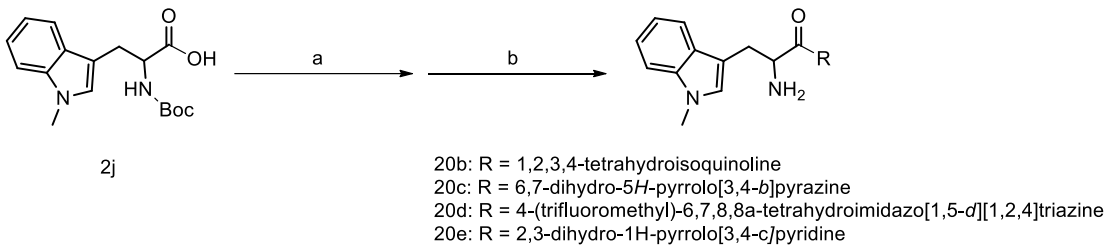


Figure 22. Reagents and conditions: (a) Substituted amine **20b-20e**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (b) TFA, DCM, ice bath.

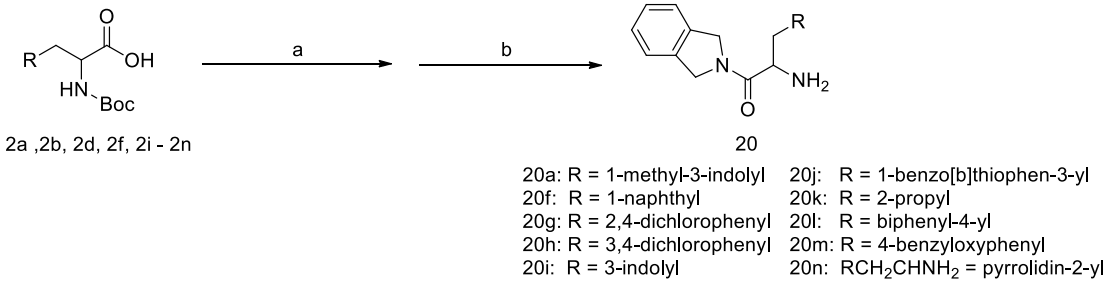


Figure 23. Reagents and conditions: (a) Isoindoline hydrochloride, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (b) TFA, DCM, ice bath.

A series of Boc-protected amino acids were condensed with isoindoline using EDCI and HOBT. A subsequent Boc-deprotection procedure afforded products **20a-20n** (Figure 22 and Figure 23). It shoud be noted that the product **20n** was obtained starting from *N*-Boc-D-proline (**2n**).

2.1.5 Synthesis of products with 5-substituted insoindoline as linker and cap groups

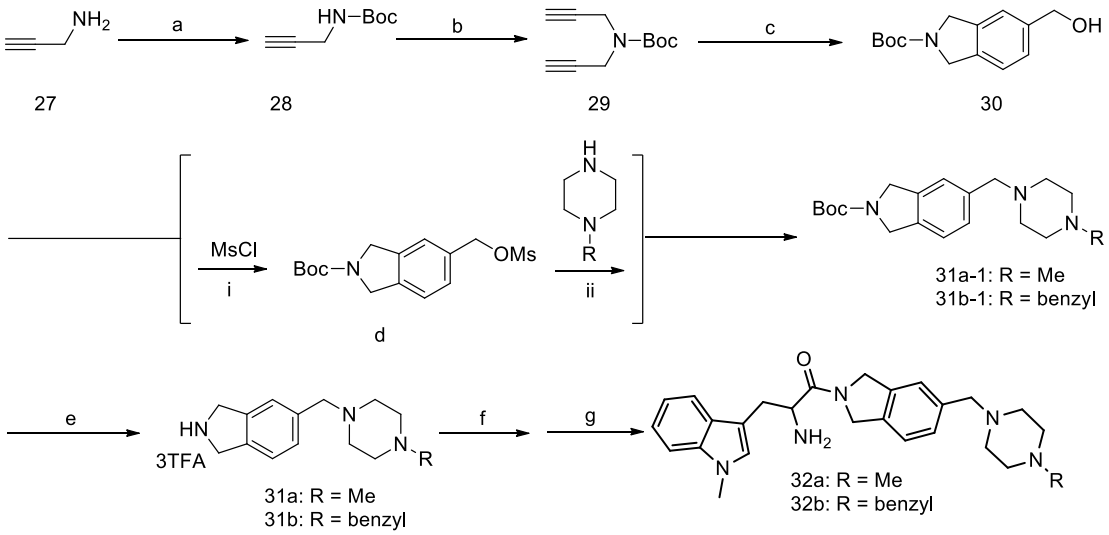


Figure 24. Reagents and conditions: (a) $(Boc)_2O$, THF, rt; (b) Propargyl bromide, NaH, THF; (c) Propargyl alcohol, $(PPh_3)_3RhCl$, THF, rt; (d) i. MsCl, DIPEA, THF, ice bath, 1.5 h; ii. Monosubstituted piperazine, rt, 3 h; (e) TFA, DCM, ice bath; (f) **2j**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (g) TFA, DCM, ice bath.

Boc-protected dipropargylamine (Figure 24, intermediate **29**) was subjected to cyclization with propargyl aclcohol using Wilkinson's catalyst to afford the key intermediate **30** which was converted to **31** by a one-pot reaction procedure (Figure

24, step d) and another followed Boc-deprotection procedure. The condensation of **31** with amino acid **2j** and a further Boc-deprotection processes yielded the targeted compounds **32a** and **32b** (Figure 24).[151]

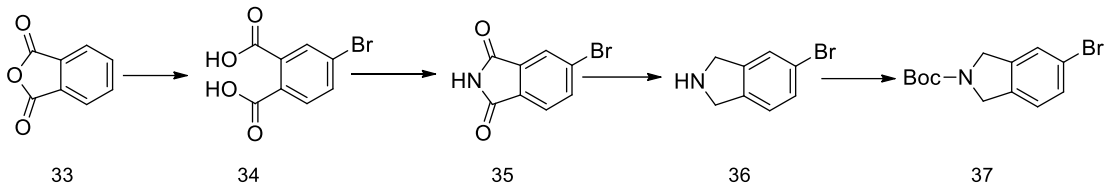


Figure 25. Reagents and conditions: (a) Br_2 liquid, NaOH, water, reflux, overnight; (b) Imidazole, DMF, 160 °C, microwave, 2.5 h; (c) $NaBH_4$, BF_3-EtO_2, THF, -10 °C to rt, reflux; (d) $(Boc)_2O$, TEA, DCM.

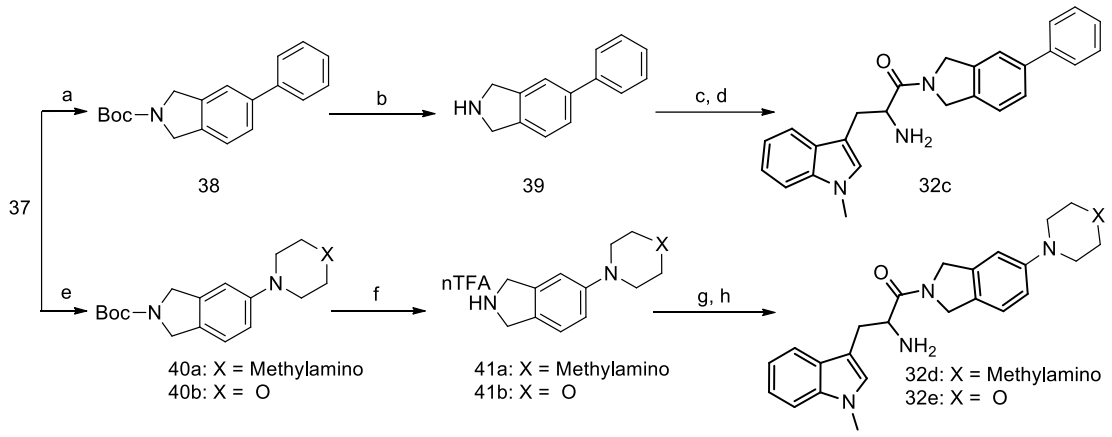


Figure 26. Reagents and conditions: (a) Phenylboronic acid, $Pd(OAc)_2$, PPh_3, Cs_2CO_3, toluene, 110 °C, reflux; (b) TFA, DCM, rt; (c) **2j**, EDCI, HOBt, DIPEA, DMF, ice bath to rt; (d) TFA, DCM, ice bath; (e) 1-Methylpiperazine or morpholine, $Pd(dba)_2$, KOtBu, tert-butyl tetraisopropylphosphorodiamidite, toluene, 110 °C, microwave, 1 h; (f) TFA, DCM, rt; (g) **2j**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (h) TFA, DCM, ice bath.

Before the the Suzuki coupling and Buchwald-Hartwig cross coupling of intermediate **37** with phenylboronic acid and different secondary amines (Figure 26), a series of bromination, microwave assisted cyclization and $NaBH_4$ reduction combined with Lewis acid[152] were conducted in sequence (Figure 25). Consequently, the 5-substituted isoindoline series **32c-e** were obtained using previously described coupling steps (Figure 26, **38** to **32c**; **40a**, **40b** to **32d**, **32e**).[153-154]

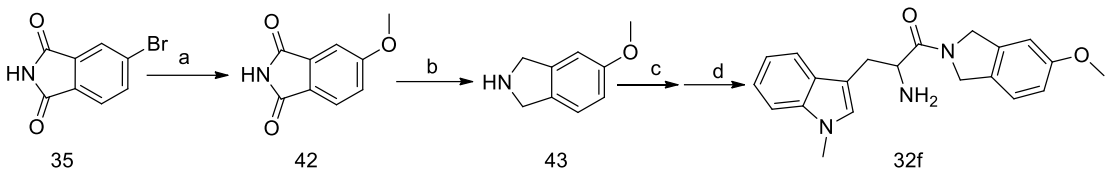


Figure 27. Reagents and conditions: (a) NaOMe, Cu_2O, MeOH, 170 °C, microwave, 30 min; (b) $NaBH_4$, BF_3-EtO_2, THF, reflux, overnight; (c) **2j**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (d) TFA, DCM, ice bath.

For the synthesis of the desired compounds **32f** and **32g** (Figure 27 and Figure 28), microwave reaction, as described in the literature,[155] was used to promote the reaction (step a of Figure 27 and Figure 28) to afford the key intermediate compounds of **42** and **44**. These were subjected to reduction reaction, condensation, cyclization, substitution, and finally condensation resulting in **32f** and **32g**.

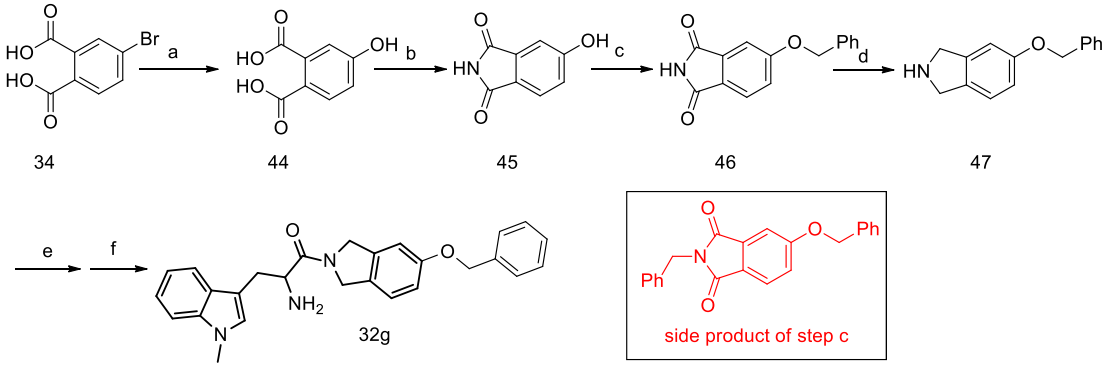


Figure 28. Reagents and conditions: (a) Cu_2O, NaOH, H_2O, 140 °C, microwave, 1.5 h; (b) Imdazole, urea, DMF, 160 °C, microwave, 2.5 h; (c) Benzyl bromide, K_2CO_3, DMF, 60-65 °C; (d) $NaBH_4$, BF_3-Et_2O, THF, relux; (e) **2j**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (f) TFA, DCM, ice bath.

In step c for intermediate **46** (Figure 28, step c) we obtained a large amount of a side product which resulted from the alkylation of the both amide-*NH* and phenolic-*OH* group in **45** by benzyl bromide (Figure 28, step c). The side product could be easily removed in procedure d but difficult in step c due to their bad solubility in organic solvent (Figure 28).

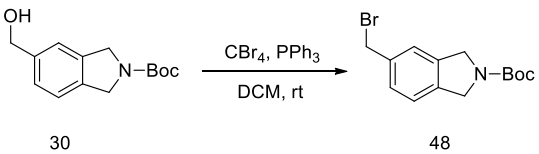


Figure 29. Hydroxyl was substituted through bromination.

To improve the substitution activity of the isoindoline derivative **30**, the hydroxyl group was replaced by bromide (Figure 29)[156] which was expected to be more easily reacted to the desired products **52a-g** (Figure 30 to Figure 33).

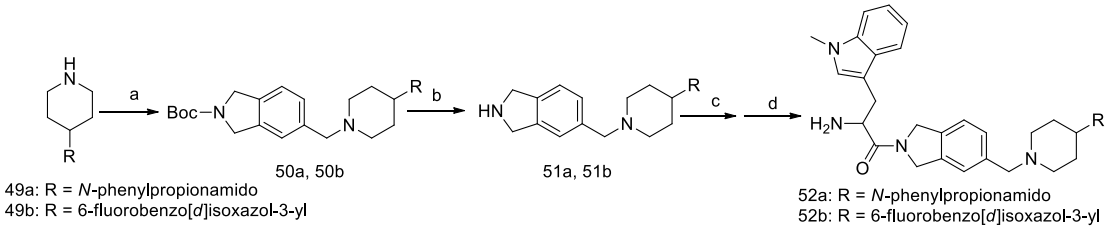


Figure 30. Reagents and conditions: (a) **48**, K_2CO_3, DMF, rt; (b) TFA, DCM, ice bath; (c) **2j**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (d) HCl/dioxane, DCM, ice bath.

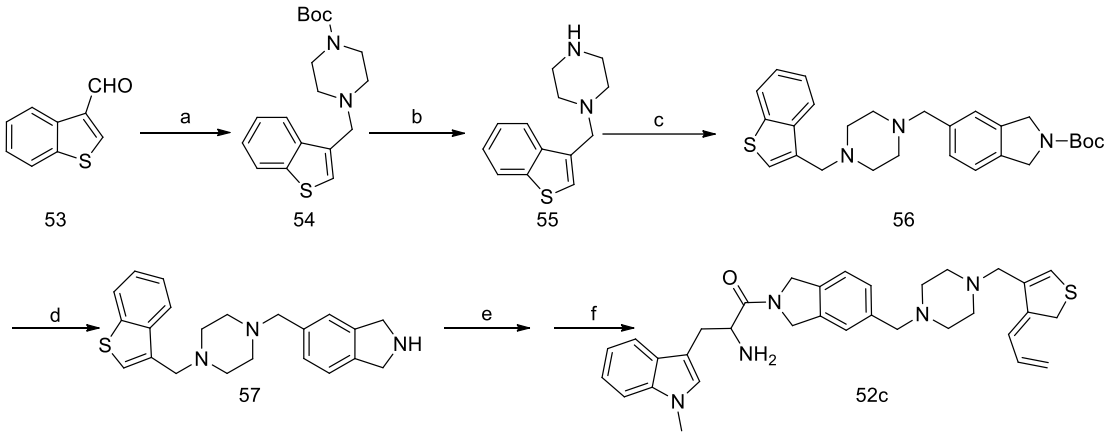


Figure 31. Reagents and conditions: (a) Tert-butyl piperazine-1-carboxylate, $NaB(AcO)_3$, DCM, rt; (b) TFA, DCM, rt; (c) **48**, K_2CO_3, DMF, rt; (d) TFA, DCM, ice bath; (e) **2j**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (f) HCl/dioxane (4M), DCM, ice bath.

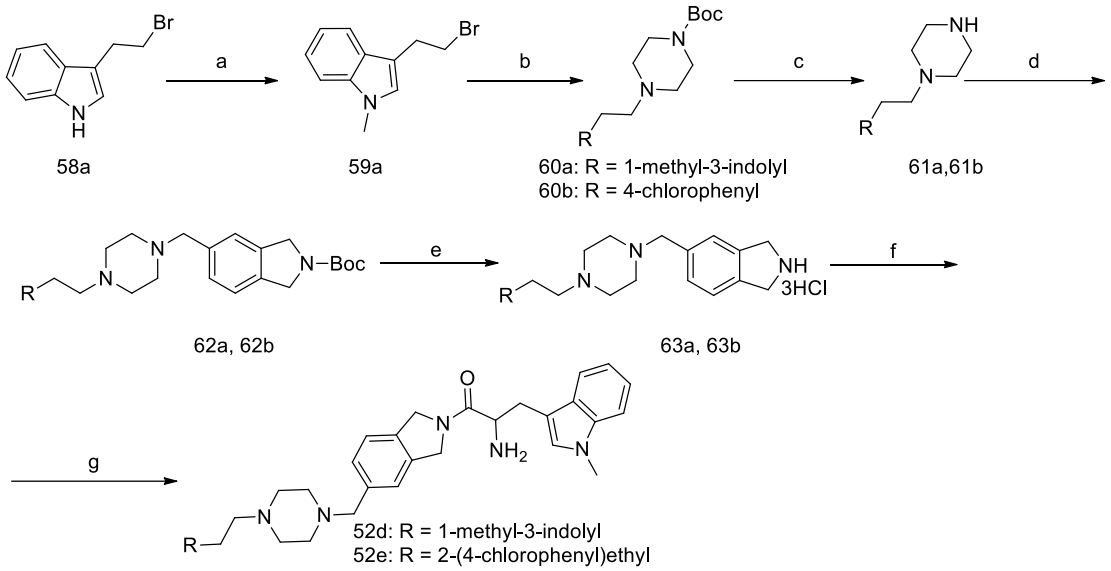


Figure 32. Reagents and conditions: (a) CH_3I, NaH, THF, rt; (b) Tert-butyl piperazine-1-carboxylate, K_2CO_3, DMF, 70-80 ^oC; (c) TFA, DCM, rt; (d) **48**, K_2CO_3, DMF, rt; (e) HCl/dioxane (4M), DCM; (f) **2j**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (g) HCl/dioxane (4M), DCM, ice bath.

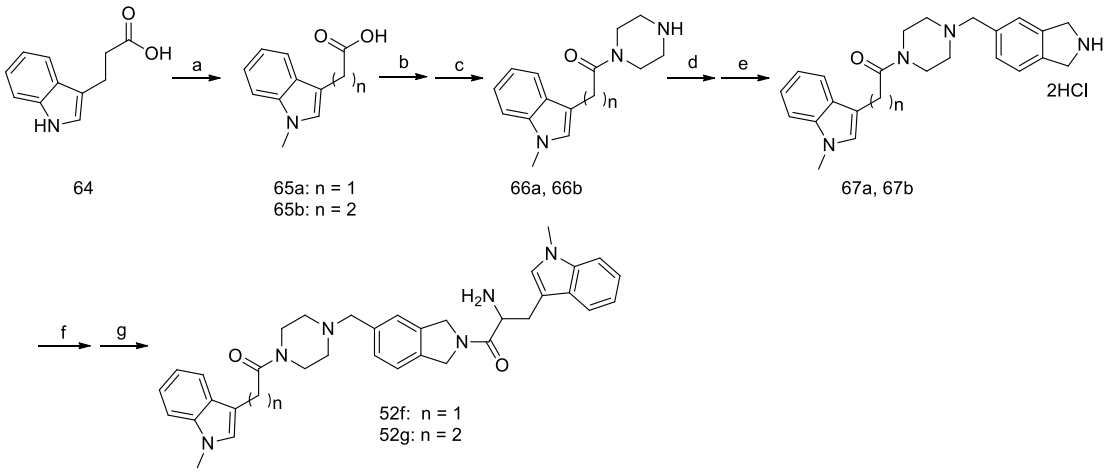


Figure 33. Reagents and conditions: (a) i. CH_3I, KOH, Me_2CO, ice bath to rt; ii. KOH, H_2O, reflux; (b) Tert-butyl piperazine-1-carboxylate, HATU, DIPEA, DMF, (c) TFA, DCM, rt; (d) **48**, K_2CO_3, DMF, rt; (e) HCl/dioxane (4M), DCM; (f) **2j**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (g) HCl/dioxane (4M), DCM, ice bath.

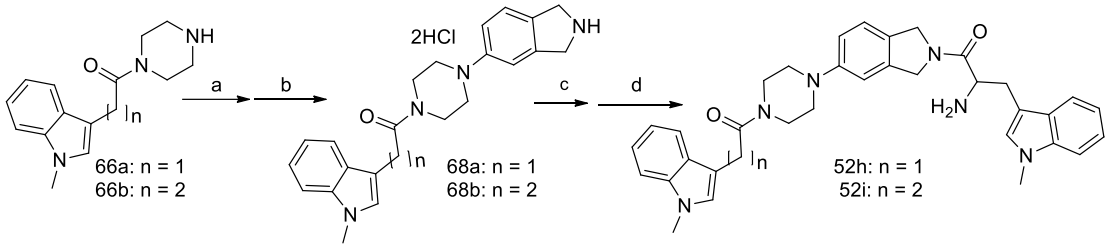


Figure 34. Reagents and conditions: (a) **37**, $Pd(dba)_2$, KOtBu, tert-butyl tetraisopropylphosphorodiamidite, toluene, 110 °C, microwave, 1 h; (b) HCl/dioxane (4M), DCM, rt; (c) **2j**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (d) HCl/dioxane (4M), DCM, ice bath.

In addition, similar procedure, as previously described for **32d** and **32e**, was performed to achieve the compounds **52h-i** (Figure 34).

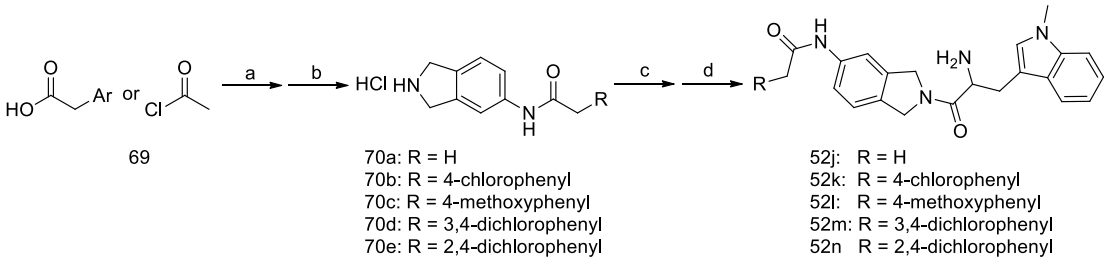
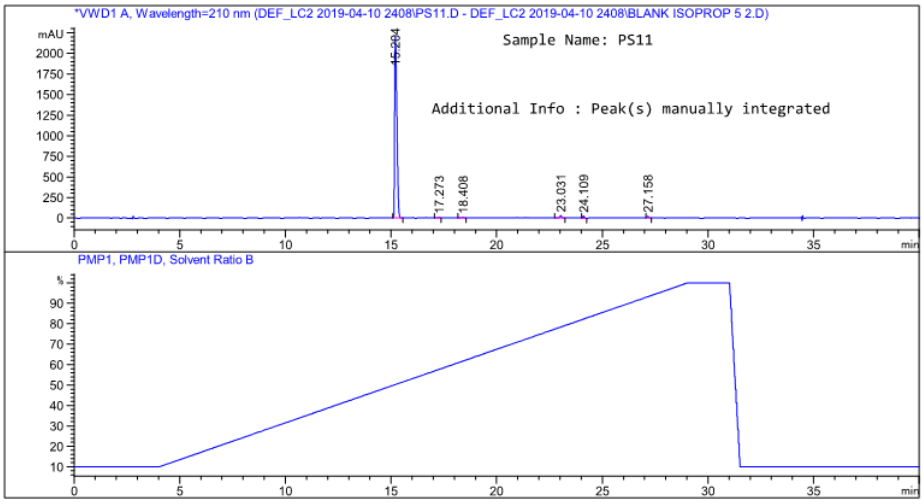


Figure 35. Reagents and conditions: (a) Tert-butyl 5-aminoisoindoline-2-carboxylate, HATU, DIPEA, DMF, rt; or Tert-butyl 5-aminoisoindoline-2-carboxylate, DIPEA, DCM, rt; (b) HCl/dioxane (4M), DCM, rt; (c) **2j**, EDCI, HOBT, DIPEA, DMF, ice bath to rt; (d) HCl/dioxane (4M), DCM, ice bath.

Other 5-(acylamino)isoindolines analogous products (**52j-52n**) were also synthesized using the commercially available tert-butyl 5-aminoisoindoline-2-carboxylate as staring material which underwent sequential procedure of acylation (Figure 35, step a), Boc-deprotection (Figure 35, step b), coupling with Boc-protected amino acid (Figure 35, step c) as well as the last Boc-deprotection process.

2.2 Purity measurement by chiral HPLC

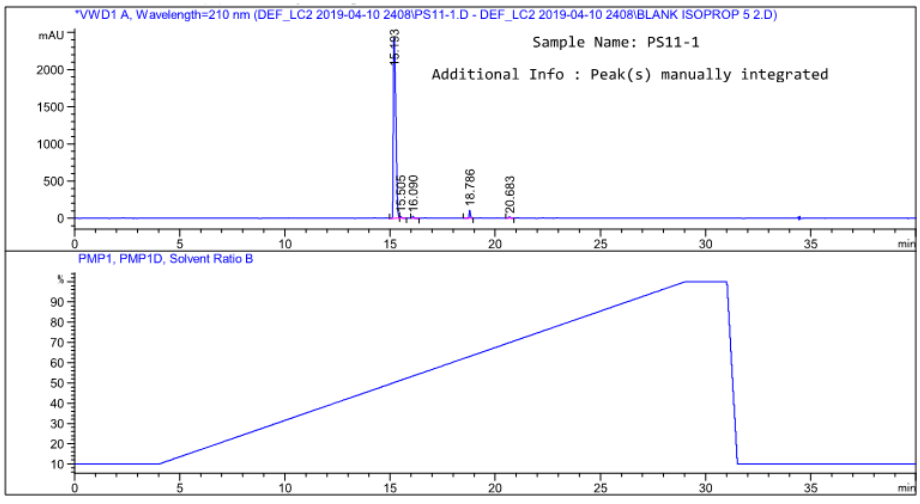


A

Area Percent Report

Signal 1: VWD1 A, Wavelength=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.204	VV	0.1208	1.64832e4	2201.54639	96.8341
2	17.273	BV	0.0680	45.96357	10.31296	0.2700
3	18.408	BB	0.0997	62.10402	8.86840	0.3648
4	23.031	BV	0.0869	190.88068	31.84731	1.1214
5	24.109	VB	0.0719	127.48119	27.10621	0.7489

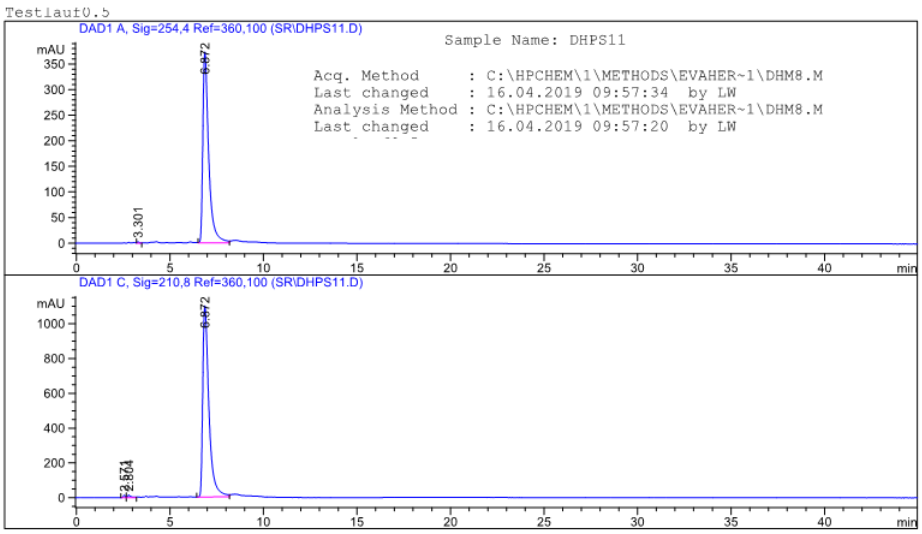


B

Area Percent Report

Signal 1: VWD1 A, Wavelength=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.193	BV	0.1349	1.99603e4	2428.68408	95.8304
2	15.505	VB	0.0844	154.62096	25.98275	0.7423
3	16.090	VB	0.0700	139.20827	28.26102	0.6683
4	18.786	BV	0.0613	444.89328	109.82298	2.1360
5	20.683	BB	0.1163	129.75906	17.54567	0.6230



C

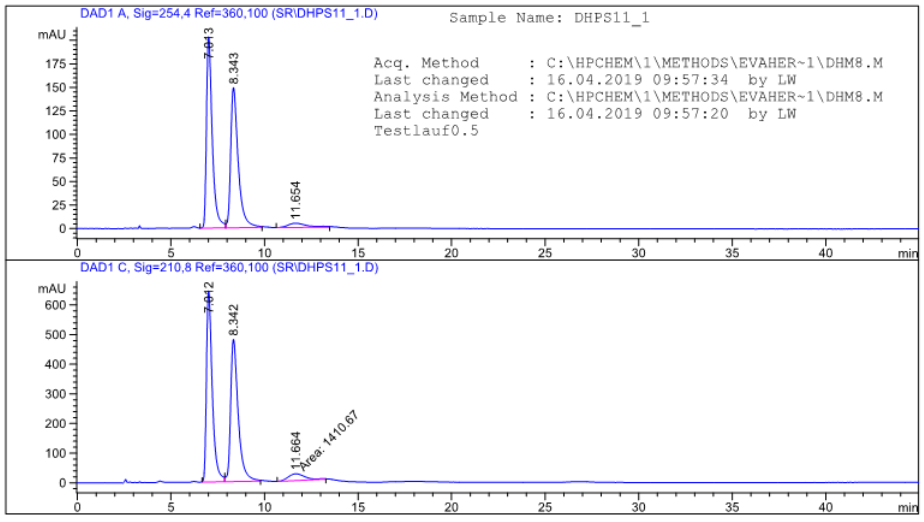
Area Percent Report

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.301	VB	0.1053	23.47791	3.20975	0.2865
2	6.872	VV	0.3233	8170.20459	373.29553	99.7135

Signal 2: DAD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.571	PV	0.1142	106.71989	14.78251	0.4188
2	2.804	VB	0.1624	194.74643	15.95697	0.7642
3	6.872	VB	0.3433	2.51814e4	1099.00415	98.8170



D

Area Percent Report

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.013	VV	0.3226	4362.08398	203.04381	49.2488
2	8.343	VB	0.4146	4163.69580	148.71628	47.0090
3	11.654	PB	0.8678	331.45929	4.54005	3.7422

Signal 2: DAD1 C, Sig=210,8 Ref=360,100

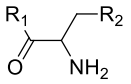
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.012	BV	0.3273	1.39941e4	644.52100	48.3578
2	8.342	VB	0.4214	1.35339e4	479.33627	46.7675
3	11.664	MM	1.0278	1410.67224	22.87512	4.8747

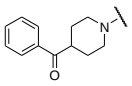
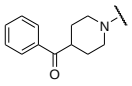
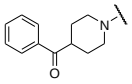
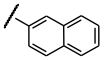
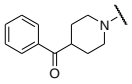
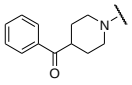
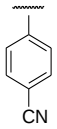
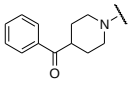
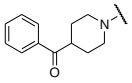
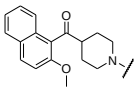
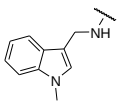
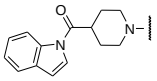
Figure 36. Chiral HPLC data. **8g** (PS11) (A) and **8f** (racemic, PS11-1) (B) in general column HPLC condition; **8g** (PS11) (C) and **8f** (racemic, PS11-1) (D) in chiral column (LiChroCART 250-4 Chiradex (Supelco)) HPLC condition. (gradient of MeOH /H_2O/TFA was used as mobile phase).

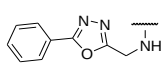
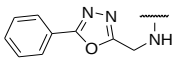
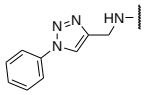
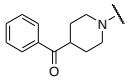
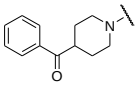
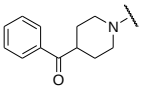
To analyze the enantiomeric purity of the synthesized amino acid derivatives we parallelly synthesized **8g** (*R* configuration, PS11) and its racemic mixture **8f** (*R, S*) using the same aforementioned methods and employed chiral HPLC. From the result we can propose that **8g** is enantiomerically pure enough (> 98.7% purity) and it also highlights the synthetic reliability for retention of the R-configuration to generate only the *R*-configured amino acid-based HDAC inhibitors. HPLC data was provided by the group of Prof. Manfred Jung, The Albert-Ludwig-University of Freiburg, Freiburg, Germany.

2.3 Structure-activity relationship of amino acid-based inhibitors against HDAC8 and their selectivity towards other HDAC subtypes

Table 1. Enzymatic activity of compounds with phenyl(piperidin-4-yl)methanone or rigid linker and R-aryl amino acid ZBG



Cpd No.	R_1	R_2	hHDAC8 inhibition (μM)[a]
8a PS1			3.22 ± 0.56
8b PS2			88.0% @ 10 μM 49.4% @ 1 μM
8c PS3			50.4% @ 10 μM 16.9% @ 1 μM
8d PS4			2.49 ± 0.22
8e PS5			24.8% @ 10 μM 5.4% @ 1 μM
8f PS11-1			44.3 % @ 10 μM 3.1 % @ 1 μM
8g PS11			66.4% @ 10 μM 16.5% @ 1 μM
8h PS6			4.10 ± 0.63
8m PS7			7.3% @ 10 μM 4.5% @ 1 μM
8n PS8			15.5% @ 10 μM
8o PS9			3.15 ± 0.44

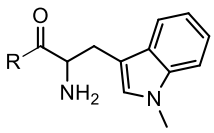
8p PS13			n.d.[b]
8q PS12			n.d.
8r PS21			n.d.
8i PS16			1.07 ± 0.26
8j PS18			0.67 ± 0.082
8k PS10			0.96 ± 0.12

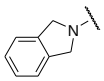
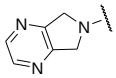
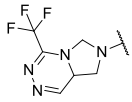
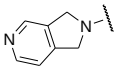
[a]: Inhibition percentage of each compound in corresponding concentration or measured IC_{50} value. [b]: n.d = not determined.

Focused on the inhouse indentified hit **8g** (PS11) as a starting point, firstly we optimized several phenylanine derivatives coupled with phenyl(piperidin-4-yl)methanone to primarily investigate the footpocket (FP) binding group with HDAC8. For our initial set of compounds, we tried to exploit the previously reported interactions between the Gly304, Trp141 residues of the hydrophobic acetate release channel and the substituted phenyl unit of the inhibitors, unfortunately these compounds of **8a-8g** (Table 1) did not exhibit dramatic improvement in activity. Modifications of the linker and cap groups could also not improve the activity; the activity was diminished sharply when rigid linkers were introduced **8m (**PS7), **8p-8r** (PS13, PS12 and PS21), since their rigid moiety steric bulk character are not accommodated in the narrow hydrophobic acetyl-lysine binding tunnel of HDAC8. In addition, *R* isomer of **8g** (PS11) is more active (66.4% @ 10 μM, 16.5% @ 1 μM) than racemic **8f** (PS11-1) (44.3% @ 10 μM 3.1% @ 1 μM), this also confirmed again the finding of Whitehead et al. and Greenwood et al. that *R*-configuration is preferable for binding affinity.[125-126] However, significant improvement was observed when benzothiophene, indole, methylindole rings were employed to replace the phenyl and naphthyl moieties, with IC_{50} values of 1.07 μM,

0.67 µM and 0.96 µM for **8i-8k** (PS16, PS18 and PS10) respectively. This improvement can be attributed to the π-π stacking interactions of the benzoheterocycles rings features with Trp141 in the FP binding pocket. The activity improvement of **8k** (PS10) with an IC_{50} of 0.96 µM to **8j** (PS18) with an IC_{50} of 0.67 µM could also be interpreted that the methyl substitution of methylindole moiety in **8j** (PS18) is more adaptable the hydrophobic pocket of FP pocket than **8k** (PS10) (Table 1).

Table 2. Enzymatic activity of compounds with isoindoline analogous linker and 1-methyl-D-tryptophan ZBG



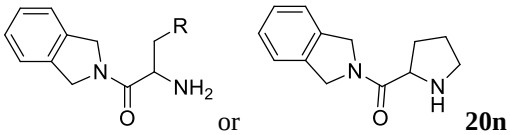
Cpd No.	R	hHDAC8 inhibition (µM)[a]
20a PS19		0.28 ± 0.61
20b PS31		1.56 ± 0.36
20c PS32		49.5% @ 10 µM 7.2% @ 1 µM
20d PS39		n.d[b].
20e PS40		4.4 ± 0.68

[a]: Inhibition percentage of each compound in corresponding concentration or calculated IC_{50} value. [b]: n.d = not determined.

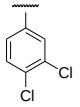
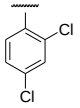
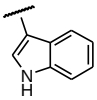
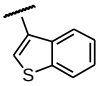
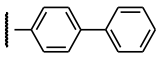
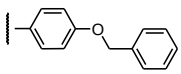
When we changed the optimized isoindoline linker to other heterobicylic linkers and attached them to above optimized 1-methyl-D-tryptophan FP binding group, compounds **20a**-**20e** (Table 2) were obtained. The isoindoline linker was found to be optimal as shown by the high potency of**20a** (PS19) (IC_{50} = 0.28 µM). Comparing **20a** (PS19) with **20b** (PS31), probably planarity, rigidity and ring size are responsible for the remarkable activity loss observed for **20b** (PS31) (IC_{50} = 1.56 µM). Among all the

aromatic rings in **20a** (PS19), **20c** (PS32) and **20e** (PS40), benzene is the most favorable moiety to form pi-pi stacking interactions with Phe152 and Phe208 residues in the narrow substrate binding pocket of HDAC8 which can explain the activity alterations from **20a** (PS19) (IC_{50} = 0.28 μM) to **20c** (PS32) (7.2% @1 μM) and **20e** (PS40) (IC_{50} = 4.4 μM). Meanwhile the previously described piperidin-4-yl linker can solely interact with Phe208 giving activity decreased from **20a** (PS19) (IC_{50} = 0.28 μM) (Table 2) to **8j** (PS18) (IC_{50} = 0.67 μM) (Table 1).

Table 3. Enzymatic activity of compounds with isoindoline linker and D-amino acid derivatives



20n

Cpd No.	R	hHDAC8 inhibition (μM)[a]
20f PS14		0.64 ± 0.17
20g PS22		74.3% @ 10 μM 28.0% @ 1 μM
20h PS23		0.39 ± 0.074
20i PS20		1.07 ± 0.26
20j PS17		0.66 ± 0.13
20k PS34		n.t.[b]
20l PS35		9.5 ± 2.3
20m PS36		n.d.
20n PS33	Shown in table header	n.d.

[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value. [b]: n.t = not tested.

Larger FP binding group like naphthalene **20f (**PS14) and biphenyl **20l** (PS35), more flexible moieties **20m** (PS36) and smaller moieties **20k** (PS34) as well as more rigid group **20n** (PS33) turned out to be active but with less stronger inhibition (Table 3). In conclusion, our optimized methylindole ring moiety as FP targeting group in compound **20a** (PS19) is preferred for HDAC8 binding affinity compared with the previous reported most promising 2,4-chlorophenyl moiety in compound **20h** (PS23).

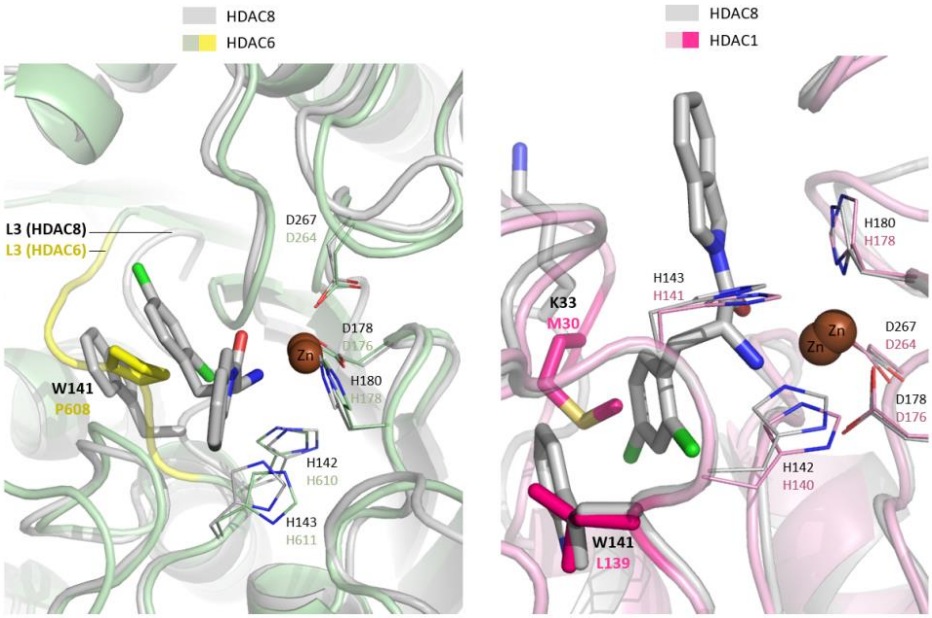
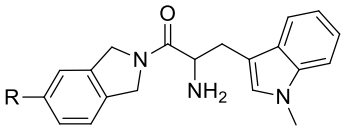


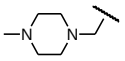
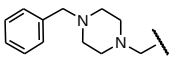
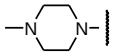
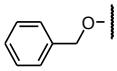
Figure 37. Superimposition of HDAC8 (PDB ID 3SFH) with HDAC6 (PDB ID 5EDU) and HDAC1 (PDB ID 5ICN). HDAC6 is colored in green (differences are highlighted in yellow), HDAC1 is magenta, HDAC8 is in grey. (Docking data provided by Lucas Praetorius).

8g (PS11), **8a** (PS1), **8d** (PS4), **8j** (PS18), **20a** (PS19) were found to be selective for HDAC8 over HDAC1 and HDAC6 (Table 6). This selectivity mainly results from the differences of amino acid residues in loops 1, 2, 3 and 7. Trp141 in HDAC8 is replaced by proline in loop3 of HDAC6 leading to the foot pocket being inaccessible to accomodate large moieties like aromatic rings (Figure 37, left). However, Trp141 replaced by leucine as well as Lys33 by the more protuberant methionine in HDAC1 which not only change the cavity shape and physicochemical features of the foot pocket but also reduce the pocket volume compared with HDAC8 (Figure 37, right). Another factor responsible for selectivity profile improvement for HDAC8 is probably oning to the incorporation of the isoindoline linker moiety since cyclic bulky

linkers are generally more preferable for selectivity toward HDAC6 and HDAC8 than slim cinnamyl and alkyl linkers.[83]

Table 4. Enzymatic activity of compounds consisted of isoindoline linker, cap group and 1-methyl-D-tryptophan ZBG



Cpd No.	R	hHDAC8 inhibition (μM)[a]
32a PS24		0.461 ± 0.056
32b PS25		0.364 ± 0.037
32c PS26		0.662 ± 0.085
32d PS27		0.158 ± 0.027
32e PS28		0.336 ± 0.046
32f PS29		0.358 ± 0.034
32g PS30		16.0 ± 2.0

[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value.

As mentioned in the previous part, another strategy for HDAC8 inhibitors selectivity and potency improvement is the modification of the cap group to regulate the interaction between the inhibitor and the rim of the substrate binding pocket of HDAC8. However, it is promiscuous and difficult to rationally design and predict the modifications. In the subsequently a series of compounds **32a-32g** (PS24-PS30) (Table 4) was synthesized that incorporate methylindole as FP group and isoindoline linker group with a series of different 5-substituted moieties. Only position-5 of the isoindoline moiety was considered since inhibitors with relatively linear and planar ring features in the substrate binding pocket are preferable but docking results showed that 4-substituted compounds do not fit well into the pocket. The inhibitory activities were not improved in compounds **32a** (PS24) and **32b** (PS25) even though a salt

bridge could be observed with Asp101 with the proximal protonated nitrogen of the piperazine moiety.

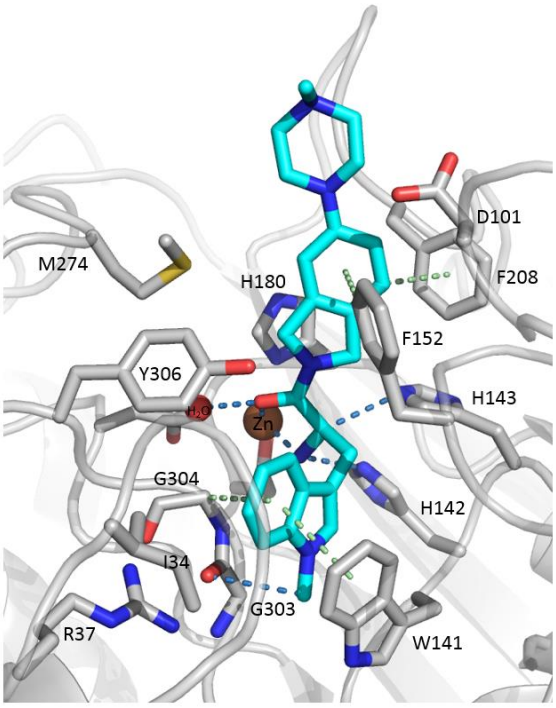
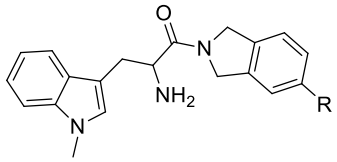
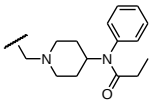
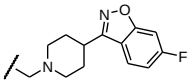
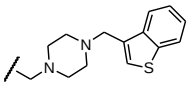
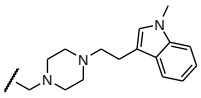
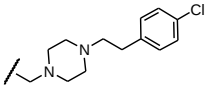
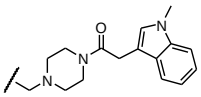
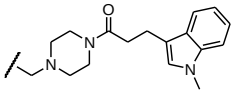
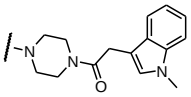
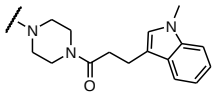
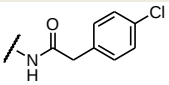
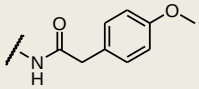
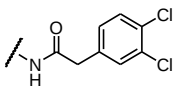


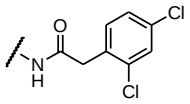
Figure 38. Docking pose of **32d** (colored cyan) in the HDAC8 binding pocket (PDB ID 3SFH). Blue dashed lines are hydrogen bonds/metal coordination, green lines are π-π stacking interaction. (Docking data provided by Lucas Praetorius).

Whereas, for **32d** (PS27) both the molecular docking pose (Figure 38) and molecular dynamics (MD) simulations postulated that the rigidly attached methylpiperazine stretched out of the substrate binding pocket where the positively charged nitrogen can interact with surrounding water molecules resulting in the only inhibitory activity improvement to an IC_{50} of 0.158 µM among these series of compounds (Table 4 and Figure 38). Moreover, based on the other compounds' inhibitory activities (**32c-32g**/PS26-PS30) it can be deduced that no direct interactions were formed between these cap groups and amino acid residues of the enzyme surface, that is why **32d** (PS27) with the most hydrophilic character displayed the best activity.

Table 5. Enzymatic activity of compounds with isoindoline linker, relatively larger cap groups and 1-methyl-D-tryptophan ZBG



Cpd No.	R	hHDAC8 inhibition (μM)[a]
52a PS44		86.7% @ 1 μM 29.2% @ 0.1 μM
52b PS48		0.140 ± 0.07
52c PS42		80.7% @ 1 μM 21.8% @ 0.1 μM
52d PS43		0.200 ± 0.020
52e PS51		0.096 ± 0.008
52f PS50		0.090 ± 0.007
52g PS56		0.095 ± 0.008
52h PS52		0.074 ± 0.008
52i PS53		0.086 ± 0.007
52j PS57		0.066 ± 0.005
52k PS54		0.072 ± 0.04
52l PS58		0.051 ± 0.004 μM
52m PS59		0.144 ± 0.014 μM

52n PS60		85.1% @ 1 μM 22.4% @ 0.1 μM

[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value.

Compound **52b** (PS48) (Table 5) showed a similar activity (IC_{50} = 0.140 μM) compared to **32d** (PS27) (IC_{50} = 0.158 μM). However, for the 5-amido based cap groups, especially for **52j**-**52l** (PS57, PS54, PS58) (Table 5), hydrogen bond interactions between the amide-*NH* at position-5 of isoindoline and Asp101 was postulated by docking studies to be responsible for the activity improvement. A similar finding was demonstrated for the reported **AA3**. Additionally, interactions of these series compounds with HDAC8 amino acid residues of the rim region might be responsible for an activity improvement of **52l** (PS58) (IC_{50} = 0.051 μM) and **52j** (PS57) (IC_{50} = 0.066 μM). In conclusion, the strategies of *R*-amino acid unit applied as ZBG, insoindoline as linker which is substituted at position-5 via an amide group yielded compound **52l** (PS58) with the most potent inhibitory activity which is about 7 folds more active than the reported compound **AA2** and about 30 fold more active than the initial in virtual screening hit **AA4** (Part 1, 1.7).

Table 6. Selectivity profiles of representative compounds

Cpd No.	hHDAC inhibition (μM)[a]					
	1	2	3	8	6	11
8a PS1	25.7% @ 25 μM 11.0% @ 10 μM	n.t.[b]	n.t.	3.22 ± 0.56	6.2% @ 25 μM	n.t.
8d PS4	40.5% @ 25 μM 14.9% @ 10 μM	n.t.	n.t.	2.49 ± 0.22	6.1% @ 25 μM	n.t.
8j PS18	46.4% @ 25 μM 25.1% @ 10 μM	n.t.	n.t.	0.67 ± 0.082	63.5% @ 25 μM 44.7% @ 10 μM	n.t.
20a PS19	54.6% @ 25 μM 37.8% @ 10 μM	n.t.	n.t.	0.28 ± 0.61	24.7% @ 25 μM 14.0% @ 10 μM	35.8% @ 10 μM
20f PS14	69.9% @ 25 μM 49.2% @ 10 μM	n.t.	n.t.	0.643 ± 0.167	34.7% @ 25 μM 14.9% @ 10 μM	n.t.

20h PS23	***n.t.***	***n.t.***	***n.t.***	***0.39 ± 0.074***	***n.t.***	***5.3% @ 10 μM***
32b PS25	n.t.	n.t.	n.t.	0.364 ± 0.037	n.t.	20% @ 10 μM
32d PS27	28.4% @ 10 μM 9.4% @ 1 μM	30.7% @ 10 μM 10.1% @ 1 μM	10.6% @ 10 μM 3.3% @ 1 μM	0.158 ± 0.027	n.t.	40.5% @ 10 μM
32f PS29	n.t.	n.t.	n.t.	0.358 ± 0.034	n.t.	n.d
32g PS30	n.t.	n.t.	n.t.	16.0 ± 2.0	n.t.	10.4% @ 10 μM
52d PS43	8.0% @ 10 μM	3.9% @ 10 μM	19.4% @ 10 μM	0.200 ± 0.02	n.t.	84.7% @ 10 μM 16.8% @ 1 μM
52e PS51	n.t.	n.t.	n.t.	0.096 ± 0.008	n.t.	70.8% @ 10 μM 9.8% @ 1 μM
52f PS50	n.t.	n.t.	n.t.	0.090 ± 0.007	n.t.	17.2% @ 10 μM
52g PS56	12.7% @ 10 μM	6.0% @ 10 μM	15.9% @ 10 μM	0.095 ± 0.008	n.t.	29.4% @ 10 μM 12.8% @ 1 μM
52h PS52	n.t.	n.t.	n.t.	0.074 ± 0.008	n.t.	52.1% @ 10 μM 47.9% @ 1 μM
52i PS53	n.t.	n.t.	n.t.	0.086 ± 0.007	n.t.	48.8% @ 10 μM 17.4% @ 1 μM
52j PS57	28.8% @ 10 μM 7.6% @ 1 μM	11.1% @ 10 μM 1.1% @ 1 μM	17.1% @ 10 μM 5.6% @ 1 μM	0.066 ± 0.005	9．2% @ 10 μM 1．6 % @ 1 μM	n.d.
52k PS54	24.7% @ 10 μM 4.0% @ 1 μM	20.7% @ 10 μM 1.6% @ 1 μM	31.3% @ 10 μM 5.0% @ 1 μM	0.072 ± 0.04	2．9% @ 10 μM 2．8% @ 1 μM	34.8% @ 10 μM 9.8% @ 1 μM
52l PS58	27.0% @ 10 μM 7.1% @ 1 μM	24.6% @ 10 μM 2.8% @ 1 μM	31.7% @ 10 μM 7.3% @ 1 μM	0.051 ± 0.004	n.t.	23.7% @ 10 μM 6.6% @ 1 μM
52m PS59	32.6% @ 10 μM 5.6% @ 1 μM	26.2% @ 10 μM	36.3% @ 10 μM 5.4% @ 1 μM	0.144 ± 0.014	n.t.	34.3% @ 10 μM 4.3% @ 1 μM
PCI-34051	28.3 ± 2.0	n.t.	n.t.	0.092 ± 0.015	48.2 ± 6.2	n.t.

[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value, [b]: n.t = not tested.

All confirmed HDAC subtypes selectivity profiles of amino acid-derived HDAC8 selective inhibitors are listed in Table 6 from which we can find that most of these inhibitors have comparable HDAC8 inhibition with PCI-34051 and excellent selectivity towards HDAC8 over other HDAC subtypes.

2.4 Cytotoxicity studies against healthy human HEK293 cells

In order to determine the cytotoxicity of our compounds against healthy human cells, human embryonic kidney cell line (HEK293) was used and its viability was evaluated using the Alamar Blue assay. HEK293 cells were incubated with the corresponding compounds at a concentration of 50 µM for 45 h and then compared with standard samples. All the results are displayed below (Table 7). Unfortunately, several of the developed HDAC8 inhibitors have strong cytotoxicity in HEK293 cell line especially those bearing larger cap group e.g. **52a-52i** and **52k-52n**. This structural feature may be attributed to certain unknown off-target effects. Further studies and synthesis of modified analogs are necessary to clearly understand this observation. The cytotoxicity data was provided by Dr. Frank Erdmann, Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Halle, Germany.

Table 7. Cytotoxicity Studies in HEK293 Cells

compounds	Viability (%)[a]	**compounds**	Viability (%)
8a	73.1 ± 4.3	**20j**	60.0 ± 5.3
8b	58.1 ± 3.5	**20k**	89.6 ± 5.8
8c	67.4 ± 2.8	**20l**	21.9 ± 1.5
8d	68.8± 4.3	**20m**	74.2 ± 3.4
8e	69.6 ± 3.5	**32a**	90.3 ± 4.2
8f	83.2 ± 6.0	**32b**	44.7 ± 1.5
8g	76.4 ± 6.6	**32c**	34.0 ± 3.3
8h	1.2 ± 0.1	**32d**	75.6 ± 1.5
8i	59.1 ± 1.8	**32e**	88.9 ± 5.3
8j	41.6 ± 1.7	**32f**	41.2 ± 0.7
8k	67.3 + 3.5	**32g**	25.8 ± 2.9
8m	51.3 ± 4.0	**52a**	13.7 ± 2.2
8n	31.7 ± 2.3	**52b**	0.92 ± 0.15
8o	2.6 ± 1.4	**52c**	0.68 ± 0.3
8p	69.2± 3.7	**52d**	0.54 ± 0.3
8q	89.3 ± 14.3	**52e**	0.58 ± 0.09
8r	88.8 ± 9.1	**52f**	n.t.
20a	73.8 ± 2.0	**52g**	0.95 ± 0.09
20b	n.t.[b]	**52h**	0.92 ± 0.15
20c	102.7 ± 2.2	**52i**	0.52 ± 0.05

20d	53.2 ± 0.7	**52j**	79.4 ± 5.5
20e	104.9 ± 1.8	**52k**	0.77 ± 0.05
20f	71.8 ± 8.4	**52l**	18.1 ± 2.4
20g	80.8 ± 4.1	**52m**	0.71 ± 0.07
20h (PS23)	71.8 ± 2.1	**52n**	0.78 ± 0.07
20i	73.5 ± 5.0	**Daunorubicin**	IC_{50} 12.55 ± 0.07 μM

[a]: Inhibition percentage of each compound in 50 μM for 45 h against HEK 293 cell line, [b]: n.t = not tested. Yellow marked compounds show significant toxicity.

2.5 Biological activity assessment for chosen amino acid-derived HDAC8 inhibitors

HDAC8 is a promising target for neuroblastoma treatment as described in part 1 (Part 1, 1.5), therefore we assessed several of the first discovered inhibitors **20a** (PS19) (HDAC8 IC_{50} = 0.28 μM), **32b** (PS25) (IC_{50} = 0.364 μM) and **32d** (PS27) (IC_{50} = 0.158 μM) in the BE(2)-C cell line for an antiproliferative activity study. All three inhibitors showed no pronounced cytotoxity in HEK293 cells, The best compound **32b** (PS25) demonstrated a dose dependent antiproliferative activity against BE(2)-C cells with an EC_{50} of 3.63 μM and was more potent than the reference HDAC8-selective inhibitor PCI-34051 (EC_{50} = 6.26 μM). In contrast, the other two compounds **20a** (PSP19) and **32d** (PS27) showed no pronounced antiproliferative activity in this cell line (Figure 39), which might due to cell permeability problems. The BE(2)-C cell line data was measured by Dr. Ina Oehme at the German Cancer Research Center, Heidelberg, Germany.

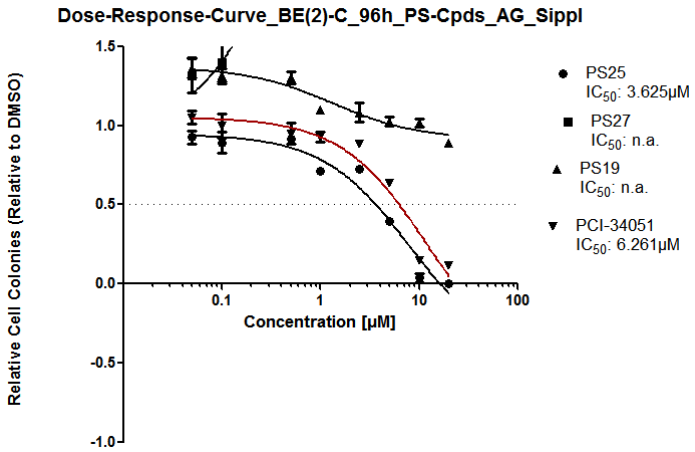


Figure 39. Dose response curve of BE(2)-C cell line treated with **20a** (PS19), **32b** (PS25), **32d** (PS27) and PCI-34051 for 96 h, DMSO was used as vehicle, PCI-34051 was used as reference.

It is reported that HDAC8 selective inhibitors can induce apoptosis in Jurkat cell line, a type of T lymphocytes cell, based on PLCγ1 (phospholipase C-γ1) activation and

calcium-induced mechanism that can be still benefical for T-cell receptor signaling defective cells[157], but is not based on the hyperacetylation of HDAC and tubulin. To evaluate the potency and HDAC substrates specificity, western blot experiments were performed as show in Figure 40. The Jurkat cell line data was provided by the group of Prof. Alfred SL Cheng, from The Chinese University of Hong Kong, Hong Kong SAR, China.

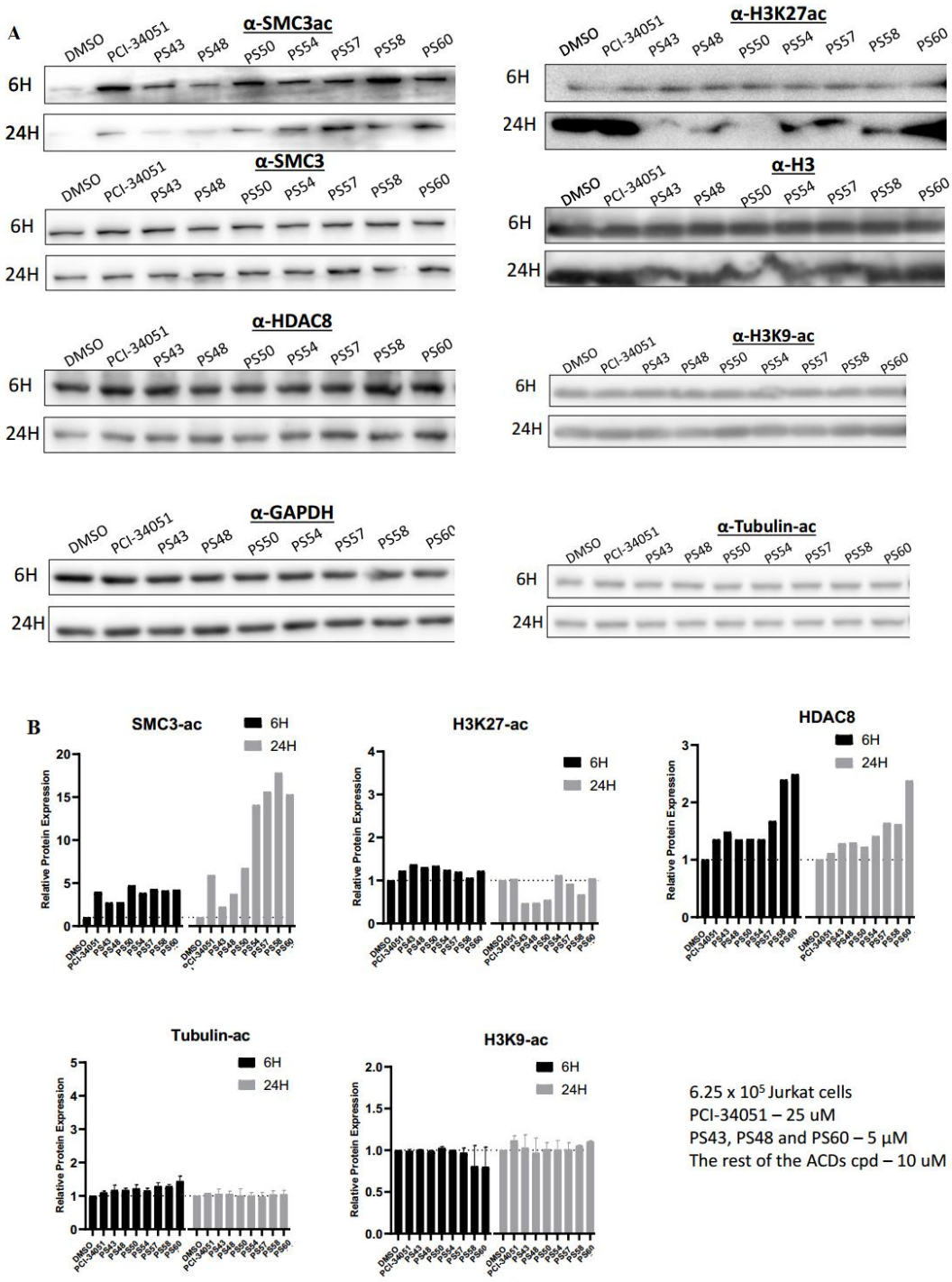


Figure 40. A: western blot of HDAC substrates acetylation and related protein level in 6.25×10^5 Jurkat cells treated with selected leads **52d** (PS43), **52b** (PS48), **52n** (PS60) at 5 µM respectively, **52f** (PS50), **52k** (PS54), **52j** (PS57), **52l** (PS58) at 10 µM respectively for 6 h and 24 h respectively. H3, tubulin, SMC3 and GAPDH were used as loading control, DMSO was used as vehicle, PCI-34051 was used as a reference. B: percentage of SMC3ac, H3K27ac, HDAC8, tubulin-ac and H3K9ac under the treatment of selected inhibitors for 6 h and 24 h repectively.

Interestingly, the chosen compounds **52f** (PS50), **52k** (PS54), **52j** (PS57), **52l** (PS58) and **52n** (PS60) can significantly and selectively upregulate the acetalytion level of

SMC3 (HDAC8 substrate) in Jurkat cells and were found to be more potent than the reference compound PCI-34051 (Figure 40). The detected SMC3 hyperacetylation is another possible SMC associated antiproliferative mechanism of action that differs with the PLCγ1 activation and calcium-induced mechanism. Moreover, of all related proteins, neither acetylation level of H3K9, H3K27 (classical class I HDAC common deacetylation targets) nor tubulin (HDAC6 substrate) acetylation were remarkably affected by these amino acid derivatives in Jurkat cells. An HDAC8 up-regulation effect was observed by **52l** (PS58) and **52n** (PS60). These obtained results confirm that the developed compounds are true HDAC8 selective inhibitors as shown in the enzymatic assay (Table 6).

2.6 Discussion and conclusion of this part

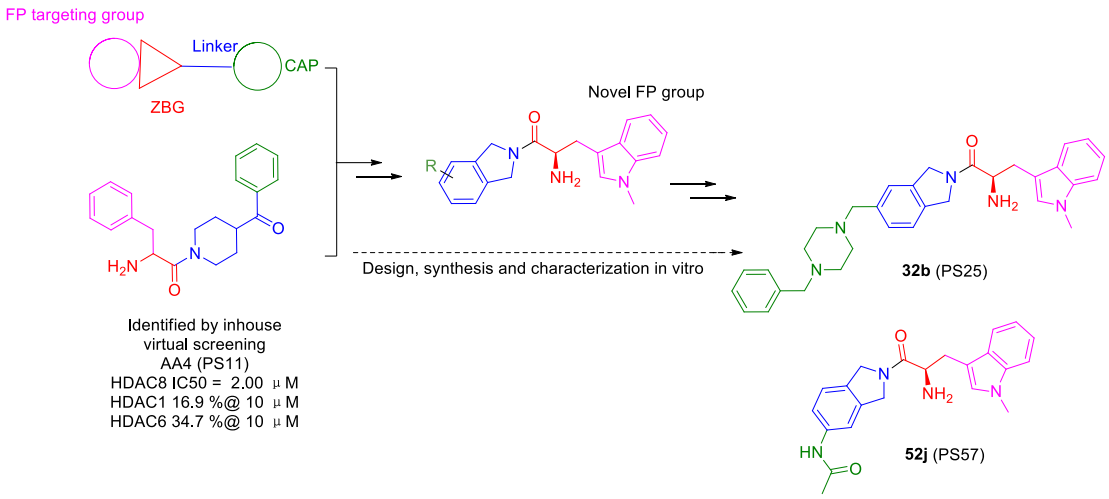


Figure 41. Structures of **32b** (PS25), **52j** (PS57) and their brief discovery process

Table 8. The summary of biological data for **32b** (PS25), **52j** (PS57)

Cpd. No.	hHDAC inhibition (μM)[a]						HEK 293 Viability[b] (%)	Function *in vitro*
	1	2	3	6	8	11		
32b PS25	n.t	n.t	n.t	n.t	0.364± 0.037	20% @ 10 μM	44.7	EC_{50} = 3.6 μM in BE(2)-C cell line
52j PS57	28.8% @10 μM 7.6% @ 1 μM	11.1% @ 10 μM 1.1% @ 1 μM	17.1% @ 10 μM 5.6% @ 1 μM	9. 2% @ 10 μM 1. 6 % @ 1 μM	0.066 ± 0.005	n.t[c]	79.4	Selectively up-regulated SMC3ac in jurkat cell line

[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value, [b]: compounds 50 μM for 45 h against HEK 293 cell line. [c]: n.t = not tested.

Starting from an inhouse virtual screening **AA4**/**8g** (PS11) and combining the reported binding mode of amino acid-based HDAC8 inhibitors, we designed and synthesized several series of amino acid derivatives including phenyl(piperidin-4-yl)methanone, heterocyclic and indoline analogous linkers, we also changed ZBG with aryl, alphatic, flexible and rigid moieties, the cap group attached to linker was altered as well. We characterized and tested these synthesized compounds in HDAC1-3, 6, 8 and 11 enzymatic assays to evaluate their potency and selectivity. Several promising

compounds were studied in BE(2)-C and Jurkat cell lines to investigate their antiproliferative activity. In addition target engagement was studied using western blot experiments. Most of the tested compounds could selectively lead to hyperacetylation of the HDAC8 substrate SMC3 in Jurkat cells. HEK293 cell line viability was also performed to analyze the safety of the compounds for healthy human cells. Of the most promising compounds, **32b** (PS25), **52j** (PS57) with a novel FP group stand out as selective and potent HDAC8 inhibitors in cellular condition. To our knowledge, this is the first time to confirm their cellular activity for this type of amino acid derived HDAC8 selective inhibitors. Importantly, these inhibitors can serve as novel useful probes for studying HDAC8 physiological functions. The optimization process and generated leads **32b** (PS25), **52j** (PS57) can be used as starting points for further improved HDAC8 selective inhibitors, which should be optimized to avoid cytotoxicity against healthy cell lines.

Part 3 Alkylated hydrazides: synthesis, characterization and biological activity as HDAC8 and HDAC3 selective inhibitors

3.1 Chemistry

3.1.1 Synthetic methods for boronic acid and hydrazine alkylation

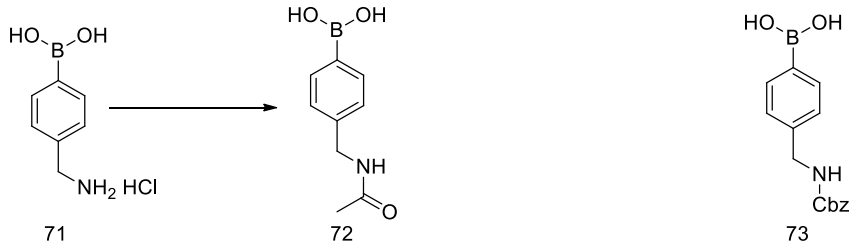


Figure 42. Reagent and conditions: AcCl 2.0 eq, DIPEA 3.5 eq, DCM, rt, 30 min.

In the first step, the key intermediate **72** was generated from the commercially available material **71** by acylation. Compound **73** was also commercially available (Figure 42).

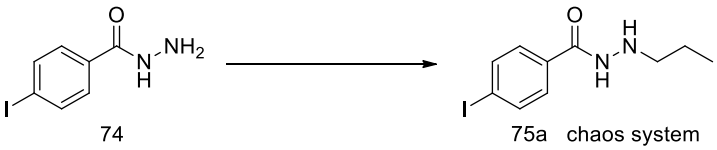


Figure 43. Reagents and conditions: Propionaldehyde 1.5 eq, MeOH/THF and then $NaBH_4$ 1.6 eq, 30 min.

For the important alkylated hydrazide intermediate **75a**, the reported method (Figure 43)[130] failed to explore because it was difficult to separate the target product from reaction mixture. Another disadvantage for this applied one-pot method is that the solvent has to be removed in the beginning of the second procedure. (Figure 43)

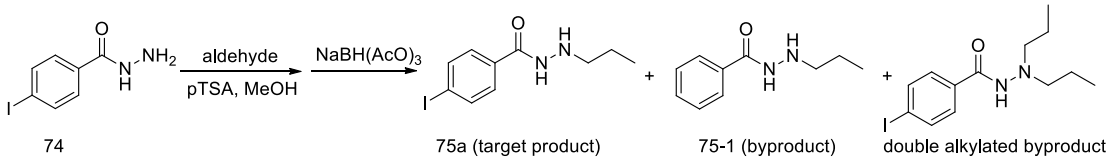


Figure 44. Alkylation of hydrazide

Therefore, we refined the method by decreasing the amount of the used aldehyde to avoid the double alkylated byproduct of **75a**, and used methanol as the sole solvent in the whole process. Additionally $NaBH_4$ was replaced by $NaBH(AcO)_3$ to reduce the intermediate Schiff base to the monoalkylated product **75a**, in oroder to avoid that the reduction of iodo substituent by $NaBH_4$ to generate byproduct **75-1**. Nevertheless, **75-1** was still observed when using $NaBH(AcO)_3$ after a long reaction time (overnight). Hence, the reaction time was decreased to 2 h to decrease the amount of the un-iodinated side product. (Figure 44)

3.1.2 Syntheis of alkylated side chains, biphenyl and phenyl linker-derived compounds

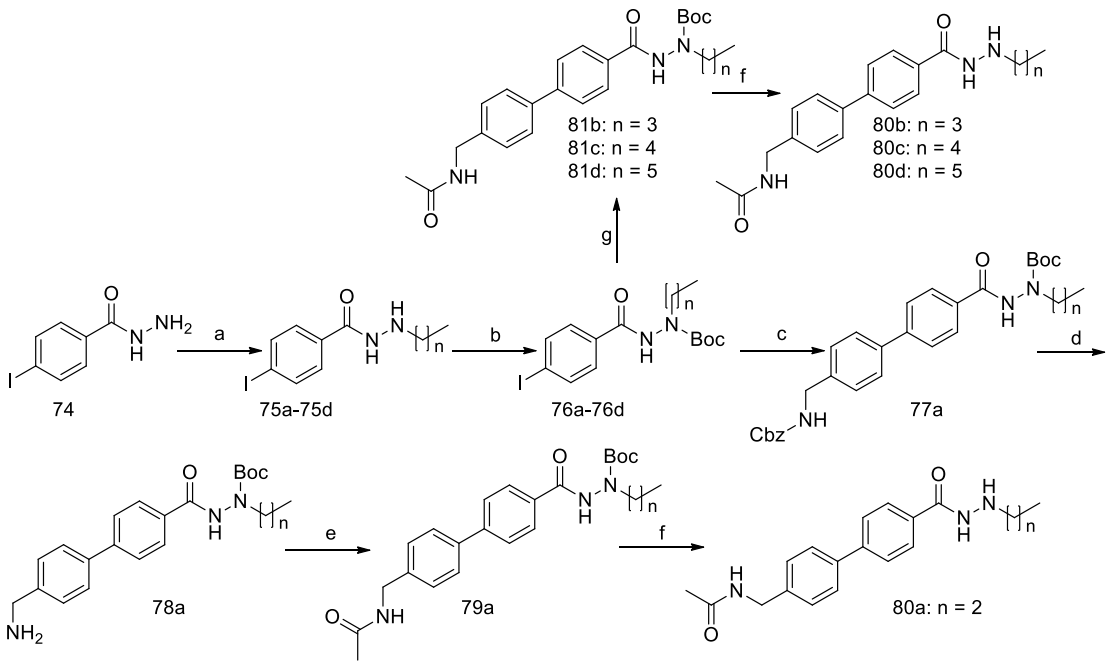


Figure 45. Reagents and conditions: (a) Respective aldehyde 1.05 eq, pTSA 0.05 eq, MeOH, rt, 2 h and then $NaBH(AcO)_3$ 2.0 eq, 1 h, rt; (b) $(Boc)_2O$ 1.1 eq, TEA 2.5 eq, THF, rt; (c) **73** 1.0 eq, $Pd(PPh_3)_4$ 0.04 eq, K_2CO_3 2.2 eq, PhMe/MeOH/H_2O, 90 °C, 7 h; (d) Pd/C, $NaBH_4$ 5.0 eq, MeOH, overnigh; (e) AcCl 1.5 eq, DIPEA 3.0 eq, DCM, rt, 30 min; (f) HCl/dioxane (4M), DCM, rt, 3 h; (g) **72** 1.0 eq, $Pd(PPh_3)_4$ 0.04 eq, K_2CO_3 2.5 eq, PhMe/MeOH/H_2O, 90 °C, 7 h.

In the subsequent procedure, Boc-protected iodophenyl derivatives were coupled with boronic acid by Suzuki coupling and a following Boc-deprotection procedure afforded

the final biphenyl products **80b-80d**. **80a** was obtained using another route involving Cbz-deprotection (Figure 45, step d), acylation (Figure 45, step e) and finally Boc-deprotection (Figure 45, step f).

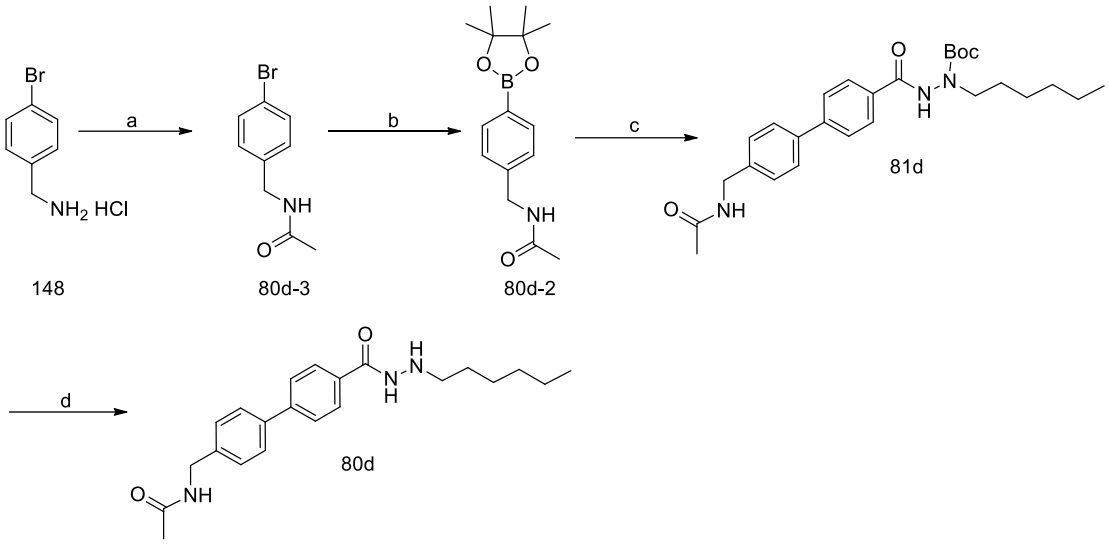


Figure 46. Reagent and conditions: (a) AcCl 1.5 eq, DIPEA 3.5 eq, DCM, rt, 30 min; (b) Bis(pinacolato)diboron 1.0 eq, KOAc 2.0 eq, $Pd(PPh_3)_2Cl_2$, dioxane, 90 °C, 6 h; (c) **76d** 1.0 eq, K_2CO_3 2.2 eq, $Pd(PPh_3)_2Cl_2$ 0.04 eq, dioxane/H_2O (5 : 1), 90 °C, 15 h; (d) HCl/dioxane (4M), DCM, rt, 3 h.

Besides, a large scale and economic method for the synthesis of **80d** was developed as follows: 4-bromobenzylamine (**148**) was acetylated and then transformed to the boronate esters **80d-2**, followed by the same above mentioned procedure (Figure 46).

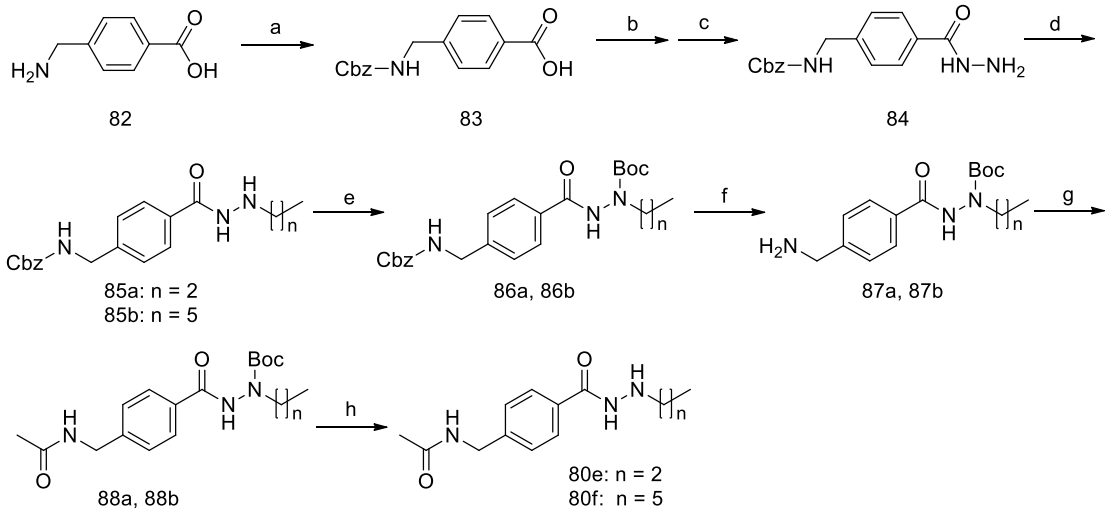


Figure 47. Reagents and conditions: (a) CbzCl 1.1 eq, Na_2CO_3 4.0 eq, H_2O, ice bath to rt, 1.5 h; (b) CDI 1.05 eq, THF, 30 min; (c) Hydrazine monohydate 10.0 eq, THF, rt; (d) Respective aldehyde 1.1 eq,

NaBH(AcO)$_3$ 3.0 eq, DCM, rt, overnight; or aldehyde 1.05 eq, pTSA 0.05 eq, MeOH, 2 h, rt and then $NaBH_4$ 4.0 eq, 1 h; (e) $(Boc)_2O$ 1.2 eq, DIPEA 2.0 eq, THF, rt; (f) Pd/C 20%, $NaBH_4$ 5.0 eq, MeOH, rt, overnight; (g) AcCl 1.5 eq, DIPEA 3.0 eq, DCM, rt, 30 min**;** (h) HCl/sioxane, DCM, rt, 3 h.

Since aminomethylbenzoic acid was utilized as a starting material, it was first protected by basic stable protective groups like Cbz at the free amino position and then reacted with hydrazine, and followed by similar procedure for **80a** (Figure 45) to give the products **80e** and **80f** (Figure 47).

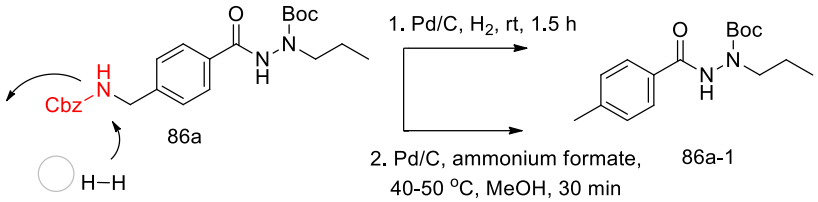


Figure 48: Failed methods for Cbz group deprotection.

But in the Cbz-deprotected step as described above (Figure 48) of the reduction reaction, when Pd/C was used as catalyzed using hydrogen or ammonium formate as hydrogen source can both lead to the removal of the amino group perhaps due to the electron withdrawing effect of the carbonyl group in para-position of **86a.** Finally, the reaction can perform smoothly to give target product (Figure 45, step d and Figure 47, step f) while sodium borohydride was used as hydrogen source.

3.1.3 Synthesis of pyrimidine linker-derived compounds

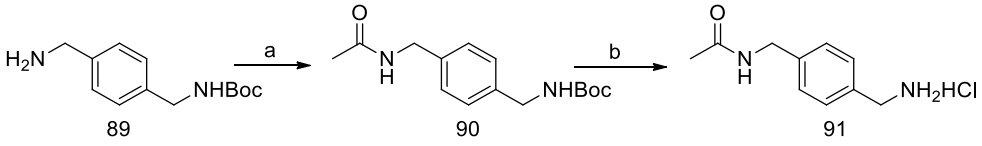
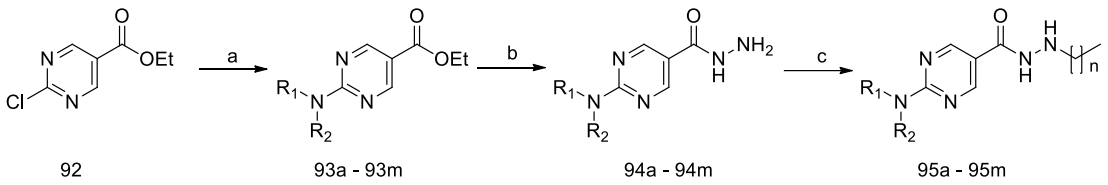


Figure 49. Reagents and conditions: (a) AcCl 1.5 eq, DIPEA 3.0 eq, DCM, 30 min, rt; (b) HCl/dioxane (4M), DCM, rt, 3 h.

In the other series of pyrimidine derivatives (Figure 50), 2-chloropyrimidinearboxylate **92** was taken as starting material, nucleophilic substitution of the chloro-group by the respective amine followed by hydrazine acylation afforded the intermediate **94a-94m** which were finally converted into the

alkylated products **95a-95m**. However, for **95i** and **95m**, their key intermediate **91** should come from starting material **89** (Figure 49).



95a R_1 = Benzyl, R_2 = H, n = 2
95b R_1 = Benzyl, R_2 = H, n = 3
95c R_1 = phenethyl, R_2 = H, n = 2
95d R_1 = 1-naphthalenylmethyl, R_2 = H, n = 2
95e R_1 = 2-chlorobenzyl, R_2 = H, n = 2
95f R_1 = 4-chlorobenzyl, R_2 = H, n = 2
95g R_1 = 2,5-dichlorobenzyl, R_2 = H, n = 2
95h R_1, R_2 = 4-methylpiperazinyl, n = 2
95i R_1 = 4-(acetamidomethyl)benzyl, R_2 = H, n = 2
95j R_1 = 1-naphthalenylmethyl, R_2 = H, n = 5
95k R_1 = phenethyl, R_2 = H, n = 5
95l R_1 = Benzyl, R_2 = H, n = 5
95m R_1 = 4-(acetamidomethyl)benzyl, R_2 = H, n = 5

Figure 50. Reagents and conditions: (a) **91** or R_1R_2NH 1.0 eq, DIPEA 3.5 (for **91**) or 2.5 eq, DCM, rt, 0.5-3 h; (b) Hydrazine monohydrate 30.0 eq, EtOH, 0.5-3 h, reflux or Hydrazine monohydrate 10.0 eq, EtOH, microwave 110 ^oC, 1 h; (c) Respective aldehyde 1.05 eq, pTSA 0.05 eq, MeOH, 2 h, rt and then $NaBH_4$ 4.0 eq, 1 h.

3.1.4 Synthesis of derivatives with indole cap group and pyrimidine linker.

For a further series of compounds focusing on piperazinylpyrimidine derivatives (Figure 52) especially indole derivatives, we first protected one piperazine-*NH* by benzylation and then coupled with pyrimidine. Further steps of acylation, alkylation and Boc-protection afforded the key intermediate **102a** and 1**02b**, which were subjected to benzyl group deprotection to produce **103a** and **103b**. The latter were converted to **104a-104h** by reductive amination or nucleophilic substitution with different indole derivatives like compound **107** (Figure 51) or indolecarbaldehyde.

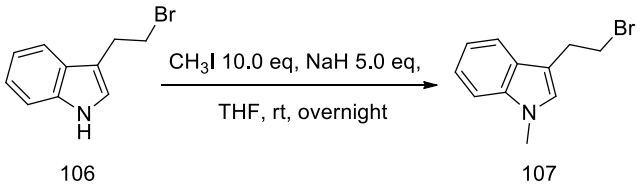


Figure 51: Methylation method for indole.

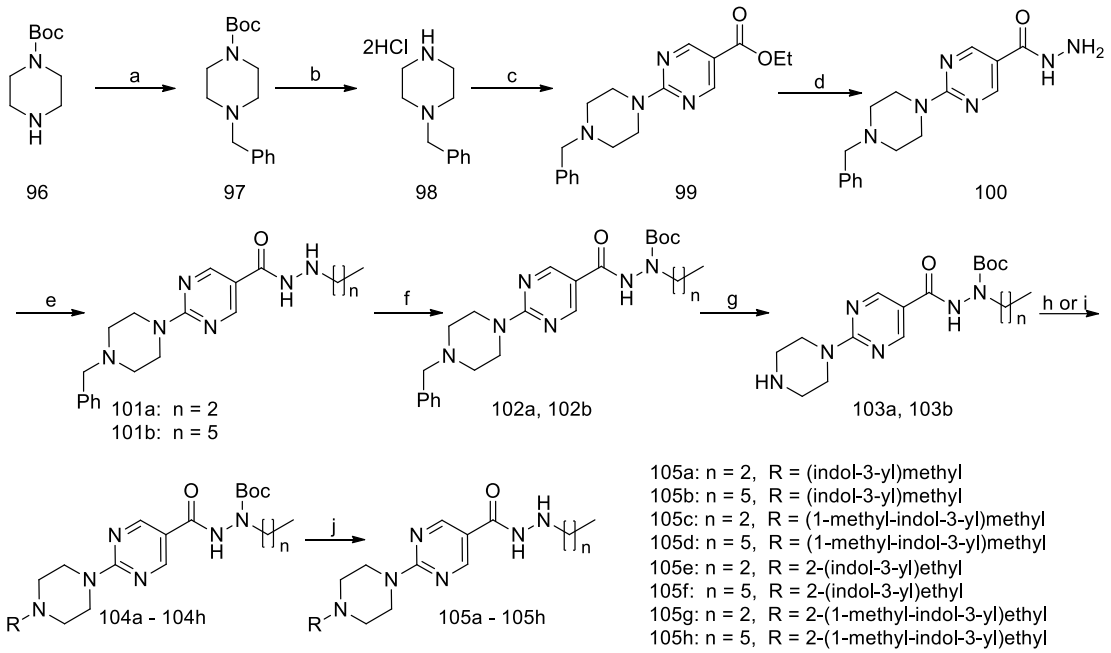


Figure 52. Reagents and conditions: (a) Benzyl chloride 2.0 eq, K_2CO_3 5.8 eq, EtOH, reflux, overnight; (b) HCl/dioxane (4M), DCM, rt, 3 h; (c) **92** 1.0 eq, DIPEA 4.5 eq, DCM, rt, 1 h; (d) Hydrazine monohydrate 30.0 eq, EtOH, reflux; (e) Respective aldehyde 1.05 eq, pTSA 0.05 eq, MeOH, 2 h, rt and then $NaBH_4$ 4.0 eq, 1 h; (f) $(Boc)_2O$ 1.1 eq, TEA 2.5 eq, DCM, rt, overnight; (g) Pd/C 10% wt, ammonium formate 4.0 eq, EtOH, 60 °C, 4 h; (h) Respective aldehyde 1.0 eq, $NaBH(AcO)_3$ 2.0 eq, DCM, rt, overnight; (i) Alkyl bromide 1.2 eq, K_2CO_3 2.5 eq, DMF, 80 °C, overnight; (j) HCl/dioxane (4M), DCM, ice bath, 3 h.

3.1.5 Synthesis of cinnamate derivatives

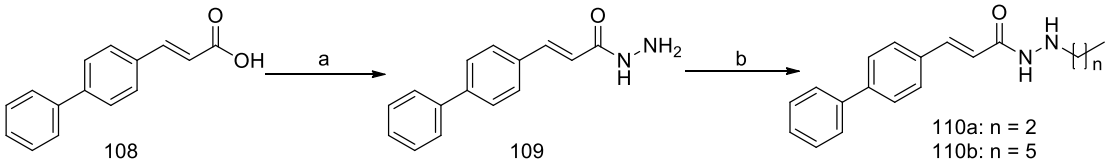


Figure 53. Reagents and conditions: (a) HATU 1.0 eq, Hydrazine monahydrate 2.0 eq, DIPEA 3.0 eq, DMF; (b) Respective aldehyde 1.05 eq, pTSA 0.05 eq, MeOH, 2 h, rt and then $NaBH_4$ 4.0 eq, 1 h, rt.

When we introduced cinnamyl moiety to the linker part of alkylated hydrazides, products **110a** and **110b** were designed and synthesized via the hydrazide derivative **100** which was subsjected to reductive amination with the respective aldehyde (Figure 53).

3.1.6 Synthesis of benzylamine cap group-derived compounds

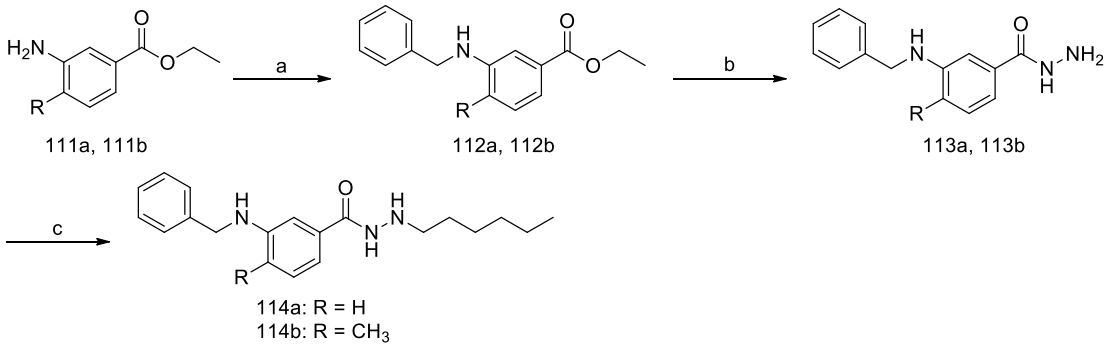


Figure 54. Reagents and conditions: (a) Benzyl chloride 0.91 eq, K_2CO_3 2.27 eq, DMF, 100 °C; (b) Hydrazine monohydrate 10.0 eq, EtOH, microwave, 110 °C, 1 h; (c) Hexanal 1.05 eq, pTSA 0.05 eq, MeOH, 2 h, rt and then $NaBH_4$ 4.0 eq, 1 h.

As described above, both the composition and the position of the cap group attached to the linker can influence the binding affinity and selectivity of the inhibitors. Compounds **114a** and **114b** were synthesized using similar steps as applied above (Figure 54).

3.1.7 Synthesis of benzothiohydrazide-derived compounds

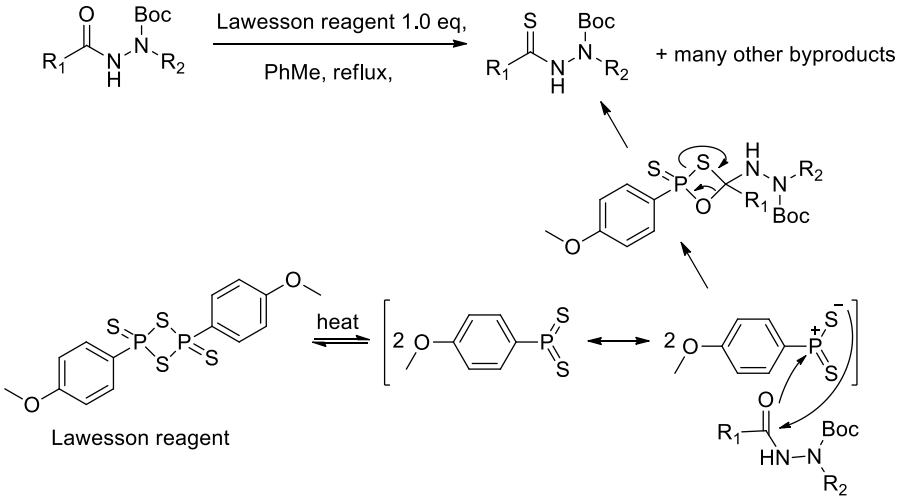


Figure 55: The formation of thioamide starting from amide and its plausible mechanism of action. The P=O is more stable than P=S bond in a cycloreversion step attributed to the converting force of amide into thioamide

We also designed several benzothiohydrazide derivatives instead of benzohydrazide-based compounds to get insights into the ZBG influence on the binding affinity. It was postulated that this group should enhance the coordination with the zinc ion owning to the stronger chelating ability of sulphur atom as compared to oxygen atom. Thereby, Lawessons' reagent was used to convert the carboxylic oxygen into sulphur under a heating condition (Figure 55). In the beginning, we used a microwave assisted procedure at 100 ^{o}C and THF was used as solvent for 1 h with one equivalent of Lawesson's reagent. However, the products were obtained in very low yields which was observed even for intermediates **115**, **121** and **124**. The latter compounds were smoothly transformed to **116**, **122** and **125** using toluene as solvent and reflux within 30 min. When reaction time was prolonged, the corresponding products were degraded.

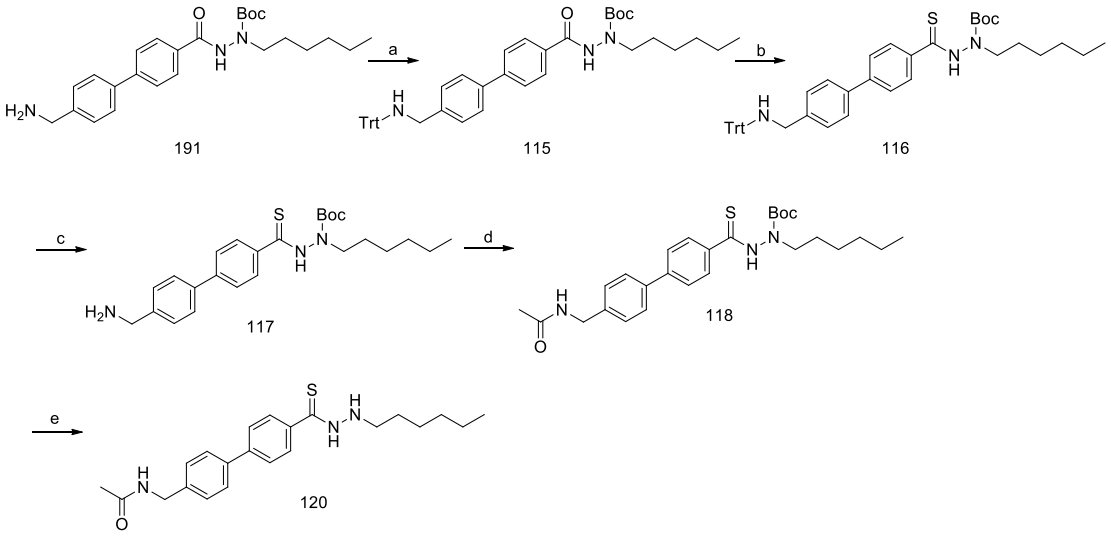


Figure 56. Reagents and conditions: (a) TrtCl 1.05 eq, TEA 2.5 eq, DCM, rt; (b) Lawesson's reagent 0.6 eq, PhMe, reflux, 25 min; (c) AcOH/H_2O (4 : 1), rt, overnight; (d) AcCl 1.0 eq, TEA 3.0 eq, DCM, rt; (e) HCl/dioxane; (4M), DCM, rt, 3 h.

In the synthesis of **120**, intermediate **191** was employed which was obtained using similar procedure like product **78a**. The starting compound was first protected by Trt group and the product was refluxed in a mixture of Lawesson's reagent for 25 min to afford the thio product **116** which was deprotected in AcOH/H_2O. Subsequently, similar steps as for **80a** (Figure 45) were adapted to generat the final product **120** (Figure 56).

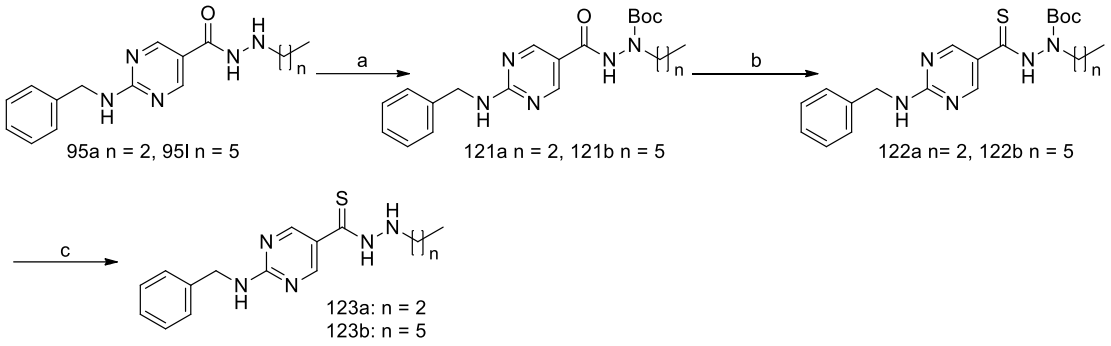


Figure 57. Reagents and conditions: (a) $(Boc)_2O$ 1.2 eq, TEA 2.5 eq, DCM, rt; (b) Lawesson's reagent 0.6 eq, toluene, reflux, 30 min; (c) HCl/dioxane (4M), DCM, rt, 3 h.

Compounds **95a** and **95l** were directly used for the synthesis of **123a** and **123b** (Figure 57) using the similar key step as **120** (Figure 56). Whereas for compound **128** (Figure 58), the staring material **103b** was again protected by Trt group, where similar steps as with **120** afforded the key intermediate **126** which was subjected to a reductive amination step d followed by Boc-deprotection step e giving the final compound **128**.

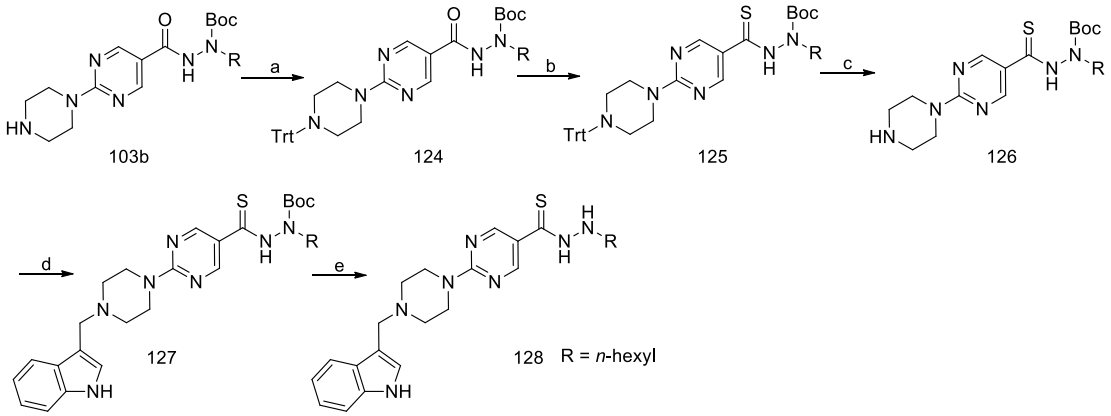
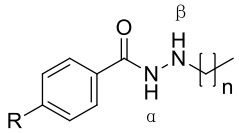


Figure 58. Reagents and conditions:(a) TrtCl 1.05 eq, TEA 2.5 eq, DCM; (b) Lawesson's reagent 0.6 eq, toluene, reflux, 20 min; (c) $AcOH/H_2O$ (4 : 1), rt, overnight; (d) Hexanal 1.0 eq, $NaBH(AcO)_3$ 2.0 eq, DCM, rt, overnight; (e) HCl/dioxane (4M), DCM, ice bath, 3 h.

3.2 Structure-activity relationship of alkylated hydrazides as inhibitors of HDAC8, HDAC3 and other HDAC subtypes

Table 9. Enzymatic activity of compounds containing a phenyl linker, acetamide cap group and alkylated hydrazide moiety of different length



Cpd No.	R	n	hHDAC inhibition (μM)[a]						
			1	2	3	8	4	6	11
80a PSP40		2	1.6 ± 0.1	0.63± 0.03	0.091±0.003	0.7 ± 0.07	0.99	> 20	1.8
80b PSP41		3	0.62 ± 0.05	1.1 ± 0.1	0.35 ± 0.02	n.t.	n.t	n.t	2.3
80c PSP42		4	0.66 ± 0.05	2.0 ± 0.2	1.5 ± 0.1	0.35	> 60	> 20	0.27
80d PSP43		5	1.8 ± 0.3	12 ± 1	> 20	0.036± 0.002	> 60	> 20	0.18
80e PSP39		2	1.6 ± 0.2	5.5 ± 0.4	1.6 ± 0.1	n.t	n.t	n.t	3.6
80f PSP67		5	4.8 ± 0.4	12 ± 1	> 20	0.023±0.001	n.t	> 20	0.34± 0.003
SR-3558	phenyl	3	0.09	0.80	0.06	2.43	n.t.	n.t.	n.t.

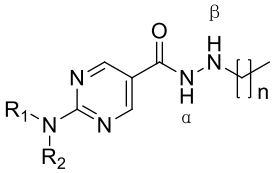
[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value. [b]: n.t = not tested.

We began the first optimization by modification of the β-nitrogen position of the biphenylhydrazine using different lengths of alkyl chains from 3-6 carbons (Table 9). This was carried out because previous studies reported that the chain length can remarkably influence the potency and selectivity of hydrazide-based HDAC inhibitors, particularly 3-carbons were shown the ideal length for HDAC1-3 selectivity and potency. Thus here, our initial compound **80a** (PSP40) displayed potent HDAC3 inhibition with an IC_{50} of 0.091 μM and improved selectivity profiles especially over HDAC1 and HDAC8 (IC_{50} = 1.6 μM and IC_{50} > 20 μM, respectively) compared to the reported referebce compound **SR-3558** with IC_{50} of HDAC1 = 0.09 μM, HDAC2 =

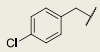
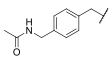
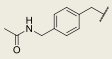
0.8 μM, HDAC3 = 0.06 μM and HDAC8 = 2.43 μM which was consistent with reported findings (Part 1, 1.7.2). Surprisingly, compound **80d** (PSP43) with a *N*-hexyl group showed potent HDAC8 inhibition and selectivity over other tested HDACs. It only possessed moderate inhibition of HDAC11 with an IC_{50} of 0.18 μM. No appreciable activities were observed from 4- and 5-carbons (**80b** (PSP41) and **80c** (PSP42)) for all the tested HDAC isoforms. This might because only 3- and 6-carbons are very suitable to fit the foot pocket size of HDAC3 and HDAC8, respectively. However, **80b** (PSP41) and **80c** (PSP42) still showed significant activity on other HDACs (including HDAC3 and 8).

In addition, we modified the HDAC3 and HDAC8 selective compounds **80a** (PSP40) and **80d** (PSP43) by truncating the phenyl cap group resulting in **80e** (PSP39) and **80f** (PSP67). Only **80f** (PSP67) showed comparable HDAC8 potency with an IC_{50} of 0.023 μM compared with parent **80d** (PSP43 with an IC_{50} of 0.036 μM) whereas the HDAC3 activity of **80e** (PSP39) with a 3-carbon akylated chain diminished. The hydrazide-based selective HDAC8 inhibitors featuring a 6-carbon alkyl group were firstly reported by us in this work (Table 9).

Table 10. Enzymatic activity of compounds containing a pyrimidine linker, aryl cap group and alkylated hydrazide moiety of different length



Cpd No.	R_1	R_2	n	hHDAC inhibition (μM)[a]					
				1	2	3	8	6	11
95a PSP48		H	2	0.25 ± 0.02	0.7 ± 0.03	0.043 ± 0.005	1.0 ± 0.11	> 20	2.0
95b NI-26		H	3	61% @ 1 μM 85% @ 10 μM	46% @ 1 μM 93% @ 10 μM	0.20 ± 0.01	66% @ 1 μM 94% @ 10 μM	n.t.[b]	3.6% @ 1 μM 34.2% @ 10 μM
95c NI-16		H	2	51% @ 1 μM 85% @ 10 μM	39% @ 1 μM 90% @ 10 μM	87% @ 1 μM 99% @ 10 μM	51% @ 1 μM 89% @ 10 μM	n.t.	-1.9% @ 1 μM 18.8% @ 10 μM

				µM	µM	µM	µM		
95d NI-15		H	2	53% @ 1 µM 91% @ 10 µM	46% @ 1 µM 93% @ 10 µM	0.081 ± 0.002	36% @ 1 µM 83% @ 10 µM	n.t.	n.t.
95e NI-23		H	2	71% @ 1 µM 89% @ 10 µM	55% @ 1 µM 92% @ 10 µM	0.060 ± 0.001	43% @ 1 µM 87% @ 10 µM	n.t.	n.t.
95f NI-32		H	2	54% @ 1 µM 92% @ 10 µM	61% @ 1 µM 96% @ 10 µM	0.037 ± 0.001	62% @ 1 µM 88% @ 10 µM	n.t.	7.3% @ 1 µM 56.6% @ 10 µM
95g NI-24		H	2	66% @ 1 µM 88% @ 10 µM	n.t.	0.058 ± 0.002	33% @ 1 µM 83% @ 10 µM	nt	n.t.
95h PSP45		-	2	0.59 ± 0.03	2.4 ± 0.1	0.12 ± 0.01	0.25 ± 0.01	> 20	1.6
95i PSP47		H	2	0.29 ± 0.02	0.92 ± 0.04	0.11 ± 0.01	n.t.	> 20	3.0
95j NI-90		H	5	8.3 ± 1.2	> 20	> 20	0.063 ± 0.004	> 20	0.75 ± 0.09
95k NI-91		H	5	2.2 ± 0.1	12 ± 1	7.2 ± 0.2	0.028 ± 0.002	> 20	1.4 ± 0.1
95l PSP70		H	5	0.56 ± 0.03	3.2 ± 0.2	3.0 ± 0.2	0.016 ± 0.001	> 20	0.41 ± 0.02
95m PSP69		H	5	0.96 ± 0.06	4.5 ± 0.3	8.5 ± 0.4	0.019 ± 0.001	> 20	0.77 ± 0.04

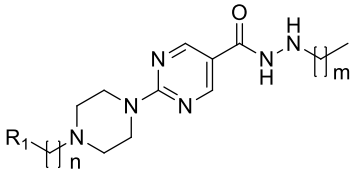
[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value. [b]: n.t = not tested.

In the second optimization step, we replaced the phenyl and biphenyl linkers with pyrimidine and incorporated arylamine and methylpiperizine as cap groups achieving compounds **95a-95m** (Table 10). Derivative **95f** (NI-32) bearing a para-chlorobenzylamine as cap group showed the best improvement in HDAC3 activity improvement (IC_{50} = 0.037 µM). In addition, chains with compounds bearing *n*-propyl group at the β-nitrogen position were superior to *n*-butyl **95b** (NI-26) and *n*-hexyl derivatives **95j-95m** (Table 10). Overall more flexible **95c** (NI-16) (87% @ 1 µM), larger **95d** (NI-15) (IC50 = 0.081 µM), longer **95i** (PSP47) (IC50 = 0.11 µM),

mono and bichloro substituted (**95e-95g,** Table 10) and hydrophilic cap groups showed nanomolar range HDAC3 activity as in **95f** (NI-32) (IC50 = 0.037 μM).

As observed with the previous series, utilizing *n*-hexyl instead of *n*-propyl group **95j-95m** (Table 10) resulted in potent HDAC8 inhibitors with selectivity over other tested HDACs. These above results not only indicated that the pyrimidine scaffold was aslo favored for HDAC3 and HDAC8 inhibition but also confirmed again that 3- and 6-carbon alkylated is determinant for potency and selectivity of the compounds.

Table 11. Enzymatic activity of compounds containing a piperazinylpyrimidine linker, indole cap groups and 3- or 6-carbon length alkylated hydrazide moiety



Cpd No.	R_1	n	m	hHDAC inhibition (μM)[a]					
				1	2	3	6	8	11
105a PSP49		1	2	0.073 ± 0.007	1.1 ± 0.1	0.030 ± 0.001	> 20	0.0082 ± 0.0006	0.570
105b PSP73		1	5	0.17 ± 0.01	0.31 ± 0.01	0.10 ± 0.04	> 20	0.0059 ± 0.0006	0.041
105c PSP50		1	2	0.011 ± 0.002	0.45 ± 0.02	0.037 ± 0.001	n.t.	0.028 ± 0.002	0.093
105d PSP74		1	5	0.17 ± 0.01	0.33 ± 0.02	0.30 ± 0.02	> 20	0.014 ± 0.001	0.027
105e PSP51		2	2	0.081 ± 0.007	1.3 ± 0.1	0.025 ± 0.001	n.t.	0.028 ± 0.002	0.39
105f PSP72		2	5	0.86 ± 0.07	1.40 ± 0.10	1.00 ± 0.10	> 20	0.013 ± 0.001	0.047
105g PSP52		2	2	0.29 ± 0.024	69% @ 1 μM 98%@ 10 μM	0.021 ± 0.001	n.t.	0.022 ± 0.001	0.31
105h PSP71		2	5	0.46 ± 0.03	1.20 ± 0.10	1.40 ± 0.10	> 20	0.023 ± 0.002	0.120

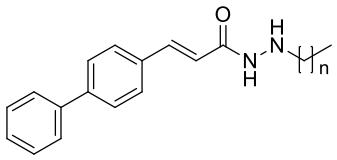
[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value. [b]: n.t = not tested.

In the subsequent optimization, indole and methyl indole were attached to a

piperizinyl pyrimidine linker via one or two carbons yielding compounds of **105a-105h** (Table 11). Almost all compounds exhibited excellent inhibiton of HDAC8 among which the best one was **105b** (PSP73) (IC_{50} = 0.0059 μM against HDAC8) which also exhibited strong HDAC11 inhibition (IC_{50} = 0.041 μM). In addition, all derivatives bearing an *n*-propyl chain linked with the β-nitrogen of the hydrazide (**105a** (PSP49), **105c** (PSP50), **105e** (PSP51) and **105g** (PSP52)) showed excellent inhibition of HDAC3. It seems like that indole and methyl indole with 2-carbon cap group (**105e** (PSP51), **105g** (PSP52)) were superior targeting HDAC3 whereas indole with one carbon cap group of **105a** (PSP49), **105b** (PSP73) displayed some preference for HDAC8.

Moreover, *N*-methylindole connected to the linker via a methylene moiety and an *n*-propylated β-nitrogen of the hydrazide resulted in the best HDA11 inhibitor **105d** (PSP74) (IC_{50} = 0.027 μM for HDAC11). In conclusion, we discovered HDAC8 selective inhibitor **105h** (PSP71), HDAC3/8 dual inhibitor **105e** (PSP51) and **105g** (PSP52) as well as HDAC8/11 dual inhibitor **105b** (PSP73), **105d (**PSP74) and **105f** (PSP72) through this optimization.

Table 12. Enzymatic activity of compounds conatining a phenylethylene linker and 3- or 6-carbon length alkylated hydrazide moiety



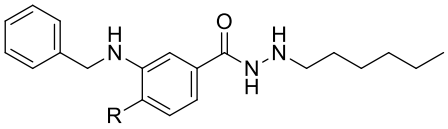
Cpd No.	n	hHDAC inhibition (μM)[a]				
		1	2	3	8	11
110a NI-105	2	4% @ 1 μM 20%@10 μM	2% @ 1 μM 9% @ 10μM	15% @10μM	> 6	n.t.[b]
110b PSP85	5	> 20	> 20	> 20	1.5 ± 0.2	6.6 ± 1.6

[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value. [b]: n.t = not tested.

Inserting one double bond between biphenyl group and the hydrazide ZBG, compounds of **110a** (NI-105) and **110b (**PSP85) resulted in strong loss in activity

against all tested HDAC isoforms (Table 12).

Table 13. Enzymatic activity of compounds containing a benzylamine cap group and n-hexylatedhydrazide moiety

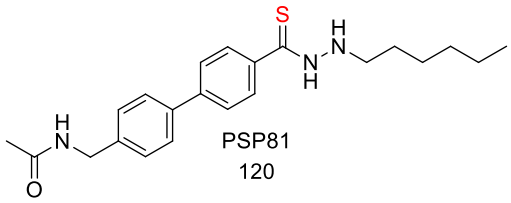


Cpd No.	R	hHDAC inhibition (μM)[a]					
		1	2	3	8	6	11
114a NI-82	H	> 20	> 20	> 20	0.046 ± 0.003	> 20	13 ± 2
114b NI-85	CH_3	35% @10 μM	26% @ 10 μM	42% @ 10 μM	2% @ 1 μM 100% @ 10 μM	n.t.[b]	n.t.

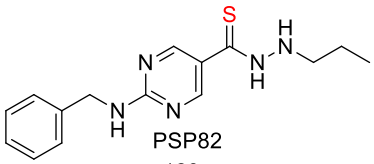
[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value. [b]: n.t = not tested.

Inspired by our previous work,[63] a benzylamine cap group was attached at position-3 of the phenyl linker yielding compound of **114a** (NI-82), which showed potent HDAC8 inhibition (IC_{50} = 0.046 μM). Addionally inserting a methyl group at position-4 of the phenyl linker resulted in a loss of HDAC8 inhibition **114b** (NI-85) (Table 13).

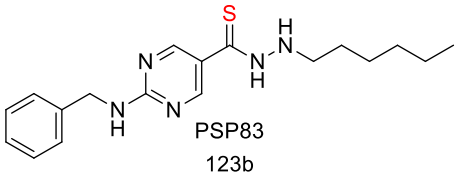
Table 14. Enzymatic activity of compounds containing a thiohydrazide moiety



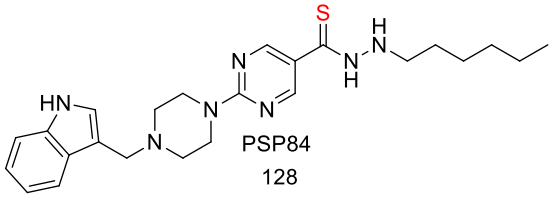
PSP81
120



PSP82
123a



PSP83
123b



PSP84
128

Cpd No.	inhibition (μM)[a]				
	HDAC1	HDAC2	HDAC3	HDAC8	HDAC11
120 PSP81	> 20	> 20	> 20	0.10 ± 0.01	> 20
123a	21.3% @ 1 μM	15.4% @ 1 μM	50.8% @ 1 μM	15.6% @ 1 μM	> 20

PSP82	45.9% @ 10 μM	32.4% @ 1 μM	80.2% @ 10 μM	36.9% @ 10 μM	
123b PSP83	> 20	> 20	> 20	0.093 ± 0.006	> 20
128 PSP84	5.2 ± 0.5	14 ± 1	3.2 ± 0.3	0.025 ± 0.002	1.0 ± 0.1

[a]: Inhibition percentage of each compound in corresponding concentration or calculated IC_{50} value.

The inhibitory potency of benzothiohydrazide derivatives against HDAC3 and HDAC8 dramatically decreased compared with their original inhibitors **120** (**80d**), **123a** (**95a**), **123b** (**95b**), **128** (**105b**), maybe because the atom size has also an important impact for inhibitors to form perfect coordination with the zinc ion. The selectivity of **123b** (PSP83) and **128** (PSP84) towards HDAC8 was interestingly improved at the same time (Table 14).

3.3 Non-enzymatic stability studies

In order to investigate the chemical stablility of selected compounds under cellular assay conditions, a 10 mM solutions of the compounds in DMSO were prepared and then diluted with the mixtures of DMEM and MeOH or ACN to afford the final solutions (**80a** of 10 µM in DMEM/DMSO/MeOH as 50/10/40 percentage; **105b**, **105c**, **110b** and **133** of 50 µM in DMEM/DMSO/ACN as 50/10/40 percentage; **80d**, **105a**, **120**, **123a**, **123b**, **128** of 10µM in DMEM/DMSO/ACN as 50/10/40 percentage). All the resulting solutions were incubated at 37 ºC for 72 h and detected by HPLC to quantify their degradation results at different time points of 0 h, 6 h, 12 h, 24 h, 28 h and 72 h. The percentage of remaining parent compound is given bellow (Table 15). This data was provided by Dr. Matthias Schmidt, Institute of Pharmacy, The Martin-Luther-University Halle-Wittenberg, Halle, Germany.

Table 15. Remaining parent compound at different time points

compounds	0h (%)	6h (%)	12h (%)	24h (%)	48h (%)	72h (%)	Compound types
80a(PSP40)	100.0	98.3	97.3	94.9	90.4	86.8	biphenyl,
80d(PSP43)	100.0	102.7	104.2	105.2	104.0	103.1	
105a(PSP49)	100.0	81.7	83.3	82.9	27.1	31.1	indole derivatives
105b(PSP73)	100.0	92.3	89.1	79.9	62.3	54.4	
105c(PSP50)	100.0	86.5	77.7	63.9	40.8	31.5	
110b(PSP85)	100.0	100.5	100.2	99.8	89.8	90.2	cinnamoyl derivatives
120(PSP81)	100.0	78.8	62.2	41.3	24.8	20.3	thio containing compounds
123a(PSP82)	100.0	70.1	52.3	27.9	6.4	5.1	
123b(PSP83)	100.0	72.9	58.8	39.5	26.2	24.6	
128(PSP84)	100.0	68.0	52.2	32.2	14.0	4.5	

The results indicate that the biphenyl-based inhibitors **80a**, **80d** and **110b** are very stable under the test conditions even after 3 days. The stability of indole-based inhibitors **105a-105c** are slightly lower than the biphenyl derivatives athough about 60% of them still remained after 24 h. However, overall the thio containing hydrazides (**120**, **123a**, **123b** and **128**) seemed to be relatively unstable compared with

the others. Structural analysis of the degradation products has to be carried out in future studies.

3.4 HDAC8 inhibitory mechanism of chosen inhibitors

3.4.1 Mechanism of inhibition of alkylhydrazide based inhibitors

Different concentrations of the HDAC8 substrate (Abz-SRGGK(STFA)FFRR-NH_2 (*S*)) were used to determine the K_i values for the chosen inhibitors **80d** (PSP43) and **105a** (PSP49) against HDAC8. A Lineweaver-Burk plot was generated based on the enzyme kinetics to predict the inhibitory type and K_i of the inhibitors. This data was provided by Matthes Zessin, Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Halle, Germany.

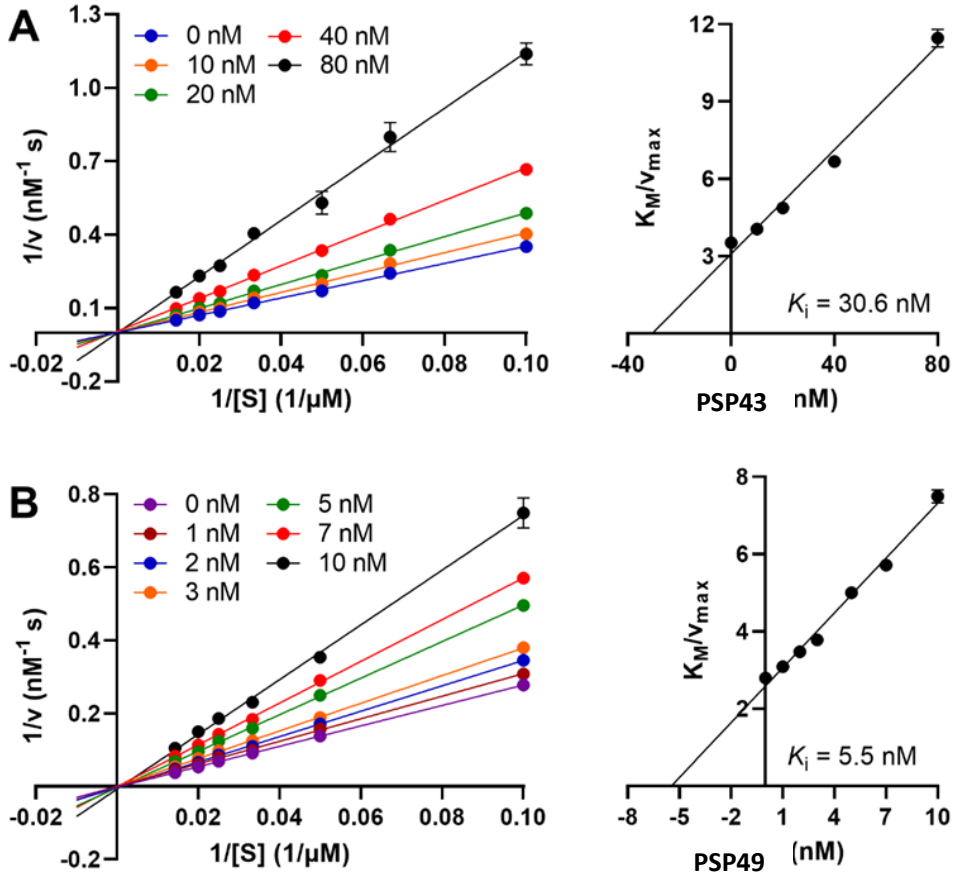


Figure 59. Different Lineweaver-Burk and tertiary plots of enzyme kinetics data for **80d** (PSP43) (A) and **105a** (PSP49) (B).Y-axis units: (pmol of substrate cleaved/min)$^{-1}$, x-axis units: (µmol^{-1}) for HDAC8.

Table 16. Calculated Ki values for **80d** (PSP43) and **105a** (PSP49) by Lineweaver-Burk plots

Compounds	K_i (nM)
80d (PSP43)	30.6
105a (PSP49)	5.5

From these plots (Figure 59) we can conclude that **80d** (PSP43) and **105a** (PSP49) are both substrate-competitive inhibitors of HDAC8 because these graphs have the same y-intercept in Lineweaver-Burk plot under different inhibitor concentrations.

3.4.2 Reversibility testing for chosen inhibitors

Lastly, a dilution experiment was carried out to determine the reversibility of **105b** (PSP73) against HDAC8 (Figure 60). The result indicated a reversible inhibitory character because the enzymatic activity was recovered after dilution with medium. This data was provided by Matthes Zessin, Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Halle, Germany.

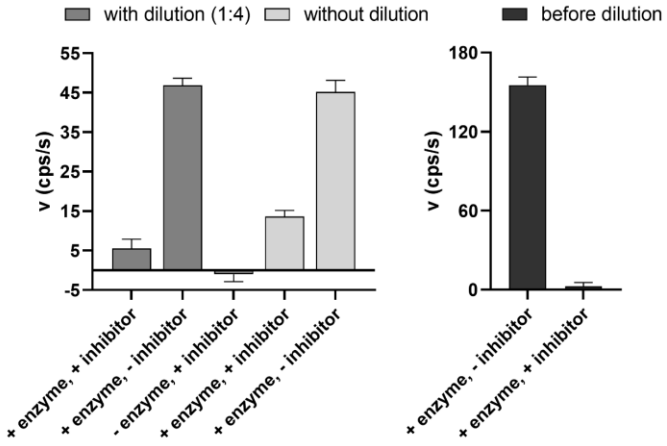


Figure 60. In this experiment, the inhibitor was incubated for 30 min with HDAC8 so that it can completely inhibit HDAC8 and later treated with substrate (Abz-SRGGK(STFA)FFRR-NH_2) and the enzymatic activity was detected. 1 nM HDAC8, 4 nM **105b** and 50 µM of substrate were used in the dilution group; a lower concentration of inhibitor, 1 nM HDAC8 and 50 µM of substrate were employed in the without dilution group whereas the before dilution group referred to 4 nM HDAC8, 16 nM **105b** and 50 µM of substrate were used.

3.5 Cytotoxicity studies against healthy human HEK293 cells

In order to determine the cytotoxicity of our compounds against healthy human cells, human embryonic kidney cell line (HEK293) was used and its viability was evaluated using the Alamar Blue assay. HEK293 cells were incubated with the corresponding compounds at a concentration of 50 µM for 45 h and then compared with standard samples. All the results are displayed below (Table 17). Unfortunately, several of the developed HDAC8 inhibitors have strong or moderate cytotoxicity in HEK293 cell line especially those bearing indole derived cap group e.g. **105a-105h** and **128**. This structural feature may be attributed to certain unknown off-target effects. Further studies and synthesis of modified analogs are necessary to clearly understand this observation. The cytotoxicity data was provided by Dr. Frank Erdmann, Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Halle, Germany.

Table 17. Cytotoxicity Studies in HEK293 Cells

compounds	Viability (%)[a]	**compounds**	Viability (%)
80a	83.89 ± 0.96	**95m**	79.44 ± 3.8
80b	80.11 ± 3.7	**105a**	47.01 ± 4.4
80c	90.83 ± 2.1	**105b**	2.18 ± 0.20
80d	121.35 ± 4.3	**105c**	45.10 ± 7.6
80e	81.18 ± 4.6	**105d**	1.64 ± 0.90
80f	113.32 ± 5.5	**105e**	11.17 ± 2.6
95a	80.95 ± 2.4	**105f**	47.08 ± 7.4
95b	88.89 ± 4.4	**105g**	20.47 ± 1.7
95c	80.55 ± 1.5	**105h**	31.84 ± 3.0
95d	78.87 ± 2.3	**110a**	79.78 ± 8.4
95e	80.71 + 2.9	**110b**	82.59 ± 4.6
95f	83.22 ± 3.3	**114a**	71.16 ± 3.9
95g	78.22 ± 5.7	**114b**	41.65 ± 7.7
95h	93.63 ± 3.7	**120**	72.20 ± 2.1
95i	93.13 ± 6.5	**123a**	79.89 ± 4.2
95j	65.67 ± 3.9	**123b**	56.70 ± 5.0
95k	90.09 ± 5.2	**128**	15.13 ± 0.84
95l	88.42 ± 0.38	**Daunorubicin**	IC_{50} 12.55 ± 0.07 µM

[a]: Inhibition percentage of each compound in 50 µM for 24 h against HEK 293 cell line. Yellow marked compounds show significant toxicity.

3.6 Biological activity assessment in cells

3.6.1 Biological activity of representative alkylated hydrazides-based HDAC8 selective inhibitors in cellular assessment

To further investigate target engagement and selectivity of these novel alkylhydrazide-based HDAC8 inhibitors, western blot experiments with 10 μM representative compounds were performed in Jurkat cells which were incubated for 6 h and 24 h. The selective HDAC8 inhibitor PCI-34051 25 μM was employed as positive control. Almost all tested inhibitors do slightly affect the acetylation level of tubulin (HDAC6 substrate) as shown quantitatively in Figure 61. As expected, all the inhibitors (**80d** (PSP43), **105a** (PSP49), **80f** (PSP67), **95m** (PSP69), **95l** (PSP70), **105h** (PSP71), **105f** (PSP72), **105b** (PSP73), **105d** (PSP74)) can significantly upregulate the acetylation of SMC3 (HDAC8 substrate) compared to PCI-34051 except of **95j** (NI-90), **95k** (NI-91) and **114a** (NI-82). H3K9 acetylation (common class I HDAC substrate) was also significantly upregulated by most inhibitors respectively compared to positive control meanwhile. **114a** (NI-82) and **80d** (PSP43) showed the weakest effect on H3K9 acetylation indicating some cellular selectivity. In addition H3K27 hyperacetylation was measured (induced by several HDAC subtypes) since this modification has been of interest in the study of immunomodulation effects. (The data was provided by the group of Prof. Alfred SL Cheng, The Chinese University of Hong Kong, Hong Kong SAR, China)

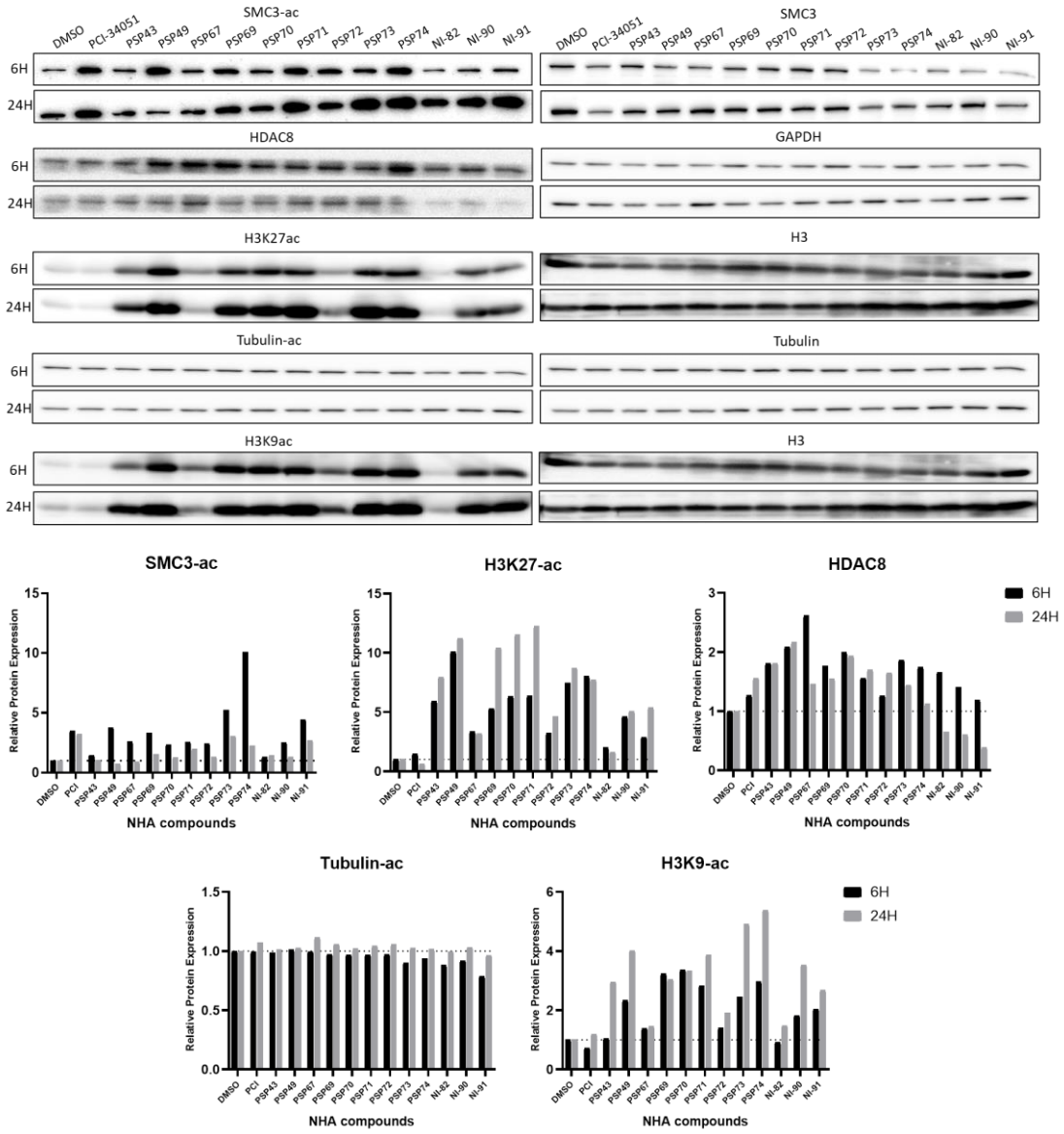


Figure 61. Upper part: Western blot results of chosen inhibitors in 10 μM against 6.25×10^5 Jurkat cells and PCI-34051 against 6.25×10^5 Jurkat cells in 25 μM after 6 h and 24 h treatment respectively. Histone H3 and GAPDH were used as a loading control, PCI-34051 and DMSO were used as positive control and vehicle respectively. Lower part: quantification of the western blot results.

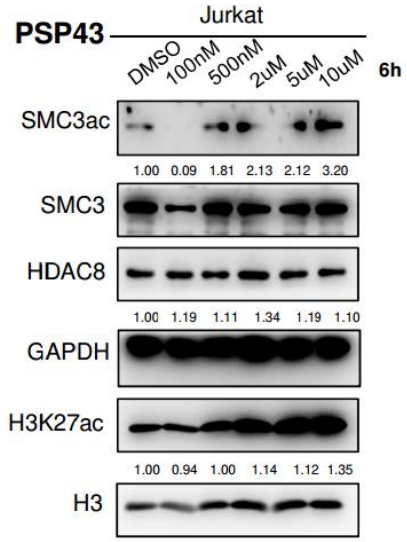


Figure 62. Dose depended activity of **80d** (PSP43) on the alteration of biomarker proteins in 6.25×10^5 Jurkat cells which were treated for 6 h under different concentrations of **80d**, Histone H3, HDAC8 and GAPDH were used as a loading control, DMSO was used as vehicle.

The lead compound **80d** (PSP43) showed a notably up-regulated acylation level of SMC3 protein and H3K27 lysine acylation at concentration above 500 nM (Figure 62).

3.6.2 Immunological anticancer activity and apoptosis in cellular assessment

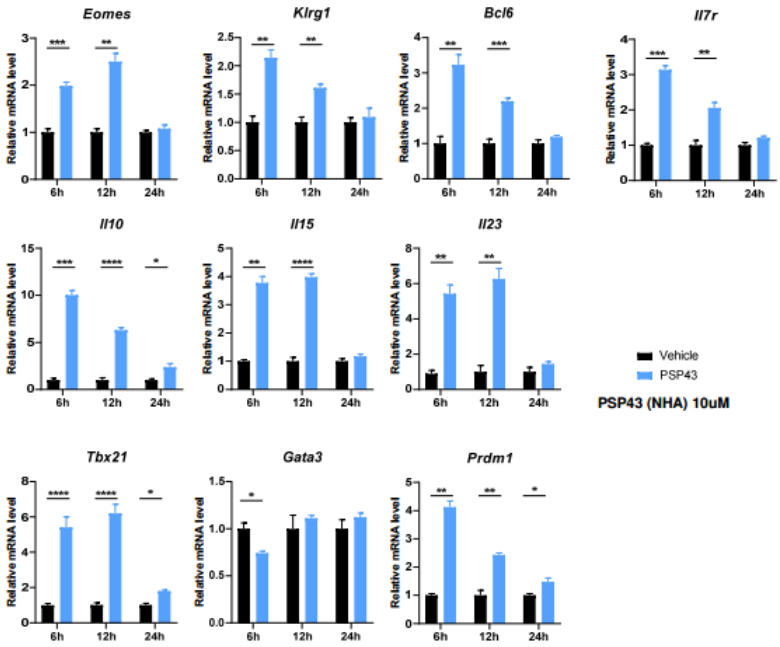


Figure 63. RT-qPCR analysis of T cell memory and effector genes in **80d** (PSP43) treated 6.25 × 10^5 Jurkat cells in 10 μM at different time point..

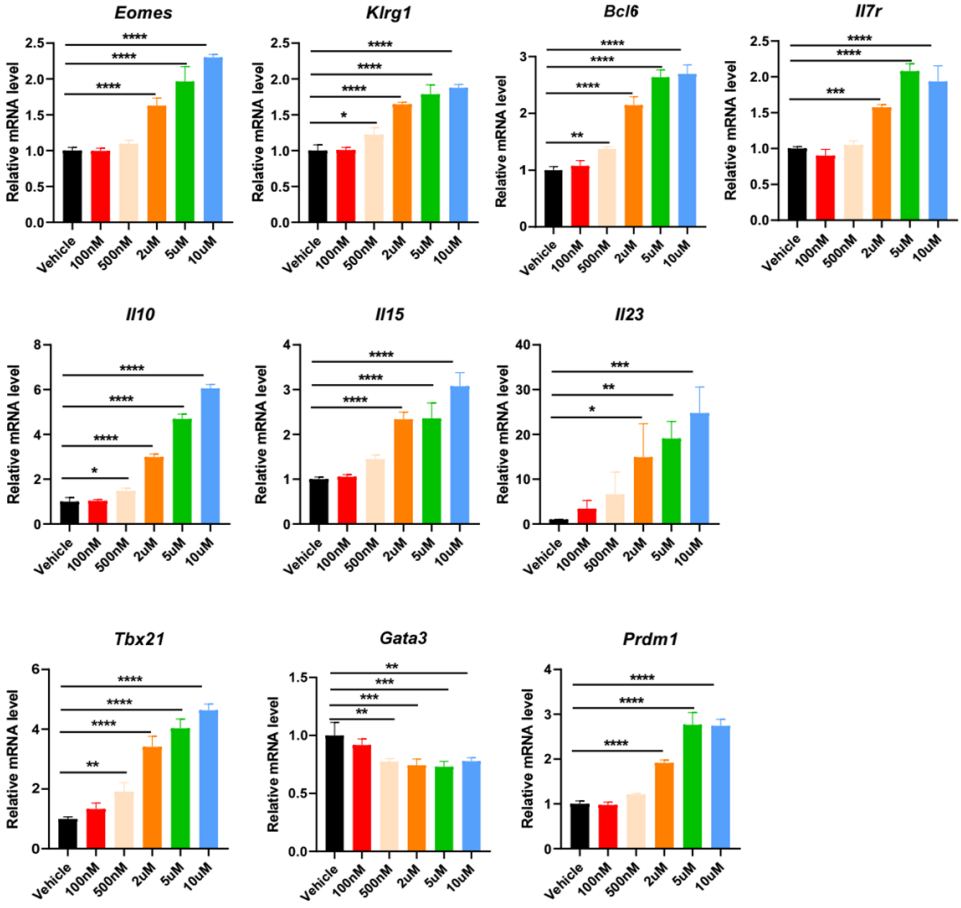


Figure 64. RT-qPCR analysis of T cell memory and effector genes in **80d** (PSP43) treated 6.25×10^5 Jurkat cells in different concentrations for 6 h.

In previous study our collaborators showed that selective HDAC8 inhibitors can significantly enhance the efficacy and durability of cancer therapy by immune-checkpoint blockade, which was accompanied by an induction of memory T cell response,[64] From the *in vitro*, stability and target engagement results **80d** (PSP43) showed the most promising effects and was selected as lead compound for further characterization. The effect of **80d** on gene expressions related to T cell functions was analyzed. (Figure 63, Figure 64)

Using RT-qPCR, we found that 10 μM **80d** (PSP43) can remarkably up-regulate nearly all measured genes involved in T cell memory (*Bcl6*, *Eomes*, *Klrg1*, *Il7r*, *Il10*, *Il15*, *Il23*) and effector (*Prdm1*, *Tbx21*) functions with the exception of *Gata3* in Jurkat T lymphocytes treated for 6 h and 12 h (Figure 63). Moreover, **80d** (PSP43) can dose-dependently up-regulate memory-related key transcription factors, surface markers (*Bcl6*, *Eome*, *Klrg*, *Il7r*), cytokines (*Il10*, *Il15*, *Il23*), and effector-related master regulators (*Tbx21* and *Prdm1*) (Figure 64).

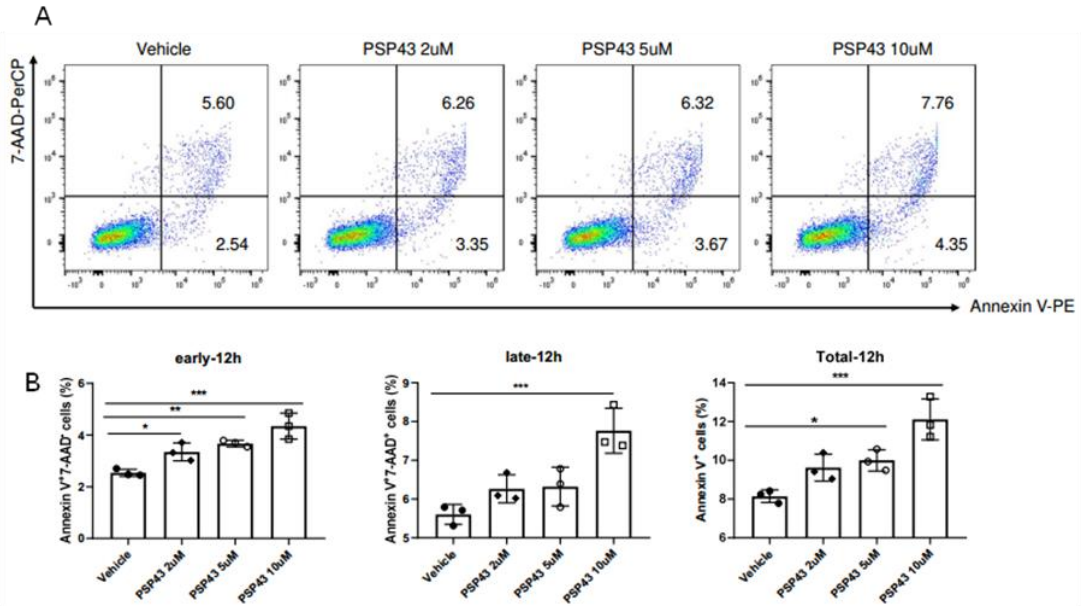


Figure 65. Apoptosis studies of **80d** (PSP43) againts Jurkat cells in different concentrations at 12 h. A: flow cytometry original scan (x-axis, annexin-V-PE; y-axis, 7-aminoactinomycin D-PerCP), DMSO was used as vehicle. B: percentage of apoptotic cells distributions treated with different inhibitor

concerntrations for 12 h (early apoptotic, late apoptotic and total apoptotic cells).

In addition, flow cytometry was used to study the apoptotic efficacy of compound **80d** (PSP43) (Figure 65). Incubation of Jurkat cells with the inhibitors in different concentrations for 12 h showed that a 10 μM concentration of **80d** (PSP43) for 12 h can induce significant apoptosis in whole Jurkat cells. Both 2 μM and 5μM can induce early apoptosis in Jurkat cells. The apoptotic trend is clear at 24 h although we can not see obvious apoptotic activity within shorter time. (The data was provided by the group of Prof. Alfred SL Cheng, The Chinese University of Hong Kong, Hong Kong SAR, China).

3.7 Immune-modulatory effect of 80d (PSP43) *in vivo*

The results of gene expression analysis in T lymphocytes suggest that **80d** (PSP43) may exert immune-modulatory effect. We studied this by intraperitoneal administration of 5, 10 and 25 mg/kg **80d** (PSP43) 5 days per week for 2 weeks in immunocompetent C57BL/6 mice. (The data was provided by the group of Prof. Alfred SL Cheng, The Chinese University of Hong Kong, Hong Kong SAR, China)

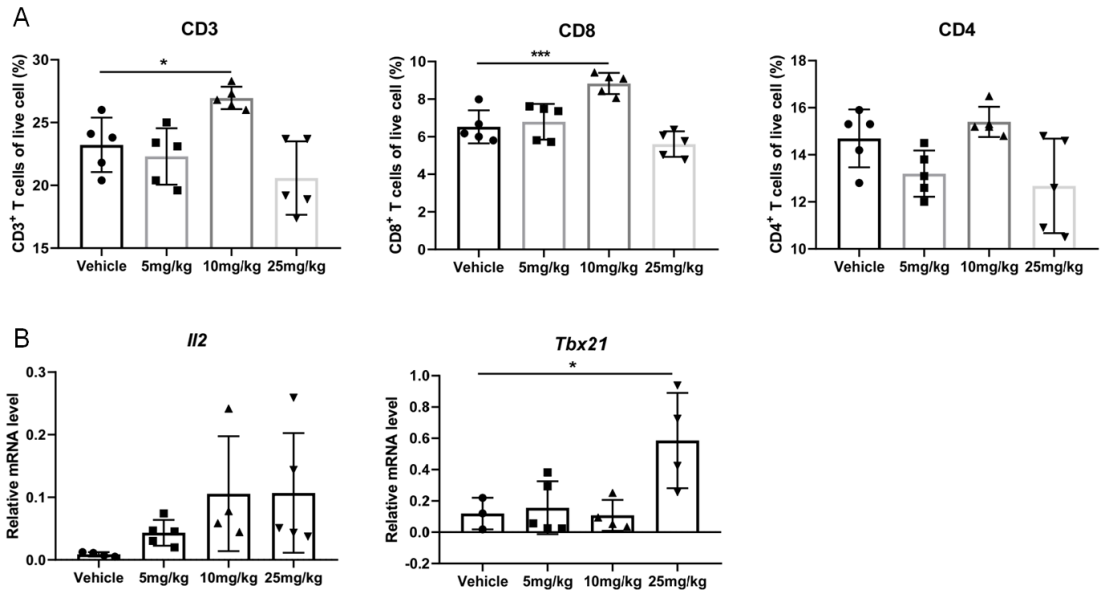
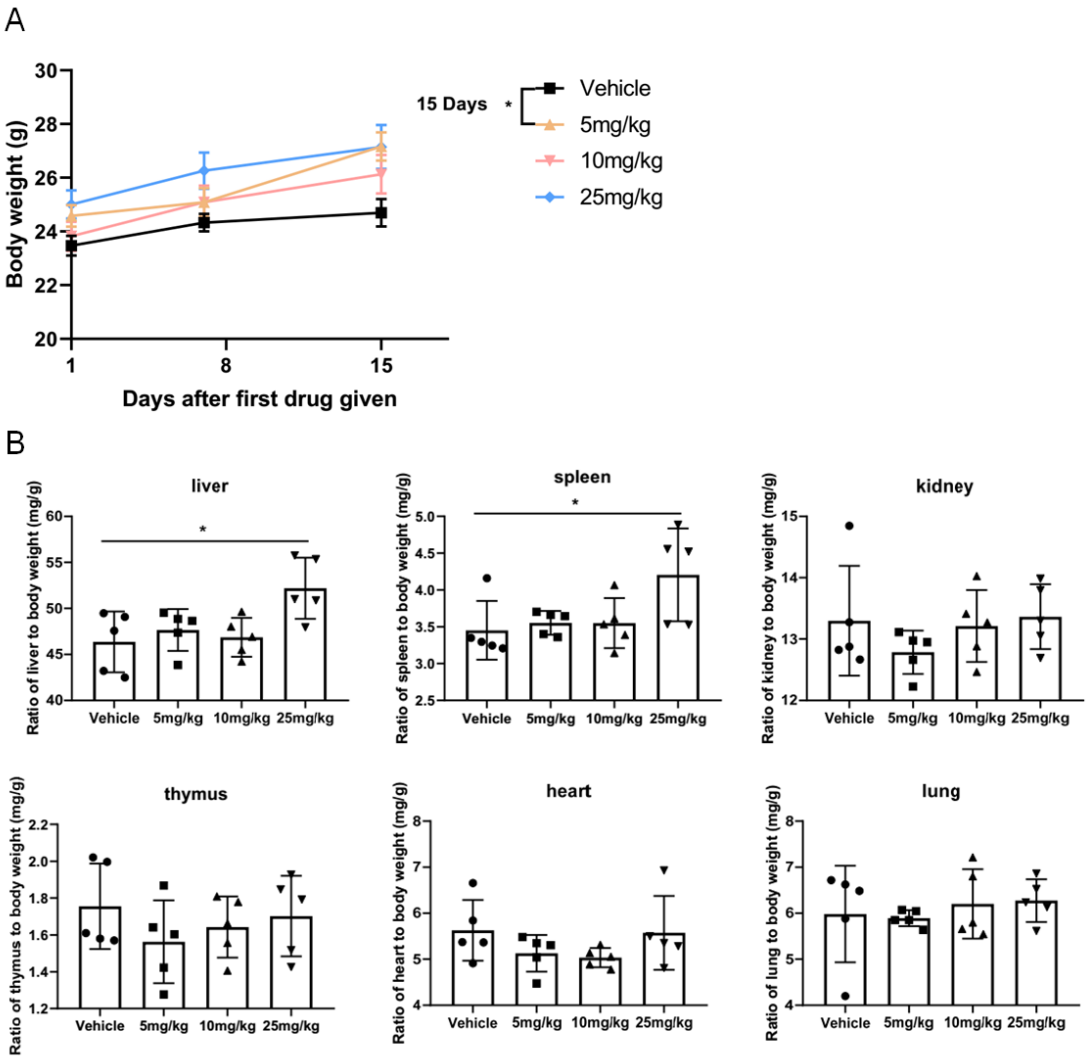


Figure 66. Immune profiling analysis through multicolor flow cytometry and RT-qPCR *in vivo* upon the treatment of **80d** (PSP43) (5, 10, 25 mg/kg) or vehicle control (30% PEG400 + 0.5% Tween 80 + 5% propylene glycol). A) Proportions of $CD3^+$, $CD8^+$ and $CD4^+$ T cells in the naïve mice spleen between the treatment of **80d** and vehicle control. B) RT-qPCR analysis of $CD4^+$ signature genes in the naïve mice spleen between the treatment of **80d** and vehicle control.

Using flow cytometry, we found that 10 mg/kg **80d** (PSP43) significantly increased the proportions of $CD3^+$ and $CD8^+$ but not $CD4^+$ T cells in the spleen of treated mice compared to vehicle control (Figure 66 A). Interestingly, RT-qPCR analysis showed that 10 and 25 mg/kg **80d** (PSP43) significantly induced the expressions of *Il2* and *Tbx21* in the sorted $CD4^+$ T cells, respectively (Figure 66 B). These data are consistent with the Jurkat T cell model and support the immune-modulatory function of **80d** (PSP43) *in vivo*. Furthermore, treatment with **80d** (PSP43) was in general

well-tolerated, as we did not observe any body weight loss (Figure 67 A) or abnormalities of internal organs such as the heart, kidney, liver, lung, spleen and thymus, except organ weight increase in liver and spleen at the highest dose (Figure 67B and 67C).



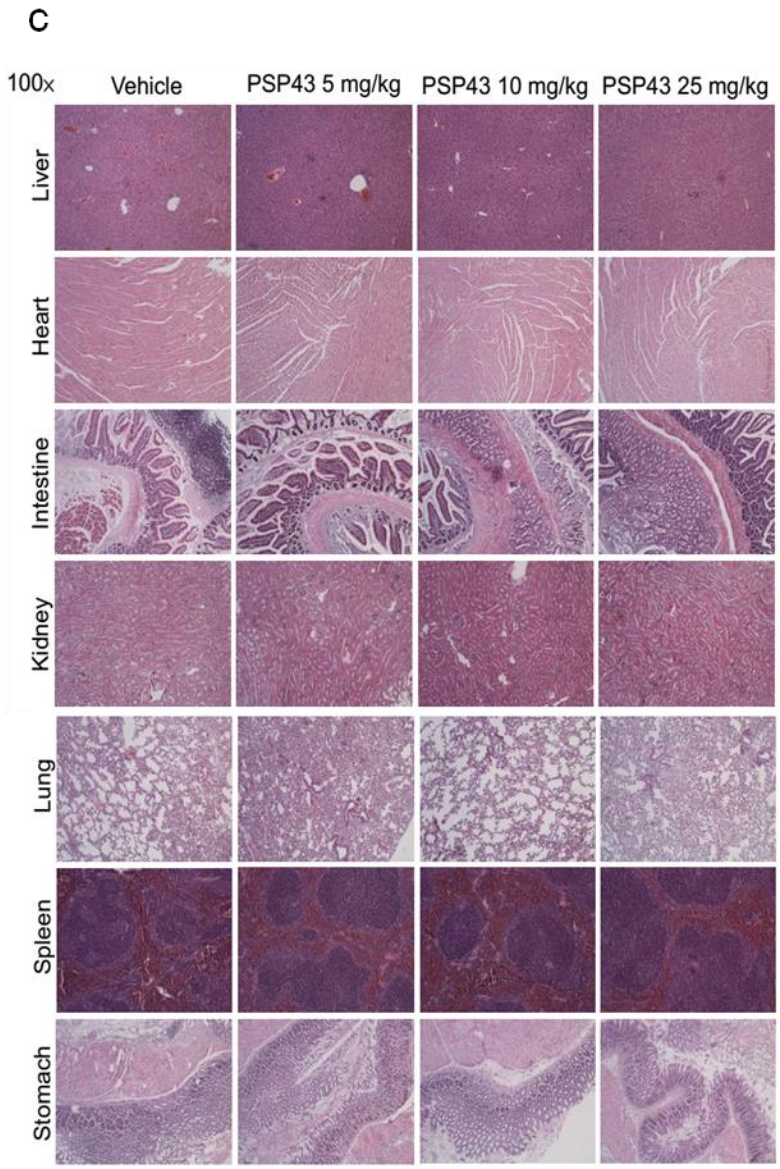


Figure 67. Toxicity evaluation of Naïve mice upon the treatment of **80d** (PSP43) (5, 10, 25 mg/kg) or vehicle control (30% PEG400 + 0.5% Tween 80 + 5% propylene glycol. A) Body weight of Naïve mice at the time point of first day, eighth day and fifteenth day after the first drug given. B) Various organs weight of Naïve Mice at the fifteenth day after the first drug given. C) Morphology study of various organs stained with hematoxylin and eosin (H&E) at the fifteenth day after the first drug given.

3.8 Discussion and conclusion of this part

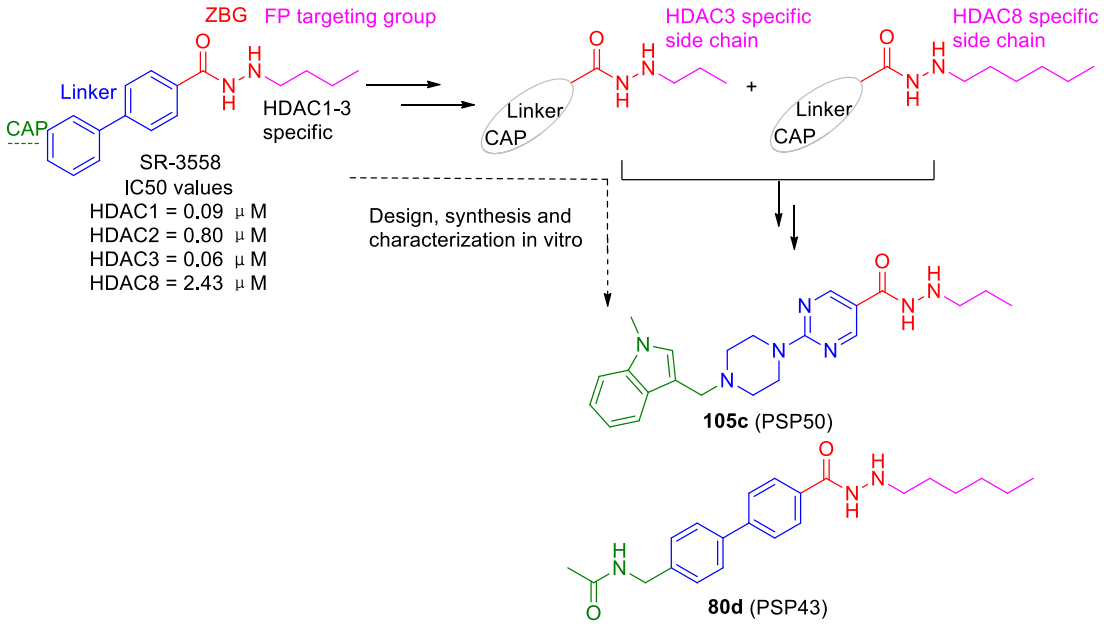


Figure 68. Structures of **80d** (PSP43), **105c** (PSP50) and their design process.

Table 18. The summary of biological data for **80d** (PSP43) and **105c** (PSP50)

Cpd. No.	hHDAC inhibition (μM)[a]							HEK 293 Viability [c]	Function *in vitro*
	1	2	3	8	4	6	11		
80d PSP43	1.8 ± 0.3	12 ± 1	>20	0.036	> 60	> 20	0.18	121.3%	Up-regulated acetylation of H3K9 and H3K27; induced apoptosis and immunological anticancer activity in Jurkat cell line. EC_{50} = 0.1155 μM against HCT116 cells
105c PSP50	0.011 ± 0.002	0.45 ± 0.02	0.037 ± 0.001	0.028 ± 0.002	n.t.[b]	n.t	0.093	45.1%	

[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value. [b]: n.t = not tested. [c]: compounds 50 μM for 24 h against HEK 293 cell line

In this part we chemically optimized several series of alkyl-hydrazide based HDAC

inhibitors which include biphenyl, phenyl and pyrimidine linker, aryl and indole derived cap group. To our best knowledge, this is the first finding that *n*-hexyl side chain linked with the hydrazide moiety can lead to HDAC8 specificity for hydrazide-based HDAC inhibitors.

Additionally, we synthesizd and characterized a series of novel class I HDAC inhibitors which were able to up-regulate H3K9 acetylation while demonstrating micromolar to submicromolar antiproliferative activity against human pancreatic cell line (PSN1 cells) and human colon cancer cell line (HCT116 cells) (**105c** (PSP50), **105e** (PSP51)). Most of alkylated hydrazide-based HDAC8 selective inhibitors (**80d** (PSP43), **105a** (PSP49), **80f** (PSP67), **95m** (PSP69), **95l** (PSP70), **105h** (PSP71), **105f** (PSP72), **105b** (PSP73), **105d** (PSP74)) can not only up-regulate HDAC8 specific SMC3 acetylation but also induce common class I HDAC target (H3K9 and H3K27) hyperacetylation. Furthermore, we tested and confirmed the inhibiory mechanism of the discovered alkyl-hydrazide based HDAC8 inhibitors as substrate competitive inhibitors against HDAC8.

Futhermore, the HDAC8 selective inhibitor **80d** displays a stable and safe profile in a non-enzymatic stability assay and a cytotoxicity assay on HEK293 cells. Interestingly, **80d** can significantly up-regulate H3K27 acetylation but also slightly affect acetylation of SMC3 in T lymphocytes. Moreover, T cell mediated immune-modulatory effect was strongly correlated with the treatment of **80d** both *in vitro* and *in vivo* compared to vehicle control. **80d** was found to be safe and without any body weight loss or internal organs' abnormalities in naive mice administered by intraperitoneal injection. In summary, this study not only identifies the alyklhydrazide as chemotype for HDAC8 selective inhibitors to overcome limitations of current hydroxamate based inhibitors, but also provides an interesting biological effect in several diseases.

Part 4 Class I HDAC targeted degraders: design, synthesis, stability study and biological evaluation

4.1 Chemistry

4.1.1 Overview of this part

The synthetic strategies for class I HDAC degraders were started using a series of known HDACi scaffolds and warheads for different ubiquitin ligases, lastly connected by different linkers (Figure 69, Figure 70 and Figure 71).

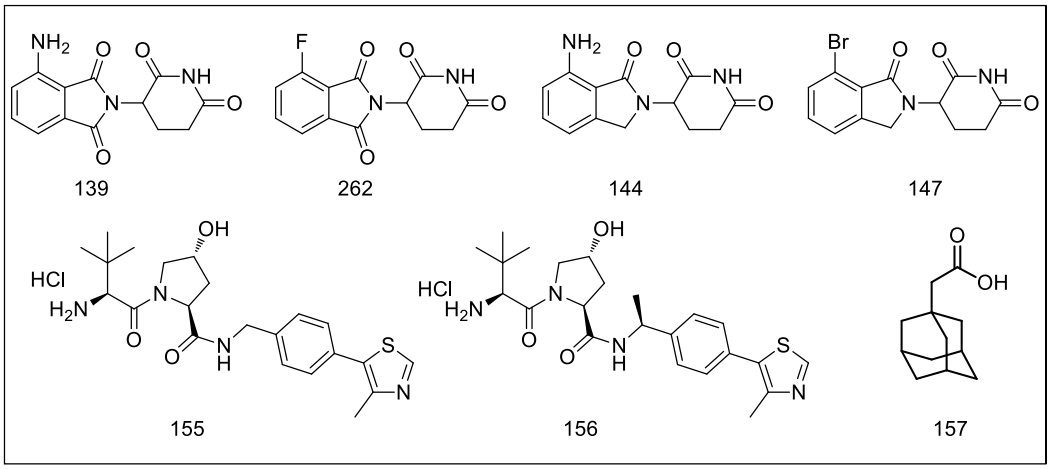


Figure 69. Representative scaffolds of the ubiquitin ligase part of designed PROTACs are shown here.

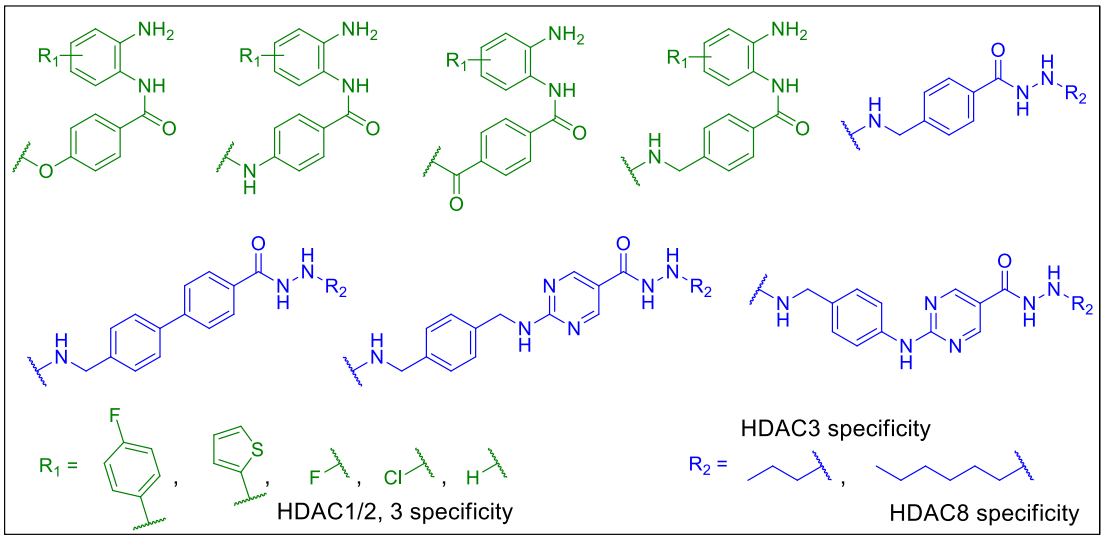


Figure 70. Representative HDAC binders used for the PROTAC design.

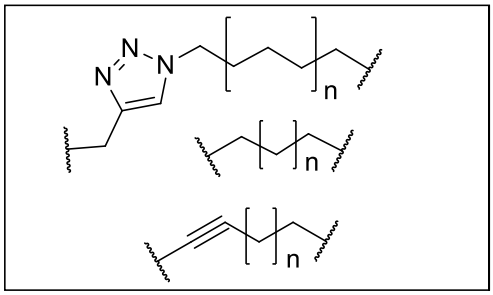


Figure 71. Representative linkers applied in this work

4.1.2 Synthesis of E3 ubiquitin ligase ligands

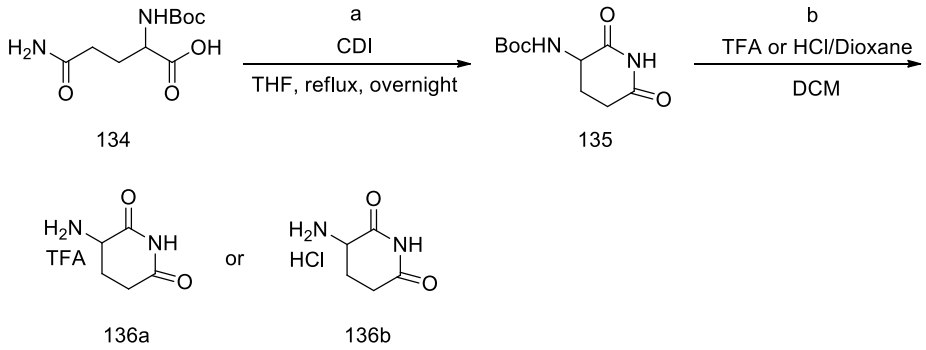


Figure 72. Synthesis method for intermediate **136a** and **136b**.

The 3-aminopiperidine-2,6-dione trifluoroacetic acid salt (**136a**) or hydrochloric acid salt (**136b**) was generally employed as one of key intermediates to achieve all the immunomodulatory drugs (IMiDs)-based E3 ligase ligands. This intermediate was obtained via a Boc-Gln-OH mediated cyclization procedure (Figure 72, step a) which was followed by a Boc-deprotection step. However, in the following procedure the hydrochloric acid salt (**136b**) was used due to its friendly work-up procedure; namely it was more easily to dry and purify it (Figure 72).

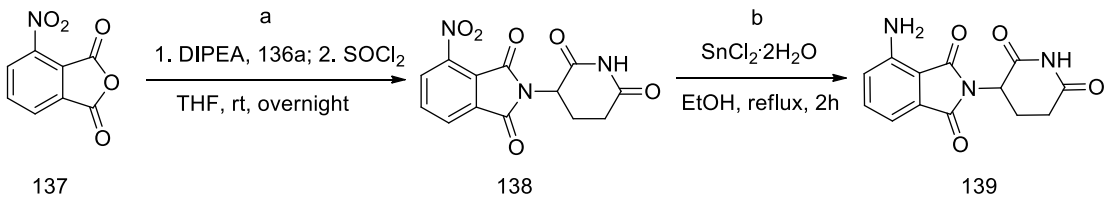
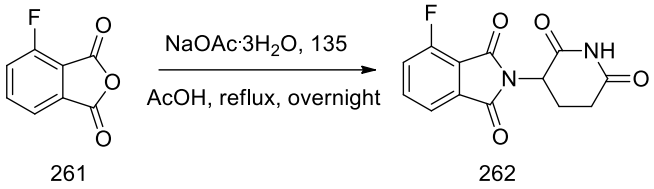


Figure 73. Procedure for the synthesis for degron **139**.

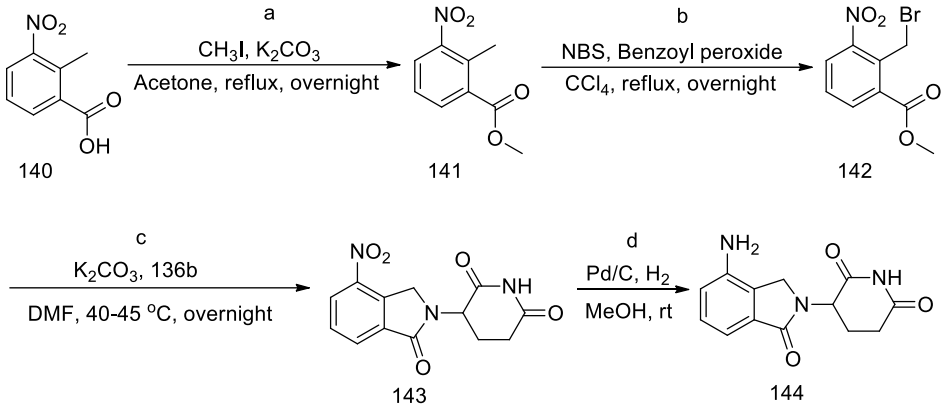
Subsequently, **136b** or **136a** were reacted with 3-nitrophthalic acid anhydride and followed by a reduction step to yield the compound **139** (Figure 73).



261 262

Figure 74. Procedure for the synthesis of **262**.

262 was directly generated from the reaction of 4-fluoroisobenzofuran-1,3-dione and intermediate **135** (Figure 74).



140 141 142 143 144

Figure 75. Procedure for the synthesis of **144**.

144 was synthesized starting from carboxylic acid **140**, which was first esterified (Figure 75, step a), followed by a bromination step in carbontetrachloride (Figure 75, step b) which was optimal compared to dichloromethane and chloroform. The product **142** underwent a cyclization with **136a** or **136b** and another reduction procedure (Figure 75, step d) to afford **144**. The synthesis method for **147** is similar to **144** and described in Figure 76.

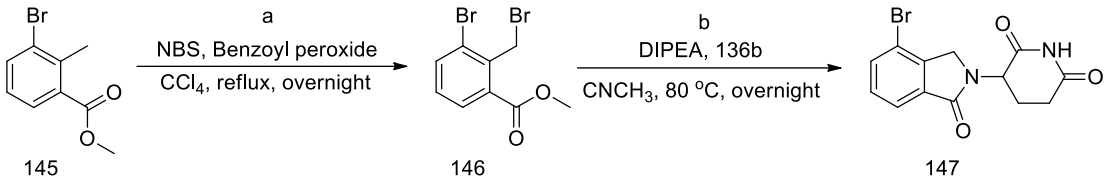


Figure 76. Procedure for the synthesis of **147**.

Following the reported methods,[158-160] the VHL ligand **155** was obtained but it should be noticed that in the work-up procedure of the Heck coupling reaction (Figure 77, step b) an extra recrystallization procedure was added using EtOAc and heptane to remove the yellow byproducts, which were not easily separated by column chromatography. Another difference is that all the amide formation steps namely procedure d and f in Figure 77 were performed under ice bath and finished within 15 min to decrease the risk of racemization of all chiral centers (Figure 77).

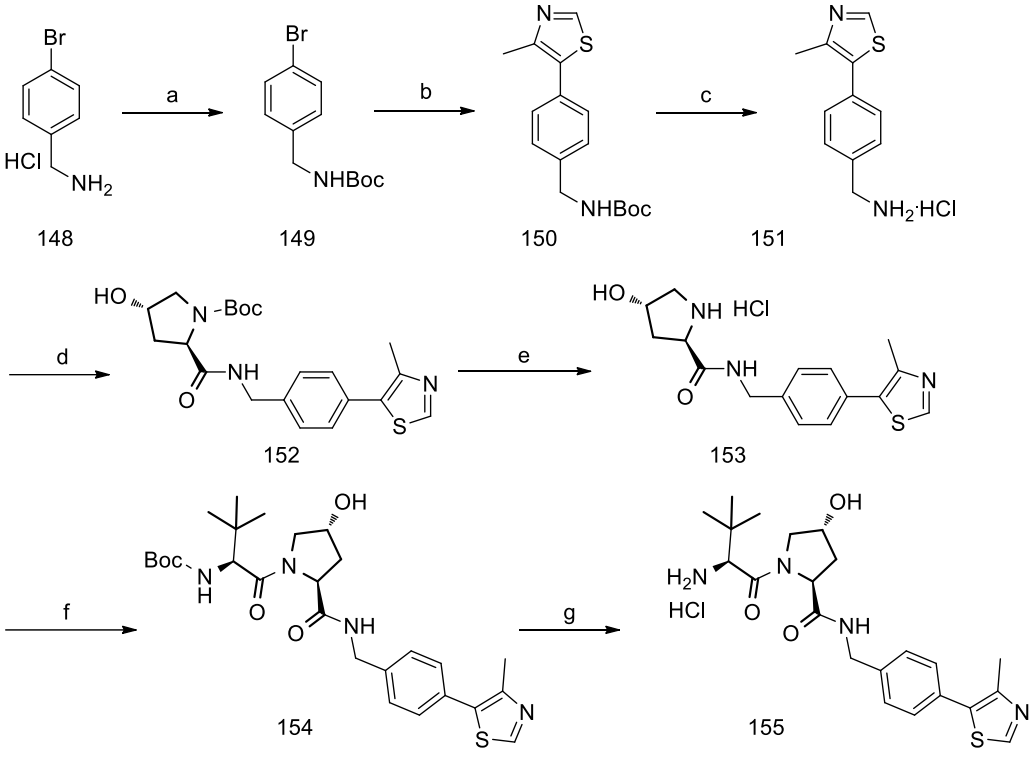


Figure 77. Procedure for the synthesis of **155**. Reagents and conditions: (a) TEA 2.2 eq, $(Boc)_2O$ 1.1 eq, CH_3CN, rt, 1 h; (b) 4-Methylthiazole 2.0 eq, KOAc 2.0 eq, $Pd(AcO)_2$ 0.01 eq, DMF, 90 °C; (c) HCl/dioxane (4M) 3.5 eq, DCM, rt, overnight; (d) N-Boc-trans-4-hydroxy-L-proline 1.05 eq, HATU 1.1 eq, DIPEA 3.5 eq, DMF, ice bath, 10 min; (e) HCl/dioxane (4M) 3.5 eq, DCM, ice bath, 3 h; (f) N-Boc-L-tert-leucine 1.0 eq, HATU 1.0 eq, DIPEA 4.0 eq, DMF, ice bath, 10 min; (g) HCl/dioxane (4M) 3.5 eq, DCM, ice bath, 3 h.

4.1.3 Synthesis of HDAC binders

4.1.3.1 Synthesis of aminobenzamide-derived HDAC binders

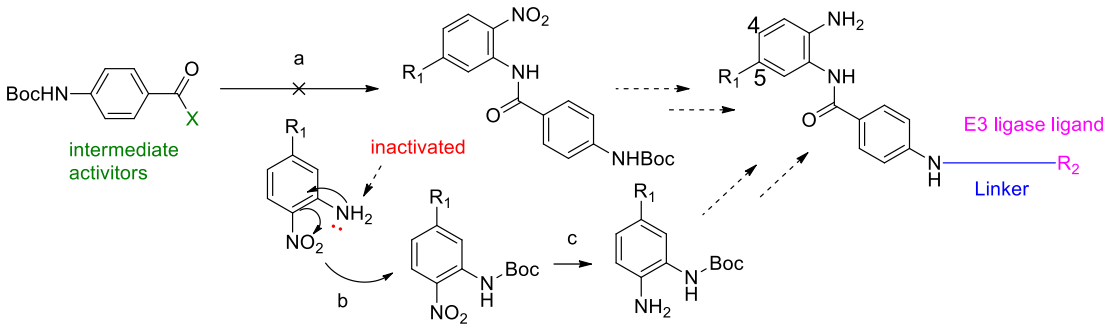


Figure 78. Synthestic strategies for benzamide-based PROTACs (X = Cl or amide condensation reagents, R_2 = E3 ligase ligand).

In the beginning, the 2-nitroaniline should be used as starting material to react with carboxylic acid which was pre-activated by amide condensation reagents or pre-transformed to acylchloride and then connected with the linker moiety attached with E3 ligase ligand (Figure 78, step a). However, almost all the amide condensation reagents failed to mediate the reaction, probably because of the strong electronwithdrawing effect of the nitro group which completely inactivated the necleophilic feature of the amine. In consequence, amine of 2-nitroaniline were generally protected by Boc anhydride followed by reduction of the nitro group into amine to recover the amine activity and subsequently coupled with the corresponding carboxylic acid smoothly (Figure 78, step b and c).

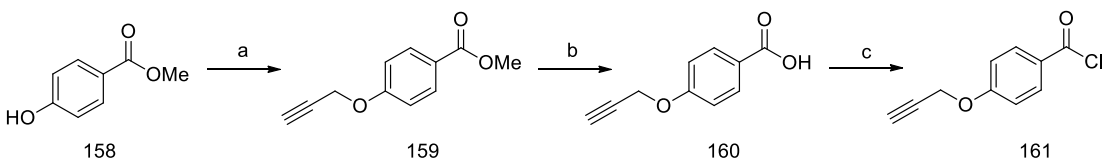


Figure 79. Reagents and conditions: (a) Propargylbromide (80%) 2.0 eq, K_2CO_3 1.3 eq, DMF, rt, 22 h; (b)

NaOH 5.0 eq, MeOH/H_2O (3 : 1), rt, 24 h; (c) $SOCl_2$ 4.0 eq, reflux, 1 h.

The key intermediate **161** was synthesized by three steps namely a phenol substitution, ester hydrolysis and conversion to the acyl chloride (Figure 79).

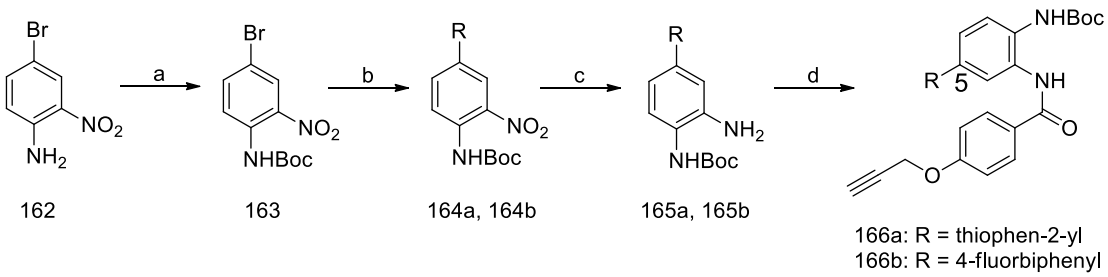


Figure 80. Reagents and conditions: (a) $(Boc)_2O$ 2.0 eq, TEA 3.0 eq, DMAP 0.01 eq, DCM, rt, 5 h; (b) Boronic acid 1.1 eq, $Pd(PPh_3)_4$ 0.05 eq, K_2CO_3 2.2 eq, THF/H_2O (5 : 1), reflux, overnight; (c) Zn 4.0 eq, AcOH, MeOH, rt; (d) **161** 1.2 eq, DIPEA 5.0 eq, THF, rt, 30 min.

The first series of HDAC binders encompassed 5-aryl-2-aminobenzamides. They were obtained by firstly protecing the amine in the starting material **162** (Figure 80, step a) and then coupling with the corresponding boronic acid by Suzuki coupling (step b) giving intermediate **164** which was reduced to **165** and condensed with **161** yielding the Boc-protected HDAC binders **166a** and **166b** (Figure 80).

Interestingly, the HDAC binders **169a** and **169b** could be directly synthesized by the coupling of **161** with **168** which were not pre-protected despite the electron withdrawing group located in position-4 (Figure 81, step b). This phenomenon might be explained by the electron withdrawing group that decreased the electron density more sharply in the meta- than para-position (Figure 81).

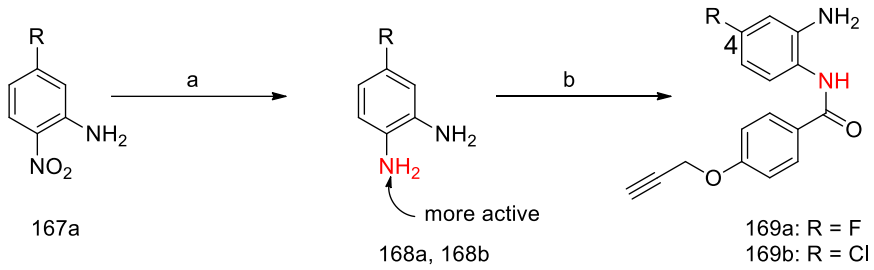


Figure 81. Reagents and conditions: (a) Pd/C 10% wt, ammonium formate 4.0 eq, EtOH, rt; (b) **160** 1.0 eq, HATU 1.2 eq, DIPEA 3.0 eq, DMF, rt, 10 min.

In contrast to **169a** and **169b** (Figure 81), the synthesis of **174a** and **174b** (Figure 82) were started from a Boc-ptotedcted step for the starting material 2-nitrophenyl (Figure 82, step a and b) and subsequently followed by a procedure that was similar as for **166**. It also needs to be noted that the chloro group was also reduced by hydrogen in the step of **172b** to **173b** (Figure 82, step c).

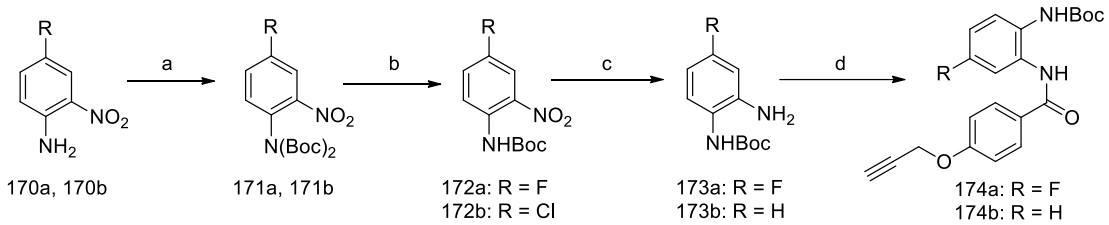


Figure 82. Reagents and conditions: (a) $(Boc)_2O$ 2.0 eq, TEA 3.0 eq, DMAP 0.01 eq, DCM, rt, 2 h; (b) TFA 1.0 eq, DCM, rt, 80 min; (c) Pd/C 10% wt, ammonium formate 4.0 eq, MeOH; (d) **161** 1.2 eq, DIPEA, 5.0 eq, THF, rt, 30 min; or **160** 1.0 eq, HATU 1.2 eq, DIPEA 3.0 eq, DMF, rt, 10 min.

Another series of 2-aminobenzamide derivatives **178a-c** were synthesized staring from p-aminomethylbenzoic acid which was pre-protected by a trifluoroacetic group and later reacted with protected or unprotected aminobenzamide to afford the Boc-protected or unprotected HDAC binders **178a-c** (Figure 83).

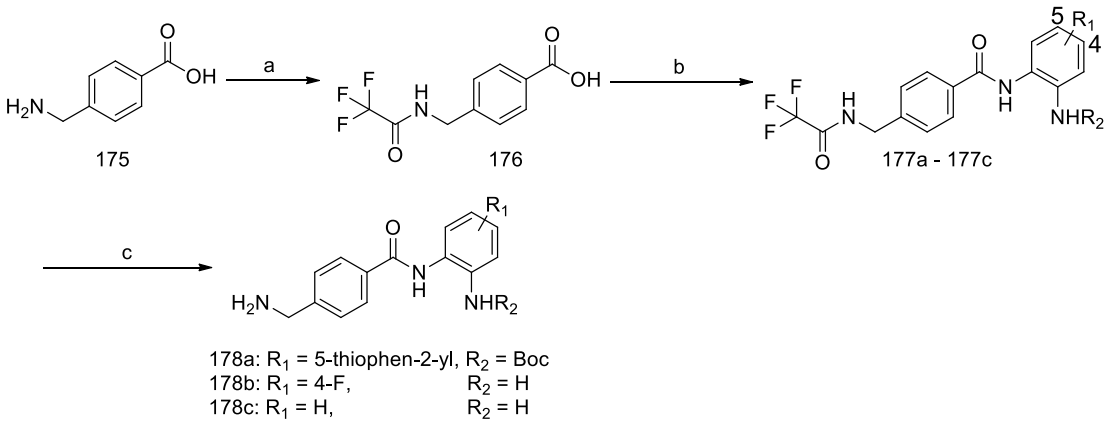


Figure 83. Reagents and conditions: (a) TFAA 2.5 eq, ice bath to rt, overnight; (b) **165a** or **168a** or o-phenylendiamine 1.2 eq, HATU 1.2 eq, DIPEA 3.0 eq, DMF; (c) K_2CO_3 4.0 eq, MeOH/H_2O (1 : 1), rt, overnight.

When para-aminobenzoic acid **179** was utilized as starting material, different synthetic methods were investigated. The first one began with a trifluoroacetic group protection procedure (Figure 84, step a), the corresponding intermediate **180** was converted to the acyl chloride **181** which was condensed with Boc-protected aminobenzamide **165a** giving target compound **183**.

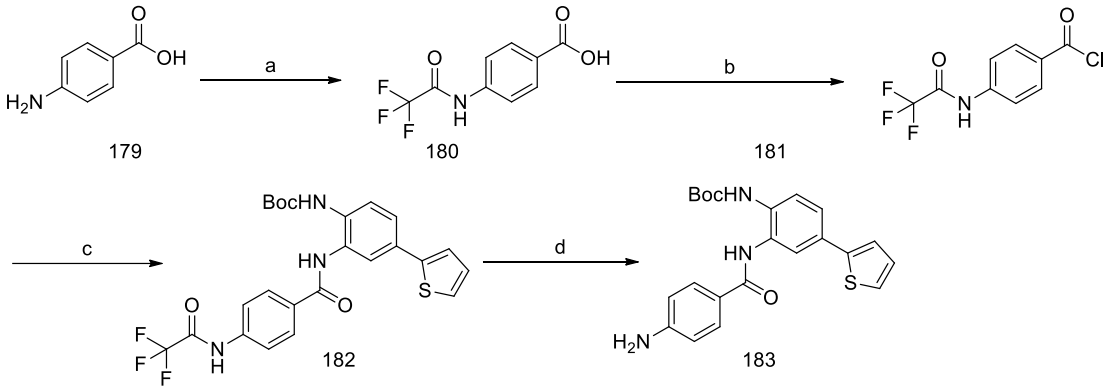


Figure 84. Reagents and conditions: (a) TFAA 2.0 eq, TFA, ice bath to rt, overnight; (b) $SOCl_2$ 5.0 eq; (c) **165a** 1.0 eq, DIPEA 4.0 eq, THF; (d) K_2CO_3 4.0 eq, MeOH/H_2O (1 : 1), rt, overnight.

The other way encompassed the direct condensation of the protected aminobenzamide **165a** with either 1-equivalent of unprotected p-aminobenzoic acid (**179**) which was mediated by the amide condensation reagent HATU (Figure 85, step c) or directly with acyl chloride (Figure 85, step b).

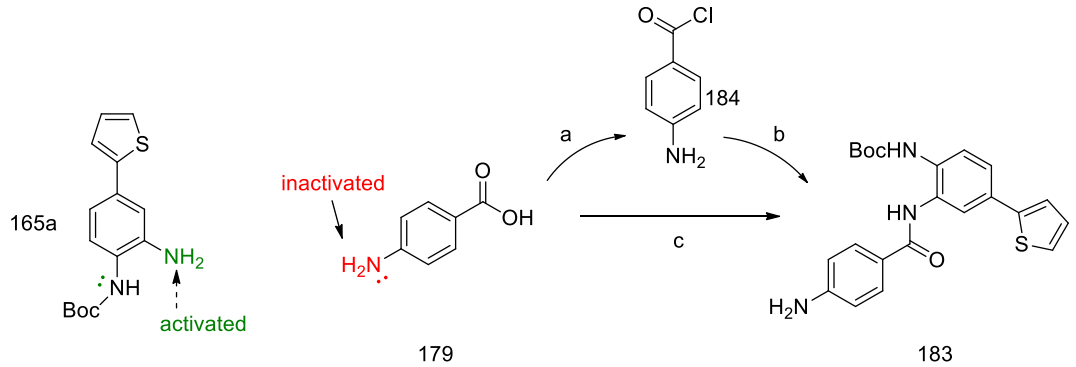


Figure 85. Reagents and conditions: (a) $SOCl_2$ 4.0 eq; (b) **165a** 1.0 eq, DIPEA 4.0 eq, THF; (c) **165a** 1.0eq, HATU 1.2 eq, DIPEA 3.0 eq, DMF, rt, overnight.

The HDAC binder **187** was obtained via a condensation and a hydrolysis procedure (Figure 86, step a and b).

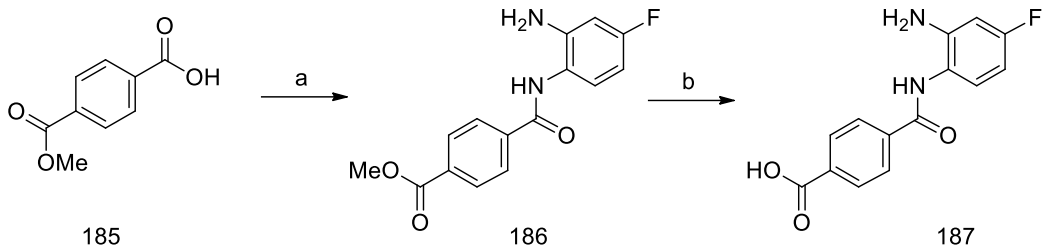


Figure 86. Reagents and conditions: (a) **168a** 1.25 eq, HATU 1.1 eq, DIPEA 3.0 eq; (b) LiOH 2.0 eq, THF/H_2O (1 : 1), reflux, 1 h.

4.1.3.2 Synthesis of alkylhydrazide-derived HDAC binders

Hydrazide-based Boc-protected HDAC binder **87a** and the negative control, Boc-protected binder **87c**, were generated from **85a** and **84** through Boc-protection and Cbz deprotection procedure (Figure 87).

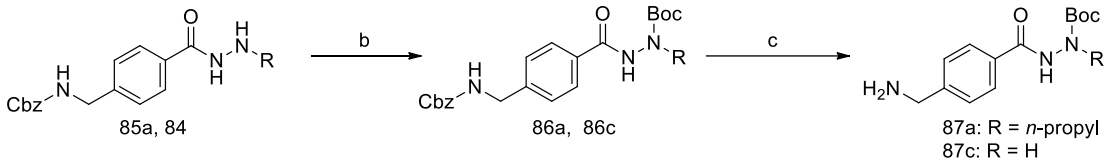


Figure 87. Reagents and conditions: (a) $(Boc)_2O$ 1.2 eq, DIPEA 2.0 eq, THF, rt; (b) Pd/C 10% wt, $NaBH_4$ 5.0 eq, MeOH, rt, overnight.

Boc-protected biphenyl containing hydrazides, **78a** and **191**, were synthesized from the starting material **148** which was protected by Cbz group and converted to the boronate ester **189** followed by a Suzuki coupling procedure (Figure 88, step c) resulting in intermediate **77a** and **190**. Lastly, a Cbz-deprotection step was carried out (Figure 88).

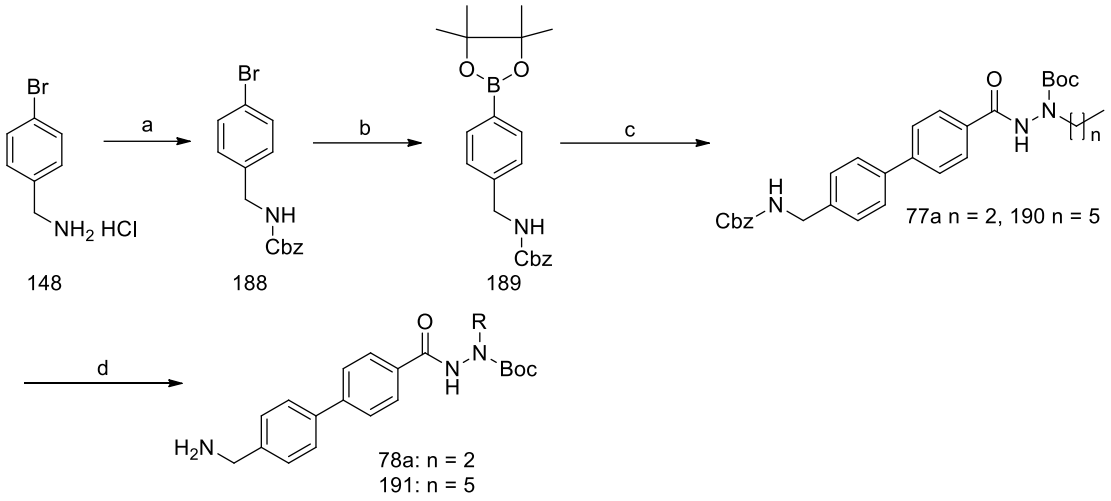


Figure 88. Reagent and conditions: (a) CbzCl 1.05 eq, NaOH 2.2 eq, THF/H_2O, 0 °C to rt; (b) Bis(pinacolato)diboron 1.0 eq, KAcO 2.0 eq, $Pd(PPh_3)_2Cl_2$, dioxane, 90 °C, 6 h; (c) **76a** or **76d** 1.0 eq, K_2CO_3 2.2 eq, $Pd(PPh_3)_2Cl_2$ 0.04 eq, dioxane/H_2O (5 : 1), 90 °C, 15 h; (d) Pd/C, $NaBH_4$ 5.0 eq, MeOH, overnight.

As mentioned in the previous part, pyrimidine fused hydrazides demonstrated excellent class I HDAC inhibitory potency and selectivity, hence we also designed and produced two different series of these Boc-protected HDAC binders (Figure 89, Figure 90).

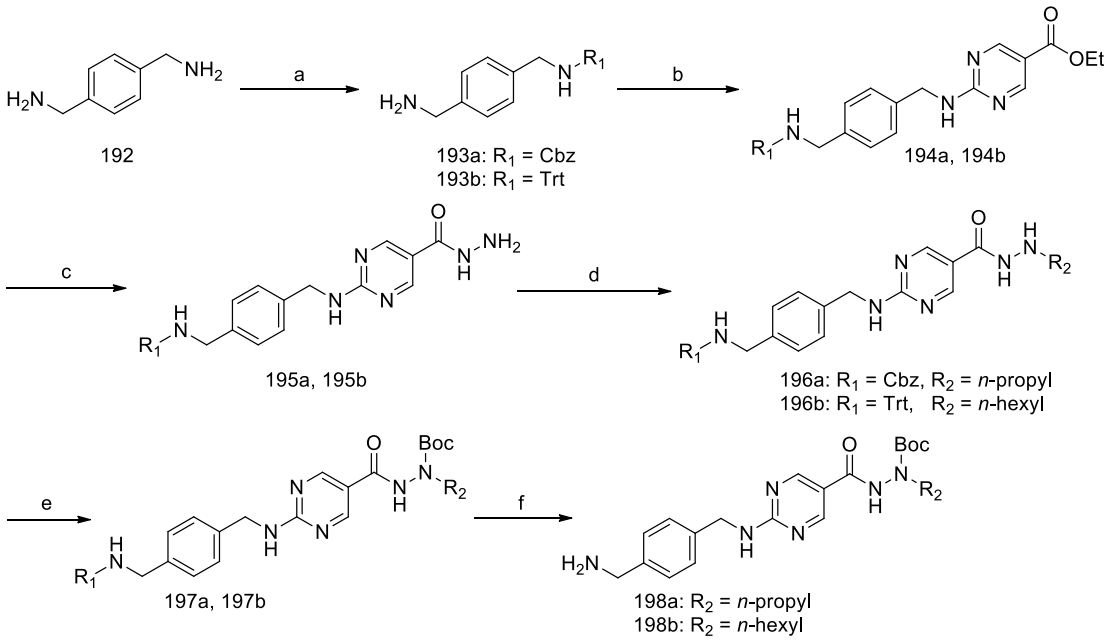


Figure 89. Reagents and conditions: (a) CbzCl 1.0 eq, TEA 1.0 eq, DCM, ice bath, 3 h for **193a**; or TrtCl 1.0 eq, dioxane, rt, overnight for **193b**; (b) **92** 1.0 eq, DIPEA 2.5 eq, DCM, rt; (c) Hydrazine monohydrate 30.0 eq, EtOH, reflux, 3 h; (d) Aldehyde 1.05 eq, pTSA 0.05 eq, MeOH, 2 h and then

$NaBH_4$ 4.0 eq, 1 h; (e) $(Boc)_2O$ 1.5 eq, TEA 3.0 eq, DCM, rt; (f) Pd/C 10% wt, H_2, MeOH, 2 h, rt for **198a**; or AcOH/H_2O (4 : 1), 60 ^oC, 1.5 h for **198b**.

The first series was synthesized by monoprotection of one amino group using Cbz and Trt (Figure 89, step a) followed by a described nucleophlic substitution reaction (step b) giving intermediate **194a** and **194b** which were converted to hydrazides **195a** and **195b**, and later alkylated resulting in **196a** and **196b**. The latter compounds were protected by Boc and then followed by Cbz- or Trt-deprotection leading to Boc-protected HDAC binders **198a** and **198b** (Figure 89).

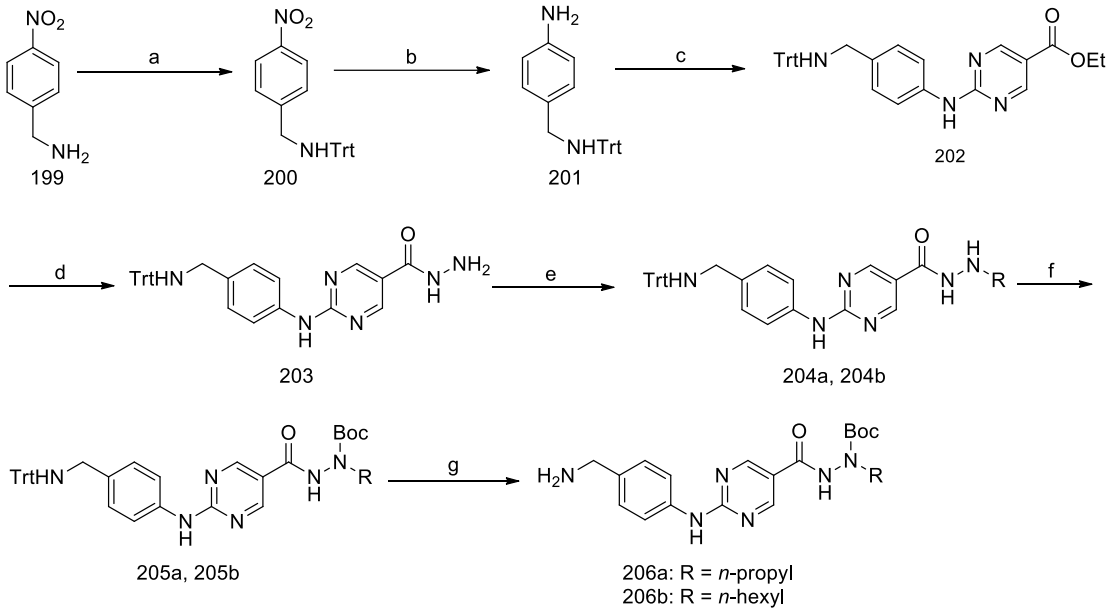


Figure 90. Reagents and conditions: (a) TrtCl 1.0 eq, TEA 2.5 eq, DCM, rt, 7 h; (b) Pd/C 10% wt, ammonium formate 4.0 eq, EtOAc/MeOH (4 : 1), 40-50 ^oC, 1 h; (c) **92** 1.0 eq, K_2CO_3 2.5 eq, DMF 80-90 ^oC, overnight; (d) Hydrazine monohydrate 30.0 eq, EtOH, reflux, 3 h; (e) Aldehyde 0.95 eq, pTSA 0.05 eq, MeOH, 2 h and then $NaBH_4$ 4.0 eq, 1 h; (f) $(Boc)_2O$ 1.1 eq, TEA 2.0 eq, THF, rt; (g) AcOH/H_2O (4 : 1), 60 ^oC, 1.5 h.

The other series were obtained starting from compound (4-nitrophenyl)methanamine (**199**) followed by Trt group protection yielding intermediate **200** which was reduced to **201** and coupled with compound **92** giving intermediate **202**. It is worth noting that step c in Figure 90 was a temperature sensitive procedure because the yield decreased and the reaction mixture become more chaotic when temperatures beyond 100 ^oC. Like the above series, **202** could be converted into the Boc-protected HDAC binders **206a** and **206b** (Figure 90).

4.1.4 E3 ubiquitin ligase ligands connected with HDAC binders via linkers

4.1.4.1 First series of degraders: aminobenzamide-triazole 6-carbon linker-pomalidomide

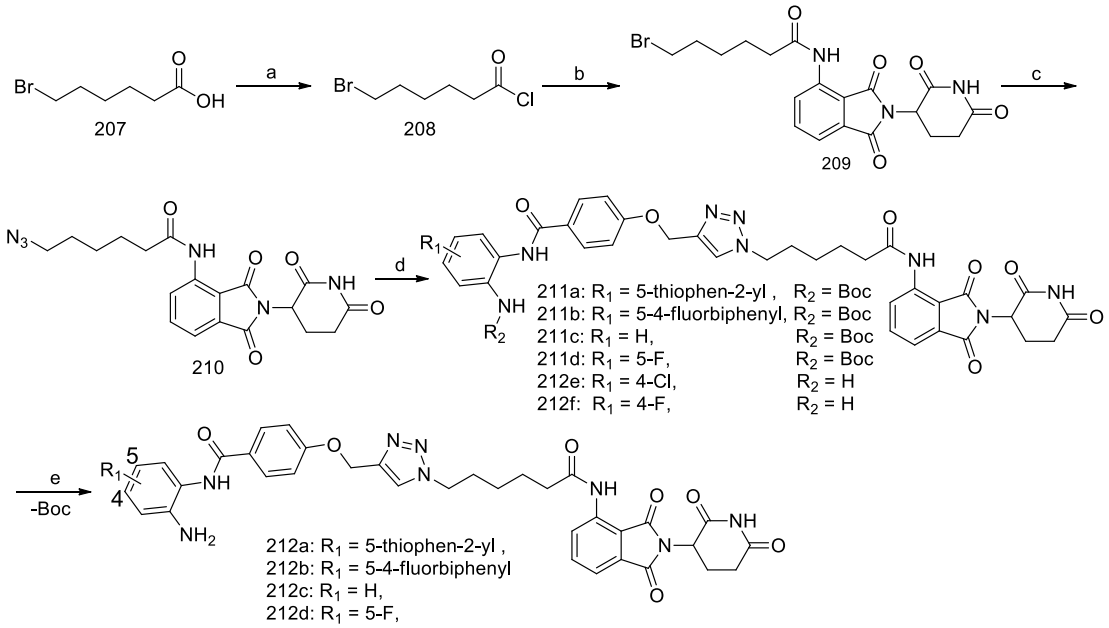


Figure 91. Reagents and conditions: (a) $SOCl_2$ 5.0 eq, reflux, 2 h, (b) **139** 0.33 eq, THF, reflux, overnight; (c) NaN_3 2.0 eq, DMF, 80 °C, overnight; (d) **166a/b** or **169a/b** or **174a/b** 0.75 eq, $CuSO_4 \cdot 5H_2O$ 0.015 eq, L-Sodium ascorbate 0.075 eq, t-BuOH/H_2O/DMF (5 : 2.5 : 1), 60 °C, 8 h; (e) TFA/DCM or HCl/dioxane (4M)/DCM, rt, 3 h.

Reported methods were followed and modified,[161-162] the synthesis was started through the acylation of 6-bromohexanoic acid (**207**) resulting in intermediate **208** which was conjugated with pomalidomide under reflux. The obtained amide **209** was substituted by sodium azide giving intermediate **210** which was converted to the triazole containing intermediates **211a-211d**. These were attained through a copper catalyzed click reaction with alkynes (Figure 91, step d) in which the solubility of the reaction mixture was improved by adding DMF and this was different from reported method.[161] For these Boc-protected intermediates **211a-211d**, a last Boc-deprotection was performed giving the corresponding PROTACs **212a-212d** (Figure 91).

4.1.4.2 Second series of degraders: aminobenzamide-CRBN-based PROTACs with alkyl linker

Amino acids (with 4- to 7-carbon spacers) **213a-213d** were utilized as starting materials, which were first protected by tert-butyl group followed by nucleophilic substitution of the fluoro thalidomide **141** under microwave assisted conditions giving intermediates **215a-215d**. Boc-deprotection and condensation with aminobenzamindes afforded the PROTACs **218a** and **218f-218i** as well as intermediates **217b-217e**, **217j-217l**. The latter were transformed to the corresponding PROTACs by a Boc-deprotection procedure (Figure 92, step d).[163]

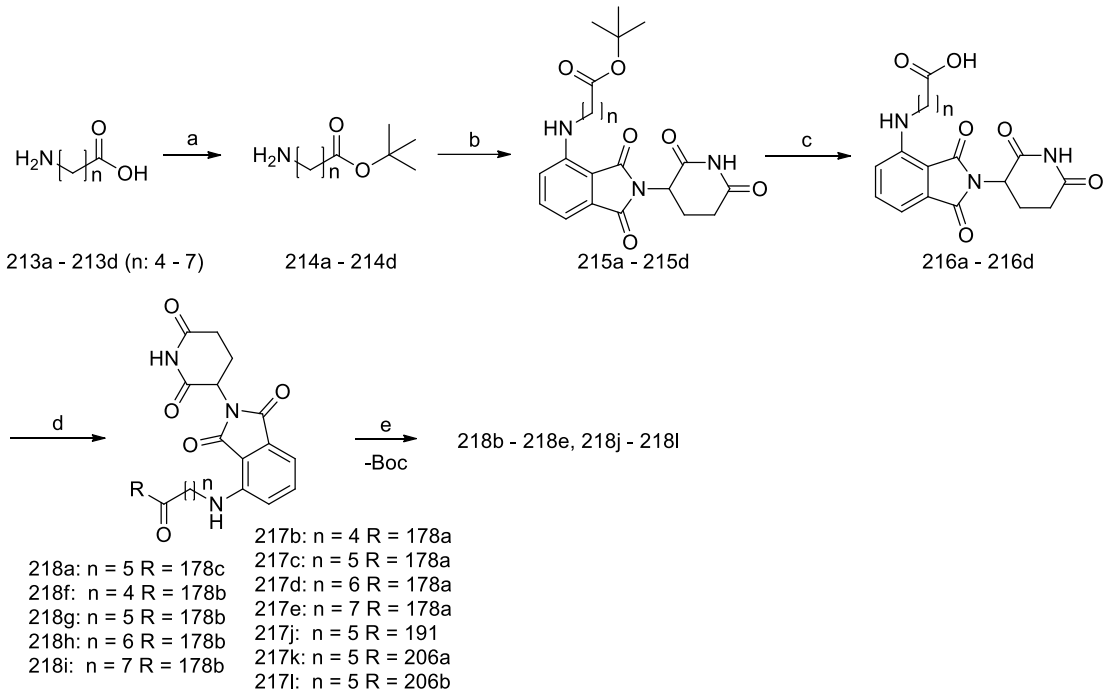


Figure 92. Reagents and conditions: (a) $SOCl_2$ 10.0 eq, rt, 2 h; then $NaHCO_3$ 2.2 eq, t-BuOH, rt, overnight; (b) **262** 0.33 eq, DIPEA 1.0 eq, NMP, 110 °C, microwave, 2 h; (c) TFA/DCM, rt, 3 h; (d) **178a-c** or **191** or **206a/b** 0.83 eq, HATU 1.25 eq, DIPEA 4.17 eq, DMF, rt, 10 min; (e) TFA/DCM or HCl/dioxane (4M), DCM, rt, 3 h; (f) **183** 1.3 eq, TCFH 1.2 eq, NMI 3.5 eq, CH_3CN.

However, the amide bond formation between **262** and substrate **216b** had to be catalyzed by a TCFH-NMI (N,N,N′,N′-tetramethylchloroformamidinium hexafluorophosphate and N-methylimidazole) system instead of HATU because their activated intermediate was more active than that of HATU, see Figure 93.[164-165]

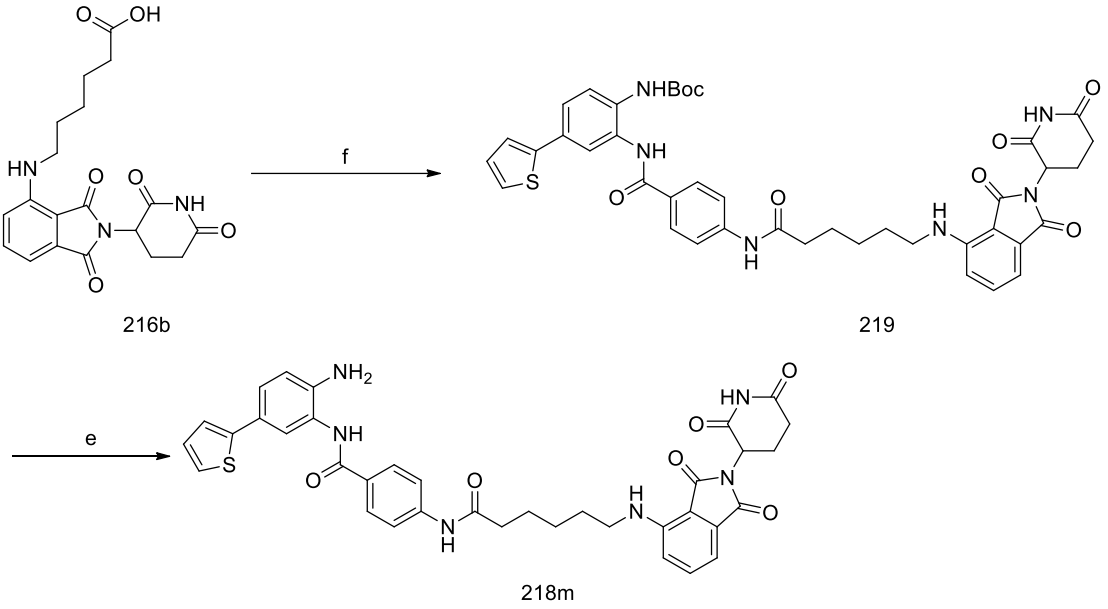


Figure 93. Alternative method for amide formation.

4.1.4.3 Third series of degraders: aminobenzamide and alkylhydrazide -CRBN based PROTACs with alkyl linker

For the challenging lenalidomide-baded degraders we could only obtain **224a** and **224b** that with an adequate purity due to the tricky purification work of this series. Briefly, 6-bromohexanoic acid was protected by tert-butyl, the resulting intermediate **220** was reacted with **144** and followed by Boc-deprotection giving intermediate **222**. This was converted to **223a** and **223b** through amide formation, and an additional Boc-deprotection yielded PROTACs **224a** and **224b** (Figure 94).[163]

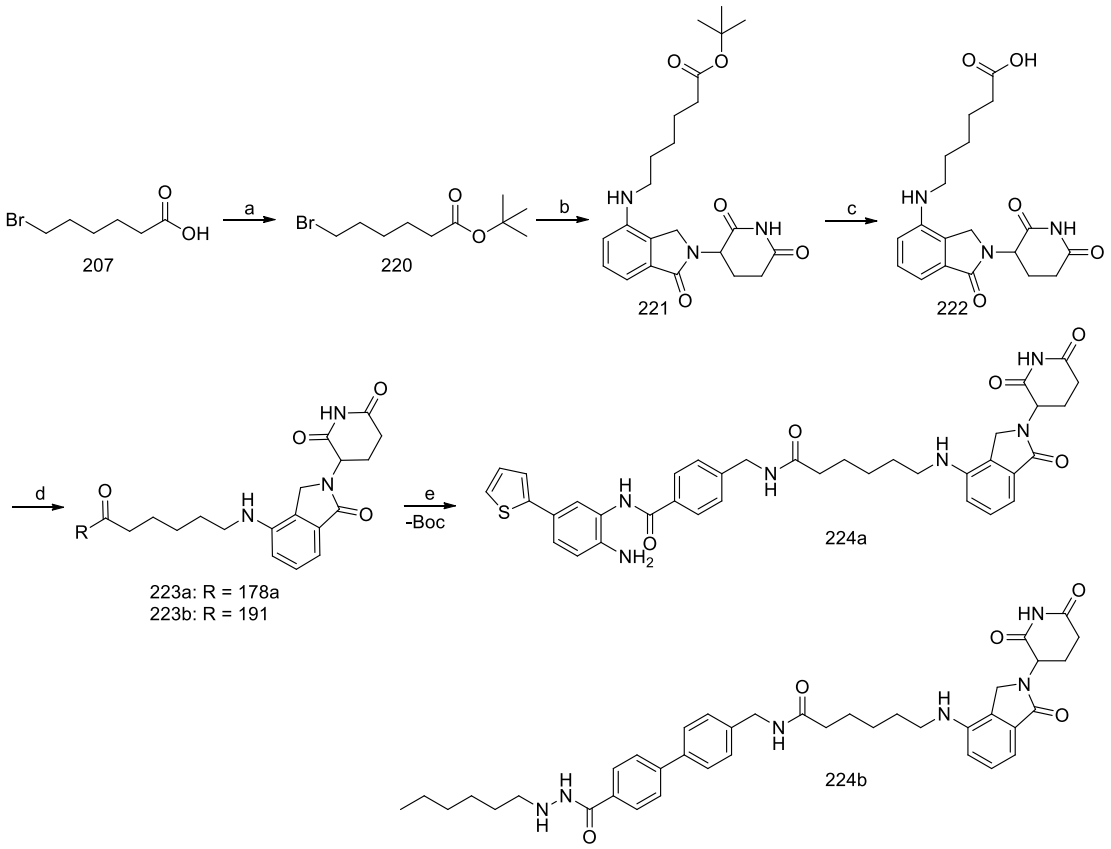


Figure 94. Reagents and conditions: (a) $SOCl_2$ 10.0 eq, rt, 2 h, $NaHCO_3$ 2.2 eq, t-BuOH, rt, overnight; (b) **144** 0.83 eq, DIPEA 2.5 eq, NMP, 110 °C, overnight; (c) TFA/DCM, rt, 3 h; (d) **178a** or **191** 0.91 eq, HATU 1.1 eq, DIPEA 2.73 eq, DMF, rt, 10 min; (e) TFA/DCM, rt, 3 h.

4.1.4.4 Fourth series of degraders: aminobenzamide and alkylhydrazide-based degraders with alkyl linker and hydrophobic adamantyl tag

227a-227i could be produced by two methods: the starting amino acids were protected by either a trifluoroacetyl group or Boc group, and the resulting linkers were coupled with the corresponding amine mediated by HATU. This was followed by a deprotected group deprotection giving the corresponding free base **227** which was condensed with adamantaneacetic acid to yield the final degraders or Boc-protected degraders. A further Boc-deprotection procedure afforded all degraders of this series (Figure 95).

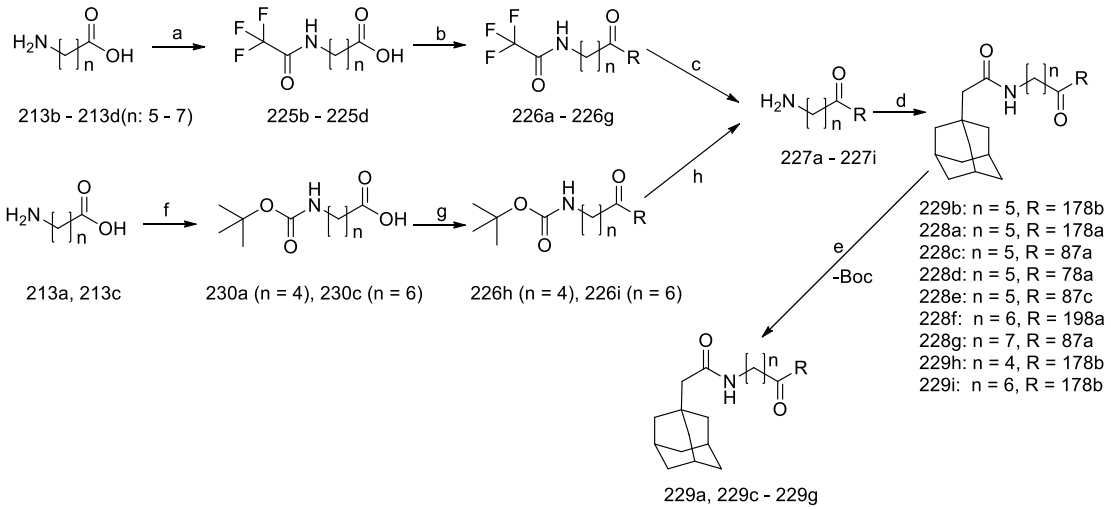


Figure 95. Reagents and conditions: (a) TFAA 2.5 eq, 80 °C, 1 h; (b) **178a/b** or **87a/c** or **78a** or **198a** 0.91 eq, HATU 1.1 eq, DIPEA 2.73 eq, DMF, rt, 10 min; (c) K_2CO_3 4.0 eq, MeOH/H_2O (1 : 1), rt, overnight; (d) **157** 1.2 eq, HATU 1.2 eq, DIPEA 3.0 eq, DMF; (e) TFA/DCM or HCl/dioxane (4M), rt, 3 h; (f) $(Boc)_2O$ 1.1 eq, NaOH 1.0 eq, dioxane/H_2O (2 : 1), rt, overnight; (g) **178b** 0.91 eq, HATU 1.1 eq, DIPEA 2.73 eq, DMF, rt, 10 min; (h) TFA/DCM, rt, 3 h.

The following alternative route was also implemented: the amino acid was protected by Cbz, then reacted with the amine **157** followed by Cbz-removal giving intermediate **233**. The latter was again condensed with adamantaneacetic acid resulting in the Boc-protected compound 2**29j-1** which underwent a Boc-deprotection procedure to yield **229j** (Figure 96).

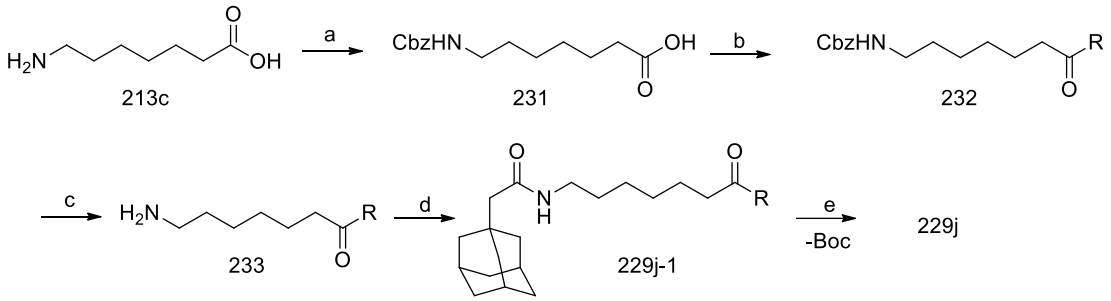


Figure 96. Reagents and conditions: (a) CbzCl 1.5 eq, K_2CO_3 2.0 eq, H_2O, rt, overnight; (b) **87a** 0.91 eq, HATU 1.0 eq, DIPEA 2.73 eq, DMF, rt, 10 min; (c) Pd/C 10% wt, H_2, rt, 2 h; (d) **157** 1.0 eq, HATU 1.2 eq, DIPEA 3.0 eq, DMF; (e) TFA/DCM, rt, 3 h.

The negative control **229k** could be easily synthesized through acylation with acetyl chloride (Figure 97).

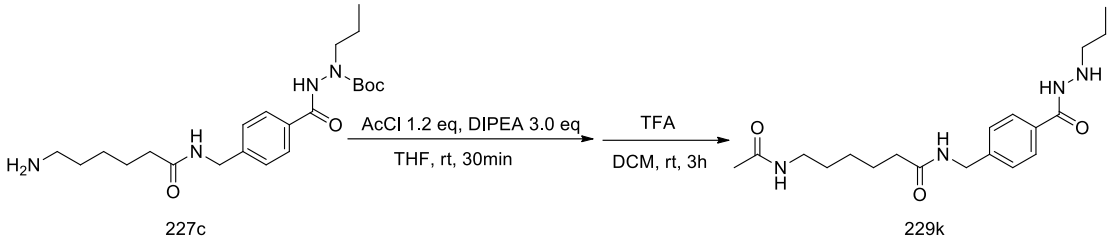


Figure 97. Synthesis method for negative control **229k**.

The adamantaneamide-*NH* was also modified by methylation using the following procedure showed in Figure 98. The amino group of the starting amino acid **213b** was protected by Boc and then the carboxylic acid tail was protected by a benzyl group yielding compound **235**. N-methylation was then performed (step c), the Boc group was cleaved and the resulting intermediate **237** was coupled with adamantaneacetic acid giving compound **238**. Afterwards, the Cbz protective group was removed, and the product was condensed with amine **87a** yielding the corresponding product **240**. Boc-deprotection afforded degrader **229l** (Figure 98).

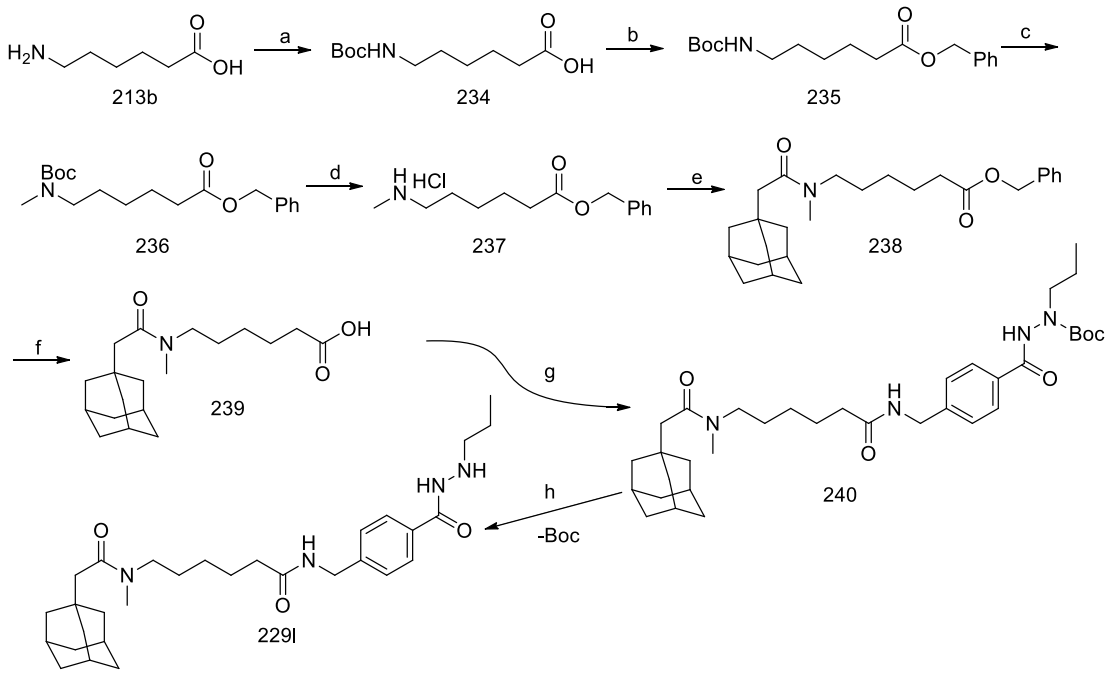


Figure 98. Reagents and conditions: (a) $(Boc)_2O$ 1.1 eq, TEA 2.0 eq, acetone/H_2O (1 : 1), rt, overnight; (b) Benzyl chloride 1.0 eq, K_2CO_3 3.0 eq, DMF, 80-90 ºC, overnight; (c) NaH 1.5 eq, CH_3I 4.0 eq, THF, rt, overnight; (d) HCl/dioxane (4M), DCM, rt, overnight; (e) **157** 1.2 eq, HATU 1.2 eq, DIPEA 4.0 eq, DMF, rt; (f) $LiOH{\cdot}H_2O$ 2.0 eq, reflux, 30 min; (g) **87a** 0.83 eq, HATU 1.0 eq, DIPEA 2.5 eq, DMF, rt, 10 min; (h) TFA/DCM, rt, 3 h.

The diamine linkers of **241** including 4- and 6-carbons needed to be first mono-protected by a Boc-group, and the free amino group was converted to the amide by reaction with carboxylic acid **187** to afford intermediate **243**. The Boc-group was removed and reacted with adamantaneacetic acid to afford final products **229m** and **229n** (Figure 99).

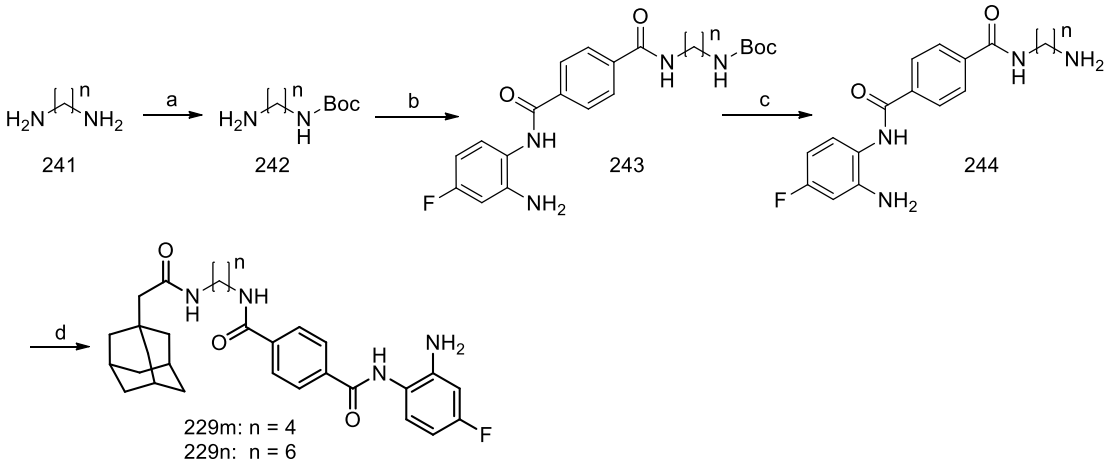


Figure 99. Reagents and conditions: (a) $(Boc)_2O$ 0.2 eq, $CHCl_3$, overnight; (b) **187** 0.91 eq, HATU 1.1 eq, DIPEA 2.73 eq, DMF, rt, 10 min; (c) TFA/DCM, rt, 3 h; (d) **157** 0.9 eq, HATU 1.0 eq, DIPEA 5.0 eq, DMF, rt, 10 min.

4.1.4.5 Fifth series degraders: aminobenzamide and alkylhydrazide-VHL-based PROTACs with alkyl linker

Two ways to produce these series compounds were developed. The first one started from dicarboxylic acid monoprotection by a benzyl group, followed by connecting with the amine position of multifarious HDAC binders resulting in intermediates **248a-248f** which were converted to PROTACs or Boc-protected PROTACs through HATU mediated amide bond formation reactions (Figure 100, step d). The corresponding intermediates underwent a Boc-removal reaction giving the degraders **250a**, **250c**, **250e** and **250f**.

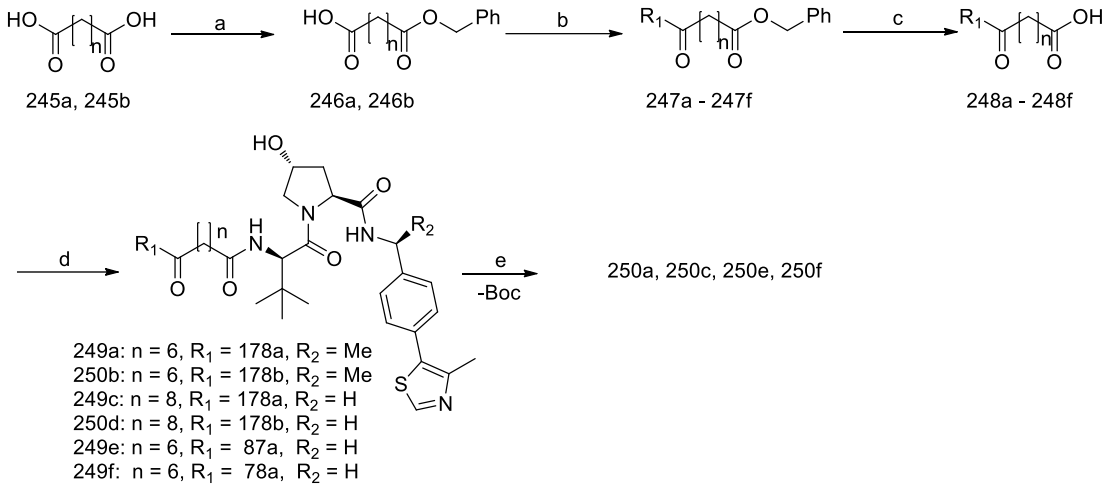


Figure 100. Reagents and conditions: (a) Benzyl bromide 0.4 eq, $NaHCO_3$ 1.6 eq, DMF/dioxane (1 : 1), 90 °C, overnight; or benzyl chloride 0.33 eq, $NaHCO_3$ 1.67 eq, DMF/dioxane (1 : 1), overnight; (b) **178a/b** or **78a** or **87a** 1.2 eq, HATU 1.2 eq, DIPEA 3.0 eq, DMF, rt, 10 min; (c) $LiOH{\cdot}H_2O$ 2.0-4.0 eq, THF/H_2O (5 : 1), reflux, 30 min; (d) **156 or 155** 1.1 eq, HATU 1.2 eq, DIPEA 4.0 eq, DMF, ice bath, 30 min; (e) HCl/dioxane (4M), DCM, ice bath, 3 h.

On the contrary, the second route started from the condensation of the VHL liand with monocarboxylic acid (Figure 101, step a), the resulting intermediate **252** was hydrolyzed by $LiOH{\cdot}H_2O$ in a mixture of MeOH/H_2O giving **253** which was reacted with the amines **206a** and **206b** to yield the compounds **225a** and **225b** (Figure 101).

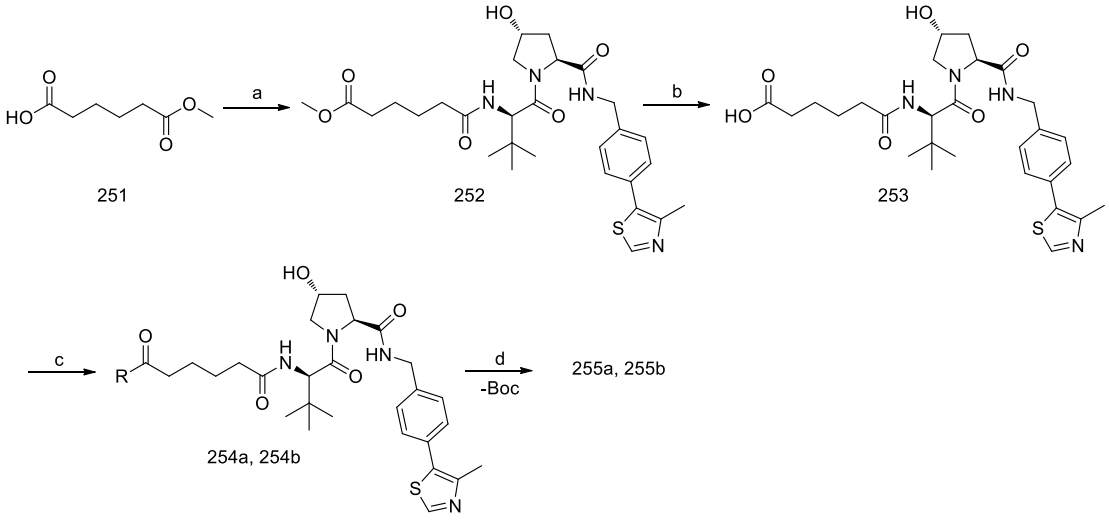


Figure 101. Reagents and conditions: (a) **155** 1.0 eq, HATU 1.1 eq, DIPEA 4.0 eq, DMF, rt, 30 min; (b) $LiOH{\cdot}H_2O$ 5.0 eq, MeOH/H_2O (5 : 1), 2.5 h; (c) **206a/b** 1.2 eq, HATU 1.1 eq, DIPEA 3.0 eq, DMF, 30 min; (d) HCl/dioxane (4M), DCM, ice bath, 3 h.

4.1.4.6 Sixth series of degraders: alkylhydrazide-CRBN-based PROTACs with alkyne linker

The final lenalidomide-based degraders were obtained starting from hex-5-ynoic acid (**256**) which was first protected and sunsequently subjected to another palladium catalyzed reduction step with **147** (Figure 102, stepb). The product **257** was hydrolyzed to the carboxylic acid **258** which was coupled with the amino group of **198a** and **198b** resulting in **259a** and **259b**. The final compounds **260a** and **260b** were generated by a Boc-deprotection procedure (Figure 102).[166]

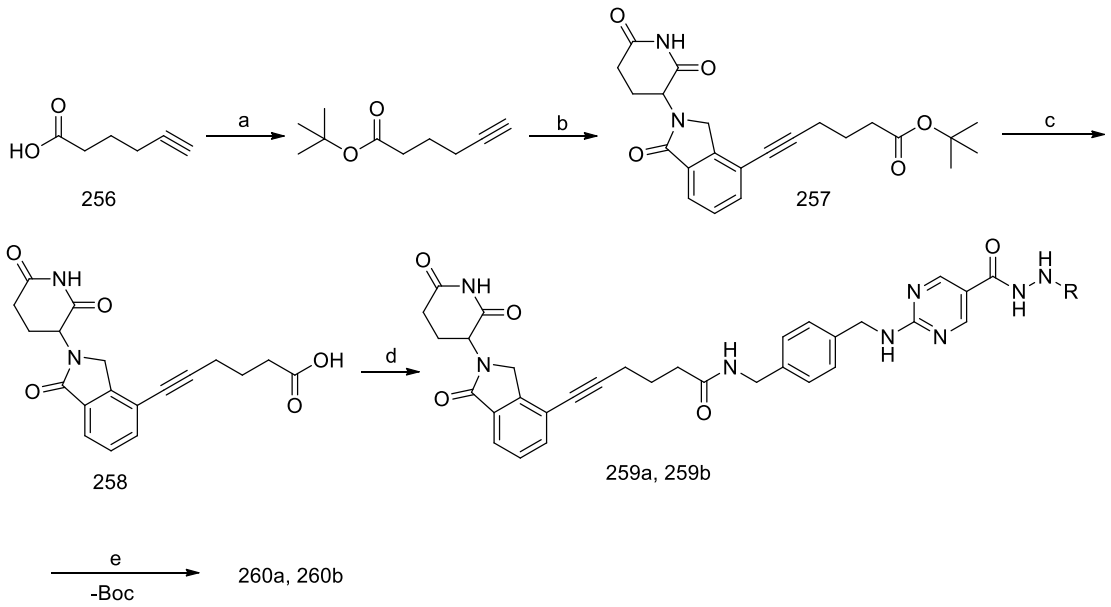
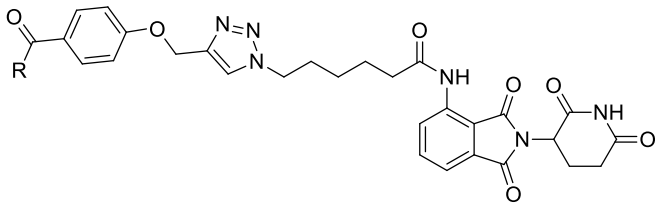
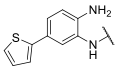
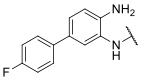
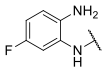
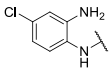
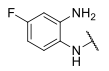


Figure 102. Reagents and conditions: (a) t-BuOH 3.5 eq, TFAA 2.2 eq, THF dry, ice bath to 3.5 h, rt, overnight, (b) **147** 0.5 eq, $Pd(PPh_3)_2Cl_2$ 0.1 eq, CuI 0.2 eq, DMF/TEA (2 : 1), 90 °C, 3 h; (c) TFA/DCM, rt, 1.5 h; (d) **198a/b** 1.2 eq, HATU 1.1 eq, DIPEA 3.0 eq, DMF, rt, 10 min; (e) HCl/dioxane (4M), MeOH/DCM (1 : 5), rt, 3 h.

4.2 HDAC inhibitory activity of designed HDAC degraders

Table 19. Enzymatic activity of PROTACs consisted of aminobenzamide-triazole linker-pomalidomide

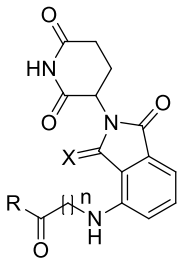


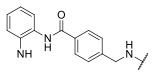
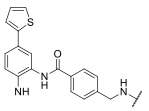
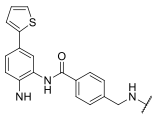
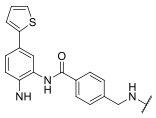
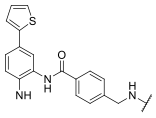
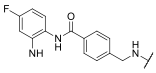
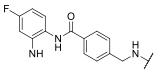
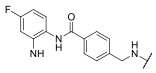
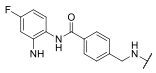
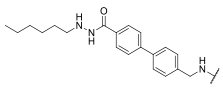
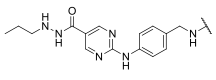
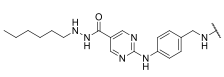
Cpd No.	R	hHDAC inhibition (μM)[a]			
		1	2	3	8
212a PSP4		0.084 ± 0.006	0.60 ± 0.04	> 20	18.3% @ 10 μM 9.9% @ 1 μM
212b PSP3		0.093 ± 0.007	0.80 ± 0.09	> 20	0% @ 10 μM
212c PSP9		> 20	> 20	17 ± 3	n.t.[b]
212d PSP1		4.76 ± 0.42	2.62 ± 0.02	1.35 ± 0.04	52,8% @ 10 μM -6,8% @ 1 μM
212e PSP8		5.8 ± 0.5	4.3 ± 0.4	5.3 ± 0.3	n.t.
212f PSP2		21.13 ± 0.184	16.11 ± 1.89	1.17 ± 0.03	2.1% @ 10 μM 0% @ 1 μM

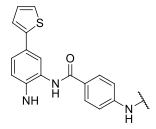
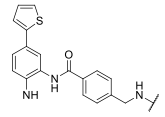
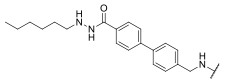
[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value. [b]: n.t = not tested.

Preliminary inhbitory activities of the first series of PROTACs on class I HDACs are shown in Table 19. These results definitely supported our hypothesis that 5-aryl-aminobenzamides showed preference for HDAC1/2 (Table 19, **212a-212d)** with excellent potency, meanwhile substitution on the aminobenzamide moiety at the 4-position with an electron withdrawing small substituent like F resulted in a slight selectivity for HDAC3 binding with only moderate inhibitory potency in **212f** (PSP2). However, the 4-position chloro-substituted aminobenzamides **212e** (PSP8) showed no evident selectivity for HDAC1-3.

Table 20. Enzymatic activity of PROTACs consisted of aminobenzamide or alkylated hydrazide-alkyl linker-pomalidomide or lenalidomide



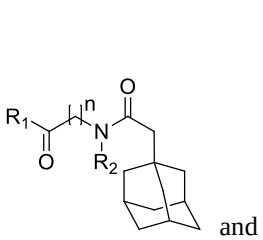
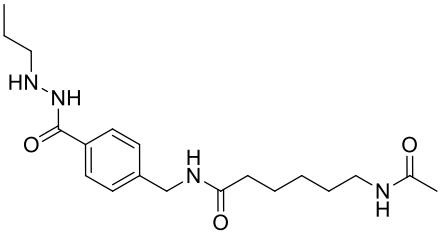
cpd No.	R	n	X	hHDAC inhibition (μM)[a]			
				1	2	3	8
218a PSP12		5	O	2.3 ± 0.2	1.3 ± 0.01	3.3 ± 0.1	n.t.[b]
218b PSP15		4	O	0.043 ± 0.008	0.12 ± 0.01	> 20	n.t.
218c PSP10		5	O	0.11 ± 0.02	0.36 ± 0.02	> 20	n.t.
218d PSP16		6	O	0.40 ± 0.09	0.65 ± 0.08	> 20	n.t.
218e PSP17		7	O	0.56 ± 0.11	3.0 ± 0.4	> 20	n.t.
218f PSP18		4	O	8.5 ± 0.6	2.6 ± 0.2	2.4 ± 0.1	n.t.
218g PSP11		5	O	8.8 ± 0.5	4.6 ± 0.3	2.7 ± 0.2	n.t.
218h PSP19		6	O	8.8 ± 0.7	5.1 ± 0.4	2.8 ± 0.2	n.t.
218i PSP20		7	O	7.4 ± 0.9	4.3 ± 0.5	3.3 ± 0.3	n.t.
218j PSP64		5	O	13% @ 1 μM 43% @ 10 μM	16% @ 1 μM 39% @ 10 μM	8% @ 1 μM 23% @ 10 μM	0.085 ± 0,06
218k PSP77		5	O	0.69 ± 0.06	1.6 ± 0.1	0.25 ± 0.01	0.32 ± 0.02
218l PSP75		5	O	44.8% @ 1 μM 71.1% @ 10 μM	34.5% @ 1 μM 64.5% @ 10μM	28.6% @ 1 μM 56.1% @ 10μM	0.031± 0.002

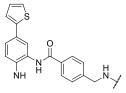
218m PSP5		5	O	0.26 ± 0.04	1.9 ± 0.3	61 ± 9	n.t.
224a PSP23		5	H	n.t.	n.t.	n.t.	n.t.
224b PSP65		5	H	28% @ 1 µM 41% @ 10 µM	22% @ 1 µM 40% @ 10 µM	7% @ 1 µM 18% @ 10 µM	0.092 ± 0.06

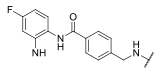
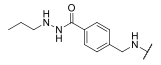
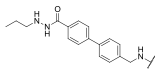
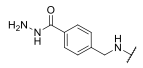
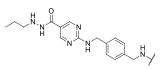
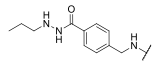
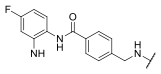
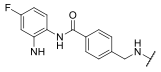
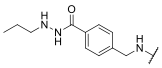
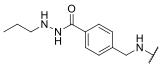
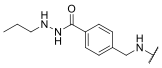
[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value. [b]: n.t = not tested.

PROTAC **218a** (PSP12), containing an unmodified aminobenzamide HDAC-ligand, showed only moderate inhibitory activity against HDAC1-3 with no obvious selectivity. When position-5 was substituted by thiophene and a 4-7 carbon long linker was used in PROTACs of **218b**-**218e** (PSP10, PSP16-PSP18) and **218m** (PSP5)**,** selectivity for HDAC1 and 2 over HDAC3 was observed. PROTACs based on fluorine 4-substituted-aminobenzamides and harnessing 4 to 7 carbon long linker did not remain selective for HDAC3 over HDAC1/2 unlike we designed in **218f**-**218i** (PSP11 and PSP18-PSP20). In addition, hydrazide-based PROTACs **218j** (PSP64), **218l** (PSP75) and **224b** (PSP65) also agreed with the potency and selectivity profiles of their ligands characters. In the case of theses PROTACs, *n*-propyl-hydrazides like **218k** (PSP77) were designed to selectively target HDAC3 as shown in Table 20.

Table 21. Enzymatic activity of HyTs consisted of aminobenzamide or alkylated hydrazide-alkyl linker-adamantane


and

229k

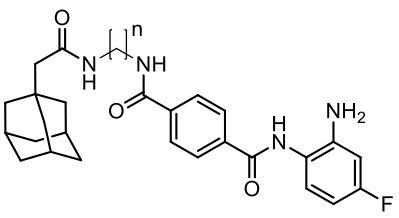
Cpd No.	R_1	n	R_2	hHDAC inhibition (µM)[a]			
				1	2	3	8
229a PSP21		5	H	0.12 ± 0.03	0.28 ± 0.03	> 20	n.t.[b]

229b PSP22		5	H	5.6 ± 0.7	3.5 ± 0.4	2.6 ± 0.3	n.t.
229c PSP33		5	H	0.46 ± 0.03	0.91 ± 0.03	0.23 ± 0.02	0.22
229d PSP34		5	H	0.78 ± 0.11	2.7 ± 0.4	0.22 ± 0.01	n.t.
229e PSP55		5	H	n.t.	n.t.	n.t..	n.t..
229f PSP58		6	H	67% @ 1 μM 82% @10 μM	53% @ 1 μM 90% @ 10 μM	88% @ 1 μM 98% @ 10 μM	72% @ 1 μM 95% @ 10 μM
229g PSP54		7	H	60% @ 1 87% @ 10	49% @ 1 μM 93% @ 10 μM	0.35 ± 0.01	n.t.
229h PSP29		4	H	> 20	15 ± 1	10 ± 1	n.t.
229i PSP30		6	H	8.3 ± 0.8	3.8 ± 0.2	2.8 ± 0.1	n.t.
229j PSP53		6	H	63% @ 1 μM 88% @ 10 μM	52% @ 1 μM 93% @ 10 μM	0.22 ± 0.01	0.089 ± 0.006
229k PSP56		-	-	31% @ 1 μM 70% @ 10 μM	25% @ 1 μM 79% @ 10 μM	48% @ 1 μM 87% @ 10 μM	28% @ 1 μM 77% @ 10 μM
229l PSP60		5	Me	57% @ 1 μM 81% @ 10 μM	48% @ 1 μM 92% @ 10 μM	82% @ 1 μM 96% @ 10 μM	86% @ 1 μM 96% @ 10 μM

[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value. [b]: n.t = not tested.

Compared with the foregoing CRBN PROTACs, inhibitory activities of adamantly-based degraders on HDACs diminished to some extent. For example **229a** (PSP21) (Table 21) still retained relatively good selectivity over HDAC3 although its activity for HDAC1/2 was comparable to **218c** (PSP10) in Table 20. Surprisingly, the *n*-propylhydrazide-based HyTs **229c** (PSP33) and **229j** (PSP53), designed to selectively target HDAC3, showed a good HDAC8 inhibitory activity (**229c** with IC_{50} = 0.22 μM for HDAC8, **229j** with IC_{50} = 0.089 μM for HDAC8) (Table 21).

Table 22. Enzymatic activity of HyTs consisted of aminobenzamide-alkyl linker-adamantane

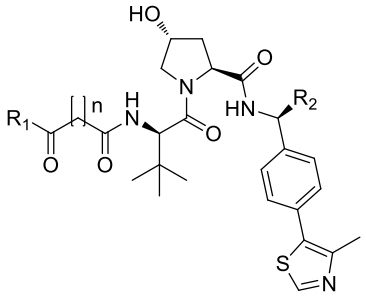


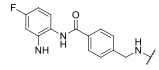
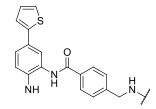
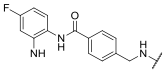
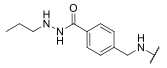
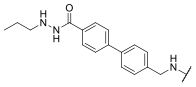
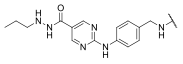
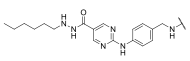
Cpd No.	n	hHDAC inhibition (μM)[a]		
		1	2	3
229m PSP31	4	5.4 ± 0.3	2.6 ± 0.2	2.1 ± 0.1
229n PSP32	6	10 ± 1	4.4 ± 0.3	3.8 ± 0.2

[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value.

HyTs **229m** (PSP31) and **229n** (PSP32) exhibited only micromolar range inhibitory activity towards HDAC1-3 without significant selectivity (Table 22).

Table 23. Enzymatic activity of PROTACs consisted of aminobenzamide or alkylatedhydrazide-alkyl linker-VHL ligand

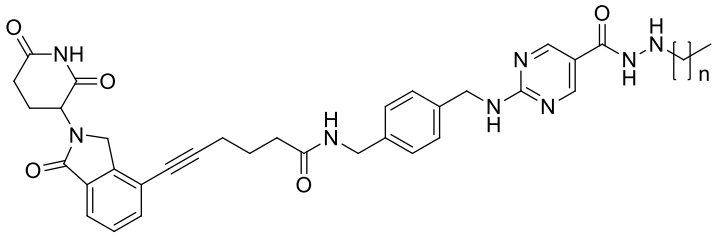


Cpd No.	R1	n	R_2	hHDAC inhibition (μM)[a]			
				1	2	3	8
250b PSP26		6	Me	14 ± 1	8.5 ± 0.7	14 ± 1	n.t.
250c PSP27		8	H	0.19 ± 0.03	0.90 ± 0.09	> 20	n.t.
250d PSP28		8	H	4.7 ± 0.6	4.2 ± 0.3	4.1 ± 0.2	n.t.
250e PSP35		6	H	0.46 ± 0.04	0.72 ± 0.04	0.46 ± 0.03	n.t.
250f PSP36		6	H	1.6 ± 0.1	2.5 ± 0.2	0.61 ± 0.05	n.t.
255a PSP78		4	H	47.0% @ 1 μM 84.3% @ 10 μM	36.7% @ 1 μM 82.9% @ 10 μM	78.1% @ 1 μM 97.9% @ 10 μM	0.13 ± 0.009
255b PSP76		4	H	2.0 ± 0.3	4.1 ± 0.2	> 20	0.041 ± 0.03

[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value. [b]: n.t = not tested.

In the VHL ligand-based PROTACs, compounds with longer linkers (8 carbons) were slightly more active than corresponding shorter linker (6 carbons) when 5-thienyl-aminobenzamides in **250c** (PSP27) or 4-fluoro-aminobenzamides were used in **250b** (PSP26) and **250d** (PSP28) as HDAC ligands. However, most alkylhydrazides-based PROTACs **250e-255b** (Table 23) exhibited submicromolar inhibitory activity on their corresponding target.

Table 24. Enzymatic activity of PROTACs consisted of alkylated hydrazide-alkyne linker-lenalidomide



Cpd No.	n	hHDAC inhibition (μM)[a]			
		1	2	3	8
259a PSP79	2	0.32 ± 0.02	0.6 ± 0.02	0.17 ± 00	0.13 ± 0.07
259b PSP80	5	59.7% @ 1 μM 89.7% @ 10 μM	61.7% @ 1 μM 93.7% @ 10 μM	48.9% @ 1 μM 82.1% @ 10 μM	0.017 ± 0.001

[a]: Inhibition percentage of each compound in corresponding concerntration or calculated IC_{50} value.

When an alkyne linker and lenalidomide part were used in PROTACs **259a** (PSP79) and **259b** (PSP80), submicromolar range inhibitory activity were obsereved against the targets. Interestingly, the planned HDAC3 PROTAC **259b** (PSP80) rather showed preference for HDAC8 inhibition (Table 24).

4.3 *In vitro* stability study

As mentioned previously we tested the chemical stability by generating a 10 mM solution in DMSO and then diluted them with the mixtures of DMEM and MeOH or ACN to affard the final solutions (**229c**, **229e**, **250c**, **250e** and **250f** of 10 μM in DMEM/DMSO/MeOH as 50/10/40 percentage; the rest of following ones 10 μM in DMEM/DMSO/ACN as 50/10/40 percentage). All the resulting solutions were incubated at 37 ºC for 72 h and at the same time corresponding solutions were detected by HPLC to quantify their degradation results at each time point of 0 h, 6 h, 12 h, 24 h, 28 h and 72 h. The final datas for each compound remained are summarised as bellow (Table 25). This data was provided by Dr. Matthias Schmidt, Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Halle, Germany.

Table 25. Remaining parent compound at different time points. The less stable compound **229e** (PSP55) is marked in blue.

Compounds	0 h (%)	6 h (%)	12 h (%)	24 h (%)	48 h (%)	72 h (%)	Degrader type
218j(PSP64)	100.0	98.5	96.9	91.0	78.9	67.5	
218k(PSP77)	100.0	94.0	89.0	80.7	64.2	54.0	A
218l(PSP75)	100.0	92.9	86.4	75.3	56.4	44.8	
224b(PSP65)	100.0	97.3	93.8	89.5	87.0	86.2	B
229c(PSP33)	100.0	99.1	98.6	92.4	90.6	83.2	C
229e(PSP55)	100.0	93.7	83.7	68.8	41.5	30.8	
250c(PSP27)	100.0	100.0	100.2	102.0	103.4	102.7	
250e(PSP35)	100.0	98.9	98.6	97.5	94.3	86.8	D
250f(PSP36)	100.0	99.3	98.8	97.9	94.2	90.1	
259a(PSP79)	100.0	92.4	83.6	72.1	54.1	45.4	E
259b(PSP80)	100.0	98.1	95.9	89.1	71.5	60.4	

The stability testing results suggested that almost all the tested compounds including the alkylated hydrazide-pomalidomide PROTACs (A), alkylated hydrazide-lenalidomide PROTACs (B), alkylated hydrazide-adamantane Hyts (C), aminobenzamide or alkylated hydrazide-VHL ligand PROTACs (D), alkylated hydrazide-alkyne linker-lenalidomide PROTACs (E) were relatively stable under the

used conditions. Even after 24 h, all the compounds still remained over 70% except the negative control (unsubtituted hydrazide) **229e**.

4.4 Biological activity assessment in cells

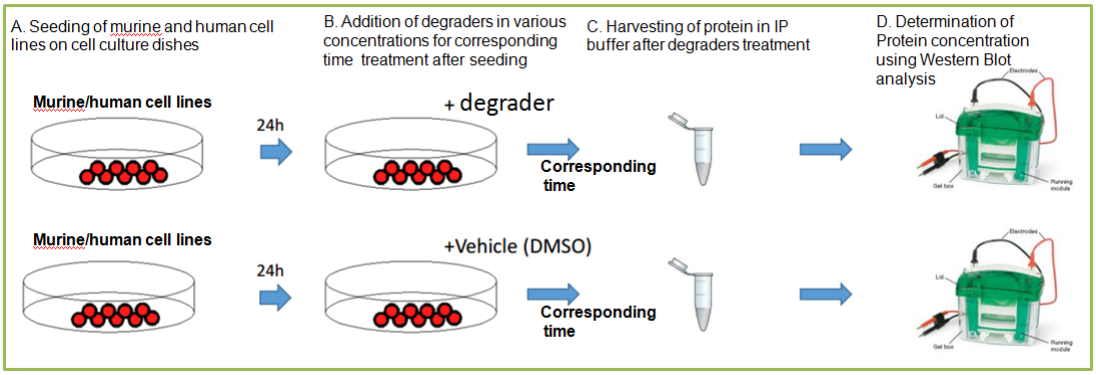


Figure 103. Cellular screening to determine the HDAC degradation potency of the synthesized degraders. The figure was provided by Prof. Günter Schneider, Technical University of Munich, Munich, Germany.

In order to determine the HDAC degradation activity of the designed degraders in the two pancreas cancer cell lines PSNI and HCT116, the following procedure was performed: murine or human cell lines were seeded on cell culture dishes which were subsequently treated by different concerntrations of degraders for a certain time. The treated cells were harvested to get their proteins in IP buffer, and the corresponding protein level was determined by western blot (Figure 103). The data was provided by the group of Prof. Günter Schneider, Technical University of Munich, Munich, Germany.

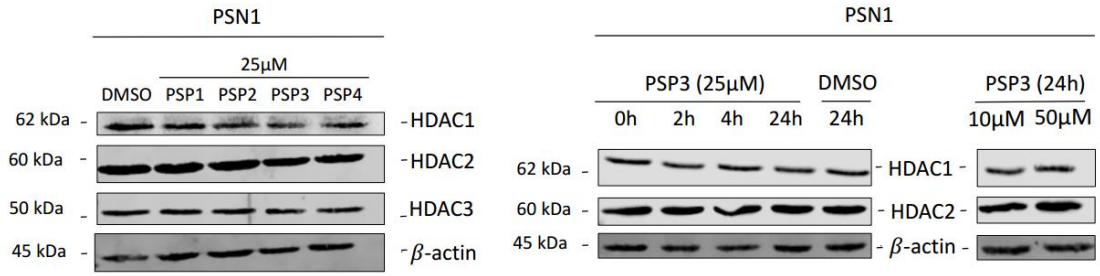


Figure 104. Left: western blot of HDAC 1-3 alteration for PSN1cells treated with **212d** (PSP1), **212f** (PSP2), **212b** (PSP3) and **212a** (PSP4) at 25 μM respectively for 24 h, actin was used as a loading control, DMSO was used as vehicle. Right: western blot of HDAC 1-3 alteration for PSN1 cells treated with **212b** (PSP3) in time and dose dependent experiment.

No obvious degradation after treatment of the triazole linker based PROTACs **212d** (PSP1), **212f** (PSP2), **212b** (PSP3) and **212a** (PSP4) was observed in PSN1 cell line

even from the dose and time dependent experiment (Figure 104, right). The incubation of PSN1 cell line with **212d** (PSP1), **212f** (PSP2), **212b** (PSP3) and **212a** (PSP4) showed also no distinct antiproliferative activity as well, maybe because of lack of cell penetration.

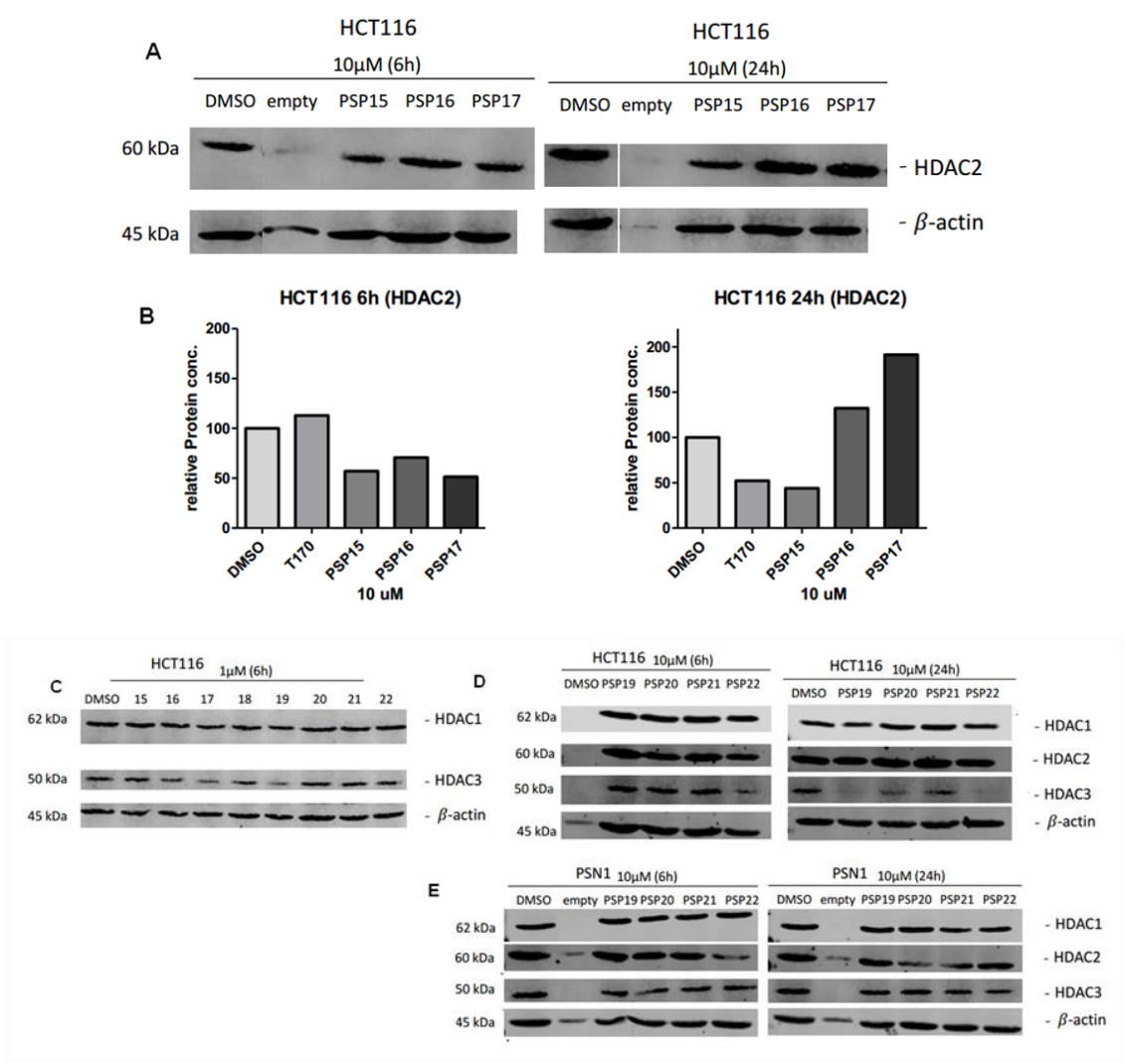


Figure 105. Western blot of HDAC2 degradation for HCT116 cells treated with **218b** (PSP15), **218d** (PSP16) and **218e** (PSP17) respectively that contain N-(2-amino-5-(thiophen-2-yl)phenyl)benzamide as HDAC binder and alkyl linker, actin was used as a loading control, DMSO was used as vehicle. A: original western blot of HDAC2 alteration for HCT116 cells treated with **218b** (PSP15), **218d** (PSP16) and **218e** (PSP17) respectively at 10 μM for 6 h and 24 h. B: percentage of HDAC2 remained after 6 h and 24 h treatment. C: HDAC1 and 3 alteration of HCT116 cells incubated with **218b** (PSP15), **218d** (PSP16), **218e** (PSP17), **218f** (PSP18), **218h** (PSP19), **218i** (PSP20), **229a** (PSP21), **229b** (PSP22) respectively at 1 μM for 6 h. D: HDAC1-3 alteration of HCT116 cells incubated with **218h** (PSP19), **218i** (PSP20), **229a** (PSP21), **229b** (PSP22) respectively at 10 μM for 6 h and 24 h respectively. E: HDAC1-3 alteration of PSN1 cells incubated with **218h** (PSP19), **218i** (PSP20), **229a** (PSP21), **229b** (PSP22) respectively at 10 μM for 6 h and 24 h respectively.

The degradation of HDAC1-2 in HCT116 cells cultured with **218b** (PSP15), **218d** (PSP16), **218e** (PSP17) confirmed that **218b** (PSP15), containing 5 carbon long linker, can selectively degrade HDAC2 in HCT116 cells with a time dependent manner compared to **218c** (PSP10), **218d** (PSP16) and **218e** (PSP17), which contain longer alkyl linkers (6-8 carbons) (Figure 105, A, B, C; B from Figure 106), albeit under a high concerntration of **218b** (PSP15) at 10 μM. Additionally, among the HDAC3 targeting PROTACs (**218f** (PSP18), **218g** (PSP11), **218h** (PSP19), **218i** (PSP20)) (Figure 105 D; E of Figure 110), **218h** (PSP19) bearing 7-carbon long alkyl linker can selectively degrade HDAC3 in HCT116 cells after 24 h treatment. However no antiproliferative effects were observed in HCT116 and PSN1 cells which were cultured with these PROTACs. Meanwhile, similar phenomena was observed for the HyT degrader **229b** (PSP22) which showed better degradation but worse antiproliferative activity than the HyT degrader **229a** (PSP21).

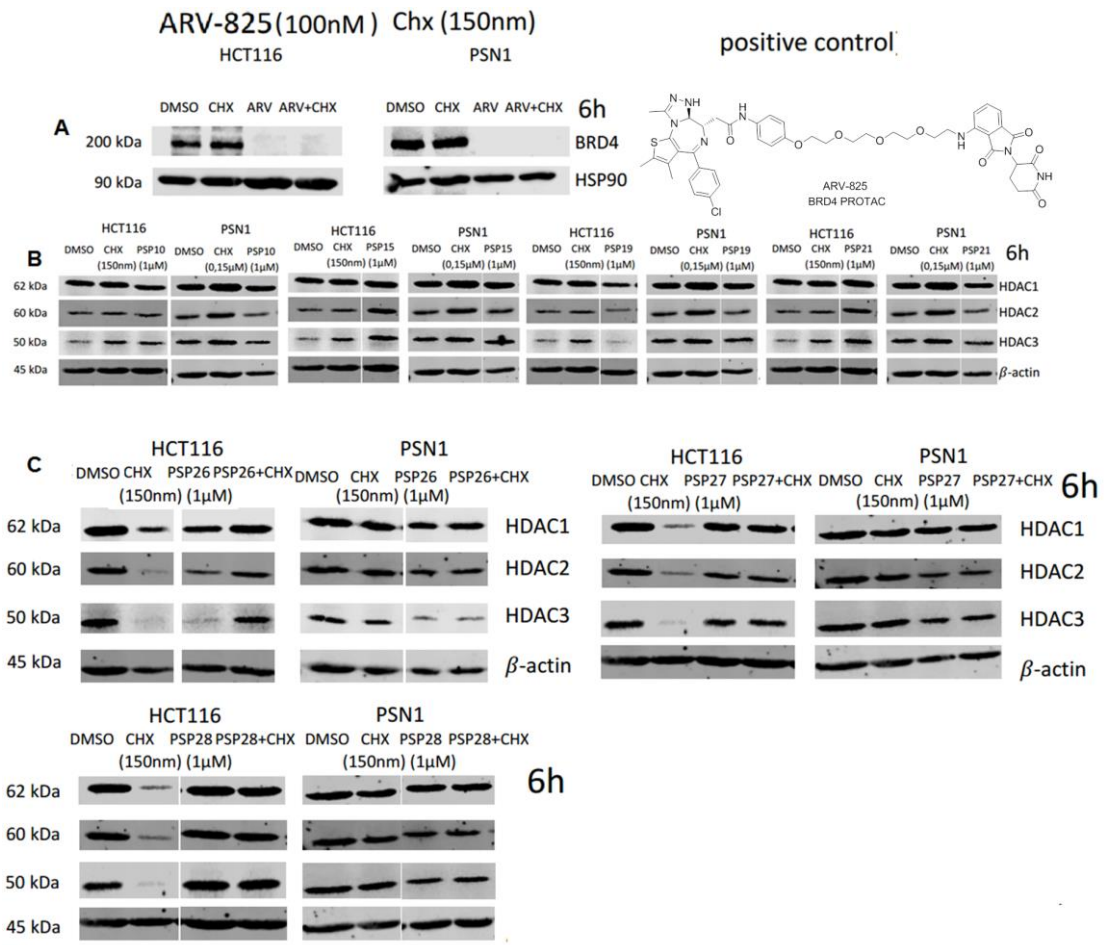


Figure 106. Western blot of cyclohexamide (Chx) co-treatment with chosen PROTACs, actin and Hsp 90 were used as a loading control, DMSO was used as vehicle, all designed PROTACs were cultured with HCT116 or PSN1 cells under 1μM for 6 h. A: methond confirmation of Chx cotreatment experiment and ARV-825 structure. B: HDAC1-3 alteration by **218c** (PSP10), **218b** (PSP15), **218h** (PSP19) and **229a** (PSP21) respectively, and cotreatment of Chx from HCT116 and PSN1 cells. C: HDAC1-3 alteration by **250b** (PSP26), **250c** (PSP27) and **250d** (PSP28) respectively and co-treatment of Chx from HCT116 and PSN1 cells.

The degrader assessment was continued with a cyclohexamide (Chx) co-treatment experiment in which Chx can block endogenous protein synthesis in PSN1 and HCT116 cells. Effective degraders can finally implement the degradation of already produced proteins. It means that the protein degradation extent of Chx co-treatment with degrader should be higher than sole degrader accomplishment and sole Chx treatment in cells (Figure 106, A). Apparently, of all tested degraders including VHL ligand-based PROTACs **250b** (PSP26), **250c** (PSP27) and **250d** (PSP28), only **218h** (PSP19) in HCT116 cells accorded with this prediction (Figure 106).

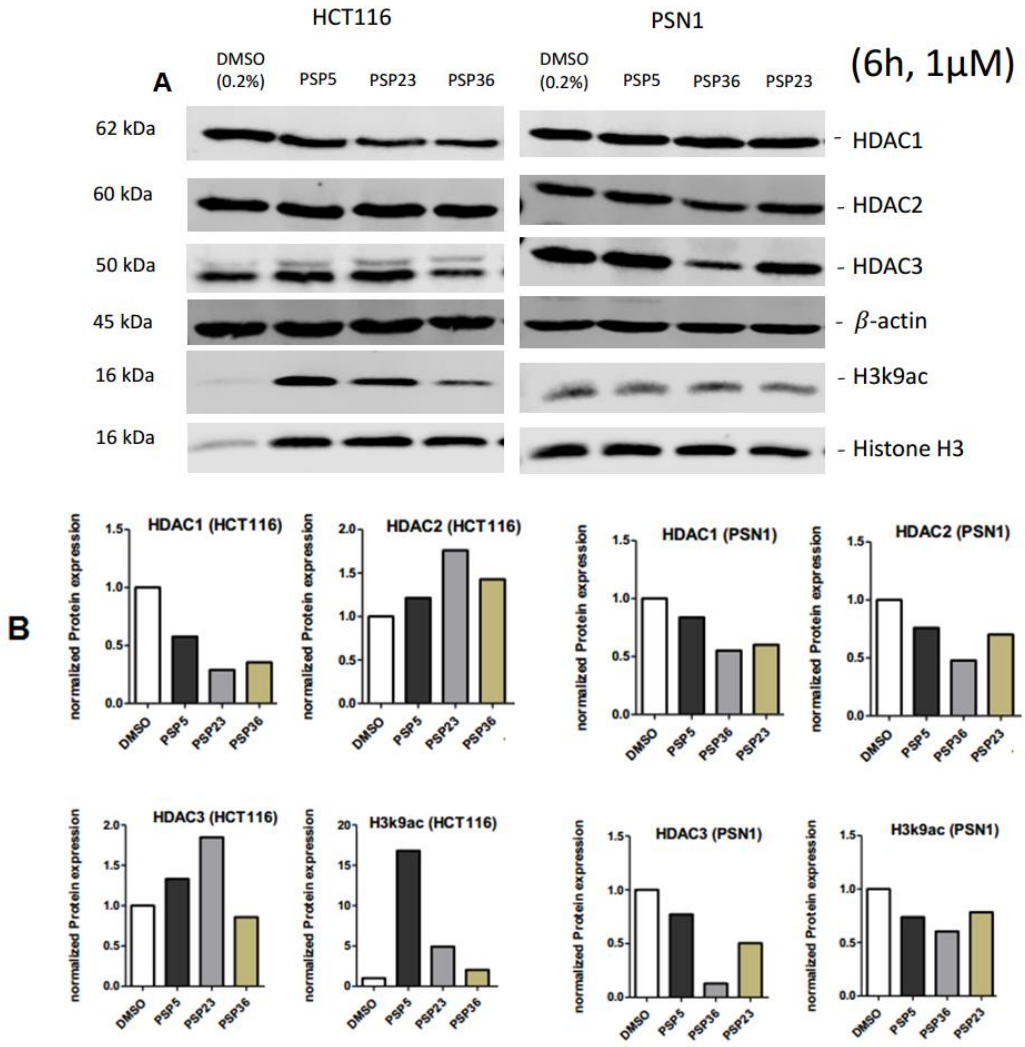


Figure 107. Western blot of HDAC1-3 alteration in PSN1 and HCT116 cells treated with PROTACs **218m (**PSP5) (pomalidomide-based), **224a** (PSP23) (lenalidomide-based) and **250f** (PSP36) (VHL ligand-based) respectively at 1 µM for 6 h treatment, actin and H3 were used as loading control, DMSO was used as vehicle, H3K9ac was used as biomarker of HDAC1-3 degradation. A: original western blot of HDAC1-3 alteration in HCT116 and PSN1 cells treated with **218m (**PSP5), **224a** (PSP23) and **250f** (PSP36) respectively at 1 µM for 6 h. B: percentage of HDAC1-3 and H3K9ac alteration in HCT116 and PSN1 cells after degraders treatment for 6 h.

The alteration of HDAC1-3 levels in HCT116 (Figure 107) implied that the lenalidomide-based PROTAC **224a** (PSP23) can selectively degrade HDAC1 to some extent compared to HDAC2 and HDAC3. **250f** (PSP36) can selectively degrade HDAC3 with a good efficacy in PSN1 cells compared to HDAC1 and HDAC2. However, only **224a** (PSP23) displayed promising antiproliferative activity with an EC_{50} of 2.07 µM in PSN1 cell lines and 1.33 µM in HCT116 cell line, respectively. The antiproliferative activity of **250f** (PSP36) only showed an EC_{50} of 5.57 µM in

PSN1 which is perhaps due to other compensatory and cross-regulatory pathways.

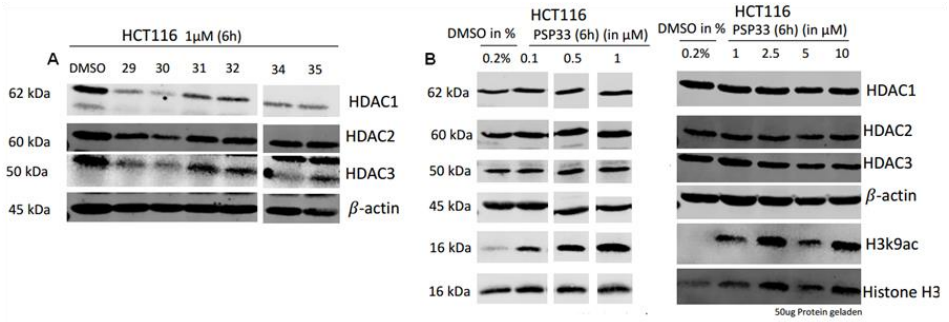


Figure 108. Western blot of HDAC1-3 alteration for HCT116 cells treated with aminobenzamide-based HyTs **229h** (PSP29), **229i** (PSP30), **229m** (PSP31) and **229n** (PSP32), alkylated hydrazide-based HyTs **229c** (PSP33) and **229d** (PSP34), VHL ligand based-PROTAC **250e** (PSP35) respectively at different concerntration for 6 h, actin and H3 were used as loading control, DMSO was used as vehicle, H3K9ac was used as biomarker of HDAC1-3 degradation. A: HDAC1-3 alteration for HCT116 cells treated with chosen degraders at 1 µM for 6 h. B: dose depended experiment of **229c** (PSP33) in HCT116 cells.

Degradation studies with **229h** (PSP29), **229i** (PSP30), **229m** (PSP31), **229n** (PSP32), **229c** (PSP33), **229d** (PSP34) and **250e** (PSP35) did not show clear and significant results (Figure 108, A). In addition, **229c** (PSP33) was tested at different concentrations (Figure 108, B). However, no obvious degradation performance was obtained from all tested concentration for 6 h.

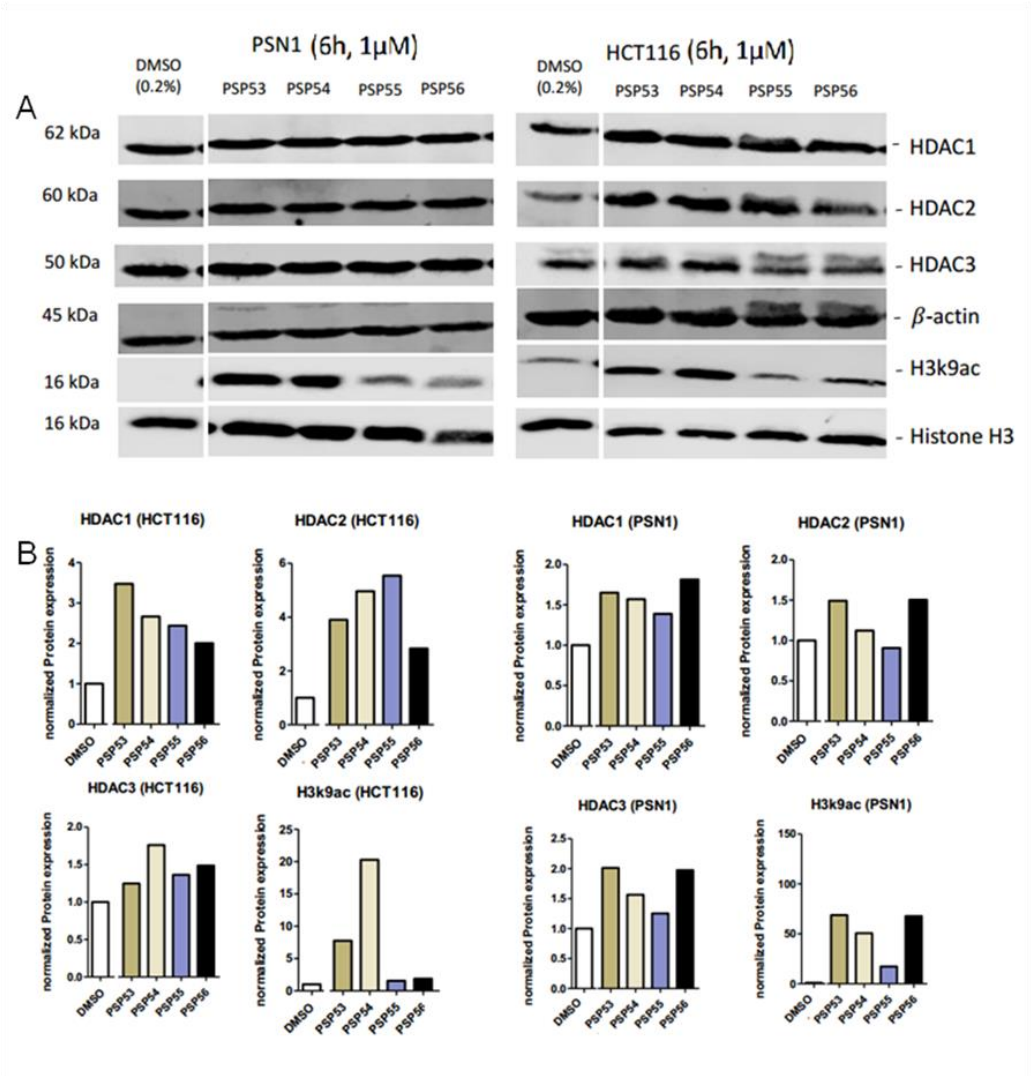


Figure 109. Western blot of HDAC1-3 level alteration for PSN1 and HCT116 cells treated with Hyts **229j** (PSP53), **229g** (PSP54) and negative control **229e** (PSP55), **229k** (PSP56) respectively at 1 μM for 6 h treatment, actin and H3 were used as loading control, DMSO was used as vehicle, H3K9ac was used as biomarker of HDAC1-3 degradation. A: original western blot of HDAC1-3 level alteration for HCT116 and PSN1 cells, B: percentage of HDAC1-3 and H3K9ac alteration in HCT116 and PSN1 cells after degraders treatment for 6 h.

Unfortunately, no HDAC degradation was observed for alkylhydrazide-based hydrophobic tagged PROTACs **229j (**PSP53) and **229g** (PSP54) in PSN1 and HCT116 cells, however both showed strong H3K9 hyperacetylation as shown in Figure 109.

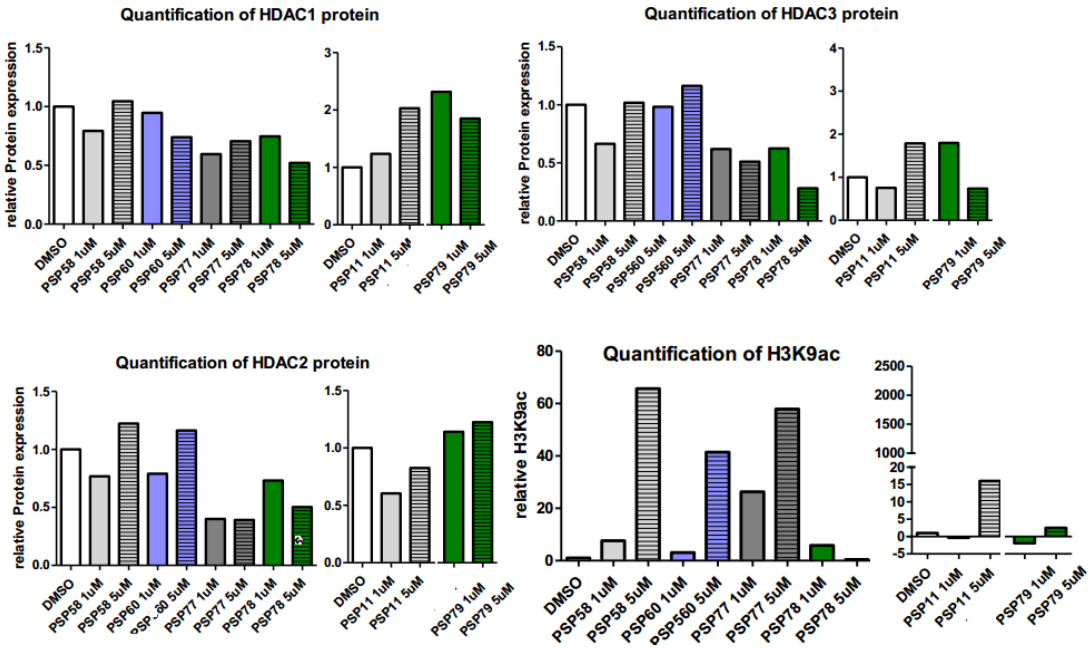


Figure 110. Percentage of HDAC1-3 and H3K9ac alteration in HCT116 cells after degraders treatment for 6 h at 1 µM and 5 µM respectively.

The degradation data in Figure 110 confirmed our previous results with only PROTAC **218k** (PSP77) which can selectively degradate HDAC2 and also display strong antiproliferative activity in HCT116 cells (EC_{50} = 0.95 µM).

4.5 Discussion and conclusion of this part

An overview of the degradation capability and antiproliferative activity of the designed and tested degraders in this part is depicted in Table 26 which is grouped on the basis of their target, linker and ubiquitin ligase type. Among all the listed compounds, several of the developed degraders harness interesting degradation effect but weak phenotypic activity such as the aminobenzamide-based degraders **218h** (PSP19) and **250b** (PSP26). This might perhaps be due to the quick endogenous compensatory protein synthesis caused by the slow degradation capability of these PROTACs. Some of them display excellent antiproliferative activity *in vitro* but weak protein degradation capability which can be observed from the alkylated hydrazide-based degraders such as **229c** (PSP33) and **229g** (PSP54). These results shed some insight into the structural features necessary to get degardation of HDAC1-3 degraders although no ideal/strong degrader was generated through this study. The results indicated that almost all the E3 ligase ligands or hydrophobic tags like pomalidomide, lenalidomide, adamantine and VHL ligand could be applied to obtain HDAC1-3 potential degraders. It was observed that most of the aminobenzamide derived degraders could not cause efficient phenotypic activity unlike some alkylated hydrazide-based degraders. The last and most challenging point is how to better combine the HDAC ligand, linker and E3 ligase ligand to generate effective degraders which are able to promote the formation of a ternary complex and induce potent degradation action. Here, a systematical linker optimization might represent a promising approach to address this problem in future work.

Table 26. Degradation and antiproliferative activity summary of designed HDAC1-3 degraders against PSN1 and HCT-116 cell lines

Compounds	Targeted HDAC (in cell)	Binder types	Linker types	Linker lengh	Degron types	Degradation Percentage	EC_{50} (μM)	
							PSN1	HCT116
212a PSP4	1	aminobenzamide	Triazole	6c	poma	E	n.t.[e]	n.t.

212b PSP3	1	aminobenzamide	Triazole	6c	poma	E	n.t.	n.t.
212c PSP9	1-3	aminobenzamide	Triazole	6c	poma	n.t.	n.t.[d]	n.t.
212d PSP1	3	aminobenzamide	Triazole	6C	poma	E	n.t.	n.t.
212e PSP8	3	aminobenzamide	Triazole	6c	poma	n.t.	n.t.	n.t.
212f PSP2	3	aminobenzamide	Triazole	6c	poma	E	n.t.	n.t.
218a PSP12	1-3	aminobenzamide	alkyl	6c	poma	n.t.	n.t.	n.t.
218b PSP15	1 **(2/HCT116)**	aminobenzamide	alkyl	5c	poma	B	7.49	3.19
218c PSP10	1	aminobenzamide	alkyl	6c	poma	D	0.765	0.392
218d PSP16	1	aminobenzamide	alkyl	7c	poma	E	5.83	1.63
218e PSP17	1	aminobenzamide	alkyl	8c	poma	E	2.99	0.798
218f PSP18	3	aminobenzamide	alkyl	5c	poma	D	n.d.	n.d.
218g PSP11	3	aminobenzamide	alkyl	6c	poma	C	n.d.	n.d.
218h PSP19	3 **(3/HCT116)**	aminobenzamide	alkyl	7c	poma	**A**	n.t.	n.t.
218i PSP20	3	aminobenzamide	alkyl	8c	poma	B	n.t.	n.t.
218k PSP77	3 **(2/HCT116)**	Alkylated hydrazide	alkyl	6c	poma	B	n.t.	0.945
218m PSP5	1	aminobenzamide	alkyl	6c	poma	C	4.36	3.66
224a PSP23	1 **(1/HCT116)** **(3/PSN1)**	aminobenzamide	alkyl	6c	lena	B	2.07	1.33
229a PSP21	1	aminobenzamide	alkyl	6c	adama	B	1.75	0.742
229b PSP22	3 **(3/HCT116)**	aminobenzamide	alkyl	6c	adama	**A**	n.d.	12.31
229c PSP33	3	Alkylated hydrazide	alkyl	6c	adama	E	0.820	0.421
229d PSP34	3	Alkylated hydrazide	alkyl	6c	adama	n.d	n.t.	0.278

229f PSP58	3	Alkylated hydrazide	alkyl	7c	adama	C	n.t.	2.31
229g PSP54	3	Alkylated hydrazide	alkyl	8c	adama	E	1.95	0.636
229h PSP29	3	aminobenzamide	alkyl	5c	adama	n.d	n.d.	9.43
229i PSP30	3	aminobenzamide	alkyl	7c	adama	n.d	n.d.	4.81
229j PSP53	3	Alkylated hydrazide	alkyl	7c	adama	E	0.705	0.387
229k PSP56	3	Alkylated hydrazide	alkyl	6c	acetyl	E	10.25	2.77
229l PSP60	3	Alkylated hydrazide	alkyl	6c	adama	D	n.t.	3.05
229m PSP31	3	aminobenzamide	alkyl	4c	adama	n.t.	n.d.	9.06
229n PSP32	3	aminobenzamide	alkyl	6c	adama	n.t.	n.t.	9.19
250b PSP26	3 **(3/HCT116) (3/PSN1)**	aminobenzamide	alkyl	8c	VHL-l	**A**	n.t.	n.t,
250c PSP27	1	aminobenzamide	alkyl	10c	VHL-l	D	35.84	5.13
250d PSP28	3	aminobenzamide	alkyl	10c	VHL-l	D	n.t.	n.t.
250e PSP35	1	Alkylated hydrazide	alkyl	8c	VHL-l	n.d.	n.d.	n.t.
250f PSP36	3 **(1/HCT116) (3/PSN1)**	Alkylated hydrazide	alkyl	8c	VHL-l	**A**	5.57	n.t.
255a PSP78	3 **(3/HCT116)**	Alkylated hydrazide	alkyl	6c	VHL-l	**A**	n.t.	n.t.
259a PSP79	3	Alkylated hydrazide	alkyne	6c	lena	E	n.t.	n.t.

E (< 10% degradated), D (10-30% degradated), C (30-50% degradated), B (50-70% degradated), A (> 70% degradated) (the maximal degradation of targeted proteins). poma = pomalidomide, lena = lenalidomide, adama = adamantane, VHL-l = VHL ligand, n.d = no determined, n.t = no tested.

Part 5 Experimental part

5.1 Enzymatic HDAC activity assay

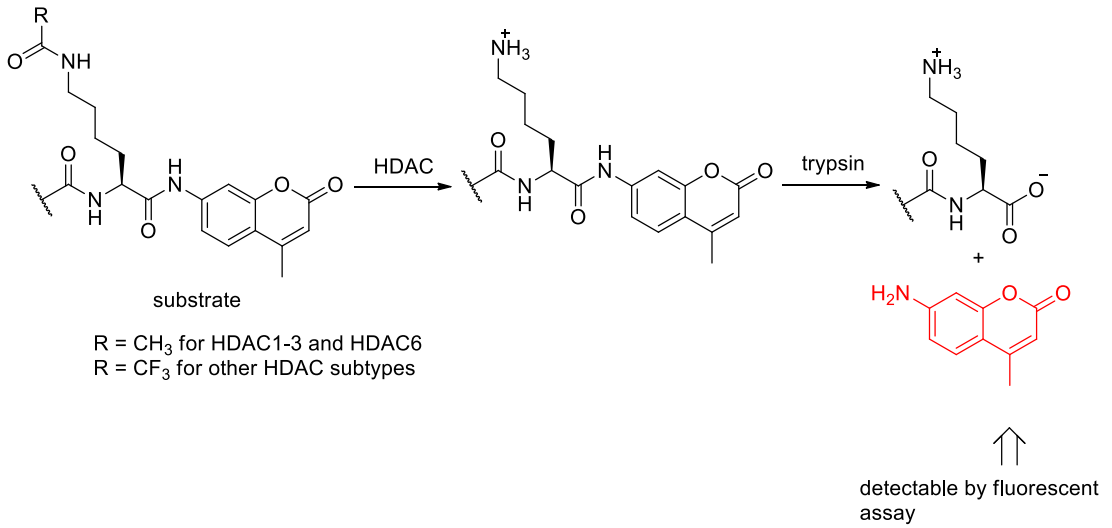


Figure 111. Mechanism of HDAC activity assay with its substrated based on fluorescence-based assay.[80]

Acetyllysine derivatives or peptides with an acetylated lysine residue are coupled with an aminomethylcoumarin fluorophore in its C-terminus to be regarded as HDAC substrates which can be first deacetylated by recombinant HDACs or HDAC with NCoR. The corresponding free lysine residue is recognized by exopeptidase trypsin to release the aminomethylcoumarin which has a different spectroscopic property compared to its parent acetylated substrate and could be quantified by fluorescence detection. As HDAC cellular substrates are proteins or protein complexes, this assay method can not accurately reflect the impact on HDAC cellular function, but can still reflect the inhibition of the catalytic activity. However, in terms of some HDACs with relatively low deacetylation activity such as e.g. HDAC8, trifluoroacetyl-lysine substrates are used instead of acetyllysine derivatives in practice.[80]

The *in vitro* enzymatic activity testing was carried out by Matthes Zessin and Patrik Zeyen, Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Halle, Germany.

5.2 Chemical Synthesis - Materials and Methods

5.2.1 Chemicals and microwave reactor

5.2.1.1 Chemicals and microwave reactor

The chemicals were purchased from abcr GmbH Karlsruhe or Sigma Aldrich Darmstadt. Microwave reactions were performed by heating to the corresponding temperature within 5 min through a microwave reactor (Monowave 450 from the manufacturer Anton Paar GmbH).

5.2.1.2 Treatment of the solvents

All of the mentioned solvents were analytically pure and used directly from chemical storage of the institute of pharmacy, Martin-Luther-University Halle-Wittenberg without further purification.

5.2.2 Chromatography

5.2.2.1 **Thin layer chromatography (TLC)**:

The TLC silica gel 60 F254 aluminum sheets 20x20 cm were purchased from the manufacturer Merck KgaA and were cuted into proper size to monitor the progress of the reactions, estimate the reaction end point, evaluate the product combinded with LC-MS, and determine the mobile phase system for column chromatographyl. The substance was dissolved in a proper solvent or taken directly from the reaction mixture and applied to the TLC aluminum sheet with a glass capillary. The subsequent TLC sheet was placed into a TLC chamber which was saturated with one of the following mobile phase and detected through a UV light under 254 nm (short wave UV light) or 366 nm (long wave UV light):

MP 1: Dichloromethane (DCM) / methanol; 50/1 with or without trimethylamine

MP 2: DCM / methanol; 30/1 with or without trimethylamine

MP 3: DCM / methanol; 25/1 with or without trimethylamine

MP 4: DCM / methanol; 15/1

MP 5: EtOAc / heptane; 10/1

MP 6: EtOAc / heptane; 5/1

MP 7: EtOAc / heptane; 1/1

5.2.2.2 **Column chromatography**: Silica gel 60 (0.015-0.04 mm) from the manufacturer Merck KgaA was used as a stationary phase. As mobile phases, a mixture of DCM, a methanol gradient and 2 or 3 drops of triethyl amine was used when there are basic moieties in the sample. Otherwise, only DCM with a methanol gradient or heptane with an EtOAc gradient was used as mobile phase. The crude substances were dry packed with a silica gel/substance ratio of 2-5 and the ratio of stationary phase with dry sample was about 10-20.

5.2.2.3 **High performance liquid chromatography (HPLC)**: The purity of all final compounds was determined by HPLC. The HPLC system consists of an XTerra RP18 column (3.5 µm 3.9 x 100 mm) form the manufacturer Waters (Milford, MA, USA), two LC-10AD pump, a SPD-M10A VP PDA detector, and a SIL-HT auto sampler all from the manufacturer Shimadzu (Kyoto, Japan). The mobile phase was in all cases a mixture of methanol/water in gradient (starting from 95 % water to 5 % water finally). The water was mixed with 0.05 % TFA.

5.2.2.4 **Preparative high performance liquid chromatography (Preparative-HPLC)**: Several compounds were purified by preparative-HPLC which contains a LiChrosorb® RP-18 (7 µm) 250-25 Merck column. The mobile phase was in all cases a mixture of acetonitrile and water (starting from 5 % water to 95 % water finally).

5.2.3 **Nuclear magnetic resonance (NMR)-Spectroscopy**: The recording of the

^{1}H-NMR and ^{13}C-NMR - spectra was performed with Varian Inova 500 or the Gemini 2000 from the manufacturer Varian. The spectra were recorded at 400 MHz or 500 MHz. The solvents used here are hexadeuterodimethyl sulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$). The chemical shift δ is expressed in ppm and is set at the axis of symmetry of the separate peaks. The signals of ^{1}HNMR spectra are specified in the following manner: (multiplicity, coupling constant J in Hz, number of protons). The following abbreviations were used for the multiplicities: s (singlet), d (doublet), t(triplet), dd (doublet of doublet), m (multiplet).

5.2.4 Mass spectrometry

5.2.4.1 **Electron-spray mass spectrometry (ESI-MS) and atmospheric pressure chemical ionization (APCI)** were used to confirm key intermediates. The dissolved samples were injected via a pump Harvard Apparatus 22 (20 µl min^{-1}). The ionization was performed via electron spray technique at 5 kV in positive or negative mode. The spectra were recorded by the Finnigan LCQClassic mass spectrometer of the manufacturer Thermo Electron (Egelsbach, Germany). It has a heatable capillary (220 °C) and a flow rate of 20 µl/min.

5.2.4.2 **High resolution mass spectrometry (HR-MS)** was also used here for all the final products. The samples were dissolved in a proper solvent such as methanol or chloroform and ionized via a Proxeon-Nano-ESI-source of the manufacturer Thermo Fisher Scientific (Bremen, Germany) at 1.3 kV. The spectra were recorded by the LTQ-Orbitrap-XL-mass spectrometer of the manufacturer Thermo Fisher Scientific (Bremen, Germany). The signal of the isotopes with the highest prevalence was given and calculated (^{35}Cl, ^{79}Br).

5.3 Synthesis procedure and compound characterization

5.3.1 Synthesis procedure and compound characterization for part 2

General procedure for the synthesis of Boc protective amino acid *2a-2m*: THF (1 mL) and water (0.5 mL) were added to the *R*-amino acid (0.43 mmol) in ice bath. $(Boc)_2O$ was then added into the mixture followed by 0.25 mL NaOH (10%) solution. The resulting mixture was stirred overnight and temperature was increased to room temperature. 2 mL water were then added to the reaction mixture with stirring followed by HCl (10%) dropwise until pH ≈ 4, and the mixture was extracted with DCM twice. The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated using rotary evaporator giving the intermediate 2a-2n as white solid or colorless oil in a quantitative yield and were directly used in the next step without further purification.

4-Methylbenzenesulfonohydrazide (4): A solution of TsCl (2.38 g, 12.48 mmol, 1.0 eq) in THF (25 mL) was slowly added to a mixture of hydrazine monohydrate (6.26 g, 125 mmol, 10.0 eq) and water (150 mL) at about 5 ^oC in ice bath under stirring. The resulting mixture was stirred for an additional 30 min, extracted three time with EtOAc. The organic layer was collected, dried by anhydrous Na_2SO_4 and concentrated to give white solid in a quantitative yield without further purification. m/z ($APCI^+$) 187.0 $(M+H)^+$.

Tert-butyl 4-(2-tosylhydrazono)piperidine-1-carboxylate (5): Tert-butyl 4-oxopiperidine-1-carboxylate was added to a solution of ***4*** (1.0 g, 5.37 mmol, 1.0 eq) in MeOH (10 mL) and the mixture was stirred for 3 h. After a white precipitate emerged, the solvent was removed by rotary evaporator, the residue was washed with a little petroleum ether and dried to yield white solid (1.95 g, yield: 99%). m/z ($APCI^+$) 368.3 $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H), 7.71 (d, *J* = 8.2 Hz,

2H), 7.38 (d, *J* = 8.1 Hz, 2H), 3.37 (dd, *J* = 13.3, 6.8 Hz, 4H), 2.40 – 2.31 (m, 5H), 2.20 (t, *J* = 6.0 Hz, 2H), 1.38 (s, 9H).

Phenyl(piperidin-4-yl)methanone TFA (7a) **and** ***(2-methoxynaphthalen-1-yl)(piperidin-4-yl)methanone TFA (7b)***: A mixture of compound ***5*** (1.60 g, 4.36 mmol, 1.0 eq), aldehyde (4.36 mmol, 1.0 eq) and Cs_2CO_3 (2.84 g, 2.0 eq) in dioxane (8 mL) was kept under argon atmosphere and heated to 110 ^oC in a microwave reactor for 2.5 h. Upon completion, the reaction was extracted with water and DCM three times, and the organic layers were collected and dried over anhydrous Na_2SO_4. After concerntration, the residue was purified by column chromatography using heptane and EtOAc as eluent to give a light yellow oil product (yield: 69% (***6a***) and 27% (***6b***)).

Tert-butyl 4-benzoylpiperidine-1-carboxylate (6a): m/z (ESI$^+$) 312.51 $(M+Na)^+$, ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (dt, *J* = 8.5, 1.7 Hz, 2H), 7.59 – 7.52 (m, 1H), 7.50 – 7.42 (m, 2H), 4.15 (d, *J* = 13.0 Hz, 2H), 3.40 (tt, *J* = 11.1, 3.7 Hz, 1H), 2.95 – 2.81 (m, 2H), 1.83 (d, *J* = 11.5 Hz, 2H), 1.75 – 1.63 (m, 2H), 1.45 (s, 9H).

Tert-butyl 4-(2-methoxy-1-naphthoyl)piperidine-1-carboxylate (6b): m/z (APCI$^+$) 370.2 $(M+H)^+$.

The Boc deprotected intermediates ***7a*** and ***7b*** were obtained through stirring ***6a*** and ***6b*** in a mixture of TFA (4 mL) and DCM (8 mL) at room temperature for 2 h followed by solvent evaporation. Intermediates ***7a*** was yellow solid and used without further purification (yield: 67%). m/z (ESI$^+$) 190.0 $(M+H)^+$, ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 – 7.90 (m, 2H), 7.55 (dd, J = 8.3, 6.4 Hz, 1H), 7.46 (t, J = 7.6 Hz, 2H), 3.39 (dd, J = 13.1, 9.5 Hz, 1H), 3.19 (dt, J = 12.6, 3.3 Hz, 2H), 2.77 (td, J = 12.3, 2.6 Hz, 2H), 1.93 – 1.81 (m, 3H), 1.75 – 1.62 (m, 2H). Intermediate ***7b*** was purified by column chromatography (EtOAc : heptane = 1 : 20 to 1 : 4 and then DCM : MeOH = 15:1 to 10 : 1 with several drops of triethylamine) to give a white solid (yield: 50%), m/z (APCI$^+$) 270.0 $(M+H)^+$.

(1-Methyl-1H-indol-3-yl)methanamine (15): A mixture of 1-methyl-indole-3-carbaldehyde (***13***) (200 mg, 1.26 mmol, 1.0 eq), hydroxylammonium chloride (0.175 g, 2.52 mmol, 2.0 eq) and sodium acetate (0.258 g, 3.15 mmol, 2.5 eq) in MeOH (1 mL) and water (0.5 mL) was stirred at room temperature for 24 h. The reaction was then extracted with water and DCM three times, and the organic layers were collected, dried over anhydrous Na_2SO_4, and concentrated to yield a light yellow solid 1-methyl-indole-3-carbaldehyde oxime **(*14*)** (160 mg, 73%) which was directly used in the next procedure without further purification.

To a mixture of compound ***14*** (100 mg, 0574 mmol, 1.0 eq) and $NiCl_2{\cdot}6H_2O$ (74.4 mg, 0.574 mmol, 1.0 eq) in MeOH (5 mL) was added $NaBH_4$ (220 mg, 5.74 mmol, 10.0 eq) slowly over several portions under ice bath with stirring until compound ***14*** was disappeared. The reaction was extracted with EtOAc and water three times, and the collected organic layers were concentrated and purified by column chromatography (EtOAc : heptane = 1 : 1 and then DCM : MeOH = 20 : 1 to 10 : 1) to give (***15***) as a white solid (50 mg, yield: 54%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.62 (d, J = 7.9 Hz, 1H), 7.37 (d, J = 8.2 Hz, 1H), 7.22 (s, 1H), 7.17 – 7.10 (m, 1H), 7.01 (t, J = 7.4 Hz, 1H), 3.91 (s, 2H), 3.73 (s, 3H), 3.65 (s, 2H). m/z (APCI$^+$) 161.0 $(M+H)^+$.

(1H-indol-1-yl)(piperidin-4-yl)methanone (19): Thionyl chloride (107 mg, 65 μL, 0.792 mmol, 1.2 eq) and TEA (80.14 mg, 110 μL, 0.792 mmol, 1.2 eq) were added to a stirred solution of 1-(tert-butoxycarbonyl)piperidine-4-carboxylic acid (***16***) (150 mg, 0.66 mmol, 1.0 eq) in DCM (5 mL) under argon atmosphere at room temperature for 20 h then the solvent was removed by evaporation and the residue tert-butyl 4-(chlorocarbonyl)piperidine-1-carboxylate (***17***) was used directly without further purification. indole (70 mg, 0.6 mmol, 1.0 eq), TEA (91 mg, 125 μL, 1.5 eq) and DMAP (7.33 mg, 0.06 mmol, 0.1 eq) were added to the solution of compound ***17*** in DCM (2 mL), and the resulting mixture was stirred for about 4 days at room

temperature then directly purified by chromatography (ethy lacetate : heptane = 0 : 1 to 1 : 4 gradually) to give tert-butyl 4-(1*H*-indole-1-carbonyl)piperidine-1-carboxylate (***18***) as a colorless solid (60 mg, yield: 31%). A mixture of 0.2 mL TFA and 2 mL DCM was dropwise added to 68 mg of compound ***18*** and the reaction was stirred for about 2 h at room temperature till ***18*** disappeared. A Na_2CO_3 saturated solution was added to the reaction until no bubbles appeared, and the mixture was extracted with EtOAc and brine. The collected organic layers were dried over anhydrous Na_2SO_4 and purified by column chromatography (DCM : MeOH = 20 : 1 to 10 : 1 with several drops of TEA) to give intermediate ***19*** as a white solid (60 mg , yield: 97%), m/z (APCI$^+$) 229.2 $(M+H)^+$.

(5-Phenyl-1,3,4-oxadiazol-2-yl)methanamine HCl salt (23)[167]: Benzohydrazide (***20***) (4.3 g, 31.40 mmol, 1.1 eq), Boc-Gly-OH (5.0 g, 28.54 mmol, 1.0 eq), DIPEA (8.12 g, 11.2 mL, 128 mmol, 2.2 eq) and EDCI (6.0 g, 31.3 mmol, 1.1 eq) were dissolved in DCM (50 mL) and the reaction was stirred for 18 h, then washed with aq HCl (10%) and saturated $NaHCO_3$ solutions. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated to get tert-butyl (2-(2-benzoylhydrazinyl)-2-oxoethyl)carbamate (***21***) as oil (6.8 g, yield: 81%), m/z (APCI$^+$) 294.1 $(M+H)^+$.

Toluenesulfonyl chloride (5.53 g, 29 mmol, 1.25 eq) was added to the solution of compound ***21*** (6.8 g, 23.2 mmol, 1.0 eq) and TEA (4.2 mL, 30.2 mmol, 1.3 eq) in DCM (70 mL) at room temperature, and the mixture was stirred for 14 h. The reaction was then washed with aq HCl (10%) and aq NaOH (10%) solutions, the collected organic layer was dried over anhydrous Na_2SO_4 and concentrated to give tert-butyl ((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)carbamate (***22***) as yellow oil (6.9 g) which was directly used in the next procedure and Boc deprotected by TFA (7 mL) and DCM (40 mL) at room temperature followed by addition of saturated Na_2CO_3 solution to the reaction mixture till no bubbles appeared. The reaction was then washed with brine three times, and the combined organic layers were dried with

anhydrous Na_2SO_4 and purified by column chromatography (DCM : MeOH = 15 : 1). The pure compound was converted to HCl salt by concentrated HCl and the resulting residue was washed with ether and pulped with IPA (isopropanol) to remove toluenesulfonic acid from last step, the residue was then dried by rotary evaporator to give (***23***) as pink solid (5.1g, yield: 97%). m/z (APCI$^+$) 176.1 $(M+H)^+$, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 – 8.00 (m, 2H), 7.56 – 7.46 (m, 3H), 4.14 (s, 2H), 1.72 (s, 2H).

(1-Phenyl-1H-1,2,3-triazol-4-yl)methanamine (26): $CuSO_4{\cdot}5H_2O$ (6.3 mg, 0.0252 mmol, 0.01 eq) was added to the mixture of water (3 mL) and t-BuOH (3 mL) at room temperature and then sodium L-ascorbate (50 mg, 0.252 mmol, 0.1 eq) and phenyl azide 0.5 N in tert-butanol methyl ether (5.04 mL, 2.52 mmol, 1.0 eq) were added dropwise to the reaction and followed by propargylamine (194 μL, 3.024 mmol, 1.2 eq). The final mixture was stirred for 3 days, and the reaction was extracted by EtOAc and water three times. The collected organic layers were dried over anhydrous Na_2SO_4 and purified by column chromatography (DCM : MeOH = 1 : 1 till 15 :1) to give ***26*** as yellow oil (142 mg, yield: 33%), m/z (APCI$^+$) 175.2 $(M+H)^+$.

TFA salt of 5-((4-methylpiperazin-1-yl)methyl)isoindoline (31a) and TFA salt of 5-((4-benzylpiperazin-1-yl)methyl)isoindoline (31b): To a solution of propargylamine (1.7 g, 31 mmol, 1.0 eq) in THF (20 mL) was added $(Boc)_2O$ (7.42 g, 34 mmol, 1.1 eq) at room temperature. The resulting mixture was stirred for about 6 h and extracted with EtOAc and brine, and the collected organic layer was washed with aq HCl (10%), dried over Anhydrous Na_2SO_4 and concentrated to give tert-butyl prop-2-yn-1-ylcarbamate (***28***) as a orange solid in a quantitative yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (s, 1H), 3.92 (d, J = 2.5Hz, 2H), 2.21 (t, J = 2.5 Hz, 1H), 1.45 (s, 9H). m/z (APCI$^+$) 157.0 $(M+H)^+$.

To a solution of intermediate ***28*** (1.2 g, 7.73 mmol, 1.0 eq) in anhydrous THF (10 mL) was added NaH (60%) (0.44 g, 10.83 mmol, 1.4 eq) and the mixture was stirred for 30 min at room temperature. Propargylbromide (1.85 g, 1.34 mL, 12.4 mmol, 1.6 eq) was

then added dropwise, and the resulting mixture was stirred overnight, then quenched with saturated NH_4Cl solution and extracted with EtOAc twice. The combined organic layers were dried over anhydrous Na_2SO_4 and the resulting residue was purified by column chromatography (EtOAc : heptane = 0 : 1 till 1 : 10) to give tert-butyl di(prop-2-yn-1-yl)carbamate (***29***) as pale yellow liquid (0.99 g, yield: 67%), m/z (APCI$^+$) 138.1 (M-tBu+2H)$^+$.

To a stirred mixure of ***29*** (0.99 g, 5.12 mmol, 1.0 eq) and propargyl alcohol (1.15 g, 1.2 mL, 20.5 mmol, 4.0 eq) in THF (30 mL) was added $(PPh_3)_3RhCl$ (142 mg, 0.1536 mmol, 0.003 eq) at room temperature and stirring was continued for 5 h. The solvent was removed and the residue was purified by column chromatography (EtOAc : heptane = 0 : 1 till 2 : 1) to give tert-butyl 5-(hydroxymethyl)isoindoline-2-carboxylate (***30***) as brown solid (0.89 g, 70%), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (m, 1H), 7.21 (m, 2H), 4.72 – 4.61 (m, 6H), 1.52 (s, 9H). m/z (APCI$^+$) 250.2 (M+H)$^+$.

To a stirred mixture of compound ***30*** (150 mmg, 0.602 mmol, 1.0 eq) and DIPEA (126 μL, 0.72 mmol, 1.2 eq) in THF (2 mL) under ice bath was added MsCl (52 μL, 0.662 mmol, 1.1 eq) and stirring was continued for 1.5 h until TLC showed complete consumption of compound ***30***. Then, 1-methylpiperazine (202.5 μL, 1.8 mmol, 3.0 eq) was added to the reaction and stirred for another 3 h under room temperature until complete reaction. The reaction was then extracted with water and EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to get brown oil (160 mg, yield: 80%), m/z (APCI$^+$) 332.4 (M+H)$^+$. The resulting product was added to a mixture of TFA (1.5 mL) and DCM (6 mL) at room temperature, and the reaction was stirred for 3 h then directly evaporated, and the residue was washed with heptane and ether, dried again by evaporator giving semisolid ***31a*** in a quantitative yield (270 mg) which was directly used in the next procedure without further purification. ***31b*** was synthesized through the same procedure, the yield of tert-butyl

5-((4-benzylpiperazin-1-yl)methyl)isoindoline-2-carboxylate before the last step was about 64%, m/z (APCI$^+$) 408.5 (M+H)$^+$.

Tert-butyl 5-bromoisoindoline-2-carboxylate (37): Liquid Br_2 (8.5 mL, 165.9 mmol, 1.1 eq) was added dropewise to a mixture of NaOH (12 g, 159.8 mmol, 2.0 eq) and phthalic anhydride (22 g, 148.5 mmol, 1.0 eq) in water (150 mL). The resulting reaction was stirred at 90 ^oC for 16 h and then cooled to room temperature with stirring. A solution of HCl (10%) was added dropwise to the reaction until pH ≈ 2. Then, the resulting precipitate was filtered. The white residue was washed with cold water and dried to give 4-bromophthalic acid (***34***) as white solid (28 g, yield: 77%). ^{1}H NMR (400 MHz, DMSO-d_6) ^{1}H δ 8.21 (s, 1H), 8.03 (d, *J* = 8.3 Hz, 1H), 7.71 (dd, *J* = 8.4, 2.3 Hz, 1H).

A mixture of compound ***34*** (4.87 g, 19.9 mmol, 1.0 eq), Urea (2.38 g, 39.3 mmol, 2.0 eq) and imdazole (1.45 g, 1.53 mL, 1.0 eq) in DMF (1.53 mL) was heated to 160 ^oC in a microwave reactor for 2.5 h. Upon completion, a solution of HCl (10%) was added to the reaction mixture until pH ≈ 4. The resulting mixture was stirred for an additional 1 h. The precipitate was then filtered and the residue was dried to give 5-bromoisoindoline-1,3-dione (***35***) as a yellow solid (2.62 g, yield: 58%), m/z (APCI$^+$) 227.1 (M+H)$^+$.

$NaBH_4$ (8.37 g, 221 mmol, 10.0 eq) was added to a stirred solution of intermediate ***35*** (5 g, 22.1 mmol, 1.0 eq) in THF (200 mL) at room temperature. The rsulting mixture was then cooled below -10 ^oC and BF_3-Et_2O (33 mL, 266 mmol, 12.0 eq) was added dropwise. The reaction was then refluxed for 24 h until TLC showed complete consumption of compound ***35***. The reaction was cooled to room temperature and quenched slowly with cold water. Most of THF was then evaporated. The resulting residue was basified until pH ≈ 10 with a solution of 6N NaOH at 0-5 ^oC followed by addition of EtOAc. The organic layer was washed with brine three times, dried over anhydrous Na_2SO_4 and concentrated to give a residue that was diluted with ether. The

mixture was acidified with 6N HCl solution until pH ≈ 2 at room temperature. The collected water layer was basified again until pH ≈ 10 with 6N NaOH solution. The aqueous layer was extracted with EtOAc three times. The combined organic layers were then washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give *5-bromoisoindoline* (***36***) as brown oil (1.59 g, yield: 36 %).[152]

To a stirred mixture of compound ***36*** (1.59 g, 5.33 mmol, 1.0 eq) in DCM (16 mL) and TEA (1.11 mL, 8 mmol, 1.5 eq) was added $(Boc)_2O$ (1.75 g, 8.0 mmol, 1.5 eq). The resulting mixture was stirred for 24 h. Then, the reation was directly concentrated and the residue was purified by column chromatography (EtOAc : heptane = 0 : 1 to 1 : 20) to give tert-butyl 5-bromoisoindoline-2-carboxylate (***37***) as a light yellow oil (1.37g, yield: 58 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (dd, J = 15.9, 7.9 Hz, 2H), 7.11 (dd, J = 20.4, 8.0 Hz, 1H), 4.63 (t, J = 15.4 Hz, 4H), 1.51 (s, 9H). m/z (APCI$^+$) 244.1 $(M+2H-tBu)^+$, 200.1 $(M+2H-Boc)^+$.

5-Phenylisoindoline (39): A stirred mixture of ***37*** (100 mg, 0.336 mmol, 1.0 eq), phenylboronic acid (62 mg, 0.404 mmol, 1.5 eq), $Pd(OAc)_2$ (3 mg, 0.013 mmol, 0.04 eq), PPh_3 (18 mg, 0.067 mmol, 0.2 eq) and Cs_2CO_3 (220 mg, 0.672 mmol, 2.0 eq) in dry toluene (2 mL) was kept under argon atmosphere and heated to 110 ^oC in a mixrowave reactor for 2.5 h. Then, the solvent was removed by evaporation and the residue was directly purified by column chromatography (EtOAc : heptane = 1 : 20 to 1 :4 gradually) to give tert-butyl 5-phenylisoindoline-2-carboxylate (***38***) as a white solid (74 mg, yield: 74%), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.55 (m, 2H), 7.45 (dd, *J* = 15.7, 7.9 Hz, 4H), 7.39 – 7.32 (m, 2H), 4.72 (dd, *J* = 16.9, 7.1 Hz, 4H), 1.53 (s, 9H), m/z (APCI$^+$) 240.2 $(M+2H-tBu)^+$. The pure ***38*** was added to a mixture of TFA (1 mL) and DCM (6 mL) and the reaction was stirred at room temperature for 1 h. Saturated Na_2CO_3 solution was then added to the reaction mixture until no bubbles appeared. The mixture was extracted with brine and EtOAc three times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (DCM : MeOH = 15 :1) to

give intermediate ***39*** as a white solid (32 mg, yield: 44%).

TFA salt of 4-(isoindolin-5-yl)morpholine (41a) and 5-(4-methylpiperazin-1-yl)isoindoline (41b): A mixture of ***37*** (150 mg, 0.5 mmol, 1.0 eq), morpholine (52 mg, 53 µL, 0.6 mmol, 1.2 eq), $Pd(dba)_2$ (23 mg, 0.025 mmol, 0.05 eq), potassium tert-butylate (113 mg, 1.0 mmol, 2.0 eq) and tert-Butoxy-bis-(diisopropylamino)-phosphin (15.4 mg, 0.05 mmol, 0.1 eq) in toluene (1.5 mL) was heated to 110 ^{o}C in a microwave reactor for 1 h. Then, the solvent was removed by evaporation and the residue was purified by column chromatography (EtOAc : heptane = 0 : 1 to 1 : 2 gradually) to give *tert-butyl 5-(4-methylpiperazin-1-yl)isoindoline-2-carboxylate* (***40a***) as a white solid (105 mg, yield: 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (dd, *J* = 20.7, 8.3 Hz, 1H), 6.82 (dd, *J* = 21.7, 13.0 Hz, 2H), 4.61 (m, 4H), 3.91 – 3.81 (m, 4H), 3.14 (d, *J* = 4.6 Hz, 4H), 1.51 (s, 9H). The pure 140 mg intermediate ***40a*** was added to a mixture of TFA (1 mL) and DCM (6 mL) at room temperature and the resulting reaction was stirred for 1 h. The solvent was then evaporated. The residue was washed with ether giving ***41a*** as brown solid in a quantitative yield (199 mg).

Intermediate ***41b*** was synthesized through the same procedure of ***41a***. Tert-butyl 5-morpholinoisoindoline-2-carboxylate (***40b***) was yellow solid (yield: 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (dd, J = 20.7, 8.4 Hz, 1H), 6.83 (m, 2H), 4.60 (dd, J = 16.1, 14.2 Hz, 4H), 3.22 (d, J = 4.6 Hz, 4H), 2.63 (m, 4H), 2.39 (s, 3H), 1.51 (s, 9H). ***41b*** was brown oil in a quantitative yield.

5-Methoxyisoindoline (43): Compound ***35*** (1.5 g, 6.64 mmol, 1.0 eq) was added to a mixture of sodium methoxide (1.79 g, 33.2 mmol, 5.0 eq) and Cu_2O (38 mg, 0.265 mmol, 0.04 eq) in MeOH (12 mL). The reaction was heated to 170 ^{o}C in a microwave reactor for 30 min. Then, the mixture was acidified by aq HCl (10%) until pH ≈ 2. The mixture was extracted with EtOAc for three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and then concentrated to give

intermediate 5-methoxyisoindoline-1,3-dione (***42***) as a yellow solid (0.98 g, yield: 83%), m/z (APCI$^+$) 178.0 (M+H)$^+$.

Intermediate ***43*** was synthesized starting from ***42*** through the same reduction procedure of intermediate ***36***. The resulting product ***43*** was a brown oil (yield: 61%), m/z (APCI$^+$) 150.1 (M+H)$^+$.

5-(Benzyloxy)isoindoline (47): A miture of compound ***34*** (2.45 g, 10 mmol, 1.0 eq), NaOH (2.0 g, 50 mmol, 5.0 eq) and Cu_2O (57.24 mg, 0.4 mmol, 0.04 eq) in water (8 mL) was heated to 140 ^oC in a microwave reactor for 1.5 h until TLC showed complete consumption of compound ***34***. Then, the reaction was filtered and the filtrate was acidified with aq HCl (15%) until pH ≈ 2. The filtrate was extracted with EtOAc three times. The collected organic layers were dried over anhydrous Na_2SO_4 and concentrated to give intermediate 4-hydroxyphthalic acid (***44***) as light yellow solid (1.48 g, yield: 81%).

A mixture of compound ***44*** (1.0 g, 5.5 mmol, 1.0 eq), Urea (0.61 g, 10.1 mmol, 2.0 eq) and imidazole (0.374 g, 5.5 mmol, 1.0 eq) in DMF (2.3 mL) was heated to 160 ^oC in a microwave reactor for 2.5 h. The reaction was acidified with aq HCl (10%) until pH ≈ 2. The resulting mixture was stirred for an additional 1 h at room temperature. The resulting precipitate was filtered and the residue was dried to give intermediate 5-hydroxyisoindoline-1,3-dione (***45***) as orange solid (0.67 g, yield: 68%), m/z (APCI$^+$) 164.1 (M+H)$^+$.

To a stirred mixture of compound ***45*** (0.6 g, 3.68 mmol, 1.0 eq) and K_2CO_3 (0.763 g, 5.52 mmol, 1.5 eq) in DMF (10 mL) was added benzylbromide (0.63 g, 3.68 mmol, 1.0 eq) and then the reaction was heated to 60 ^oC for 3 h. Upon completion, water was added to the reaction mixture. The reaction was extracted with EtOAc. The collected organic layer was washed with HCl (10%) solution, dried over anhydrous Na_2SO_4 and concentrated to give a white solid (0.9 g) which was consisted of two compounds.

One of them was intermediate 5-(benzyloxy)isoindoline-1,3-dione (***46***) with a stronger polarity, m/z (APCI$^+$) 254.1 $(M+H)^+$ and the other one was double alkylate byproduct with a smaller polarity, m/z (APCI$^+$) 344.3 $(M+H)^+$. The mixture was used directly without further purification.

A stirred mixture of $NaBH_4$ (1.35 g, 35.5 mmol, 10.0 eq) and the above mentioned mixed product in THF (30 mL) was cooled to about 0 ^oC followed by addition of BF_3-Et_2O dropwise. The resulting reaction was heated to reflux overnight and then cooled to room temperature. MeOH was added to the reaction mixture and reflux was continued for 1 h. The final reaction was concentrated and the residue was directly purified by column chromatography (DCM : MeOH = 1 : 0 till 20 : 1 gradually with several drops of TEA) to give compound ***47*** as white solid (450 mg, yield: 50%), m/z (APCI$^+$) 226.2 $(M+H)^+$.

Tert-butyl 5-(bromomethyl)isoindoline-2-carboxylate (48): A mixture of compound tert-butyl 5-(hydroxymethyl)isoindoline-2-carboxylate (***30***) (100 mg, 0.401 mmol, 1.0 eq), CBr_4 (160 mg, 0.482 mmol, 1.2 eq) and PPh_3 (127 mg, 0.484 mmol, 1.2 eq) in DCM (2 mL) was stirred at room temperature for 30 min. The reaction was then concentrated and the residue was directly purified by column chromatography (EtOAc : heptane = 1 : 5) to give ***48*** as white solid (99 mg, yield: 79%), m/z (APCI$^+$) 313.8 $(M+H)^+$.

N-(1-(isoindolin-5-ylmethyl)piperidin-4-yl)-N-phenylpropionamide (51a) and ***6-fluoro-3-(1-(isoindolin-5-ylmethyl)piperidin-4-yl)benzo[d]isoxazole (51b)***: A mixture of 5-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole hydrochloride (***49b***) (137 mg, 0.53 mmol, 1.1 eq), intermediate ***48*** (150 mg, 0.48 mmol, 1.0 eq) and K_2CO_3 (200 mg, 1.45 mmol, 3.0 eq) in DMF (10 mL) was stirred at room temperature overnight until compound ***48*** was complete consumption. Then, water was added to the reaction. The mixture was extracted with EtOAc three times. The combined organic layers were washed with brine three times, dried over anhydrous Na_2SO_4 and concentrated to give

intermediate tert-butyl 5-((4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)isoindoline-2-carboxylate (***50b***) as orange oil (180 mg, yield: 83%), m/z (APCI$^+$) 452.8 $(M+H)^+$. A mixture of the pure intermediate ***50b*** in TFA (2 mL) and DCM (10 mL) under ice bath was stirred for 2 h. Saturated Na_2CO_3 solution was then added to the reaction mixture till no bubbles appeared. The final reaction was extracted with brine and EtOAc three times. The collected organic layers were dried over anhydrous Na_2SO_4 and concentrated to give ***51b*** as brown semisolid (120 mg, yield: 85%) that was used without further purification.

The synthesis procedure of ***49a*** to ***51a*** was same with the procedure of ***49b*** to ***51b***. ***50b*** was orange oil (175 mg, yield: 79%), m/z (APCI$^+$) 464.9 $(M+H)^+$. ***51a*** was brown semisolid (120 mg, yield:87 %).

5-((4-(Benzo[b]thiophen-3-ylmethyl)piperazin-1-yl)methyl)isoindoline (57): A mixture of $NaB(AcO)_3$ (1.96 g, 92.46 mmol, 3.0 eq), benzo[b]thiophene-3-carbaldehyde (0.5 g, 30.82 mmol, 1.0 eq) and tert-butyl piperazine-1-carboxylate (0.64 g, 34.4 mmol, 1.1 eq) in DCM (10 mL) was stirred at room temperature overnight. The reaction was then extracted with DCM and brine. The collected organic layer was washed with brine 3 times, dried over anhydrous Na_2SO_4 and concentrated to give intermediate tert-butyl 4-(benzo[b]thiophen-3-ylmethyl)piperazine-1-carboxylate (***54***) as colorless oil (1.0 g, yield: 98%).

The resulting intermediate ***54*** was added to a stirred mixture of TFA (2 mL) and DCM (10 mL) at room temperature and the mixture was stirred for 2 h. Then, saturated Na_2CO_3 sollution was added to the reaction mixture till no bubbles appeared. The final reaction was extracted with brine and DCM three times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give intermediate 1-(benzo[b]thiophen-3-ylmethyl)piperazine (***55***) as a light orange solid (0.6 g, yield:

86%), part of which was converted to HCl salt by a solution of HCl/dioxane (4M) and solvent evaporation. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 2H), 7.97 (dd, J = 6.8, 2.3 Hz, 2H), 7.62 (s, 1H), 7.42 – 7.34 (m, 2H), 3.79 (s, 2H), 3.09 – 3.03 (m, 4H), 2.61 (t, J = 4.1 Hz, 4H).

The synthesis procedure of intermediate ***57*** starting from intermediate ***55*** was same with the procedure of above mentioned ***49b*** to ***51b***. The intermediate tert-butyl 5-((4-(benzo[b]thiophen-3-ylmethyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate (***56***) was purified by column chromatography (DCM: MeOH = 1 : 0 to 30 : 1 gradually) in 83% yield, m/z (APCI$^+$) 464.2 $(M+H)^+$, 408.2 $(M+2H\text{-}tBu)^+$. ***57*** was orange semisolid (yield: 77 %) that was directly used without further purification.

3-(2-(4-(Isoindolin-5-ylmethyl)piperazin-1-yl)ethyl)-1-methyl-1H-indole (63a) **and** ***5-((4-(4-chlorophenethyl)piperazin-1-yl)methyl)isoindoline (63b)***: A mixture of CH_3I (3.1 g, 21.9 mmol, 10.0 eq) and NaH (0.44 g, 11.0 mmol, 5.0 eq) in anhydrous THF (5 mL) was stirred for about 5 min and was added a solution of 3-(2-bromoethyl)-indole (***58a***) (490 mg, 2.19 mmol, 1.0 eq) in THF (20 mL). The stirring was continued overnight until ***58a*** was disappeared. Cold water was added dropwise to the reaction mixture till no bubbles appeared. The resulting mixture was concentrated and the residue was diluted with EtOAc. The solution was washed with brine three times. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was prurified by column chromatography (EtOAc : heptane = 1 : 15) to give 3-(2-bromoethyl)-1-methyl-indole (***59a***) as a yellow oil (0.30 g, yield: 59%)[168], m/z (APCI$^+$) 238.0 $(M+H)^+$.

A stirred mixture of compound ***59a*** (0.52 g, 2.18 mmol, 1.0 eq), tert-butyl piperazine-1-carboxylate (0.488 g, 2.62 mmol, 1.2 eq) and K_2CO_3 (0.61 g, 4.36 mmol, 2.0 eq) in DMF (10 mL) was heated to 70-80 ^oC for 8 h. The reaction was extracted with EtOAc and brine. The collected organic layer was concentrated and the resulting residue wa purified by column chromatography (EtOAc : heptane = 0 : 1, 1 : 5 and 1 :

1 gradually) to give tert-butyl 4-(2-(1-methyl-*1H*-indol-3-yl)ethyl)piperazine-1-carboxylate (***60a***) as a light orange oil (0.7 g, yield: 93%), m/z (APCI$^+$) 344.3 (M+H)$^+$. ***61a*** was synthesized starting from ***60a*** through the same procedure of ***54*** to ***55***. ***61a*** was brown semisolid (yield: 80%), m/z (APCI$^+$) 244.3 (M+H)$^+$. ***61a*** was converted to ***63a*** through the same procedure of ***57***. ***63a*** was brown semisolid (yield: 90%). The intermediate ***62***a was light orange solid (yield: 69%), m/z (APCI$^+$) 475.3 (M+H)$^+$.

Intermediate ***63b*** was synthesized starting from 1-(2-bromoethyl)-4-chlorobenzene (***59b***) through the same procedure of ***59a*** to ***63a***: tert-butyl 4-(4-chlorophenethyl)piperazine-1-carboxylate (***60b***) was colorless oil (yield: 87%); 1-(4-chlorophenethyl)piperazine (***61b***) was colorless oil in a quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.21 (m, 2H), 7.15 – 7.10 (m, 2H), 2.91 (dd, *J* = 9.5, 4.6 Hz, 4H), 2.76 (dd, *J* = 9.7, 6.5 Hz, 2H), 2.59 – 2.42 (m, 6H), 1.86 (s, 1H); tert-butyl 5-((4-(4-chlorophenethyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate (***62b***) was white solid (yield: 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.10 (m, 7H), 4.65 (d, *J* = 16.8 Hz, 4H), 3.51 (s, 2H), 2.76 (m, 2H), 2.66 – 2.22 (m, 10H), 1.51 (s, 9H); ***63b*** was HCl salt (yield: 82%) and synthesized starting from ***62b*** which was stirred in a mixture of HCl/dioxane (4M) (2 mL) and DCM (5 mL) at room temperature for 2 h follwed by solvent evaporation. ***63b*** was used without further purification, m/z (APCI$^+$) 356.4 (M+H)$^+$.

1-(4-(Isoindolin-5-ylmethyl)piperazin-1-yl)-2-(1-methyl-1H-indol-3-yl)ethanone 2 HCl (67a) **and** ***1-(4-(isoindolin-5-ylmethyl)piperazin-1-yl)-3-(1-methyl-1H-indol-3-yl)propan-1-one (67b)***: A mixture of CH_3I (3.75 g, 1.7 mL, 26.4 mmol, 5.0 eq), compound ***64*** (1.0 g, 5.28 mmol, 1.0 eq) and KOH (1.8 g, 30.1 mmol, 5.7 eq) in acetone (25 mL) was stirred under ice bath for 10 min and the stirring was then continued at room temperature for 4 h. Part of solvent was removed by evaporation and water (20 mL) was added to the residue followed by another portion of KOH (1.48 g, 26.4 mmol, 5.0

eq). The resulting mixture was heated to reflux until intermediate (ester) was disappeared. The reaction was acidified by HCl (10%) until pH ≈ 1 and the precipitate was filtered. The residue was washed with water several times and dried at room temperature for several days[169] to give 3-(1-methyl-*1H*-indol-3-yl)propanoic acid (***65b***) as light yellow solid(yield: 98%), m/z (APCI$^+$) 204.3 $(M+H)^+$. The intermediate 2-(1-methyl-*1H*-indol-3-yl)acetic acid (***65a***) was commercial available.

A mixture of ***65a*** (1.0 g, 5.28 mmol, 1.0 eq), tert-butyl piperazine-1-carboxylate (0.98 g, 5.28 mmol, 1.0 eq), HATU (2.41 g, 6.33 mmol, 1.2 eq) and DIPEA(1.37 g, 10.6 mmol, 2.0 eq) in DMF (10mL) was stirred at room temperature overnight. Water was then added to the mixture. The mixture was then extracted with EtOAc three times. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give an orange oil (1.7 g) that was added to a mixture of TFA (2 mL) and DCM (10 mL) and the mixture was stirred at room temperature for 1 h. Then, saturated Na_2CO_3 solution was added to the mixture till no bubbles appeared. The reaction was extracted with EtOAc and brine three times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give 2-(1-methyl-*1H*-indol-3-yl)-1-(piperazin-1-yl)ethanone (***66a***) as a yellow oil (1.2 g, yield: 88%).

Intermediate ***67a*** as HCl salt was synthesized starting from ***66a*** through the same procedure of ***61b*** to ***63b***. Intermediate tert-butyl 5-((4-(2-(1-methyl-*1H*-indol-3-yl)acetyl)piperazin-1-yl)methyl)isoindoline-2-carboxyl ate before the Boc deprotection procedure was light black solid (yield: 85%), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 7.9 Hz, 1H), 7.29 (d, J = 8.2 Hz, 1H), 7.23 (d, J = 7.1 Hz, 1H), 7.21 – 7.14 (m, 3H), 7.11 (t, J = 7.5 Hz, 1H), 6.95 (s, 1H), 4.64 (d, J = 15.8 Hz, 4H), 3.81 (s, 2H), 3.75 (s, 3H), 3.69 – 3.64 (m, 2H), 3.50 – 3.43 (m, 4H), 2.43 – 2.37 (m, 2H), 2.30 – 2.24 (m, 2H), 1.51 (s, 9H). ***67a*** was in 90% yield, m/z (APCI$^+$) 389.5 $(M+H)^+$, 777.7 $(2M+H)^+$.

Intermediate ***67b*** was synthesized through the same procedure of ***67a***. Intermediate 3-(1-methyl-*1H*-indol-3-yl)-1-(piperazin-1-yl)propan-1-one (***66b***) was yellow oil in a quantitive yield. Intermediate tert-butyl 5-((4-(3-(1-methyl-*1H*-indol-3-yl)propanoyl)piperazin-1-yl)methyl)isoindoline-2-carb oxylate before the Boc deprotection procedure was a colorless solid (yield: 87%), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, J = 7.9 Hz, 1H), 7.29 (d, J = 8.2 Hz, 1H), 7.24 – 7.15 (m, 4H), 7.10 (t, J = 7.4 Hz, 1H), 6.88 (s, 1H), 4.65 (d, J = 16.0 Hz, 4H), 3.74 (s, 3H), 3.65 – 3.59 (m, 2H), 3.44 (s, 2H), 3.38 – 3.32 (m, 2H), 3.13 – 3.08 (m, 2H), 2.70 – 2.64 (m, 2H), 2.38 – 2.32 (m, 2H), 2.19 (s, 2H), 1.51 (s, 9H). ***67b*** was in 94% yield, m/z (APCI$^+$) 403.5 $(M+H)^+$, 805.7 $(2M+H)^+$.

1-(4-(Isoindolin-5-yl)piperazin-1-yl)-2-(1-methyl-1H-indol-3-yl)ethanone 2 HCl (68a) **and** ***1-(4-(isoindolin-5-yl)piperazin-1-yl)-3-(1-methyl-1H-indol-3-yl)propan-1-one 2 HCl (68b)***: ***68a*** and ***68b*** were synthesized starting from ***66a*** and ***66b*** through the same procedure of ***37*** to ***40a*** while HCl/dioxane (4M) solution was used instead of TFA.

The yield of intermediate tert-butyl 5-(4-(2-(1-methyl-*1H*-indol-3-yl)acetyl)piperazin-1-yl)isoindoline-2-carboxylate before the Boc deprotection procedure was 26%, ^{1}H NMR (400 MHz, DMSO-D_6) δ 7.57 – 7.53 (m, 1H), 7.38 – 7.35 (m, 1H), 7.23 – 7.06 (m, 4H), 7.02 – 6.97 (m, 1H), 6.88 – 6.84 (m, 1H), 4.47 (t, J = 11.5 Hz, 2H), 3.78 (d, J = 15.1 Hz, 2H), 3.73 (s, 3H), 3.66 – 3.59 (m, 2H), 3.50 – 3.42 (m, 2H), 3.27 – 3.19 (m, 2H), 3.03 (m, 2H), 2.51 (m, 2H), 1.40 (d, J = 22.9 Hz, 9H). m/z (APCI$^+$) 475.6 $(M+H)^+$. A mixture of compound tert-butyl 5-(4-(2-(1-methyl-*1H*-indol-3-yl)acetyl)piperazin-1-yl)isoindoline-2-carboxylate in HCl/dioxane (4M) (2 mL) and DCM (10 mL) was stirred at room temperature for 1.5 h. Then, the solvent was removed directly by evaporation to give ***68a*** that was used without further purification while byproduct will increase dramatically if the reaction was stirred longer than 1.5 h.

The yield of intermediate tert-butyl 5-(4-(3-(1-methyl-*1H*-indol-3-yl)propanoyl)piperazin-1-yl)isoindoline-2-carboxylate before the Boc deprotection procedure was about 38%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54 – 7.49 (m, 1H), 7.34 (m, 1H), 7.21 – 6.96 (m, 5H), 6.86 – 6.83 (m, 1H), 4.51 – 4.44 (m, 2H), 3.70 (d, J = 3.2 Hz, 3H), 3.55 (m, 2H), 3.44 – 3.33 (m, 2H), 3.21 (m, 2H), 3.07 – 2.81 (m, 6H), 2.71 – 2.66 (m, 2H), 2.50 (d, J = 1.9 Hz, 2H), 1.41 (d, J = 20.2 Hz, 9H). m/z (APCI$^+$) 489.5 $(M+H)^+$. The next Boc deprotection step was same with ***68a*** and the product ***68b*** was used without further purification.

N-(isoindolin-5-yl)acetamide hydrochloride (70a): Acetyl chloride (202 mg, 2.57 mmol, 2.0 eq) was added dropwise to a stirred mixture of tert-butyl 5-aminoisoindoline-2-carboxylate (300 mg, 1.28 mmol, 1.0 eq) and DIPEA (497 mg, 3.85 mmol, 3.0 eq) in DCM (5 mL) at room temperature and the stirring was continued for about 10 min. The reaction was directly evaporated. The residue was purified by column chromatography (EtOAc : heptane = 0 : 1 to 2 : 1 gradually) to give a white solid (300 mg, yield: 84%) that was added to a mixture of HCl/dioxane (4M) and DCM at room temperature and the reaction was stirred for 2 h followed by solvent evaporation to yield ***70a*** as a gray solid (210 mg, yield: 90%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 10.01 (s, 2H), 7.74 (s, 1H), 7.45 (d, J = 8.3 Hz, 1H), 7.28 (d, J = 8.3 Hz, 1H), 4.42 (dt, J = 17.4, 5.5 Hz, 4H), 2.04 (s, 3H).

General procedure for the synthesis of intermediate *2-(4-chlorophenyl)-N-(isoindolin-5-yl)acetamide HCl (70b)*, *N-(isoindolin-5-yl)-2-(4-methoxyphenyl)acetamide HCl (70c)*, *2-(3,4-dichlorophenyl)-N-(isoindolin-5-yl)acetamide HCl (70d)*, *2-(2,4-dichlorophenyl)-N-(isoindolin-5-yl)acetamide HCl (70e)*: A mixture of 2-(4-chlorophenyl)acetic acid (200 mg, 1.17 mmol, 1.0 eq), tert-butyl 5-aminoisoindoline-2-carboxylate (275 mg, 1.17 mmol, 1.0 eq), HATU (535 mg, 1.4 mmol, 1.2 eq) and DIPEA (455 mg, 3.52 mmol, 3.0 eq) in DMF (5 mL) was stirred at

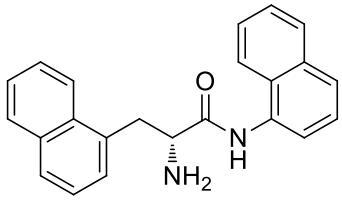
room temperature for about 30 min. The reaction was then extracted with EtOAc and water. The collected organic layer was washed with brine three times followed by concentration. The residue was purified by column chromatography (EtOAc : heptane = 2 : 1 to 1 : 1) to give an intermediate as white solid (410 mg, yield: 90%), m/z (APCI$^+$) 387.0 $(M+H)^+$, 331.0 $(M+2H\text{-}tBu)^+$, 673.0 $(2M+H)^+$. The intermediate was treated same with ***70a*** to deprotect Boc group giving ***70b*** as a gray solid (200 mg, yield: 58%).

Gray solid of ***70c-70e*** were synthesized through the above same procedure: ***70c*** was an orange solid (yield: 96%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H), 9.93 (s, 2H), 7.74 (s, 1H), 7.49 (d, J = 8.3 Hz, 1H), 7.27 (dd, J = 16.9, 8.4 Hz, 3H), 6.86 (d, J = 8.6 Hz, 2H), 4.48 – 4.38 (m, 4H), 3.71 (s, 3H), 3.58 (s, 2H); ***70d*** (yield: 88%), m/z (APCI$^+$) 321.2 $(M+H)^+$, 643.2 $(2M+H)^+$; ***70e*** (yield: 92%), m/z (APCI$^+$) 321.1 $(M+H)^+$, 643.2 $(2M+H)^+$.

General procedure for the synthesis of *8a-8r, 20a-20n, 32a-32g and 52a-52n*: Boc protected amino acid (1.5 mmol) was added to a stirred mixture of EDCI (*N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimid -hydrochlorid) (1.25 mmol) and HOBT (1-Hydroxy-1H-benzotriazole monohydrate) (1.25 mmol) in DMF (3 mL) under ice bath followed by DIPEA (3.5 mmol) (One more equivelant of DIPEA should be used in case TFA or HCl salts). The reaction was stirred for 30 min under ice bath. Then amine (1.0 mmol) was added to the resulting mixture and the stirring was continued overnight at room temperature. Upon completion, the reaction was extracted with water and DCM three times. The collected organic layers were washed with brine, dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography (EtOAc/heptane and then DCM/Methanol) to give corresponding product which was added to a mixture of TFA and DCM (2 : 5) or 4M HCl/dioxane and DCM (2 : 5) under ice bath and the mixture was stirred for 3 h (if the mixture was not easy to be stirred, another 1 mL MeOH was added). Then, saturated Na_2CO_3 solution was added to the reaction mixture until no bubbles

appeared. The reaction was extracted with brine and DCM three times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (EtOAc/heptane and then DCM/Methanol with several drops of TEA) to give final product.

(*R*)-2-amino-*N*,3-di(naphthalen-1-yl)propanamide 8m (PS7):



A stirred mixture of *(R)-2-((tert-butoxycarbonyl)amino)-3-(naphthalen-1-yl)propanoic acid* (***2d***) (0.095 g, 0.3 mmol, 1.0 eq) and dry TEA (triethylamine) in dry THF (1 mL) was cooled to about 0 ^oC in ice bath and then ethyl chloroformate (ECF) (39.07 mg, 35 μL, 0.36 mmol, 1.2 eq) was added dropwise to the mixture. The reaction was stirred for an additional 30 min followed by addition of naphthalen-1-amine (52 mg, 0.36 mmol, 1.2 eq) to the mixture and stirring was continued for 1 h under ice bath and then at room temperature overnight. Water (5 mL) were added to the reaction and the resulting mixture was extracted with EtOAc 3 times. The collected organic layers were washed with 1N HCl solution followed by saturated Na_2CO_3 solution and concentrated. The residue was purified by column chromatography (EtOAc : hepatne = 15 : 1, 2 : 1 gradually) to give (*R*)-tert-butyl (3-(naphthalen-1-yl)-1-(naphthalen-1-ylamino)-1-oxopropan-2-yl)carbamate as a white solid (84 mg, yield: 64%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, *J* = 8.4 Hz, 1H), 7.99 (s, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.76 (dd, *J* = 21.6, 6.5 Hz, 3H), 7.67 – 7.56 (m, 2H), 7.51 (t, *J* = 7.4 Hz, 1H), 7.47 – 7.27 (m, 5H), 7.22 (d, *J* = 8.5 Hz, 1H), 5.44 (s, 1H), 4.84 (d, *J* = 6.5 Hz, 1H), 3.69 (s, 2H), 1.45 (s, 9H). A mixture of the pure product in TFA (2 mL) and DCM (5 mL) under ice bath was stirred for 2 h. Then saturated Na_2CO_3 solution was added to the reaction mixture till no bubbles appeared. The final reaction was extracted with brine three times. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by

column chromatography (EtOAc : heptane = 1 : 1 and then DCM: MeOH = 20 : 1 with several drops of TEA) to give (***8m***) as a white solid (35 mg, yield: 54%).

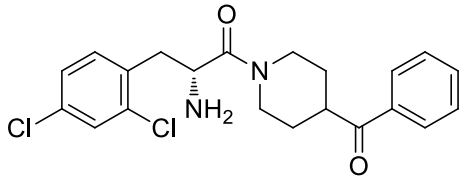
^{1}H NMR (500 MHz, $CDCl_3$) δ 10.29 (s, 1H), 8.33 (dd, *J* = 10.5, 8.1 Hz, 2H), 7.94 – 7.85 (m, 3H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.68 (d, *J* = 8.2 Hz, 1H), 7.60 (ddd, *J* = 8.3, 6.9, 1.3 Hz, 1H), 7.57 – 7.48 (m, 4H), 7.48 – 7.39 (m, 2H), 4.21 (dd, *J* = 14.2, 3.3 Hz, 1H), 4.04 (dd, *J* = 10.5, 3.4 Hz, 1H), 3.04 (dd, *J* = 14.2, 10.5 Hz, 1H), 1.67 (s, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.92, 134.26, 134.23, 134.16, 132.52, 132.00, 129.01 128.91, 128.04, 127.64, 126.65, 126.20, 126.10, 126.09, 125.96, 125.49, 124.85, 123.95, 120.34, 118.26, 56.71, 38.63.

HRMS calculated for $C_{23}H_{21}N_2O^+$ (M+H): 341.1648, found: 341.1641.

HPLC: rt = 12.39 min (98.58 %).

(*R*)-2-amino-1-(4-benzoylpiperidin-1-yl)-3-(2,4-dichlorophenyl)propan-1-one 8a (PS1):



White solid (21 mg, yield: 30%).

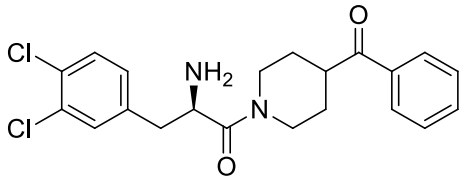
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 – 7.89 (m, 2H), 7.60 – 7.55 (m, 1H), 7.47 (dd, *J* = 10.6, 4.8 Hz, 2H), 7.39 (s, 1H), 7.23 – 7.17 (m, 2H), 4.54 (dd, *J* = 36.0, 13.3 Hz, 1H), 4.09 (dd, *J* = 12.2, 6.4 Hz, 1H), 3.95 (dd, *J* = 25.0, 13.6 Hz, 1H), 3.45 (ddd, *J* = 14.6, 10.7, 3.9 Hz, 1H), 3.24 – 3.13 (m, 1H), 3.07 – 2.97 (m, 1H), 2.89 – 2.80 (m, 2H), 1.93 – 1.65 (m, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 201.39, 201.19, 172.88, 135.67, 135.64, 134.70, 134.53, 134.13, 133.94, 133.63, 133.35, 133.21, 133.06, 129.36, 129.22, 128.78, 128.20, 127.28, 127.04, 49.91, 49.82, 44.76, 44.45, 43.00, 42.93, 41.66, 41.59, 40.25, 39.84, 28.56, 28.50, 28.28, 28.26.

HRMS calculated for $C_{21}H_{23}Cl_2N_2O_2^+$ (M+H): 405.1131, found: 405.1126.

HPLC: rt = 12.23 min (97.89 %).

(*R*)-2-amino-1-(4-benzoylpiperidin-1-yl)-3-(3,4-dichlorophenyl)propan-1-one 8b (PS2):



Colorless sticky oil (28 mg, yield: 29%).

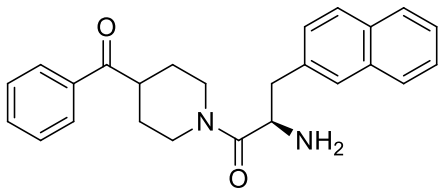
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 – 7.87 (m, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 2H), 7.37 (dd, *J* = 8.1, 3.7 Hz, 1H), 7.31 (s, 1H), 7.05 (dd, *J* = 8.2, 1.4 Hz, 1H), 4.52 (dd, *J* = 12.6, 9.1 Hz, 1H), 3.99 – 3.99 (m, 2H), 3.52 – 3.40 (m, 1H), 3.21 (t, *J* = 11.5 Hz, 1H), 3.03 – 2.64 (m, 4H), 2.03 – 1.67 (m, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 201.36, 201.19, 172.63, 172.56 , 138.15, 137.91, 135.65, 135.59, 133.26, 133.22, 132.54, 132.37, 131.18, 131.15, 131.04, 130.78, 130.50, 130.33, 128.84, 128.79, 128.75, 128.21, 52.17, 44.72, 44.59, 42.88, 41.64, 41.59, 41.52, 28.42, 28.41, 28.35, 28.22.

HRMS calculated for $C_{21}H_{23}Cl_2N_2O_2^+$ (M+H): 405.1131, found: 405.1131.

HPLC: rt = 12.36 min (98.25 %).

(*R*)-2-amino-1-(4-benzoylpiperidin-1-yl)-3-(naphthalen-2-yl)propan-1-one 8c (PS3):



White solid (21 mg, yield: 24%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.74 (m, 5H), 7.65 (s, 1H), 7.55 (m, 1H), 7.45 (m, 4H), 7.33 (dd, *J* = 8.4, 1.5 Hz, 1H), 4.60 – 4.48 (m, 1H), 4.07 (t, *J* = 6.9 Hz, 1H), 3.82 (dd, *J* = 32.5, 13.5 Hz, 1H), 3.45 – 3.25 (m, 1H), 3.20 – 3.09 (m, 1H), 2.99 – 2.58 (m, 3H), 1.89 – 1.55 (m, 6H).

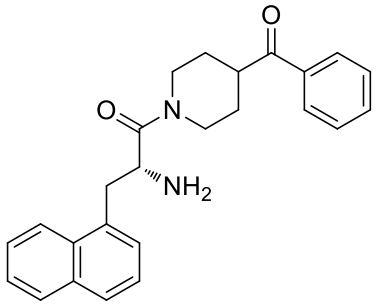
^{13}C NMR (101 MHz, $CDCl_3$) δ 201.43, 201.18, 173.14, 172.98, 135.68, 135.62,

135.21, 135.00, 133.48, 133.17, 133.08, 132.36, 132.31, 128.72, 128.39, 128.17, 128.12, 127.86, 127.65, 127.58, 127.53, 127.43, 127.38, 126,20, 126.17, 125.60, 52.51, 44.71, 44.45, 43.00, 42.99, 42.97, 42.84, 41.54, 41.47, 28.28, 28.15, 28.13.

HRMS calculated for $C_{25}H_{27}N_2O_2^+$ (M+H): 387.207, found: 387.206.

HPLC: rt = 12.45 min (97.98 %).

(*R*)-2-amino-1-(4-benzoylpiperidin-1-yl)-3-(naphthalen-1-yl)propan-1-one 8d (PS4):



Colorless sticky solid (33 mg, yield: 49%).

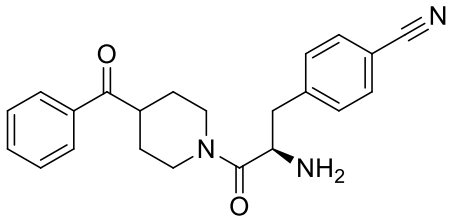
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.3 Hz, 1H), 7.88 – 7.74 (m, 4H), 7.57 – 7.33 (m, 7H), 4.57 – 4.13 (m, 3H), 3.48 – 3.28 (m, 3H), 3.20 – 3.12 (m, 1H), 2.90 – 2.54 (m, 2H), 2.30 (s, 2H), 2.06 – 1.91 (m, 1H), 1.71 (d, *J* = 13.7 Hz, 1H), 1.62 – 1.44 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 201.46, 201.04, 173.25, 173.15, 135.67, 135.61, 133.97, 133.80, 133.73, 133.33, 133.11, 133.08, 132.21, 132.09, 129.08, 128.82, 128.69, 128.12, 128.06, 127.92, 127.83, 127.67, 127.53, 126.28, 125.74, 125.65, 125.60, 125.43, 123.47, 123.20, 51.40, 51.15, 44.80, 44.04, 42.88, 42.58, 41.49, 41.25, 40.22, 40.11, 28.07, 28.01, 27.90, 27.38.

HRMS calculated for $C_{25}H_{27}N_2O_2^+$ (M+H): 387.207, found: 387.206.

HPLC: rt = 12.57 min (97.54 %).

(*R*)-4-(2-amino-3-(4-benzoylpiperidin-1-yl)-3-oxopropyl)benzonitrile 8e (PS5):



White solid (36 mg, yield: 48%).

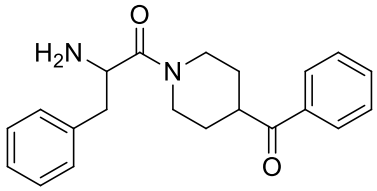
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (dd, *J* = 20.9, 7.5 Hz, 2H), 7.57 (dd, *J* = 17.1, 7.5 Hz, 3H), 7.42 (dt, *J* = 14.2, 6.8 Hz, 4H), 4.61 – 4.26 (m, 3H), 4.14 (s, 2H), 3.77 – 3.64 (m 1H), 3.49 – 3.36 (m, 2H), 3.28 – 3.16 (m, 1H), 3.13 – 3.06 (m, 1H), 2.95 – 2.48 (m, 2H), 1.92 – 1.49 (m, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 201.33, 201.11, 170.51, 169.83, 142.02, 141.97, 135.49, 135.36, 133.41, 133.29, 132.46, 132.26, 130.54, 130.37, 128.83, 128.22, 128.18, 118.69, 118.57, 111.23, 111.06, 70.52, 70.50, 51.58, 51.50, 44.94, 44.90, 42.85, 42.34, 41.81, 41.69, 40.75, 40.28, 28.47, 28.11, 28.05.

HRMS calculated for $C_{22}H_{24}N_3O_2^+$ (M+H): 362.186, found: 362.185.

HPLC: rt = 10.05 min (99.31 %).

(*R*,*S*)-2-amino-1-(4-benzoylpiperidin-1-yl)-3-phenylpropan-1-one 8f (PS11-1):

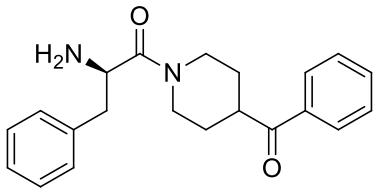


Colorless oil (98 mg, yield: 77%).

HRMS calculated for $C_{21}H_{25}N_2O_2^+$ (M+H): 337.1910, found: 337.1904.

HPLC (254nm): rt_1 = 7.01 min (49.25 %), rt_2 = 8.34 min (47.01 %).

(*R*)-2-amino-1-(4-benzoylpiperidin-1-yl)-3-phenylpropan-1-one 8g (PS11):



White solid (36 mg, yield: 28%).

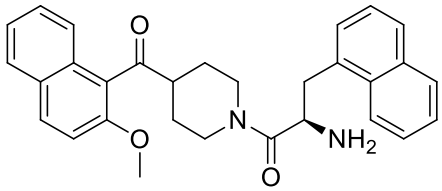
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (dd, *J* = 26.0, 7.4 Hz, 2H), 7.49 (q, *J* = 7.4 Hz, 1H), 7.44 – 7.33 (m, 2H), 7.32 – 7.14 (m, 5H) , 4.98 – 4.85 (m, 1H), 4.34 (dt, *J* = 23.2, 11.6 Hz, 1H), 3.59 – 3.38 (m, 2H), 3.35 – 3.08 (m, 3H), 2.94 – 2.55 (m, 2H), 2.32 – 1.99 (m, 1H), 1.84 – 1.64 (m, 2H), 1.52 – 1.33 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 201.49, 201.43, 167.40, 167.08, 135.53, 135.50, 134.51, 134.17, 133.15, 133.05, 129.98, 129.80, 128.97, 128.69, 128.64, 128.21, 128.17, 127.77, 127.50, 51.28, 51.13, 45.38, 44.84, 42.71, 42.16, 41.75, 41.71, 38.04, 37.91, 29.67, 28.04, 27.42, 27.33.

HRMS calculated for $C_{21}H_{25}N_2O_2^+$ (M+H): 337.1910, found: 337.1918.

HPLC (254nm): rt = 6.87 min (99.71 %).

(*R*)-2-amino-1-(4-(2-methoxy-1-naphthoyl)piperidin-1-yl)-3-(naphthalen-1-yl)propan-1-one 8h (PS6):



White solid (40 mg, yield: 63%).

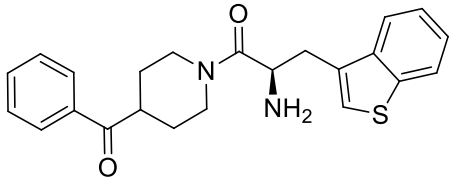
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (dd, *J* = 20.1, 8.3 Hz, 1H), 7.95 – 7.27 (m, 11H), 7.20 (dd, *J* = 9.0, 3.7 Hz, 1H), 4.48 – 4.26 (m, 2H), 3.85 (d, *J* = 10.1 Hz, 3H), 3.68 – 3.59 (m, 1H), 3.55 – 3.33 (m, 4H), 3.28 – 3.15 (m, 1H), 2.92 – 2.64 (m, 2H), 2.54 – 2.34 (m, 1H), 1.87 – 1.42 (m, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 208.26, 207.70, 172.28, 171.72, 153.62, 153.59, 133.91, 133.66, 133.35, 132.78, 132.15, 132.08, 131.37, 130.92, 130.79, 128.98, 128.79, 128.76, 128.74, 128.15, 128.07, 127.74, 127.70, 127.60, 127.53, 126.44, 126.36, 125.72, 125.61, 125.38, 124.13, 123.94, 123.83, 123.73, 123.48, 123.44, 123.33, 112.57 , 56.39, 56.32, 51.25, 50.82, 49.21, 49.13, 45.16, 44.40, 41.69, 41.61, 39.42, 39.10, 27.42, 26.83, 26.58, 26.27.

HRMS calculated for $C_{30}H_{31}N_2O_3^+$ (M+H): 467.2329, found: 467.2325.

HPLC: rt = 13.52 min (99.63 %).

(*R*)-2-amino-3-(benzo[b]thiophen-3-yl)-1-(4-benzoylpiperidin-1-yl)propan-1-one 8i (PS16):



White solid (25 mg, yield: 21%).

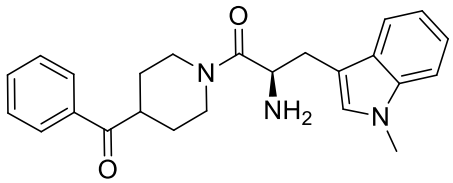
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.81 (m, 3H), 7.77 (d, *J* = 7.5 Hz, 1H), 7.55 (t, *J* = 7.3 Hz, 1H), 7.39 (ddd, *J* = 25.1, 15.6, 7.4 Hz, 4H), 7.24 (d, *J* = 3.2 Hz, 1H), 4.46 (dd, *J* = 33.4, 13.3 Hz, 1H), 4.13 (d, *J* = 6.4 Hz, 1H), 3.63 (dd, *J* = 25.6, 13.7 Hz, 1H), 3.35 – 2.97 (m, 4H), 2.82 – 2.39 (m, 2H), 1.99 (s, 2H), 1.85 – 1.73 (m, 1H), 1.66 – 1.45 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 201.46, 201.15, 173.16, 173.12, 140.54, 140.27, 138.87, 138.72, 135.67, 135.62, 133.17, 133.11, 132.40, 132.05, 128.73, 128.16, 128.13, 124.38, 124.18, 123.95, 123.74, 123.18, 122.95, 121.52, 121.29, 50.86, 50.70, 44.84, 44.22, 42.93, 42.72, 41.53, 41.44, 35.78, 35.58, 28.21, 28.18, 28.13, 27.86.

HRMS calculated for $C_{23}H_{25}N_2O_2S^+$ (M+H): 393.1631, found: 393.164.

HPLC: rt = 10.23 min (98.54 %).

(*R*)-2-amino-1-(4-benzoylpiperidin-1-yl)-3-(1-methyl-1*H*-indol-3-yl)propan-1-one 8j (PS18):



White solid (35 mg, yield: 30%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (t, J = 6.2 Hz, 2H), 7.67 – 7.49 (m, 2H), 7.42 (t, J = 7.6 Hz, 2H), 7.29 – 7.17 (m, 1H), 7.16 – 6.91 (m, 3H), 4.85 – 4.25 (m, 6H), 3.74 (d, J = 7.0 Hz, 3H), 3.54 (d, J = 13.3 Hz, 1H), 3.34 – 3.20 (m, 2H), 3.01 (t, J = 12.0 Hz, 1H), 2.77 – 2.63 (m, 1H), 1.87 – 1.45 (m, 3H).

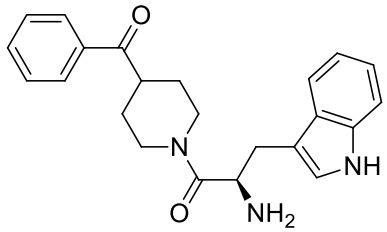
^{13}C NMR (101 MHz, $CDCl_3$) δ 201.42, 136.98, 135.67, 135.63, 133.11, 133.04,

128.69, 128.50, 128.21, 128.18, 128.10, 127.91, 127.73, 121.73, 121.67, 119.23, 119.09, 118.68, 118.51, 109.60, 109.29, 108.73, 108.11, 51.48, 50.86, 45.08, 44.73, 42.91, 42.57, 41.66, 32.70, 32.66, 29.67, 28.23, 28.03, 27.97, 27.23.

HRMS calculated for $C_{24}H_{28}N_3O_2^+$ (M+H): 390.2176, found: 390.2172.

HPLC: rt = 10.02 min (96.04 %).

(*R*)-2-amino-1-(4-benzoylpiperidin-1-yl)-3-(1*H*-indol-3-yl)propan-1-one 8k (PS10):



White solid (104 mg, yield: 83%).

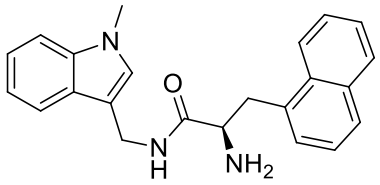
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.73 (s, 0.46H), 9.37 (s, 0.57H), 7.80 – 7.70 (m, 2H), 7.63 – 7.32 (m, 5H), 7.28 (d, J = 8.3 Hz, 0.79H), 7.21 (s, 0.46H), 7.07 – 6.89 (m, 2H), 5.79 (s, 2H), 4.43 (d, J = 3.6 Hz, 1H), 4.16 (d, J = 7.6 Hz, 1H), 3.65 – 3.44 (m, 1H), 3.26 – 3.07 (m, 3H), 2.91 (s, 1H), 2.69 – 2.37 (m, 2H), 1.61 – 1.35 (m, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 201.73, 201.67, 169.47, 169.20, 162.03, 161.76, 136.29, 135.50, 135.44, 133.18, 133.13, 128.70, 128.18, 127.25, 127.16, 125.10, 124.74, 121.90, 121.78, 119.41, 119.26, 118.00, 115.75, 111.84, 107.98, 107.94, 51.12, 50.74, 44.80, 44.65, 42.59, 42.32, 41.69, 41.48, 29.67, 28.85, 28.62, 28.09, 27.73, 27.61, 27.43.

HRMS calculated for $C_{23}H_{26}N_3O_2^+$ (M+H): 376.2020, found: 376.202.

HPLC: rt = 9.08 min (98.21 %).

(*R*)-2-amino-*N*-((1-methyl-1*H*-indol-3-yl)methyl)-3-(naphthalen-1-yl)propanamide 8n (PS8):



Colorless solid (47 mg, yield: 45%).

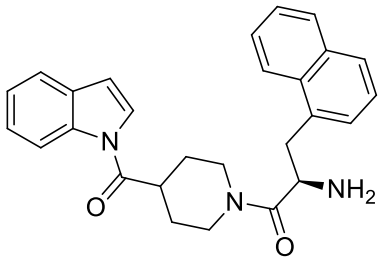
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 8.4 Hz, 1H), 7.85 (d, *J* = 8.3 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.58 – 7.46 (m, 3H), 7.40 (t, *J* = 4.9 Hz, 1H), 7.37 – 7.27 (m, 3H), 7.26 – 7.21 (m, 1H), 7.14 – 7.09 (m, 1H), 6.94 (s, 1H), 4.58 (dd, *J* = 5.3, 2.9 Hz, 2H), 3.98 (dd, *J* = 13.9, 4.3 Hz, 1H), 3.85 (dd, *J* = 9.5, 4.4 Hz, 1H), 3.72 (s, 3H), 3.00 (dd, *J* = 13.9, 9.6 Hz, 1H), 2.34 (s, 2H).

^{13}C NMR (101 MHz,$CDCl_3$) δ 173.53, 137.06, 134.04, 134.02, 131.91, 128.75, 127.79, 17.69, 127.59, 127.06, 126.40, 125.86, 125.32, 123.97, 121.89, 119.33, 118.98, 111.22, 109.31, 55.80, 38.27, 34.61, 32.63.

HRMS calculated for $C_{23}H_{24}N_3O^+$ (M+H): 358.1919, found: 358.1916.

HPLC: rt = 12.54 min (93.12 %).

(*R*)-1-(4-(1*H*-indole-1-carbonyl)piperidin-1-yl)-2-amino-3-(naphthalen-1-yl)propan-1-one 8o (PS9):



White oil (58 mg, yield: 61%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 – 8.15 (m, 2H), 7.88 – 7.68 (m, 1H), 7.58 – 7.28 (m, 5H), 7.24 – 7.09 (m, 2H), 7.00 (s, 2H), 6.50 (dd, J = 21.9, 3.6 Hz, 1H), 5.11 – 4.90 (m, 1H), 4.41 – 4.09 (m, 2H), 3.80 – 3.50 (m, 2H), 3.47 – 3.18 (m, 1H), 3.10 – 2.20 (m, 4H), 1.82 – 1.56 (m, 2H).

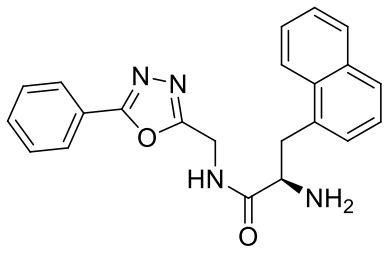
^{13}C NMR (101 MHz, $CDCl_3$) δ 172.33, 172.88, 169.06, 168.97, 135.47, 135.44, 133.85, 133.59, 132.24, 132.03, 131.94, 131.19, 130.25, 130.14, 129.10, 128.68, 128.51, 128.08, 126.85, 126.00, 125.94, 125.58, 125.18, 125.04, 124.40, 123.88,

123.80, 123.76, 123.65, 123.41, 120.69, 116.66, 116.63, 109.45, 109.16, 50.92, 50.57, 44.96, 44.16, 41.29, 41.24, 40.58, 40.11, 36.42, 36.12, 29.68, 29.62, 27.96, 27.75, 27.27, 26.86.

HRMS calculated for $C_{27}H_{28}N_3O_2^+$ (M+H):: 426.2176, found: 426.217.

HPLC: rt = 14.64 min (98.56 %).

(*R*)-2-amino-3-(naphthalen-1-yl)-*N*-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)propanamide 8p (PS13):



White solid (28 mg, yield: 20%).

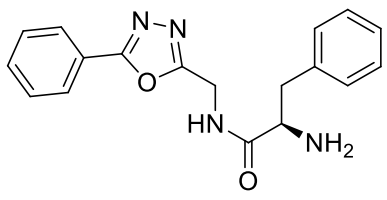
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 – 8.14 (m, 2H), 8.06 – 8.00 (m, 2H), 7.85 (d, *J* = 7.9 Hz, 1H), 7.76 (d, *J* = 8.1 Hz, 1H), 7.57 – 7.47 (m, 5H), 7.41 – 7.33 (m, 2H), 4.79 (d, *J* = 5.9 Hz, 2H), 4.03 (dd, *J* = 14.1, 3.6 Hz, 1H), 3.90 (dd, *J* = 10.3, 3.6 Hz, 1H), 2.95 (dd, *J* = 14.1, 10.4 Hz, 1H), 1.69 (s, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.99, 165.47, 163.56, 134.20, 133.97, 132.02, 131.97, 129.19, 129.01, 128.02, 127.70, 127.10, 126.58, 126.06, 125.46, 123.88, 123.71, 55.82, 38.50, 34.36.

HRMS calculated for $C_{22}H_{21}N_4O_2^+$ (M+H): 373.1659, found: 373.1655.

HPLC: rt = 9.44 min (98.43 %).

(*R*)-2-amino-3-phenyl-*N*-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)propanamide 8q (PS12):



White solid (38 mg, yield: 32%).

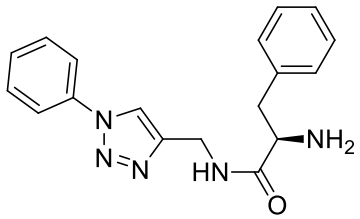
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (t, *J* = 5.2 Hz, 1H), 8.04 – 7.94 (m, 2H), 7.57 – 7.46 (m, 3H), 7.31 – 7.26 (m, 1H), 7.26 – 7.16 (m, 4H), 4.75 (d, *J* = 6.0 Hz, 2H), 3.71 (dd, *J* = 9.2, 4.1 Hz, 1H), 3.29 (dd, *J* = 13.8, 4.1 Hz, 1H), 2.77 (dd, *J* = 13.8, 9.3 Hz, 1H), 1.71 (s, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.66, 165.29, 163.45, 137.50, 131.87, 129.25, 129.04, 128.70, 126.92, 126.88, 123.53, 56.31, 40.74, 34.14.

HRMS calculated for $C_{18}H_{18}N_4O_2Na^+$ (M+Na): 345.1322, found: 345.1318.

HPLC: rt = 8.10 min (99.07 %).

(*R*)-2-amino-3-phenyl-*N*-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)propanamide 8r (PS21):



White solid (150 mg, yield: 49%).

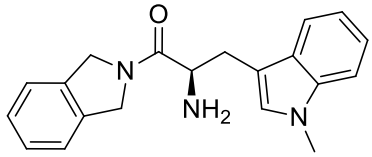
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.46 (s, 1H), 7.85 (dd, *J* = 8.5, 0.9 Hz, 2H), 7.61 (t, *J* = 7.8 Hz, 2H), 7.50 (t, *J* = 7.4 Hz, 1H), 7.36 (b, 2H), 7.26 – 7.15 (m, 5H), 4.47 – 4.36 (m, 2H), 3.90 (s, 1H), 3.05 (dd, *J* = 13.6, 6.3 Hz, 1H), 2.93 (dd, *J* = 13.6, 7.3 Hz, 1H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.57, 145.61, 137.03, 136.07, 130.38, 129.86, 129.13, 128.77, 127.29, 121.64, 120.46, 54.51, 38.23, 34.60.

HRMS calculated for $C_{18}H_{20}N_5O^+$ (M+Na): 322.1662, found: 322.1655.

HPLC: rt = 11.34 min (99.98 %).

(*R*)-2-amino-1-(isoindolin-2-yl)-3-(1-methyl-1*H*-indol-3-yl)propan-1-one 20a (PS19):



Colorless oil (60 mg, yield: 56%).

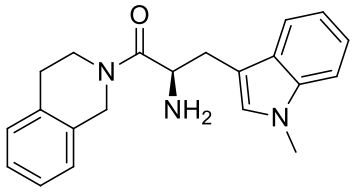
^{1}H NMR (400 MHz, $CDCl_3$). δ 7.63 (d, J = 7.9 Hz, 1H), 7.31 – 7.18 (m, 5H), 7.16 – 7.07 (m, 2H), 6.94 (s, 1H), 4.79 (dd, J = 32.0, 15.9 Hz, 3H), 4.44 (d, J = 13.4 Hz, 1H), 3.95 (t, J = 6.8 Hz, 1H), 3.66 (s, 3H), 3.20 (dd, J = 14.3, 6.2 Hz, 1H), 3.00 (dd, J = 14.3, 7.7 Hz, 1H), 1.99 (br, s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.98, 137.01, 136.11, 136.00, 127.86, 127.72, 127.70, 127.42, 122.85, 122.51, 121.72, 119.03, 118.50, 110.05, 109.39, 53.78, 52.27, 52.02, 32.55, 31.92.

HRMS calculated for $C_{20}H_{22}N_3O^+$ (M+H): 320.1757, found: 320.1757.

HPLC: rt = 8.01 min (98.58 %).

(*R*)-2-amino-1-(3,4-dihydroisoquinolin-2(1*H*)-yl)-3-(1-methyl-1*H*-indol-3-yl)propan-1-one 20b (PS31):



White solid about (80 mg, yield: 48%).

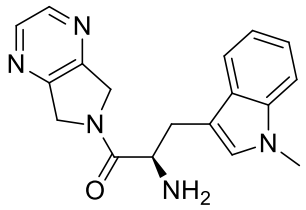
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (t, *J* = 7.9 Hz, 1H), 7.28 (s, 0.16H), 7.59 – 7.05 (m, 4.87H), 7.05 – 6.99 (m, 1.5H), 6.89 (s, 0.5H), 6.77 (s, 0.5H), 6.43 (d, *J* = 7.5 Hz, 0.5H), 4.75 – 4.64 (m, 1H), 4.33 (d, *J* = 15.9 Hz, 0.5H), 4.18 – 4.03 (m, 2H), 3.65 – 3.53 (m, 2H), 3.43 – 3.35 (m, 2H), 3.15 (dd, *J* = 14.2, 6.6 Hz, 0.5H), 3.07 – 2.95 (m, 1.5H), 2.68 (dd, *J* = 12.3, 6.5 Hz, 1.5H), 2.40 – 2.29 (m, 0.5H), 2.08 (s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.29, 174.17, 136.98, 136.83, 134.45, 134.03, 133.19, 132.20, 128.45, 128.14, 127.85, 127.74, 127.66, 127.31, 126.59, 126.47, 126.46, 126.13, 125.70, 121.71, 121.56, 119.05, 118.99, 118.52, 118.34, 110.09, 109.73, 109.45, 109.33, 52.11, 46.80, 44.61, 42.89, 40.21, 32.67, 32.51, 32.46, 32.24, 29.10, 28.23.

HRMS calculated for $C_{21}H_{24}N_3O^+$ (M+H): 334.1914, found: 334.1919.

HPLC: rt = 7.58 min (95.36 %).

(*R*)-2-amino-3-(1-methyl-1*H*-indol-3-yl)-1-(5*H*-pyrrolo[3,4-b]pyrazin-6(7*H*)-yl)propan-1-one 20c (PS32):



White solid (66 mg, yield: 65%).

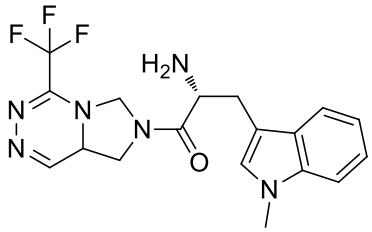
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (dd, *J* = 17.3, 2.7 Hz, 2H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.20 (d, *J* = 8.1 Hz, 1H), 7.17 – 7.12 (m, 1H), 7.10 – 7.05 (m, 1H), 6.95 (s, 1H), 4.89 – 4.79 (m, 2H), 4.72 (d, *J* = 17.6 Hz, 1H), 4.38 (d, *J* = 15.2 Hz, 1H), 3.92 (t, *J* = 6.8 Hz, 1H), 3.69 (s, 3H), 3.22 (dd, *J* = 14.2, 6.8 Hz, 1H), 3.00 (dd, *J* = 14.2, 7.3 Hz, 1H), 1.77 (s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.48, 151.86, 151.51, 143.91, 143.52, 136.95, 127.70, 127.63, 121.82, 119.14, 118.48, 109.90, 109.29, 53.93, 50.93, 50.84, 32.64, 32.37.

HRMS calculated for $C_{36}H_{39}N_{10}O_2^+$ (2M+H): 643.3252, found: 643.325.

HPLC: rt = 4.50 min (94.79 %).

(2*R*)-2-amino-3-(1-methyl-1*H*-indol-3-yl)-1-(4-(trifluoromethyl)-8,8a-dihydroimidazo[1,5-d][1,2,4]triazin-7(6*H*)-yl)propan-1-one 20d (PS39):



White solid (30 mg, yield: 30%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.40 (m, 1H), 7.23 – 6.80 (m, 5H), 4.73 – 4.45 (m, 2H), 4.27 (d, *J* = 16.6 Hz, 1H), 4.07 – 3.98 (m, 1H), 3.66 – 3.56 (m, 3H), 3.15 – 3.04 (m, 2H), 3.02 – 2.79 (m, 2H), 1.89 (s, 2H).

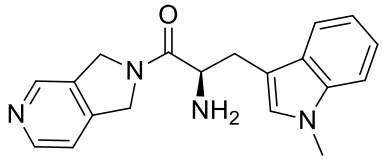
^{13}C NMR (101 MHz, $CDCl_3$) δ 174.92, 148.48, 142.82, 136.11, 129.43, 127.37,

127.04, 121.96, 118.91, 118.46, 109.01, 53.32, 42.41, 41.71, 37.72, 33.02, 32.56, 29.66.

HRMS calculated for $C_{36}H_{39}F_6N_{12}O_2^+$ (2M+H): 785.3212, found: 785.321.

HPLC: rt = 5.20 min (92.76 %).

(*R*)-2-amino-3-(1-methyl-1*H*-indol-3-yl)-1-(1*H*-pyrrolo[3,4-c]pyridin-2(3*H*)-yl)propan-1-one 20e (PS40):



White solid (38 mg, yield: 27%).

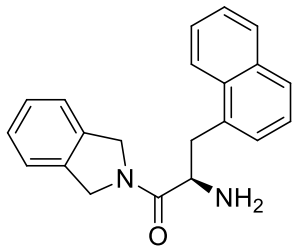
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 – 8.35 (m, 2H), 7.62 – 7.50 (m, 1H), 7.32 – 6.92 (m, 5H), 4.94 – 4.65 (m, 3H), 4.32 (dd, *J* = 31.4, 13.8 Hz, 1H), 4.07 (s, 1H), 3.66 (d, *J* = 5.3 Hz, 3H), 3.27 – 3.19 (m, 1H), 3.16 – 3.07 (m, 1H), 2.34 (s, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 148.61, 148.37, 145.21, 144.61, 144.24, 137.00, 132.44, 132.20, 127.96, 127.93, 127.61, 121.97, 119.25, 118.37, 118.35, 118.01, 117.67, 109.49, 109.46, 53.72, 52.05, 51.56, 50.47, 49.99, 32.62, 32.60, 31.95.

HRMS calculated for $C_{19}H_{21}N_4O^+$ (M+H): 321.1710, found: 321.1701.

HPLC: rt = 2.04 min (91.71 %).

(*R*)-2-amino-1-(isoindolin-2-yl)-3-(naphthalen-1-yl)propan-1-one 20f (PS14):



Colorless oil (50 mg, yield: 50%).

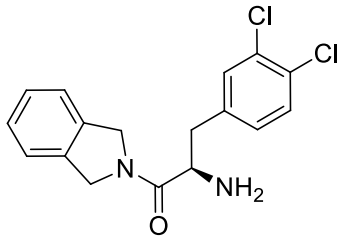
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 8.5 Hz, 1H), 7.80 (d, J = 8.1 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.56 (ddd, J = 8.4, 6.8, 1.3 Hz, 1H), 7.50 – 7.44 (m, 1H), 7.42 – 7.31 (m, 2H), 7.25 – 7.12 (m, 3H), 6.96 (d, J = 7.3 Hz, 1H), 4.83 – 4.72 (m, 1H), 4.61 (dd, J = 13.3, 11.3 Hz, 2H), 4.03 (t, J = 7.0 Hz, 1H), 3.79 (d, J = 13.3 Hz, 1H), 3.45 (qd, J = 13.7, 7.2 Hz, 2H), 1.88 (s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.68, 135.84, 135.74, 133.75, 133.64, 132.07, 128.92, 127.63, 127.60, 127.58, 127.31, 126.19, 125.65, 125.39, 123.31, 122.75, 122.34, 54.00, 52.15, 51.71, 39.65.

HRMS calculated for $C_{21}H_{21}N_2O^+$ (M+H): 317.1648, found: 317.1646.

HPLC: rt = 10.58 min (96.13 %).

(*R*)-2-amino-3-(3,4-dichlorophenyl)-1-(isoindolin-2-yl)propan-1-one 20g (PS22):



White solid (65 mg, yield: 43%).

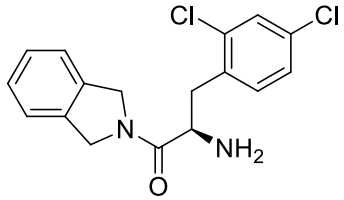
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.29 (m, 2H), 7.29 – 7.23 (m, 3H), 7.21 – 7.17 (m, 1H), 7.07 (dd, *J* = 8.2, 2.0 Hz, 1H), 4.89 (d, *J* = 13.2 Hz, 1H), 4.82 (dd, *J* = 16.0, 1.4 Hz, 1H), 4.75 – 4.69 (m, 1H), 4.49 (d, *J* = 13.3 Hz, 1H), 3.79 (t, *J* = 6.7 Hz, 1H), 2.99 (dd, *J* = 13.6, 6.3 Hz, 1H), 2.76 (dd, *J* = 13.6, 7.6 Hz, 1H), 1.80 (s, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.96, 138.05, 135.90, 135.60, 132.40, 131.14, 130.83, 130.35, 128.78, 127.94, 127.61, 122.96, 122.55, 54.47, 52.32, 52.06, 41.08.

HRMS calculated for $C_{17}H_{17}Cl_2N_2O^+$ (M+H): 335.0713, found: 335.0712.

HPLC: rt = 13.42 min (98.42 %).

(*R*)-2-amino-3-(2,4-dichlorophenyl)-1-(isoindolin-2-yl)propan-1-one 20h (PS23):



White solid (28 mg, yield: 17%).

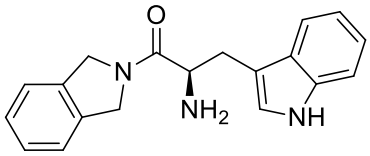
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 2.1 Hz, 1H), 7.29 (dd, *J* = 5.7, 2.7 Hz, 3H), 7.26 – 7.15 (m, 3H), 4.88 (dd, *J* = 27.7, 14.8 Hz, 2H), 4.73 (d, *J* = 15.9 Hz, 1H), 4.62 (d, *J* = 13.6 Hz, 1H), 3.98 (s, 1H), 3.14 (dd, *J* = 13.4, 6.0 Hz, 1H), 2.92 (dd, *J* = 13.4, 8.2 Hz, 1H), 1.69 (br, s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.10, 135.95, 135.78, 134.64, 133.95, 133.45, 133.03, 129.29, 127.89, 127.58, 127.14, 122.95, 122.61, 52.34, 52.24, 52.06, 39.40.

HRMS calculated for $C_{17}H_{17}Cl_2N_2O^+$ (M+H): 335.0713, found: 335.0706.

HPLC: rt = 12.30 min (98.13 %).

(*R*)-2-amino-3-(1*H*-indol-3-yl)-1-(isoindolin-2-yl)propan-1-one 20i (PS20):



White solid (40 mg, yield: 42%).

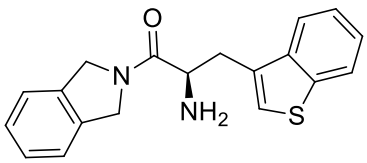
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, *J* = 14.9 Hz, 1H), 7.64 (d, *J* = 7.6 Hz, 1H), 7.32 (dd, *J* = 13.0, 6.1 Hz, 2H), 7.25 – 7.06 (m, 5H), 4.84 (dd, *J* = 14.6, 4.9 Hz, 2H), 4.73 (d, *J* = 15.9 Hz, 1H), 4.41 (d, *J* = 13.6 Hz, 1H), 3.98 (t, *J* = 6.7 Hz, 1H), 3.23 (dd, *J* = 14.3, 6.4 Hz, 1H), 3.03 (dd, *J* = 14.3, 7.5 Hz, 1H), 1.99 (s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.01, 136.27, 136.00, 135.93, 123.03, 123.01, 122.87, 122.52, 122.10, 119.49, 118.35, 111.43, 111.40, 111.37, 53.68, 52.31, 52.03, 32.04.

HRMS calculated for $C_{19}H_{20}N_3O^+$ (M+H): 306.1601, found: 306.160.

HPLC: rt = 8.61 min (97.25 %).

(*R*)-2-amino-3-(benzo[b]thiophen-3-yl)-1-(isoindolin-2-yl)propan-1-one 20j (PS17):



White solid (40 mg, yield: 21%).

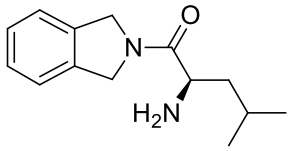
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.74 (m, 2H), 7.43 – 7.26 (m, 3H), 7.25 – 7.16 (m, 3H), 7.06 (d, *J* = 6.8 Hz, 1H), 4.78 (t, *J* = 13.7 Hz, 2H), 4.64 (d, *J* = 15.8 Hz, 1H), 4.19 (d, *J* = 12.8 Hz, 1H), 3.98 (t, *J* = 6.6 Hz, 1H), 3.29 (dd, *J* = 14.1, 7.0 Hz, 1H), 3.15 (dd, *J* = 14.2, 7.1 Hz, 1H), 1.97 (br, s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.46, 140.35, 138.74, 135.86, 135.74, 132.21, 127.74, 127.43, 124.36, 124.10, 123.79, 122.99, 122.83, 122.43, 121.34, 53.22, 52.28, 51.93, 35.12.

HRMS calculated for $C_{19}H_{19}N_2OS^+$ (M+H): 323.1213, found: 323.1210.

HPLC: rt = 9.93 min (96.63 %).

(*R*)-2-amino-1-(isoindolin-2-yl)-4-methylpentan-1-one 20k (PS34):



White solid (52 mg, yield: 40%).

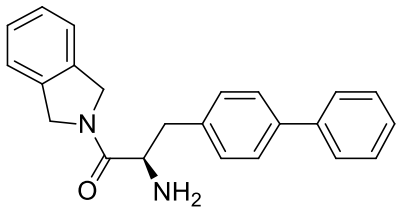
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.22 (m, 4H), 4.83 (ddd, *J* = 23.2, 20.4, 11.7 Hz, 4H), 3.65 (s, 1H), 1.97 – 1.40 (m, 5H), 0.98 (dd, *J* = 11.7, 6.6 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 175.02, 136.23, 135.95 127.87, 127.53, 123.00, 122.61, 52.44, 51.90, 24.72, 23.60, 21.71.

HRMS calculated for $C_{14}H_{21}N_2O^+$ (M+H): 233.1648, found: 233.1648.

HPLC: rt =4.89 min (97.06 %).

(*R*)-3-([1,1'-biphenyl]-4-yl)-2-amino-1-(isoindolin-2-yl)propan-1-one 20l (PS35):



Light yellow solid (89 mg, yield: 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.44 (m, 4H), 7.43 – 7.37 (m, 2H), 7.35 – 7.29 (m, 3H), 7.28 (d, J = 3.8 Hz, 2H), 7.26 – 7.21 (m, 1H), 7.15 (d, J = 7.2 Hz, 1H), 4.92 – 4.74 (m, 3H), 4.39 (d, J = 13.4 Hz, 1H), 3.90 (t, J = 6.3 Hz, 1H), 3.10 (dd, J = 13.3, 6.5 Hz, 1H), 2.89 (dd, J = 13.4, 7.5 Hz, 1H), 2.21 (br, s, 2H).

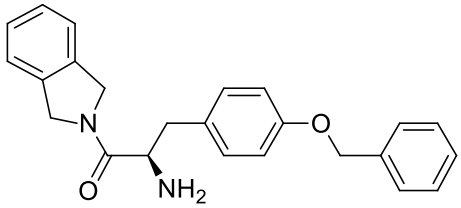
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.39, 140.72, 139.82, 136.58, 135.91, 135.83, 129.69, 128.69, 127.81, 127.53, 127.27, 127.19, 126.98, 122.92, 122.50, 54.84, 52.32,

51.98, 41.96.

HRMS calculated for $C_{23}H_{23}N_2O^+$ (M+H): 343.1805, found: 343.1803.

HPLC: rt = 8.80 min (96.79 %).

(*R*)-2-amino-3-(4-(benzyloxy)phenyl)-1-(isoindolin-2-yl)propan-1-one 20m (PS36):



White solid (55 mg, yield: 18%).

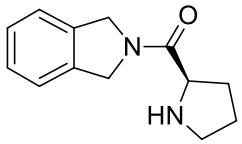
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.26 (m, 7H), 7.25 – 7.10 (m, 4H), 6.87 (d, *J* = 8.5 Hz, 2H), 5.00 – 4.93 (m, 2H), 4.83 (dd, *J* = 14.6, 8.4 Hz, 2H), 4.71 (d, *J* = 16.0 Hz, 1H), 4.30 (d, *J* = 13.4 Hz, 1H), 3.86 (s, 1H), 3.00 (dd, *J* = 13.3, 6.7 Hz, 1H), 2.83 (dd, *J* = 13.4, 7.2 Hz, 1H), 2.39 (br, s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.28, 157.76, 136.95, 135.94, 135.92, 130.30, 129.58, 128.52, 127.89, 127.77, 127.48, 127.37, 122.90, 122.52, 114.99, 70.01, 54.90, 52.28, 51.95, 41.29.

HRMS calculated for $C_{24}H_{25}N_2O_2^+$ (M+H): 373.1911, found: 373.1908.

HPLC: rt = 8.56 min (98.82 %).

(*R*)-isoindolin-2-yl(pyrrolidin-2-yl)methanone 20n (PS33):



Colorless sticky semisolid (78 mg, yield: 77%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.13 (m, 4H), 5.99 (br, s, 1H), 5.08 (d, *J* = 13.4 Hz, 1H), 4.87 – 4.67 (m, 3H), 3.78 – 3.52 (m, 3H), 2.56 (dt, *J* = 14.3, 7.4 Hz, 1H),, 2.21 – 1.95 (m, 3H).

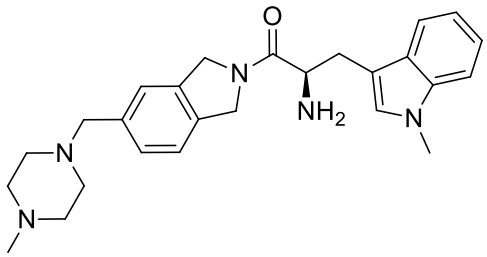
^{13}C NMR (101 MHz, $CDCl_3$) δ 167.92, 135.25, 135.19, 128.32, 128.09, 123.05,

122.94, 72.59, 64.11, 58.92, 53.03, 52.39, 46.77, 29.15, 24.90.

HRMS calculated for $C_{13}H_{17}N_2O^+$ (M+H): 217.1335, found: 217.1334.

HPLC: rt = 2.76 min (97.18 %).

(*R*)-2-amino-3-(1-methyl-1*H*-indol-3-yl)-1-(5-((4-methylpiperazin-1-yl)methyl)isoindolin-2-yl)propan-1-one 32a (PS24):



Gray solid (34 mg, yield: 18%).

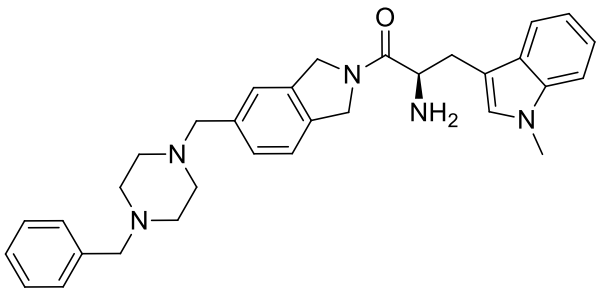
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 7.9 Hz, 1H), 7.26 – 7.04 (m, 6H), 6.94 (s, 1H), 4.86 – 4.69 (m, 3H), 4.47 (t, *J* = 13.7 Hz, 1H), 3.94 (t, *J* = 6.9 Hz, 1H), 3.68 (d, *J* = 2.9 Hz, 3H), 3.48 (d, *J* = 4.9 Hz, 2H), 3.19 (ddd, *J* = 14.2, 5.9, 1.3 Hz, 1H), 2.97 (ddd, *J* = 14.2, 7.9, 3.5 Hz, 1H), 2.47 (br, s, 8H), 2.29 (s, 3H), 2.21 (br, s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.00, 138.12, 137.82, 137.03, 137.01, 136.28, 136.16, 134.98, 134.82, 128.81, 128.50, 127.83, 127.81, 127.74, 127.70, 123.50, 123.14, 122.57, 122.22, 121.71, 121.70, 119.04, 119.02, 118.48, 110.05, 109.36, 62.72, 62.71, 55.01, 54.99, 53.73, 53.71, 52.97, 52.20, 52.10, 51.98, 51.87, 45.91, 45.90, 32.58, 32.57, 31.88, 29.67.

HRMS calculated for $C_{26}H_{34}N_5O^+$ (M+H): 432.27579, found: 432.2752.

HPLC: rt = 4.63 min (96.25 %).

(*R*)-2-amino-1-(5-((4-benzylpiperazin-1-yl)methyl)isoindolin-2-yl)-3-(1-methyl-1*H*-indol-3-yl)propan-1-one 32b (PS25):



White solid (54 mg, yield: 35%).

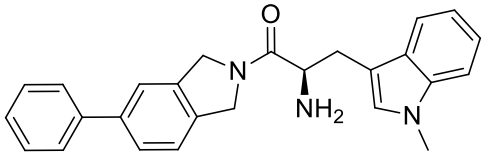
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.9 Hz, 1H), 7.31 (d, *J* = 4.4 Hz, 4H), 7.25 – 7.03 (m, 7H), 6.95 (s, 1H), 4.86 – 4.70 (m, 3H), 4.47 (t, *J* = 12.5 Hz, 1H), 3.97 – 3.91 (m, 1H), 3.68 (d, *J* = 2.3 Hz, 3H), 3.52 (s, 2H), 3.49 (d, *J* = 4.5 Hz, 2H), 3.20 (dd, *J* = 14.4, 5.9 Hz, 1H), 2.97 (ddd, *J* = 14.3, 7.8, 3.4 Hz, 1H), 2.48 (br, s, 8H), 2.08 (br, s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.01, 138.10, 138.02, 138.00, 137.78, 137.03, 137.01, 136.28, 136.16, 134.97, 134.80, 129.20, 128.84, 128.53, 128.17, 127.85, 127.83, 127.71, 127.67, 127.02, 127.01, 123.55, 123.17, 122.55, 121.99, 121.71, 119.05, 119.02, 118.50, 110.11, 109.37, 63.01, 62.78, 62.75, 53.77, 53.75, 53.07, 53.04, 52.98, 52.19, 52.09, 51.98, 51.88, 32.58, 32.57, 31.95, 29.68.

HRMS calculated for $C_{32}H_{38}N_5O^+$ (M+H): 508.3071, found: 508.307.

HPLC: rt = 6.53 min (97.40 %).

(*R*)-2-amino-3-(1-methyl-1*H*-indol-3-yl)-1-(5-phenylisoindolin-2-yl)propan-1-one 32c (PS26):



White solid (26 mg, yield: 44%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 – 7.26 (m, 9H), 7.25 – 7.12 (m, 3H), 7.00 (d, *J* = 1.9 Hz, 1H), 4.95 – 4.73 (m, 3H), 4.50 (d, *J* = 13.3 Hz, 1H), 4.13 – 4.03 (m, 1H), 3.68 (d, *J* = 4.5 Hz, 3H), 3.24 (dd, *J* = 14.1, 5.8 Hz, 1H), 3.08 (dd, *J* = 14.3, 7.8 Hz, 1H), 2.55 (br, s, 2H).

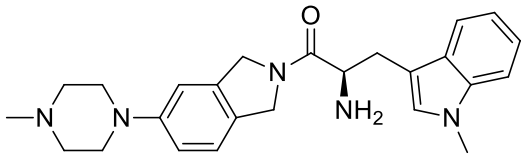
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.42, 141.27, 140.97, 140.63, 137.05, 136.64,

136.58, 134.99, 134.86, 128.83, 128.82, 127.98, 127.73, 127.50, 127.48, 127.11, 127.07, 126.97, 126.67, 123.13, 122.82, 121.82, 121.51, 121.18, 119.17, 119.16, 118.42, 109.45, 109.31, 53.37, 52.40, 52.19, 52.04, 51.86, 32.59, 32.58, 31.91, 29.64.

HRMS calculated for $C_{26}H_{26}N_3O^+$ (M+H): 396.2070, found: 396.207.

HPLC: rt = 11.20 min (95.16 %).

(*R*)-2-amino-3-(1-methyl-1*H*-indol-3-yl)-1-(5-(4-methylpiperazin-1-yl)isoindolin-2-yl)propan-1-one 32d (PS27):



White solid (48 mg, yield: 32%).

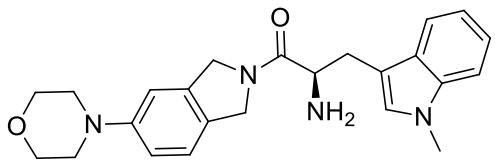
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 7.9 Hz, 1H), 7.26 – 7.17 (m, 2H), 7.11 (dddd, *J* = 8.0, 6.9, 2.2, 1.1 Hz, 1.5H), 7.00 (d, *J* = 8.3 Hz, 0.5H), 6.93 (d, *J* = 3.3 Hz, 1H), 6.83 (ddd, *J* = 12.6, 8.5, 3.2 Hz, 1.5H), 6.64 (d, *J* = 1.7 Hz, 0.5H), 4.80 – 4.62 (m, 3H), 4.44 – 4.36 (m, 1H), 3.92 (t, *J* = 6.9 Hz, 1H), 3.66 (d, *J* = 6.6 Hz, 3H), 3.23 – 3.11 (m, 5H), 2.96 (ddd, *J* = 14.3, 7.8, 3.2 Hz, 2H), 2.61 – 2.52 (m, 4H), 2.34 (s, 3H), 2.18 (br, s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.95, 173.91, 151.48, 151.28, 137.22, 137.09, 137.00, 127.84, 127.71, 127.69, 127.23, 126.94, 123.28, 122.99, 121.67, 119.00, 118.99, 118.49, 118.47, 116.38, 115.86, 110.12, 110.08, 110.04, 109.81, 109.39, 109.37, 55.00, 53.67, 53.65, 52.47, 52.25, 51.79, 51.64, 49.48, 49.37, 46.05, 32.58, 32.55, 31.90, 31.86, 31.84, 29.67.

HRMS calculated for $C_{25}H_{32}N_5O^+$ (M+H): 418.2601, found: 418.2593.

HPLC: rt = 5.39 min (93.21 %).

(*R*)-2-amino-3-(1-methyl-1*H*-indol-3-yl)-1-(5-morpholinoisoindolin-2-yl)propan-1-one 32e (PS28):



White solid (54 mg, yield: 35%).

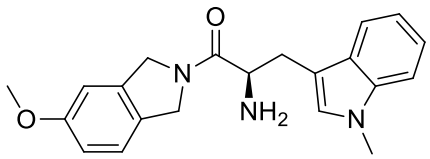
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 7.9 Hz, 1H), 7.29 – 7.19 (m, 2H), 7.18 – 7.10 (m, 1.5H), 7.04 (d, *J* = 8.3 Hz, 0.5H), 6.95 (d, *J* = 2.3 Hz, 1H), 6.87 – 6.78 (m, 1.6H), 6.65 (s, 0.4H), 4.83 – 4.66 (m, 3H), 4.44 (d, *J* = 13.1 Hz, 1H), 3.99 – 3.93 (m, 1H), 3.89 – 3.82 (m, 4H), 3.69 (d, *J* = 5.4 Hz, 3H), 3.23 – 3.09 (m, 5H), 2.98 (ddd, *J* = 14.3, 7.8, 3.0 Hz, 1H), 2.31 (br, s, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.86, 173.80, 151.54, 151.33, 137.31, 137.17, 137.03, 127.82, 127.76, 127.73, 127.63, 127.34, 123.39, 123.09, 121.72, 121.71, 119.03, 118.49, 118.46, 116.06, 115.59, 109.96, 109.92, 109.85, 109.57, 109.40, 109.38, 66.82 (s), 53.54, 52.49, 52.26, 51.81, 51.64, 49.78, 49.69, 32.60, 32.58, 31.90, 31.75, 29.67.

HRMS calculated for $C_{24}H_{29}N_4O_2^+$ (M+H): 405.2285, found: 405.229.

HPLC: rt = 7.35 min (99.08 %).

(*R*)-2-amino-1-(5-methoxyisoindolin-2-yl)-3-(1-methyl-1*H*-indol-3-yl)propan-1-one 32f (PS29):



Yellow solid (65 mg, yield: 35%).

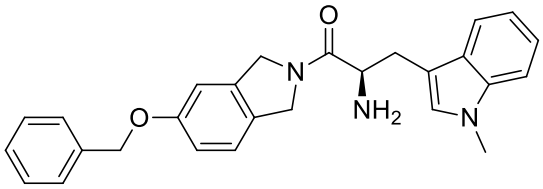
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.8 Hz, 1H), 7.29 – 7.19 (m, 2H), 7.17 – 7.11 (m, 1.5H), 7.03 (d, *J* = 8.2 Hz, 0.5H), 6.96 (d, *J* = 1.7 Hz, 1H), 6.84 – 6.63 (m, 2H), 4.86 – 4.65 (m, 3H), 4.43 (d, *J* = 13.3 Hz, 1H), 3.99 – 3.92 (m, 1H), 3.79 (d, *J* = 7.0 Hz, 3H), 3.68 (d, *J* = 4.2 Hz, 3H), 3.20 (dd, *J* = 14.4, 5.8 Hz, 1H), 3.00 (ddd, *J* = 14.2, 7.9, 3.9 Hz, 1H), 2.11 (br, s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.94, 173.89, 159.70, 159.45, 137.51, 137.35, 137.03, 128.04, 127.82, 127.77, 127.73, 127.43, 123.59, 123.30, 122.85, 122.51, 121.73, 119.05, 119.04, 118.49, 118.47, 114.13, 114.08, 109.94, 109.91, 109.39, 107.71, 107.59, 55.48, 53.62, 53.59, 52.45, 52.16, 51.76, 51.55, 32.58, 32.57, 31.74, 31.73, 29.68.

HRMS calculated for $C_{21}H_{24}N_3O_2^+$ (M+H): 350.1863, found: 350.187.

HPLC: rt = 7.28 min (95.47 %).

(*R*)-2-amino-1-(5-(benzyloxy)isoindolin-2-yl)-3-(1-methyl-1*H*-indol-3-yl)propan-1-one 32g (PS30):



Light yellow solid (54 mg, yield: 29%).

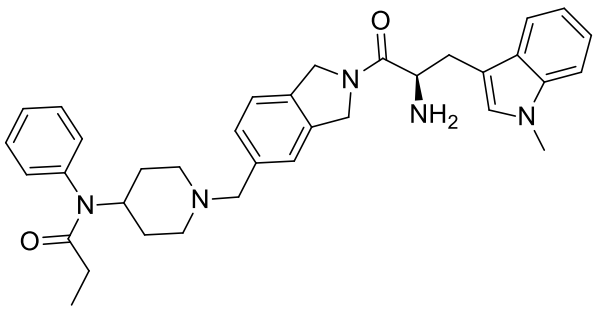
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.9 Hz, 1H), 7.46 – 7.30 (m, 5H), 7.27 – 7.10 (m, 3H), 7.04 – 6.69 (m, 4H), 5.05 (d, *J* = 6.1 Hz, 2H), 4.83 – 4.64 (m, 3H), 4.40 (dd, *J* = 12.8, 6.2 Hz, 1H), 3.95 (t, *J* = 6.1 Hz, 1H), 3.67 (d, *J* = 3.5 Hz, 3H), 3.20 (dd, *J* = 14.3, 6.1 Hz, 1H), 3.00 (ddd, *J* = 14.3, 7.7, 3.0 Hz, 1H), 2.18 (br, s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.85, 173.80, 158.83, 158.59, 137.56, 137.39, 137.02, 137.01, 136.76, 136.74, 128.61, 128.60, 128.37, 128.17, 128.03, 127.83, 127.75, 127.41, 127.35, 123.64, 123.34, 121.73, 119.04, 118.48, 115.07, 114.92, 109.94, 109.39, 108.88, 108.69, 70.26, 53.66, 53.62, 52.43, 52.15, 51.77, 51.56, 32.57, 32.56, 31.74, 29.65.

HRMS calculated for $C_{27}H_{28}N_3O_2^+$ (M+H): 426.2176, found: 426.217.

HPLC: rt = 1.18 min (94.52 %).

(*R*)-*N*-(1-((2-(2-amino-3-(1-methyl-1*H*-indol-3-yl)propanoyl)isoindolin-5-yl)methyl)piperidin-4-yl)-*N*-phenylpropionamide 52a (PS44):



Brown semisolid (120 mg, yield: 84%).

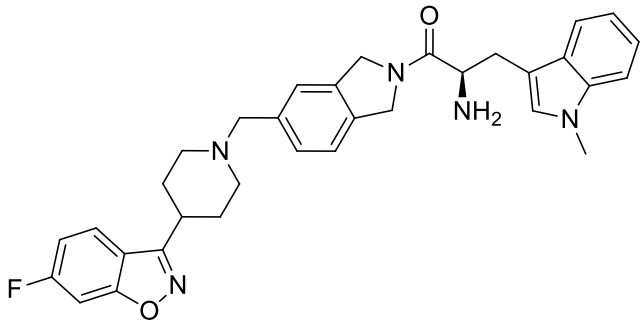
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (dd, *J* = 7.8, 4.7 Hz, 1H), 7.42 – 7.36 (m, 3H), 7.27 – 7.24 (m, 1H), 7.21 (t, *J* = 7.5 Hz, 1H), 7.17 – 7.10 (m, 3H), 7.06 (dd, *J* = 13.2, 7.2 Hz, 3H), 6.99 (s, 1H), 4.86 (d, *J* = 14.1 Hz, 1H), 4.79 – 4.63 (m, 3H), 4.48 (dd, *J* = 13.3, 6.8 Hz, 1H), 4.00 (s, 1H), 3.69 (s, 3H), 3.45 (d, *J* = 1.5 Hz, 2H), 3.21 (dd, *J* = 14.3, 5.4 Hz, 1H), 3.02 (dd, *J* = 11.9, 8.2 Hz, 1H), 2.88 (d, *J* = 10.9 Hz, 2H), 2.33 (br, s, 2H), 2.13 (t, *J* = 11.5 Hz, 2H), 1.91 (q, *J* = 7.4 Hz, 2H), 1.76 (d, *J* = 11.6 Hz, 2H), 1.43 (dd, *J* = 24.2, 12.2 Hz, 2H), 1.01 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.62, 173.56, 138.89, 137.06, 136.16, 136.07, 134.95, 134.79, 130.35, 129.30, 128.91, 128.61, 128.29, 127.96, 127.92, 127.72, 127.70, 123.54, 123.24, 122.55, 122.25, 121.80, 119.15, 119.12, 118.44, 109.42, 109.39, 62.62, 62.57, 53.44, 53.03, 52.99, 52.29, 52.20, 51.97, 51.88, 32.61, 30.36, 28.49, 9.58.

HRMS calculated for $C35H42N5O2^{+}$ (M+H): 564.3333, found: 564.333.

HPLC: rt = 10.89 min (98.61 %).

(*R*)-2-amino-1-(5-((4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)isoindolin-2-yl)-3-(1-methyl-1*H*-indol-3-yl)propan-1-one 52b (PS48):



Gray solid (120 mg, yield: 62%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.59 (d, *J* = 6.7 Hz, 1H), 7.33 – 6.89 (m, 9H), 4.95 – 4.51 (m, 4H), 4.36 – 4.18 (m, 2H), 3.63 (dd, *J* = 28.1, 10.5 Hz, 5H), 3.36 – 2.97 (m, 6H), 2.39 (s, 2H), 2.25 – 2.05 (m, 4H).

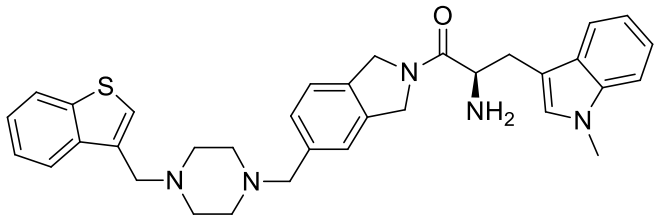
^{13}C NMR (126 MHz, $CDCl_3$) δ 165.14, 163.90, 163.79, 163.14, 160.63, 136.91, 136.47, 136.38, 136.23, 136.17, 135.46, 129.20, 128.97, 128.29, 128.24, 128.21, 127.77, 127.75, 125.49, 123.95, 122.75, 122.73, 122.65, 122.46, 121.71, 119.12,

118.49, 118.47, 117.11, 117.09, 112.62, 112.61, 112.42, 112.41, 109.37, 109.32, 97.48, 97.27, 62.39, 62.33, 52.82, 52.27, 52.17, 52.06, 51.89, 32.58. 32.55, 30.32, 29.68.

HRMS calculated for $C_{33}H_{35}FN_5O_2^+$ (M+H): 552.2769, found: 552.276.

HPLC: rt = 11.15 min (100 %).

(*R*)-2-amino-1-(5-((4-(benzo[b]thiophen-3-ylmethyl)piperazin-1-yl)methyl)isoindolin-2-yl)-3-(1-methyl-1*H*-indol-3-yl)propan-1-one 52c (PS42):



Gray solid (55 mg, yield: 31%).

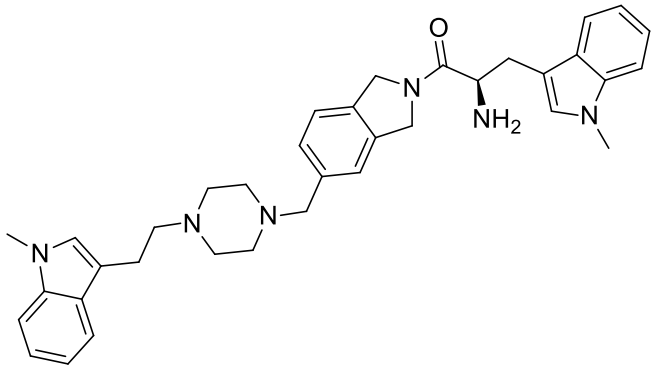
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, *J* = 7.5 Hz, 1H), 7.84 (d, *J* = 7.3 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.41 – 7.31 (m, 3H), 7.25 – 7.04 (m, 6H), 6.98 (s, 1H), 5.00 – 4.65 (m, 3H), 4.53 – 4.43 (m, 1H), 4.00 (s, 1H), 3.79 (s, 2H), 3.68 (d, *J* = 3.1 Hz, 3H), 3.53 (s, 2H), 3.22 (d, *J* = 9.4 Hz, 1H), 3.05 – 2.99 (m, 1H), 2.56 (br, d, *J* = 32.6 Hz, 10H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.63, 151.50, 140.58, 138.91, 137.06, 137.04, 136.27, 136.23, 135.79, 135.18, 135.00, 132.59, 132.56, 129.03, 128.72, 128.24, 127.91, 127.87, 127.78, 127.77, 125.50, 124.76, 124.31, 123.89, 123.42, 122.70, 122.64, 122.55, 122.54, 122.33, 121.78, 119.12, 119.10, 118.48, 109.66, 109.41, 109.40, 62.57, 62.50, 56.19, 52.90, 52.86, 52.82, 52.77, 52.28, 52.19, 52.01, 51.90, 32.62, 32.61, 31.47, 30.33.

HRMS calculated for $C_{68}H_{75}N_{10}O_2S_2^+$ (2M+H): 1127.5510, found: 1127.550.

HPLC: rt = 11.84 min (98.99 %).

(*R*)-2-amino-3-(1-methyl-1*H*-indol-3-yl)-1-(5-((4-(2-(1-methyl-1*H*-indol-3-yl)ethyl)piperazin-1-yl)methyl)isoindolin-2-yl)propan-1-one 52d (PS43):



Gray solid (60 mg, yield: 33%).

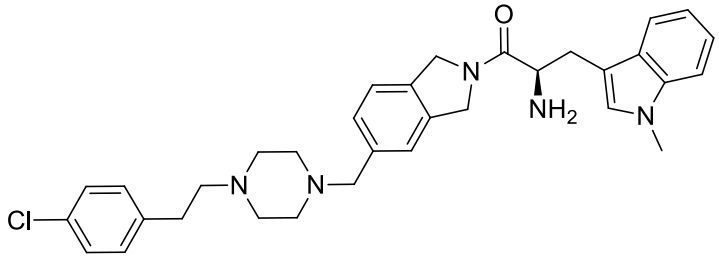
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (dd, *J* = 13.7, 8.1 Hz, 2H), 7.28 (d, *J* = 8.1 Hz, 2H), 7.26 – 7.00 (m, 8H), 6.91 (s, 1H), 5.01 – 4.94 (m, 1H), 4.85 – 4.71 (m, 2H), 4.63 – 4.55 (m, 1H), 4.10 (d, *J* = 15.4 Hz, 1H), 3.73 (dd, *J* = 4.2, 2.6 Hz, 6H), 3.56 (s, 2H), 3.23 (s, 1H), 3.09 (d, *J* = 6.0 Hz, 3H), 2.95 – 2.51 (m, 12H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.66, 137.18, 137.16, 136.98, 136.17, 136.06, 128.97, 128.69, 128.15, 127.61, 127.59, 127.56, 126.64, 123.55, 123.33, 122.72, 122.46, 122.23, 121.94, 121.69, 119.28, 119.25, 118.88, 118.73, 118.42, 109.51, 109.27, 62.36, 62.13, 58.89, 52.75, 52.51, 52.44, 52.06, 51.96, 32.69, 32.67, 32.60, 31.98, 31.91, 31.85, 31.76, 30.03, 29.68, 21.93.

HRMS calculated for $C_{36}H_{43}N_6O^+$ (M+H): 575.3493, found: 575.349.

HPLC: rt = 11.29 min (95.28 %).

(*R*)-2-amino-1-(5-((4-(4-chlorophenethyl)piperazin-1-yl)methyl)isoindolin-2-yl)-3-(1-methyl-1*H*-indol-3-yl)propan-1-one 52e (PS51):



White solid (65 mg, yield: 37%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.8 Hz, 1H), 7.31 – 7.27 (m, 2H), 7.26 – 7.06 (m, 8H), 7.02 (d, *J* = 2.7 Hz, 1H), 4.92 (t, *J* = 12.1 Hz, 1H), 4.77 (dd, *J* = 41.5, 16.0 Hz, 2H), 4.54 (dd, *J* = 18.8, 13.7 Hz, 1H), 4.04 (s, 1H), 3.71 (d, *J* = 4.1 Hz, 3H),

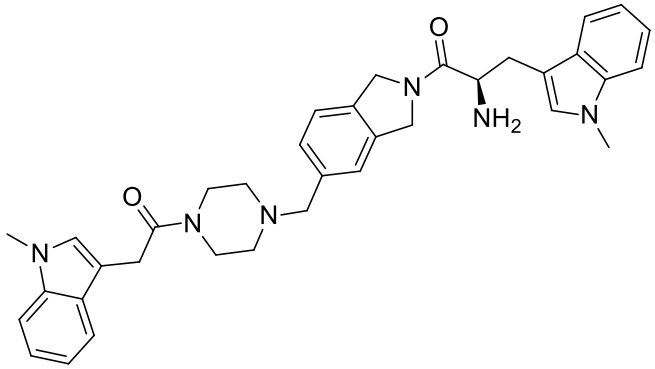
3.55 (s, 2H), 3.24 (d, *J* = 13.7 Hz, 1H), 3.08 – 3.01 (m, 1H), 2.84 (d, *J* = 5.4 Hz, 2H), 2.78 – 2.42 (m, 12H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.60, 173.58, 137.11, 137.09, 136.25, 136.18, 134.98, 134.97, 132.04, 130.23, 130.00, 128.97, 128.67, 128.57, 128.52, 128.04, 127.99, 127.71, 123.60, 123.35, 122.69, 122.68, 122.39, 121.83, 119.17, 119.15, 118.45, 109.44, 62.50, 62.47, 59.77, 53.43, 52.78, 52.77, 52.36, 52.26, 52.03, 51.92, 32.65, 32.63, 32.37, 31.21, 29.68.

HRMS calculated for $C_{33}H_{39}ClN_5O^+$ (M+H):556.2838, found: 556.284.

HPLC: rt = 11.56 min (95.58 %).

(*R*)-2-methyl-3-(1-methyl-1*H*-indol-3-yl)-1-(5-((4-(2-(1-methyl-1*H*-indol-3-yl)acetyl)piperazin-1-yl)methyl)isoindolin-2-yl)propan-1-one 52f (PS50):



Gray solid (89 mg, yield: 48%).

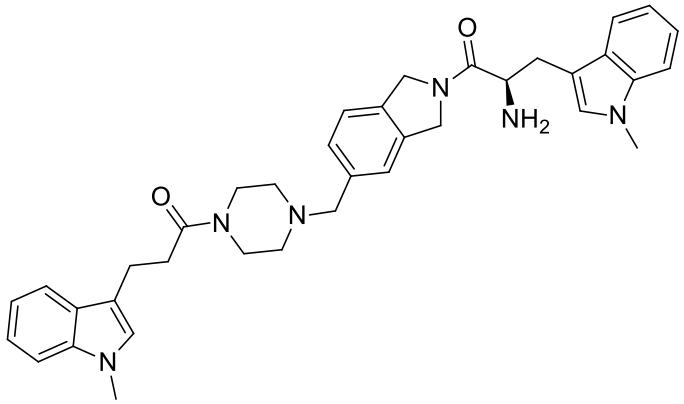
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (dd, *J* = 13.1, 7.8 Hz, 2H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.31 (dd, *J* = 7.7, 4.1 Hz, 1H), 7.26 – 7.07 (m, 7H), 7.00 (dd, *J* = 13.2, 6.8 Hz, 2H), 4.89 (d, *J* = 14.0 Hz, 1H), 4.64 (d, *J* = 16.0 Hz, 1H), 4.55 – 4.39 (m, 2H), 3.89 (s, 1H), 3.73 (s, 4H), 3.66 (d, *J* = 2.5 Hz, 3H), 3.54 – 3.40 (m, 7H), 3.07 (dd, *J* = 14.0, 6.4 Hz, 1H), 2.89 (dd, *J* = 13.3, 6.0 Hz, 1H), 2.26 (s, 4H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.91, 169.50, 137.66, 137.57, 137.11, 136.95, 136.40, 135.81, 135.11, 128.72, 128.70, 128.58, 128.54, 128.17, 128.10, 127.91, 123.71, 123.41, 123.03, 122.82, 121.56, 121.45, 119.36, 118.99, 118.98, 118.86, 109.92, 109.90, 109.80, 107.90, 62.05, 61.99, 53.36, 53.11, 52.61, 52.17, 52.05, 51.75, 51.66, 46.02, 41.62, 32.71, 32.63, 32.62, 30.74, 30.69.

HRMS calculated for $C_{36}H_{41}N_6O_2^+$ (M+H): 589.3286, found: 589.329.

HPLC: rt = 10.70 min (99.32 %).

(*R*)-2-amino-3-(1-methyl-1*H*-indol-3-yl)-1-(5-((4-(3-(1-methyl-1*H*-indol-3-yl)propanoyl)piperazin-1-yl)methyl)isoindolin-2-yl)propan-1-one 52g (PS56):



Gray solid (80 mg, yield: 42%).

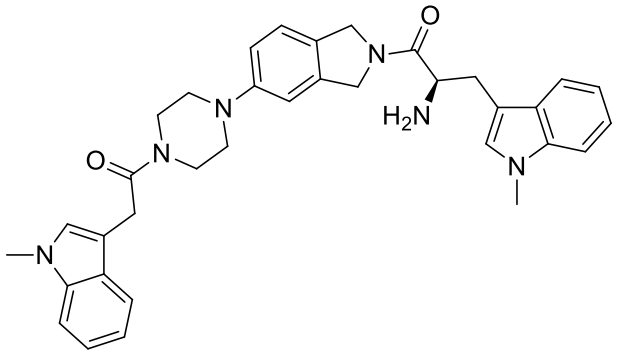
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (t, *J* = 7.7 Hz, 2H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.25 – 6.81 (m, 10H), 4.91 (t, *J* = 12.0 Hz, 1H), 4.75 (dd, *J* = 15.8, 5.4 Hz, 1H), 4.61 (dd, *J* = 15.8, 5.5 Hz, 1H), 4.41 – 4.31 (m, 1H), 4.22 (s, 1H), 3.86 (br, s, 2H), 3.71 (d, *J* = 11.6 Hz, 3H), 3.68 – 3.55 (m, 5H), 3.39 (d, *J* = 6.9 Hz, 2H), 3.34 (d, *J* = 3.9 Hz, 2H), 3.29 – 3.16 (m, 2H), 3.10 (t, *J* = 7.4 Hz, 2H), 2.68 (t, *J* = 6.4 Hz, 2H), 2.32 (d, *J* = 4.6 Hz, 2H), 2.16 (s, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.92, 171.89, 171.30, 137.58, 137.33, 137.09, 137.07, 136.17, 136.09, 134.96, 134.92, 128.80, 128.54, 128.51, 128.43, 127.76, 127.72, 126.59, 123.48, 123.22, 122.69, 122.45, 121.88, 121.64, 119.27, 118.86, 118.83, 118.51, 113.92, 109.49, 109.30, 108.49, 108.45, 62.64, 62.60, 53.18, 52.98, 52.82, 52.78, 52.44, 52.35, 52.13, 52.02, 45.55, 41.57, 34.21, 32.69, 32.68, 32.65, 30.01, 29.76, 21.06.

HRMS calculated for $C_{37}H_{43}N_6O_2^+$ (M+H): 603.3442, found: 603.345.

HPLC: rt = 11.32 min (100.00 %).

(*R*)-2-amino-3-(1-methyl-1*H*-indol-3-yl)-1-(5-(4-(2-(1-methyl-1*H*-indol-3-yl)acetyl)piperazin-1-yl)isoindolin-2-yl)propan-1-one 52h (PS52):



Gray solid (62 mg, yield: 30%).

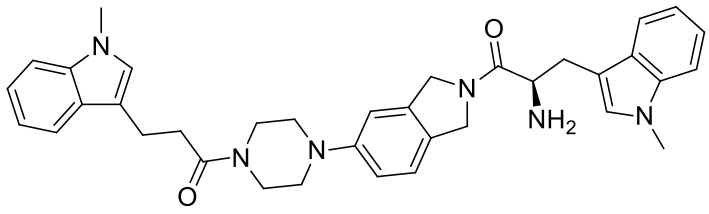
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (dd, *J* = 9.4, 4.8 Hz, 2H), 7.40 – 7.28 (m, 2H), 7.19 – 6.97 (m, 6H), 6.81 (dd, *J* = 28.2, 19.8 Hz, 3H), 4.81 (t, *J* = 14.0 Hz, 1H), 4.61 – 4.52 (m, 1H), 4.45 – 4.29 (m, 2H), 3.91 (t, *J* = 6.7 Hz, 1H), 3.79 (s, 2H), 3.72 (s, 3H), 3.68 – 3.49 (m, 7H), 3.12 – 2.84 (m, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.14, 169.61, 151.11, 151.05, 137.84, 136.97, 136.96, 128.83, 128.82, 128.18, 128.04, 128.02, 127.95, 127.90, 127.00, 126.97, 123.66, 123.44, 121.60, 121.50, 119.36, 118.92, 118.90, 116.10 115.30, 110.44, 110.20, 109.96, 109.32, 107.85, 53.10, 52.48, 52.18, 52.06, 51.78, 51.40, 49.50, 49.05, 45.78, 45.77, 41.40, 32.74, 32.67, 32.66, 30.81, 30.14.

HRMS calculated for $C_{35}H_{39}N_6O_2^+$ (M+H): 575.3129, found: 575.3118.

HPLC: rt = 13.52 min (96.54 %).

(*R*)-2-amino-3-(1-methyl-1*H*-indol-3-yl)-1-(5-(4-(3-(1-methyl-1*H*-indol-3-yl)propanoyl)piperazin-1-yl)isoindolin-2-yl)propan-1-one 52i (PS53):



Gray solid (58 mg, yield: 30%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (t, *J* = 6.8 Hz, 2H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.27 – 6.96 (m, 7H), 6.91 (s, 1H), 6.81 – 6.74 (m, 1H), 6.71 (s, 0.5H), 6.60 (s, 0.5H), 4.86 (t, *J* = 12.7 Hz, 1H), 4.70 (dt, *J* = 31.1, 15.8 Hz, 2H), 4.44 (dd, *J* = 12.9, 5.5 Hz, 1H), 4.05 (s, 1H), 3.77 (s, 2H), 3.72 (s, 3H), 3.69 (d, *J* = 5.4 Hz, 3H), 3.47 (s, 2H), 3.23 (dd,

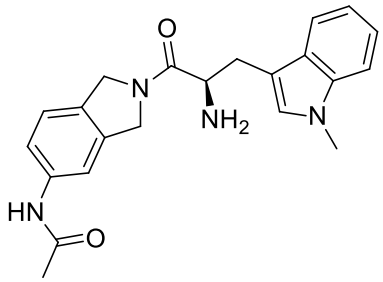
J = 13.5, 4.3 Hz, 1H), 3.16 (t, *J* = 7.5 Hz, 2H), 3.05 (dt, *J* = 9.5, 7.6 Hz, 3H), 2.82 (s, 2H), 2.75 (t, *J* = 7.5 Hz, 2H), 2.60 (br, s, 4H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.40, 173.28, 171.39, 171.38, 151.21, 151.00, 137.11, 137.08, 137.05, 128.11, 128.07, 127.84, 127.73, 127.65, 127.61, 126.59, 123.40, 123.16, 121.81, 121.63, 119.16, 119.14, 118.83, 118.82, 118.74, 118.47, 116.90, 116.54, 113.68, 110.71, 110.51, 109.47, 109.46, 109.29, 53.31, 52.59, 52.27, 51.96, 51.69, 49.84, 49.82, 49.79, 49.67, 45.41, 41.45, 34.08, 34.07, 32.69, 32.63, 32.58, 31.03, 21.07, 21.05.

HRMS calculated for $C_{36}H_{41}N_6O_2^+$ (M+H): 589.3286, found: 589.3281.

HPLC: rt = 13.92 min (98.16 %).

(*R*)-*N*-(2-(2-amino-3-(1-methyl-1*H*-indol-3-yl)propanoyl)isoindolin-5-yl)acetamide 52j (PS57):



Gray solid (58 mg, yield: 43%).

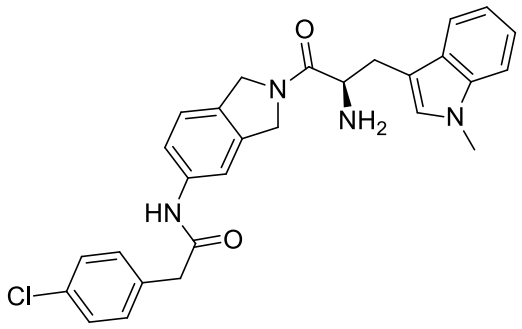
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (d, *J* = 14.0 Hz, 1H), 7.59 – 7.54 (m, 1.5H), 7.46 (s, 0.5H), 7.29 (d, *J* = 8.1 Hz, 0.5H), 7.24 (dd, *J* = 8.1, 3.8 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.09 (dt, *J* = 15.2, 8.0 Hz, 2H), 6.98 (d, *J* = 8.2 Hz, 0.5H), 6.91 (d, *J* = 6.5 Hz, 1H), 4.63 (ddd, *J* = 38.3, 18.9, 10.9 Hz, 3H), 4.37 (dd, *J* = 19.9, 13.5 Hz, 1H), 3.87 (dd, *J* = 14.4, 7.6 Hz, 1H), 3.67 (d, *J* = 11.9 Hz, 3H), 3.17 (dd, *J* = 14.3, 5.3 Hz, 1H), 2.97 – 2.88 (m, 1H), 2.11 (t, *J* = 13.8 Hz, 5H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.60, 173.58, 169.00, 168.96, 138.12, 137.82, 137.06, 137.04, 136.68, 131.38, 131.33, 127.77, 127.76, 127.73, 127.69, 122.96, 122.74, 121.85, 121.82, 119.63, 119.58, 119.17, 119.11, 118.42, 118.40, 114.60, 114.37, 109.69, 109.69, 109.47, 53.60, 53.55, 52.29, 52.09, 51.90, 51.68, 32.62, 32.59, 32.00, 31.97, 31.85, 24.30, 24.28.

HRMS calculated for $C_{22}H_{25}N_4O_2^+$ (M+H): 377.1972, found: 377.198.

HPLC: rt = 10.07 min (99.65 %).

(*R*)-*N*-(2-(2-amino-3-(1-methyl-1*H*-indol-3-yl)propanoyl)isoindolin-5-yl)-2-(4-chlorophenyl)acetamide 52k (PS54):



Gray solid (56 mg, yield: 20%).

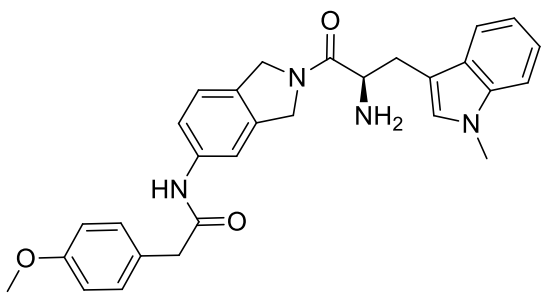
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.22 (d, *J* = 1.8 Hz, 1H), 7.62 – 7.53 (m, 2H), 7.47 – 6.97 (m, 9H), 4.87 (t, *J* = 13.9 Hz, 1H), 4.66 – 4.58 (m, 1H), 4.46 (t, *J* = 15.5 Hz, 1H), 4.40 – 4.31 (m, 1H), 3.96 (dd, *J* = 13.4, 6.8 Hz, 1H), 3.68 – 3.59 (m, 5H), 3.09 (dd, *J* = 14.2, 6.7 Hz, 1H), 2.94 (dd, *J* = 14.3, 6.9 Hz, 1H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.15, 138.01, 138.95, 137.43, 136.97, 136.69, 135.38, 132.28, 131.72, 131.43, 130.85, 128.92, 128.90, 128.65, 128.23, 128.00, 127.95, 123.54, 123.30, 121.53, 119.04, 118.92, 113.95, 113.66, 109.99, 109.06, 109.04, 53.04, 53.01, 52.43, 52.01, 51.56 , 42.86, 32.68 29.79, 29.78.

HRMS calculated for $C_{28}H_{28}ClN_4O_2^+$ (M+H): 487.1895, found: 487.1892.

HPLC: rt = 13.32 min (97.07 %).

(*R*)-*N*-(2-(2-amino-3-(1-methyl-1*H*-indol-3-yl)propanoyl)isoindolin-5-yl)-2-(4-methoxyphenyl)acetamide 52l (PS58):



Gray semisolid (95 mg, yield: 62%).

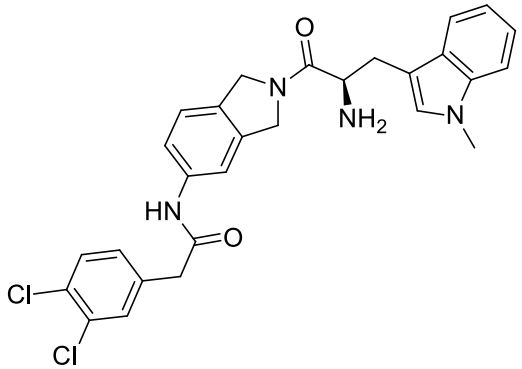
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, *J* = 41.1 Hz, 1H), 7.57 (t, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 50.0 Hz, 1H), 7.25 – 6.85 (m, 10H), 4.71 – 4.55 (m, 3H), 4.39 – 4.31 (m, 1H), 3.90 – 3.82 (m, 1H), 3.78 (s, 3H), 3.66 (d, *J* = 10.5 Hz, 3H), 3.60 (d, *J* = 9.0 Hz, 2H), 3.16 (dd, *J* = 14.2, 5.9 Hz, 1H), 2.92 (td, *J* = 14.8, 8.0 Hz, 1H), 1.87 (s, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.87, 173.77, 169.92, 158.98, 137.76, 137.46, 137.05, 137.03, 136.83, 136.78, 131.72, 131.66, 130.51, 130.47, 127.79, 127.73, 127.71, 126.48, 126.47, 122.96, 122.74, 121.81, 121.77, 121.73, 119.63, 119.52, 119.14, 119.07, 119.04, 118.46, 114.56, 114.49, 114.48, 114.34, 109.93, 109.90, 109.45, 109.44, 55.30, 53.70, 52.25, 52.04, 51.87, 51.67, 43.63, 32.61, 32.59, 32.11, 32.04.

HRMS calculated for $C_{29}H_{30}N_4O_3Na^+$ (M+H): 505.2210, found: 505.2208.

HPLC: rt = 12.25 min (99.51 %).

(*R*)-*N*-(2-(2-amino-3-(1-methyl-1*H*-indol-3-yl)propanoyl)isoindolin-5-yl)-2-(3,4-dichlorophenyl)acetamide 52m (PS59):



Gray solid (240 mg, yield: 81%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (d, *J* = 36.5 Hz, 1H), 7.60 – 7.45 (m, 2H), 7.44 – 7.29 (m, 2H), 7.28 – 7.25 (m, 1H), 7.21 (dd, *J* = 13.1, 6.1 Hz, 6H), 4.72 – 4.53 (m, 3H), 4.39 (t, *J* = 13.5 Hz, 1H), 3.87 (dt, *J* = 13.5, 7.1 Hz, 1H), 3.72 – 3.65 (m, 3H), 3.60 – 3.50 (m, 2H), 3.18 (dd, *J* = 14.3, 5.7 Hz, 1H), 2.98 – 2.87 (m, 1H), 2.06 (br, s, 2H).

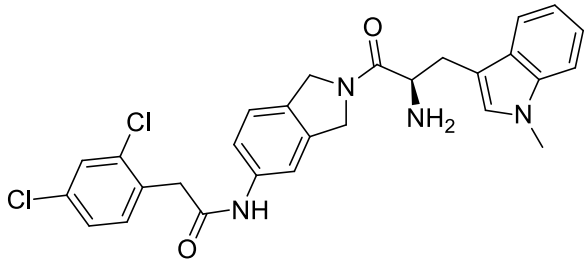
^{13}C NMR (126 MHz, $CDCl_3$) δ 173.86, 173.81, 173.69, 173.59, 168.61, 168.59, 146.50, 146.21, 137.82, 137.50, 137.09, 137.07, 136.98, 136.72, 136.68, 134.95, 134.94, 132.57, 131.72, 131.65, 131.35, 131.21, 131.17, 130.57, 130.56, 128.72,

128.65, 127.81, 127.79, 127.73, 127.70, 127.65, 125.57, 125.39, 123.48, 123.19, 123.01, 122.77, 121.89, 121.86, 121.79, 119.76, 119.63, 119.21, 119.14, 119.09, 118.43, 118.40, 118.38, 115.13, 114.77, 114.69, 114.46, 109.80, 109.65, 109.62, 109.51, 109.47, 109.46, 108.89, 108.54, 53.56, 52.33, 52.27, 52.14, 52.06, 51.90, 51.85, 51.71, 51.68, 43.00, 42.98, 32.64, 32.61, 32.60, 32.59, 32.05, 32.02, 31.92, 31.90.

HRMS calculated for $C_{28}H_{27}Cl_2N_4O_2^+$ (M+H): 521.1506, found: 521.1514.

HPLC: rt = 14.04 min (99.92 %).

(*R*)-*N*-(2-(2-amino-3-(1-methyl-1*H*-indol-3-yl)propanoyl)isoindolin-5-yl)-2-(2,4-dichlorophenyl)acetamide 52n (PS60):



Gray solid (250 mg, yield: 81%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (d, *J* = 57.5 Hz, 1H), 7.59 – 7.45 (m, 2H), 7.37 (d, *J* = 1.9 Hz, 1H), 7.30 – 7.17 (m, 4H), 7.15 – 6.87 (m, 4H), 4.69 – 4.55 (m, 3H), 4.37 (t, *J* = 14.4 Hz, 1H), 3.90 – 3.82 (m, 1H), 3.69 (dd, *J* = 17.1, 12.1 Hz, 5H), 3.17 (dd, *J* = 14.1, 5.1 Hz, 1H), 2.92 (ddd, *J* = 18.7, 14.3, 8.0 Hz, 1H), 1.99 (br, s, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.73, 173.59, 167.93, 137.76, 137.42, 137.08, 137.05, 136.78, 136.70, 134.99, 134.96, 133.96 (s), 132.45, 132.40, 131.76, 131.68, 131.46, 131.45, 129.38, 127.77, 127.74, 127.66, 127.49, 127.48, 122.99, 122.76, 121.87, 121.82, 119.81, 119.65, 119.20, 119.12, 118.40, 118.38, 114.72, 114.54, 109.72, 109.68, 109.49, 109.47, 53.58, 52.25, 52.03, 51.87, 51.67, 41.22, 41.19, 32.62, 32.60, 32.10, 32.04, 31.98, 31.91.

HRMS calculated for $C_{28}H_{27}Cl_2N_4O_2^+$ (M+H): 521.1506, found: 521.1501; $C_{28}H_{26}Cl_2N_4O_2Na^+$ (M+Na): 543.1325, found: 543.1320.

HPLC: rt = 12.18 min (99.77 %).

5.3.2 Synthesis procedure and compound characterization for part 3

(4-(Acetamidomethyl)phenyl)boronic acid (72): Acetylchloride (0.84 g, 0.77 mL, 10.7 mmol, 2.0 eq) was added dropwise to a stirred mixture of (4-(aminomethyl)phenyl)boronic acid hydrochloride (***71***) (1.0 g, 5.33 mmol, 1.0 eq) and DIPEA (2.42 g, 18.7 mmol, 3.5 eq) in DCM (10 mL) at room temperature. The reaction was stirred for an additional 0.5 h at room temperature. Then, the reaction was concentrated and purified by column chromatography (EtOAc : heptane and then DCM : MeOH gradually) to give compound ***72*** as white solid (0.6 g, yield: 59%), m/z ($APCI^+$) 150.1 ($M-B(OH)_2^-$).

N'-propylbenzohydrazide (byproduct 75-1): This compound was white solid. ^1H NMR (400 MHz, DMSO-d_6) δ 11.42 (s, 1H), 7.87 (d, J = 8.4 Hz, 2H), 7.71 (t, J = 5.5 Hz, 1H), 7.61 (d, J = 8.4 Hz, 2H), 2.22 (dd, J = 12.9, 7.2 Hz, 2H), 1.50 (dq, J = 14.7, 7.3 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H).

General procedure for the synthesis of *tert-butyl 2-(4-iodobenzoyl)-1-propylhydrazinecarboxylate (76a)*, *tert-butyl 1-butyl-2-(4-iodobenzoyl)hydrazinecarboxylate (76b)*, *tert-butyl 2-(4-iodobenzoyl)-1-pentylhydrazinecarboxylate (76c)* and *tert-butyl 1-hexyl-2-(4-iodobenzoyl)hydrazinecarboxylate (76d)*: To a stirred mixture of 4-iodobenzohydrazide (***74***) (1.0 mmol), anhydrous Na_2SO_4 (0.5 g) and pTSA (5% mmol) in MeOH (5 mL) was added dropwise the corresponding aldehyde (1.05 mmol). The mixture was stirred at room temperature for 2 h followed by addition of $NaBH(AcO)_3$ (2.0 mmol) to the reaction. The resulting mixture was stirred for an additional 1 h. Then, saturated Na_2CO_3 solution was added to the mixture until pH ≈ 10. The mixture was extracted with DCM and water three times. The collected organic layers were washed with brine, dried over anhydrous Na_2SO_4 and

concentrated. The residue was purified by column chromatography (EtOAc : heptane = 1 : 10 to 1 : 3 gradually) to give ***75a-75d*** as white solids. To a stirred mixture of the corresponding ***75a-75d*** (1.0 mmol) and TEA (2.5 mmol) in THF (6 mL) was added $(Boc)_2O$ (1.1 mmol). The reaction was stirred at room temperature overnight. The reaction was concentrated and the residue was purified by column chromatography (EtOAc : heptane = 1 : 15, 1 : 10 to 1 : 5) to give ***76a-76d*** as white solid or colorless oil.

4-Iodo-N'-propylbenzohydrazide (75a) was white solid in 78% yield. m/z ($APCI^+$) 305.4 $(M+H)^+$.

Tert-butyl 2-(4-iodobenzoyl)-1-propylhydrazinecarboxylate (76a) was white solid in 81% yield. ^1H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.77 (s, 2H), 7.49 (d, *J* = 8.4 Hz, 2H), 3.59 – 3.50 (m, 2H), 1.62 (dt, *J* = 14.7, 7.4 Hz, 2H), 1.47 (s, 9H), 0.93 (t, *J* = 7.4 Hz, 3H).

N'-butyl-4-iodobenzohydrazide (75b) was white solid in 87% yield. m/z ($APCI^+$) 319.1 $(M+H)^+$, 637.3 $(2M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 10.04 (d, *J* = 3.7 Hz, 1H), 7.87 – 7.79 (m, 2H), 7.61 – 7.55 (m, 2H), 5.05 (s, 1H), 2.76 (s, 2H), 1.45 – 1.28 (m, 4H), 0.87 (t, *J* = 7.2 Hz, 3H).

Tert-butyl 1-butyl-2-(4-iodobenzoyl)hydrazinecarboxylate (76b) was sticky oil in 68% yield.

4-Iodo-N'-pentylbenzohydrazide (75c) was white solid in 47% yield. m/z ($APCI^+$) 333.2 $(M+H)^+$, 665.2 $(2M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 10.04 (d, *J* = 4.2 Hz, 1H), 7.86 – 7.77 (m, 2H), 7.61 – 7.54 (m, 2H), 5.04 (d, *J* = 6.2 Hz, 1H), 2.74 (dd, *J* = 10.5, 6.6 Hz, 2H), 1.47 – 1.24 (m, 6H), 0.86 (dd, *J* = 9.3, 4.8 Hz, 3H).

Tert-butyl 2-(4-iodobenzoyl)-1-pentylhydrazinecarboxylate (76c) was white solid in 63% yield. ^1H NMR (500 MHz, DMSO-d_6) δ 10.56 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.66 – 7.55 (m, 2H), 3.39 (s, 2H), 1.43 (dd, *J* = 25.7, 16.3 Hz, 6H), 1.29 (d, *J* = 20.6 Hz, 9H), 0.85 (s, 3H).

N'-hexyl-4-iodobenzohydrazide (75d) was white solid in 90% yield. m/z ($APCI^+$) 347.2 $(M+H)^+$, 693.3 $(2M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 10.03 (d, *J* = 4.6

Hz, 1H), 7.88 – 7.80 (m, 2H), 7.63 – 7.55 (m, 2H), 5.04 (d, *J* = 5.0 Hz, 1H), 2.74 (dd, *J* = 11.4, 6.8 Hz, 2H), 1.43 (dt, *J* = 13.9, 7.4 Hz, 2H), 1.34 – 1.21 (m, 5H), 0.84 (t, *J* = 6.9 Hz, 3H).

Tert-butyl 1-hexyl-2-(4-iodobenzoyl)hydrazinecarboxylate (76d) was white solid in 63% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.54 (s, 1H), 7.87 (d, *J* = 8.4 Hz, 2H), 7.66 – 7.56 (m, 2H), 3.40 (s, 2H), 1.54 – 1.39 (m, 6H), 1.28 (d, *J* = 31.0 Hz, 11H), 0.84 (d, *J* = 6.6 Hz, 3H).

Tert-butyl 2-(4'-((((benzyloxy)carbonyl)amino)methyl)-[1,1'-biphenyl]-4-carbonyl)-1-propylhydrazinecarboxylate (77a): A stirred mixture of (4-((((benzyloxy)carbonyl)amino)methyl)phenyl)boronic acid (***73***) (400 mg, 1.4 mmol, 1.1 eq), ***76a*** (514 mg, 1.27 mmol, 1.0 eq), $Pd(PPh_3)_4$ (59 mg, 0.05 mmol, 0.04 eq) and K_2CO_3 (387 mg, 2.8 mmol, 2.2 eq) in toluene (18 mL), MeOH (2 mL) and H_2O (2 mL) was kept under argon atmosphere and heated to 90 °C for 7 h. The reaction was directly concentrated and the residue was purified by column chromatography (EtOAc : heptane = 1 : 4 to 1 : 2 gradually) to give ***77a*** as white solid (0.6 g, yield: 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 7.8 Hz, 2H), 7.70 – 7.61 (m, 3H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.49 – 7.44 (m, 1H), 7.37 (dd, *J* = 12.0, 7.9 Hz, 5H), 5.16 (s, 2H), 4.44 (d, *J* = 6.0 Hz, 2H), 3.58 (t, *J* = 7.3 Hz, 2H), 1.65 (dd, *J* = 14.6, 7.3 Hz, 2H), 1.48 (s, 9H), 0.95 (t, *J* = 7.4 Hz, 3H).

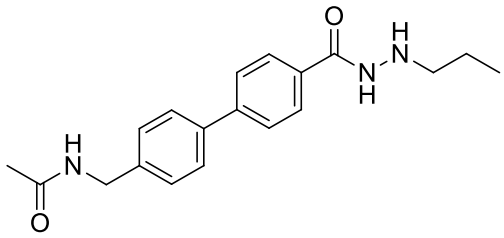
Tert-butyl 2-(4'-(aminomethyl)-[1,1'-biphenyl]-4-carbonyl)-1-propylhydrazinecarboxylate (78a): Pd/C (10%) (140 mg) was added to a mixture of ***77a*** (0.7 g, 1.85 mmol, 1.0 eq) in MeOH (15 mL) at room temperature and followed by $NaBH_4$ (350 mg, 9.25 mmol, 5.0 eq) over three portions. The reaction was charged with a balloon and stirred overnight. The mixture was filtered. The collected filtrate was extracted with EtOAc and brine three times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give ***78a*** as colorless oil (0.46 g, yield: 80%). m/z

(APCI$^+$) 328.5 (M-tBu+2H)$^+$, 384.7 (M+H)$^+$.

Procedure for the synthesis of *N-((4'-(2-propylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl)methyl)acetamide 80a (PSP40)*:

Tert-butyl 2-(4'-(acetamidomethyl)-[1,1'-biphenyl]-4-carbonyl)-1-propylhydrazinecarboxylate (79a): This compound was synthesized starting from ***78a*** through the same procedure of ***72*** to give***79a*** as a white solid (150 mg, yield: 69%). Compound ***79a*** was added to a mixture of HCl/dioxane (4M) (2 mL) and DCM (5 mL). The resulting mixture was stirred at room temparature for 3 h. Then, saturated Na_2CO_3 solution was added to the mixture until no bubbles appeared. The mixture was extracted with DCM and brine three times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (EtOAc : heptane = 1 : 5 and then DCM: MeOH = 1 : 0 to 25 : 1 with several drops of TEA) to give ***80a*** as a white solid (100 mg, yield: 87%).

***N*-((4'-(2-propylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl)methyl)acetamide 80a (PSP40)**:



^{1}H NMR (500 MHz, DMSO-d$_6$) δ 10.05 (d, *J* = 6.0 Hz, 1H), 8.37 (t, *J* = 5.9 Hz, 1H), 7.94 – 7.88 (m, 2H), 7.75 – 7.71 (m, 2H), 7.69 – 7.64 (m, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 5.11 (dd, *J* = 11.6, 5.6 Hz, 1H), 4.29 (d, *J* = 5.9 Hz, 2H), 2.76 (dd, *J* = 12.6, 6.9 Hz, 2H), 1.88 (s, 3H), 1.51 – 1.43 (m, 2H), 0.91 (t, *J* = 7.4 Hz, 3H).

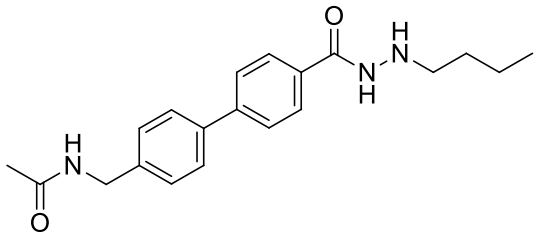
^{13}C NMR (126 MHz, DMSO-d$_6$) δ 169.61, 165.37, 142.97, 140.04, 138.09, 132.37, 128.39, 128.13, 127.19, 126.81, 53.58, 42.27, 23.03, 21.32, 12.13.

HRMS calculated for $C_{19}H_{24}N_3O_2^+$ (M+H): 326.1863, found: 326.1858.

HPLC: rt = 5.96 min (97.57 %).

Procedure for the synthesis of *N-((4'-(2-butylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl)methyl)acetamide 80b (PSP41), N-((4'-(2-pentylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl)methyl)acetamide 80c (PSP42)* and *N-((4'-(2-hexylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl)methyl)acetamide 80d (PSP43)*: The intermediates ***81b-81d*** were synthesized through the same procedure as ***78a*** using ***72*** instead of ***73***. The final compounds ***80b-80d*** were synthesized from the corresponding intermediates ***81b-81d*** through the same procedure of ***79a*** to ***80a. 81b*** was light pink solid (176 mg, yield: 85%), m/z (APCI$^+$) 440.3 $(M+H)^+$; ***81c*** was light pink solid (237 mg, yield: 86%), m/z (APCI$^+$) 454.6 $(M+H)^+$; ***81d*** was light pink solid (190 mg, yield: 85%), m/z (APCI$^+$) 468.6 $(M+H)^+$.

***N*-((4'-(2-butylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl)methyl)acetamide 80b (PSP41)**:



White solid (110 mg, yield: 83%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H), 8.37 (s, 1H), 7.97 – 7.52 (m, 6H), 7.35 (d, *J* = 7.4 Hz, 2H), 5.13 (s, 1H), 4.28 (d, *J* = 5.1 Hz, 2H), 2.78 (d, *J* = 6.3 Hz, 2H), 1.88 (s, 3H), 1.56 – 1.24 (m, 4H), 0.88 (t, *J* = 6.9 Hz, 3H).

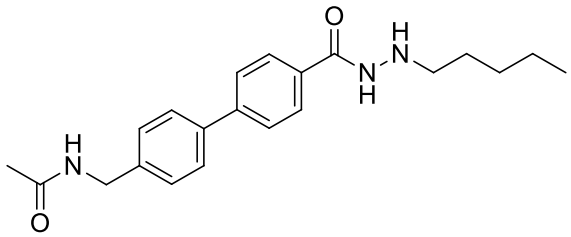
^{13}C NMR (101 MHz, DMSO-d_6) δ 169.61, 165.36, 142.97, 140.03, 138.09, 132.36, 128.39, 128.12, 127.18, 126.80, 51.38, 42.27, 30.26, 23.02, 20.29, 14.36.

HRMS calculated for $C_{20}H_{26}N_3O_2^+$ (M+H): 340.2020, found: 340.202.

HPLC: rt = 10.94 min (95.35 %).

***N*-((4'-(2-pentylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl)methyl)acetamide 80c**

(PSP42):



White solid (150 mg, yield: 81%).

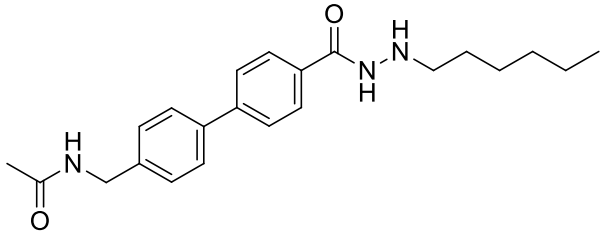
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (s, 1H), 8.36 (t, *J* = 5.7 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 2H), 7.69 (dd, *J* = 25.0, 8.2 Hz, 4H), 7.34 (d, *J* = 8.1 Hz, 2H), 5.07 (s, 1H), 4.28 (d, *J* = 5.9 Hz, 2H), 2.77 (t, *J* = 7.1 Hz, 2H), 1.87 (s, 3H), 1.25 – 1.50 (m, 6H), 0.87 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.59, 165.34, 142.96, 140.03, 138.08, 132.36, 128.38, 128.11, 127.18, 126.80, 51.66, 42.26, 29.33, 27.76, 23.03, 22.50, 14.38.

HRMS calculated for $C_{21}H_{28}N_3O_2^+$ (M+H): 354.2176, found: 354.2176.

HPLC: rt = 11.80 min (98.23 %).

***N*-((4'-(2-hexylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl)methyl)acetamide 80d (PSP43)**:



White solid (120 mg, yield: 80%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (d, *J* = 5.7 Hz, 1H), 8.35 (t, *J* = 5.9 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.66 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 8.3 Hz, 2H), 5.06 (dd, *J* = 11.8, 6.2 Hz, 1H), 4.27 (d, *J* = 5.9 Hz, 2H), 2.77 (dd, *J* = 12.3, 6.8 Hz, 2H), 1.87 (s, 3H), 1.48 – 1.40 (m, 2H), 1.36 – 1.23 (m, 6H), 0.85 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.58, 165.33, 142.95, 140.03, 138.07, 132.35, 128.38, 128.11, 127.18, 126.80, 51.70, 42.26, 31.68, 28.06, 26.80, 23.02, 22.53, 14.38.

HRMS calculated for $C_{22}H_{30}N_3O_2^+$ (M+H): 368.2333, found: 368.2333.
HPLC: rt = 12.70 min (99.61 %).

Another method for large scale synthesis of *80d (PSP43)*:

N-(4-bromobenzyl)acetamide (80d-3): Acetyl chloride (1.06 g, 13.5 mmol, 1.5 eq) was added dropwise to a stirred mixture of (4-bromophenyl)methanamine hydrochloride (***148***) (2.0 g, 9.05 mmol, 1.0 eq) and DIPEA (4.06 g, 31.4 mmol, 3.5 eq) in DCM (20 mL) at room temperature. The reaction was stirred for an additional 30 min. The reaction was washed with HCl (10%) solution followed by saturated NH_4Cl solution and water respectively. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated to give *N*-(4-bromobenzyl)acetamide (***80d-3***) as white solid (1.82 g, yield: 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (s, 1H), 7.53 – 7.42 (m, 2H), 7.19 (dd, *J* = 8.8, 2.2 Hz, 2H), 4.19 (d, *J* = 6.0 Hz, 2H), 1.85 (s, 3H).

N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)acetamide (80d-2): A stirred mixture of ***80d-3*** (1.8 g, 7.9 mmol, 1.0 eq), bis(pinacolato)diboron (2.0 g, 7.9 mmol, 1.0 eq), KOAc (1.55 g, 15.8 mmol, 2.0 eq) and $Pd(PPh_3)_4Cl_2$ (276 mg, 0.4 mmol, 0.05 eq) in dioxane (20 mL) was heated to about 90 ^{o}C for 6 h. The reaction was concentrated and the residue was purified by column chromatography (DCM : MeOH = 1 : 0 to 30 : 1 gradually) to give ***80d-2*** as white solid (1.72 g, yield: 80%). m/z (APCI$^+$) 276.2 $(M+H)^+$, 551.6 $(2M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (t, *J* = 5.7 Hz, 1H), 7.62 (d, *J* = 7.9 Hz, 2H), 7.25 (d, *J* = 7.8 Hz, 2H), 4.26 (d, *J* = 6.0 Hz, 2H), 1.87 (s, 3H), 1.27 (s, 12H).

A stirred mixture of ***80d-2*** (1.54 g, 5.6 mmol, 1.0 eq), tert-butyl 1-hexyl-2-(4-iodobenzoyl)hydrazinecarboxylate (***76d***) (2.5 g, 5.6 mmol, 1.0 eq), K_2CO_3 (1.7 g, 12.3 mmol, 2.2 eq) and $Pd(PPh_3)_4Cl_2$ (157 mg, 0.22 mmol, 0.04 eq) in dioxane (15 mL) and H_2O (3 mL) was kept under argon atmosphere. The reaction was heated to 90 ^{o}C and stirred for 7 h. Then, the reaction was concentrated and the residue was purified by column chromatography (DCM : MeOH = 1 : 0, 40 : 1, 30 : 1

gradually) to give tert-butyl 2-(4'-(acetamidomethyl)-[1,1'-biphenyl]-4-carbonyl)-1-hexylhydrazinecarboxylate (***81d***) as brown oil (2.4 g, yield: 91%). ***80d*** (***PSP43***) was finally synthesized through the same procedure of ***79a*** to ***80a*** in 85%a yield.

Procedure for the synthesis of *N-(4-(2-propylhydrazinecarbonyl)benzyl)acetamide 80e (PSP39)* and *N-(4-(2-hexylhydrazinecarbonyl)benzyl)acetamide 80f (PSP67)*: Benzyl chloroformate (2.48 g, 2.08 mL, 14.5 mmol, 1.1 eq) was added dropwise to a stirred mixture of 4-(aminomethyl)benzoic acid (***82***) (2.0 g, 13.2 mmol, 1.0 eq) and Na_2CO_3 (5.61 g, 53 mmol, 4.0 eq) in water (100 mL) under ice bath. The resulting mixture was stirred for an additional 1.5 h and precipitate was appeared. Then, aq HCl (1N) was added dropwise to the mixture until pH ≈ 3. The precipitate was filtered. The white residue was dried to yield intermediate 4-((((benzyloxy)carbonyl)amino)methyl)benzoic acid (***83***) as white solid (3.39 g, yield: 90%). m/z (APCI$^+$) 286.4 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.84 (s, 1H), 7.88 (d, *J* = 8.2 Hz, 3H), 7.41 – 7.26 (m, 7H), 5.04 (s, 2H), 4.26 (d, *J* = 6.2 Hz, 2H).

Benzyl 4-(hydrazinecarbonyl)benzylcarbamate (84): CDI (Carbonyldiimidazole) (1.79 g, 11 mmol, 1.05 eq) was added to a stirred mixture of ***83*** (3.0 g, 10.51 mmol, 1.0 eq) in THF (40 mL). The reaction was stirred for an additional 30 min at room temperature. Hydrazine monohydrate (5.26 g, 105 mmol, 10.0 eq) was then added dropwise to the reaction and the resulting mixture was stirred at room temperature overnight. The reaction was evaporated and diluted with water (50 mL) until a precipitate appeared. The precipitate was filtered and washed with cold water two times. The white solid was dried to give ***84*** as white solid (1.95 g, yield: 64%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (s, 1H), 7.85 (t, *J* = 5.9 Hz, 1H), 7.75 (d, *J* = 8.1 Hz, 2H), 7.41 – 7.13 (m, 7H), 5.03 (s, 2H), 4.44 (s, 2H), 4.23 (d, *J* = 6.2 Hz, 2H).

Benzyl 4-(2-propylhydrazinecarbonyl)benzylcarbamate (85a)* and *benzyl

4-(2-hexylhydrazinecarbonyl)benzylcarbamate (85b): $NaBH(AcO)_3$ (4.14 g, 19.5 mmol, 3.0 eq) was added to a stirred mixture of propionaldehyde (0.416 g, 0.514 mL, 7.16 mmol, 1.1 eq) and intermediate ***84*** (1.95 g, 6.52 mmol, 1.0 eq) in DCM (20 mL). The resulting mixture was stirred at room temperature overnight. Saturated Na_2CO_3 solution was then added dropwise to the reaction until no bubbles appeared. The reaction was washed with water two times. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (EtOAc: heptane = 1 : 15, 1 : 10 and then DCM : MeOH = 30 : 1 gradually) to give ***85a*** as white solid (1.67 g, yield: 75%). m/z ($APCI^+$) 342.8 $(M+H)^+$.

To a stirred mixture of intermediate ***84*** (400 mg, 1.34 mmol, 1.0 eq), p-toluenesulfonic acid (13 mg, 0.007 mmol, 0.05 eq) and anhydrous Na_2SO_4 (0.8 g) in MeOH (5 mL) was added hexanal (141 mg, 1.41 mmol, 1.05 eq). An additional amount of DCM (2.5 mL) was added to the reaction if the reaction was not easy to be stirred. The resulting mixture was stirred for 2 h at room temperature. $NaBH_4$ (204 mg, 5.36 mmol, 4.0 eq) was then added to the reaction over several portions and the strring was continued for 1 h. Saturated Na_2CO_3 solution was added to the mixture until no bubbles appeared. The reaction was extracted with water and DCM two times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (DCM : MeOH = 1 : 0, 40 : 1, 30 : 1 gradually) to give ***85b*** as white solid (360 mg, yield: 65%). ^1H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 7.85 (t, *J* = 6.2 Hz, 1H), 7.74 (d, *J* = 8.3 Hz, 2H), 7.39 – 7.27 (m, 7H), 5.10 – 4.96 (m, 3H), 4.23 (d, *J* = 6.2 Hz, 2H), 2.75 (t, *J* = 7.0 Hz, 2H), 1.47 – 1.38 (m, 2H), 1.18 – 1.35(m, 6H), 0.88 – 0.82 (m, 3H).

Tert-butyl 2-(4-((((benzyloxy)carbonyl)amino)methyl)benzoyl)-1-propylhydrazinecarboxylate (86a) **and** ***tert-butyl 2-(4-((((benzyloxy)carbonyl)amino)methyl)benzoyl)-1-hexylhydrazinecarboxylate***

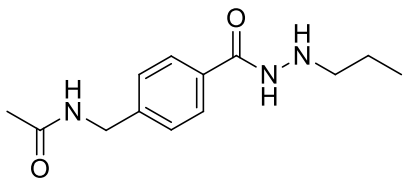
(86b)*:** A mixture of ***85a (1.49 g, 4.364 mmol, 1.0 eq), $(Boc)_2O$ (1.14 g, 5.24 mmol, 1.2 eq) and *N*,*N*-Diisopropylethylamine (DIPEA) (1.13 g, 8.74 mmol, 2.0 eq) or triethylamine (TEA) 2.0 eq in THF (15 mL) was stirred overnight. The mixture was directly concentrated and the residue was purified by column chromatography (EtOAc : heptane = 0 : 1, 1 : 5, 1 : 3 gradually) to give ***86a*** as white solid (1.85 g, yield: 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.74 (d, *J* = 7.7 Hz, 2H), 7.45 – 7.27 (m, 7H), 5.15 (s, 2H), 4.43 (d, *J* = 6.0 Hz, 2H), 3.59 – 3.52 (m, 2H), 1.62 (dd, *J* = 14.6, 7.3 Hz, 2H), 1.46 (s, 9H), 0.94 (t, *J* = 7.4 Hz, 3H).

The synthesis procedure of ***86b*** was the same as ***86a*** starting from ***85b*** to give ***86b*** as white semisolid (360 mg, yield: 79%).

Tert-butyl 2-(4-(aminomethyl)benzoyl)-1-propylhydrazinecarboxylate (87a)* and *tert-butyl 2-(4-(aminomethyl)benzoyl)-1-hexylhydrazinecarboxylate (87b): ***87a*** and ***87b*** were synthesized through the same procedure of ***77a*** to ***78a*** starting from ***86a*** and ***86b***. ***87a*** was light green oil (0.98 g, yield: 95%); ***87b*** was colorless oil in a quantitative yield. m/z (APCI$^+$) 350.2 $(M+H)^+$, 250.1 $(M+2H\text{-}Boc)^+$.

N-(4-(2-propylhydrazinecarbonyl)benzyl)acetamide ***80e*** (***PSP39***) and *N*-(4-(2-hexylhydrazinecarbonyl)benzyl)acetamide ***80f*** (***PSP67***) were synthesized through the same procedure of ***78a*** to ***80a*** starting from ***87a*** and ***87b***.

***N*-(4-(2-propylhydrazinecarbonyl)benzyl)acetamide 80e (PSP39)**:



White solid (95 mg, yield: 54%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.40 (t, *J* = 5.5 Hz, 1H), 7.76 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 4.27 (d, *J* = 5.9 Hz, 2H), 2.74 (t, *J* = 7.0 Hz,

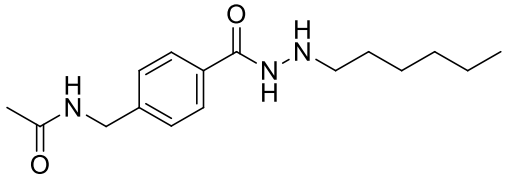
2H), 2.49 (s, 1H), 1.87 (s, 3H), 1.49 – 1.41 (m, 2H), 0.89 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 169.73, 165.56, 143.42, 132.12, 127.49, 127.42, 53.55, 42.28, 22.98, 21.29, 12.09.

HRMS calculated for $C_{13}H_{19}N_3O_2Na^+$ (M+H): 272.1369, found: 272.1367.

HPLC: rt = 2.18 min (97.46 %).

***N*-(4-(2-hexylhydrazinecarbonyl)benzyl)acetamide 80f (PSP67)**:



White solid (152 mg, yield: 74%).

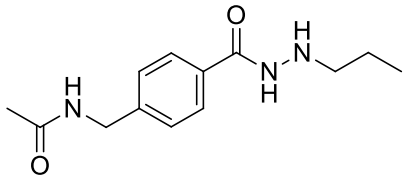
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.37 (t, *J* = 5.8 Hz, 1H), 7.76 (d, *J* = 8.1 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 5.04 (s, 1H), 4.28 (d, *J* = 6.0 Hz, 2H), 2.76 (t, *J* = 7.1 Hz, 2H), 1.88 (s, 3H), 1.47 – 1.40 (m, 2H), 1.35 – 1.23 (m, 6H), 0.86 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 169.67, 165.54, 143.43, 132.16, 127.49, 127.43, 51.69, 42.28, 31.68, 28.06, 26.80, 23.01, 22.53, 14.39.

HRMS calculated for $C_{32}H_{51}N_6O_4^+$ (2M+H): 583.3972, found: 583.3962.

HPLC: rt = 10.44 min (99.26 %).

Tert-butyl 2-(4-methylbenzoyl)-1-propylhydrazinecarboxylate (86a-1) (byproduct)



Colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H), 7.72 (s, 2H), 7.28 (d, *J* = 8.1 Hz, 2H), 3.36 (s, 2H), 2.35 (s, 3H), 1.54 – 1.28 (m, 11H), 0.86 (t, *J* = 6.9 Hz, 3H).

Intermediate N-(4-(aminomethyl)benzyl)acetamide hydrochloride (91): Acetyl

chloride (0.25 g, 3.18 mmol, 1.5 eq) was added to a mixture of tert-butyl 4-(aminomethyl)benzylcarbamate (***89***) (0.5 g, 2.11 mmol, 1.0 eq) and DIPEA (0.82 g, 6.34 mmol, 3.0 eq) in DCM (5 mL) at room temperature. The resulting reaction was stirred for 30 min. The reaction was concentrated and the residue was purified by column chromatography (DCM : MeOH = 1 : 0, 30 : 1 to 20 gradually) to give tert-butyl 4-(acetamidomethyl)benzylcarbamate (***90***) as orange oil (0.68 g). m/z (APCI$^+$) 223.0 $(M+H)^+$, 557.2 $(2M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (s, 4H), 5.71 (s, 1H), 4.83 (s, 1H), 4.41 (d, *J* = 5.7 Hz, 2H), 4.29 (d, *J* = 5.7 Hz, 2H), 2.02 (s, 3H), 1.46 (s, 9H). The intermediate ***90*** was added to a mixture of HCl/dioxane (4M) (2 mL) and DCM (10 mL) at room temperature. The reaction was stirred for 3 h at room temperature. The reaction was evaporated directly to give compound ***91*** as light yellow solid (0.40 g, yield: 93% over two steps).

General procedure for the synthesis of *95a-95m*:

Ethyl 2-(benzylamino)pyrimidine-5-carboxylate (93a): A mixture of ethyl 2-chloropyrimidine-5-carboxylate (***92***) (200 mg, 1.07 mmol, 1.0 eq), benzylamine (115 mg, 1.07 mmol, 1.0 eq) and DIPEA (0.34 g, 2.63 mmol, 2.5 eq) (The amount of DIPEA should be increased to 3.5 eq when amine was HCl salt) in DCM (10 mL) was stirred at room temperature for 30 min. The reaction was extracted with DCM and water three times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (EtOAc : heptane = 1 : 5 and then DCM : MeOH = 1 : 40) to give intermediate ***93a*** as white solid (240 mg, yield: 87%). Another work-up procedure of ***93a***: Upon completion, the reaction was washed with aq HCl (10%) three times and brine. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated to give ***93a***. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (d, *J* = 1.3 Hz, 2H), 8.60 (t, *J* = 6.2 Hz, 1H), 7.31 – 7.16 (m, 5H), 4.56 (d, *J* = 6.4 Hz, 2H), 4.24 (q, *J* = 7.1 Hz, 2H), 1.26 (t, *J* = 7.1 Hz, 3H).

2-(Benzylamino)pyrimidine-5-carbohydrazide (94a):

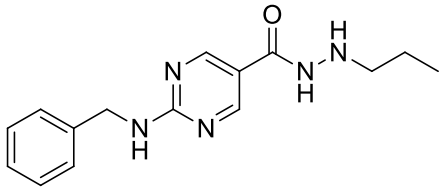
Method 1: A stirred mixture of hydrazine monohydrate (1.4 g, 28 mmol, 30.0 eq) and

93a (0.24 g, 0.93 mmol, 1.0 eq) in EtOH (3 mL) was refluxed for 3 h. The reaction was cooled to room temperature. The precipitate was filtered. The white residue was washed with cold water and dried to give ***94a*** as white solid (150 mg, yield: 66%).

Method 2: A mixture of ***93a*** (1.0 mmol) and hydrazine monohydrate (10.0 mmol) in EtOH (1 mL) was heated to 110 ^{o}C in a microwave reactor for 1 h. The reaction was then evaporated. The resulting residue was washed with cold water and dried to give the targeted compound.

2-(Benzylamino)-*N*'-propylpyrimidine-5-carbohydrazide 95a (PSP48):

This compound was synthesized through the same procedure of ***84*** to ***85b*** starting from ***94a***.



White solid (38 mg, yield: 33%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H), 8.64 (s, 2H), 8.25 (t, *J* = 6.3 Hz, 1H), 7.32 – 7.16 (m, 5H), 4.99 (s, 1H), 4.53 (d, *J* = 6.4 Hz, 2H), 2.69 (t, *J* = 7.1 Hz, 2H), 1.41 (dt, *J* = 14.5, 7.3 Hz, 2H), 0.86 (t, *J* = 7.4 Hz, 3H).

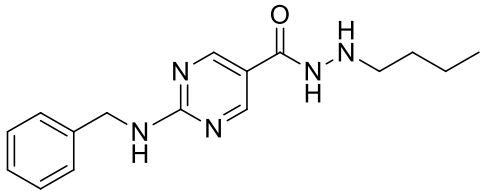
^{13}C NMR (101 MHz,DMSO-d_6) δ 163.55, 163.28, 158.16, 157.70, 140.09, 128.68, 127.42, 127.12, 115.94, 53.57, 44.39, 21.22, 12.04.

HRMS calculated for $C_{15}H_{20}N_5O^+$ (M+H): 286.1662, found: 286.166.

HPLC: rt = 4.95 min (95.03 %).

The following compounds ***95b-95m*** were synthesized using the same procedure of ***95a*** starting from the corresponding substituted amine and ***92***.

2-(Benzylamino)-*N*'-butylpyrimidine-5-carbohydrazide 95b (NI-26):



White solid (136 mg, yield: 58%).

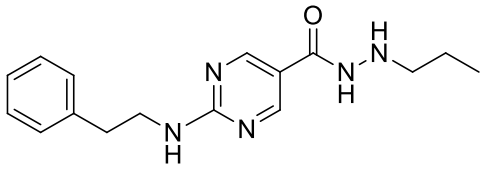
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (s, 1H), 8.65 (s, 2H), 8.26 (t, *J* = 6.3 Hz, 1H), 7.32 – 7.15 (m, 5H), 5.00 (s, 1H), 4.53 (d, *J* = 6.4 Hz, 2H), 2.73 (t, *J* = 7.0 Hz, 2H), 1.44 – 1.26 (m, 4H), 0.86 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.50, 163.33, 158.11, 157.68, 140.16, 128.66, 127.44, 127.09, 115.98, 51.40, 44.41, 30.22, 20.25, 14.33.

HRMS calculated for $C_{16}H_{22}N_5O^+$ (M+H): 300.1819, found: 300.182.

HPLC: rt = 11.53 min (97.94 %).

2-(Phenethylamino)-*N*'-propylpyrimidine-5-carbohydrazide 95c (NI-16):



White solid (133 mg, yield: 53%).

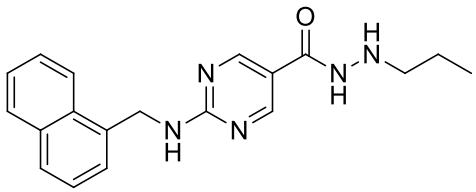
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (s, 1H), 8.66 (d, *J* = 18.8 Hz, 2H), 7.79 (t, *J* = 5.6 Hz, 1H), 7.29 – 7.14 (m, 5H), 5.01 (s, 1H), 3.52 (dd, *J* = 14.0, 6.6 Hz, 2H), 2.82 (t, *J* = 7.3 Hz, 2H), 2.71 (t, *J* = 7.0 Hz, 2H), 1.48 – 1.37 (m, 2H), 0.88 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.58, 163.19, 157.95, 157.76, 139.92, 129.09, 128.72, 126.47, 115.61, 53.62, 42.77, 35.23, 21.29, 12.08.

HRMS calculated for $C_{16}H_{22}N_5O^+$ (M+H): 300.1819, found: 300.182.

HPLC: rt = 5.75 min (99.21 %).

2-((Naphthalen-1-ylmethyl)amino)-*N*'-propylpyrimidine-5-carbohydrazide 95d (NI-15):



White solid (174 mg, yield: 67%).

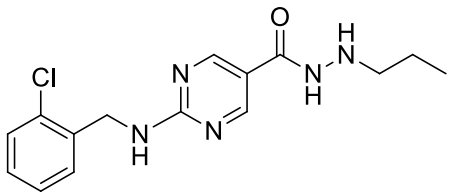
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80 (s, 1H), 8.67 (s, 2H), 8.32 (t, *J* = 6.0 Hz, 1H), 8.16 – 8.11 (m, 1H), 7.95 – 7.90 (m, 1H), 7.83 – 7.78 (m, 1H), 7.57 – 7.50 (m, 2H), 7.45 – 7.40 (m, 2H), 5.00 (d, *J* = 6.1 Hz, 3H), 2.70 (t, *J* = 7.1 Hz, 2H), 1.47 – 1.37 (m, 2H), 0.88 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.50, 163.33, 158.18, 157.74, 135.06, 133.73, 131.30, 128.96, 127.71, 126.59, 126.18, 125.84, 125.03, 123.80, 116.04, 53.59, 42.58, 21.29, 12.09.

HRMS calculated for $C_{19}H_{22}N_5O^+$ (M+H): 336.1819, found: 336.1818.

HPLC: rt = 11.64 min (93.64 %).

2-((2-Chlorobenzyl)amino)-*N*'-propylpyrimidine-5-carbohydrazide 95e (NI-23):



White solid (128 mg, yield: 64%).

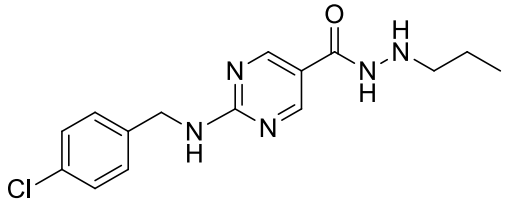
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s, 1H), 8.66 (d, *J* = 12.2 Hz, 2H), 8.26 (t, *J* = 6.1 Hz, 1H), 7.45 – 7.39 (m, 1H), 7.29 – 7.22 (m, 3H), 5.01 (s, 1H), 4.59 (d, *J* = 6.2 Hz, 2H), 2.70 (t, *J* = 6.9 Hz, 2H), 1.49 – 1.36 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.43, 163.28, 158.21, 157.70, 136.94, 132.37, 129.56, 128.86, 128.60, 127.54, 116.37, 53.58, 42.51, 21.28, 12.08.

HRMS calculated for $C_{15}H_{19}ClN_5O^+$ (M+H): 320.1273, found: 320.1271.

HPLC: rt = 11.93 min (96.16 %).

2-((4-Chlorobenzyl)amino)-*N*'-propylpyrimidine-5-carbohydrazide 95f (NI-32):



White solid (99 mg, yield: 66%).

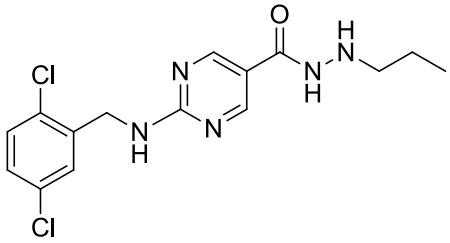
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (s, 1H), 8.65 (s, 2H), 8.28 (t, *J* = 6.3 Hz, 1H), 7.36 – 7.27 (m, 4H), 5.01 (s, 1H), 4.51 (d, *J* = 6.4 Hz, 2H), 2.70 (t, *J* = 7.1 Hz, 2H), 1.47 – 1.37 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.44, 163.23, 158.14, 157.66, 139.25, 131.63, 129.33, 128.62, 116.16, 53.58, 43.81, 21.28, 12.08.

HRMS calculated for $C_{15}H_{18}Cl_2N_5O^+$ (M+H): 320.1273, found: 320.1272.

HPLC: rt = 11.90 min (95.72 %)

2-((2,5-Dichlorobenzyl)amino)-*N*'-propylpyrimidine-5-carbohydrazide 95g (NI-24):



White solid (130 mg, yield: 72%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s, 1H), 8.67 (d, *J* = 9.7 Hz, 2H), 8.28 (t, *J* = 6.1 Hz, 1H), 7.57 (d, *J* = 2.0 Hz, 1H), 7.37 – 7.26 (m, 2H), 5.01 (s, 1H), 4.55 (d, *J* = 6.1 Hz, 2H), 2.70 (t, *J* = 6.4 Hz, 2H), 1.48 – 1.36 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.38, 163.17, 158.24, 157.71, 136.22, 133.29, 132.48, 130.06, 129.00, 127.70, 116.51, 53.57, 42.18, 21.28, 12.07.

HRMS calculated for $C_{15}H_{18}Cl_2N_5O^+$ (M+H): 354.0883, found: 354.0883.

HPLC: rt = 13.43 min (96.61 %)

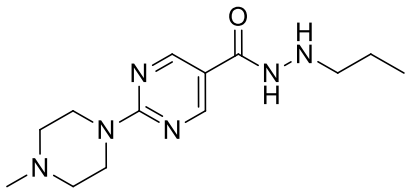
Intermediates of *95h (PSP45)*:

Ethyl 2-(4-methylpiperazin-1-yl)pyrimidine-5-carboxylate (93h) was white solid

(0.24 g, yield: 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (s, 2H), 4.25 (q, *J* = 7.1 Hz, 2H), 3.87 – 3.80 (m, 4H), 2.38 – 2.33 (m, 4H), 2.20 (s, 3H), 1.27 (t, *J* = 7.1 Hz, 3H).

2-(4-Methylpiperazin-1-yl)pyrimidine-5-carbohydrazide (94h) was white solid (0.25 g, yield: 91%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.64 (s, 1H), 8.72 (s, 2H), 4.37 (d, *J* = 66.4 Hz, 2H), 3.84 – 3.70 (m, 4H), 2.39 – 2.27 (m, 4H), 2.19 (s, 3H).

2-(4-Methylpiperazin-1-yl)-*N*'-propylpyrimidine-5-carbohydrazide 95h (PSP45):



White solid (78mg, yield: 44%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.92 (s, 1H), 8.75 (s, 2H), 3.89 (s, 4H), 2.73 (s, 2H), 2.63 (s, 4H), 2.38 (s, 3H), 1.44 (dd, *J* = 14.2, 7.0 Hz, 2H), 0.89 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 163.22, 161.77, 160.07, 157.80, 53.91, 53.57, 44.97, 42.87, 21.31, 12.09.

HRMS calculated for $C_{13}H_{23}N_6O^+$ (M+H): 279.1928, found: 279.1926.

HPLC: rt = 5.07 min (98.13 %).

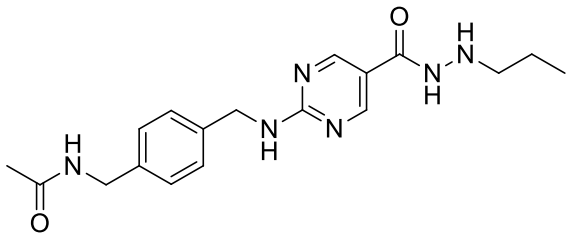
Intermediates of *95i (PSP47)*:

Ehyl 2-((4-(acetamidomethyl)benzyl)amino)pyrimidine-5-carboxylate (93i) was white solid (313 mg, yield: 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (d, *J* = 0.9 Hz, 2H), 8.58 (t, *J* = 6.4 Hz, 1H), 8.29 – 8.22 (m, 1H), 7.19 (dd, *J* = 26.9, 8.2 Hz, 4H), 4.52 (d, *J* = 6.4 Hz, 2H), 4.23 (q, *J* = 7.1 Hz, 2H), 4.18 (d, *J* = 5.8 Hz, 2H), 1.83 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 3H).

N-(4-(((5-(hydrazinecarbonyl)pyrimidin-2-yl)amino)methyl)benzyl)acetamide (94i) was white solid (210 mg, yield: 70%). m/z (APCI$^+$) 315.2 (M+H)$^+$. ^{1}H NMR (400

MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.63 (s, 2H), 8.27 – 8.20 (m, 2H), 7.19 (dd, *J* = 27.7, 8.1 Hz, 4H), 4.49 (d, *J* = 6.3 Hz, 2H), 4.37 (s, 2H), 4.18 (d, *J* = 5.9 Hz, 2H), 1.82 (s, 3H).

***N*-(4-(((5-(2-propylhydrazinecarbonyl)pyrimidin-2-yl)amino)methyl)benzyl)acetamide 95i (PSP47)**:



White solid (158 mg, yield: 66%).

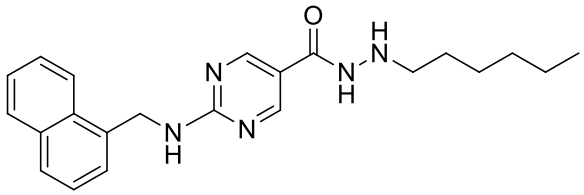
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.66 (s, 2H), 8.28 (dt, *J* = 12.7, 5.9 Hz, 2H), 7.19 (dd, *J* = 26.8, 8.0 Hz, 4H), 4.51 (t, *J* = 8.6 Hz, 2H), 4.18 (d, *J* = 5.9 Hz, 2H), 2.71 (s, 2H), 1.83 (s, 3H), 1.41 (dt, *J* = 14.4, 7.2 Hz, 2H), 0.87 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.50, 163.48 163.28, 158.13, 157.71, 138.63, 138.43, 127.68, 127.43, 53.56, 44.19, 42.33, 22.98, 21.28, 12.07.

HRMS calculated for $C_{18}H_{25}N_6O_2^+$ (M+H): 357.2034, found: 357.2034.

HPLC: rt = 8.54 min (98.36 %).

***N*'-hexyl-2-((naphthalen-1-ylmethyl)amino)pyrimidine-5-carbohydrazide 95j (NI-90)**:



White solid (244 mg, yield: 72%).

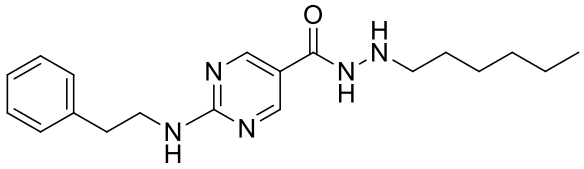
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s, 1H), 8.67 (s, 2H), 8.33 (t, *J* = 6.0 Hz, 1H), 8.13 (d, *J* = 8.6 Hz, 1H), 7.92 (dd, *J* = 6.7, 2.6 Hz, 1H), 7.83 – 7.77 (m, 1H), 7.57 – 7.40 (m, 4H), 5.00 (d, *J* = 6.0 Hz, 3H), 2.72 (t, *J* = 6.8 Hz, 2H), 1.45 – 1.36 (m, 2H), 1.34 – 1.19 (m, 6H), 0.83 (dd, *J* = 8.7, 4.7 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d$_6$) δ 163.50, 163.33, 158.16, 157.76, 135.06, 133.73, 131.30, 128.96, 127.71, 126.59, 126.18, 125.83, 125.03, 123.79, 116.03, 51.72, 42.58, 31.65, 28.04, 26.77, 22.52, 14.36.

HRMS calculated for $C_{22}H_{28}N_5O^+$ (M+H): 378.2288, found: 378.2290.

HPLC: rt = 14.43 min (96.91 %)

_N_'-hexyl-2-(phenethylamino)pyrimidine-5-carbohydrazide 95k (NI-91):



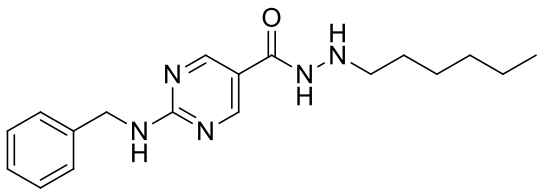
White solid (193 mg, yield: 69%).

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.78 (s, 1H), 8.64 (d, *J* = 18.8 Hz, 2H), 7.79 (t, *J* = 5.7 Hz, 1H), 7.32 – 7.11 (m, 5H), 4.98 (s, 1H), 3.52 (dd, *J* = 14.4, 6.3 Hz, 2H), 2.87 – 2.79 (m, 2H), 2.73 (t, *J* = 7.0 Hz, 2H), 1.41 (dt, *J* = 14.0, 7.2 Hz, 2H), 1.35 – 1.19 (m, 6H), 0.85 (t, *J* = 6.8 Hz, 3H).

HRMS calculated for $C_{19}H_{28}N_5O^+$ (M+H): 342.2288, found: 342.2287.

HPLC: rt = 13.99 min (98.67 %)

2-(Benzylamino)-_N_'-hexylpyrimidine-5-carbohydrazide 95l (PSP70):



White solid (260 mg, yield: 74%).

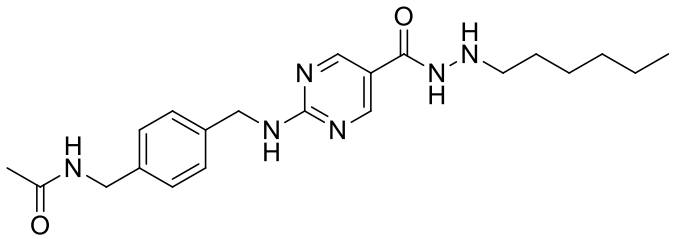
^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.80 (s, 1H), 8.68 (d, *J* = 13.0 Hz, 2H), 8.27 (t, *J* = 6.3 Hz, 1H), 7.39 – 7.13 (m, 5H), 5.05 (s, 1H), 4.54 (d, *J* = 6.3 Hz, 2H), 2.73 (t, *J* = 7.0 Hz, 2H), 1.46 – 1.35 (m, 2H), 1.33 – 1.18(m, 6H), 0.83 (t, *J* = 6.7 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d$_6$) δ 163.50, 163.34, 158.13, 157.70, 140.16, 128.64, 127.44, 127.07, 115.98, 51.74, 44.43, 31.66, 28.05, 26.78, 22.53, 14.34.

HRMS calculated for $C_{18}H_{26}N_5O^+$ (M+H): 328.2132, found: 328.2131.

HPLC: rt = 13.59 min (97.34 %).

_N_-(4-(((5-(2-hexylhydrazinecarbonyl)pyrimidin-2-yl)amino)methyl)benzyl)acetamide 95m (PSP69):



White solid (345 mg, yield: 55%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.80 (s, 1H), 8.66 (s, 2H), 8.27 (t, *J* = 6.0 Hz, 2H), 7.21 (dd, *J* = 33.1, 7.9 Hz, 4H), 5.02 (s, 1H), 4.51 (d, *J* = 6.3 Hz, 2H), 4.20 (d, *J* = 5.9 Hz, 2H), 2.74 (t, *J* = 7.1 Hz, 2H), 1.85 (s, 3H), 1.47 – 1.38 (m, 2H), 1.34 – 1.23 (m, 6H), 0.86 (t, *J* = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 169.48, 163.52, 163.30, 158.10, 157.68, 138.65, 138.45, 127.71, 127.45, 115.98, 51.74, 44.21, 42.35, 31.67, 28.06, 26.79, 23.01, 22.54, 14.38.

HRMS calculated for $C_{21}H_{31}N_6O_2^+$ (M+H): 399.2503, found: 399.2502.

HPLC: rt = 11.82 min (92.68 %).

3-(2-Bromoethyl)-1-methyl-indole (107): NaH (60%) (0.36 g, 8.9 mmol, 5.0 eq) was added to a stirred solution of 3-(2-bromoethyl)-indole (***106***) (0.4 g, 1.78 mmol, 1.0 eq) in THF (5 mL). The mixture was stirred for several minutes followed by addition of CH_3I (2.68 g, 1.4 mL, 17.8 mmol, 10.0 eq). The resulting reaction was stirred at room temperature overnight. Then, 10% HCl was added dropwise to the mixture until no bubbles appeared. The reaction was extracted with EtOAc and brine three times. The combined organic layers were dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography (EtOAc : heptane = 1 : 15) to give ***107*** as light yellow oil (0.25 g, yield: 59%). m/z (APCI$^+$) 238.0 $(M+H)^+$.

Procedure for the synthesis of *105a* to *105h*:

1-Benzylpiperazine dihydrochloride (98): Benzylchloride (1.36 g, 1.24 mL, 1.74 mmol, 1.0 eq) was added to a mixture of tert-butyl piperazine-1-carboxylate (***96***) (2.0 g, 10.74 mmol, 1.0 eq) and K_2CO_3 (8.6 g, 62.3 mmol, 5.8 eq) in EtOH (20 mL). The resulting mixture was refluxed overnight. The mixture was filtered. The filtrate was concentrated and the resulting residue was purified by column chromatography to give an intermediate, m/z (APCI$^+$) 277.3 $(M+H)^+$. This intermediate was added to a mixture of HCl/dioxane (4M) (5 mL) and DCM (10 mL). The reaction was stirred at room temperature for 3 h. The precipitate was filtered and dried to give compound ***98*** as white solid (1.83 g, yield: 68%).

Ethyl 2-(4-benzylpiperazin-1-yl)pyrimidine-5-carboxylate (99): This compound was synthesized through the same procedure of ***92*** to ***93a*** starting from ***98*** while 4.5 eq DIPEA was used. ***99*** was a white solid in 97% yield. m/z (APCI$^+$) 327.4 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (s, 2H), 7.34 – 7.22 (m, 5H), 4.24 (q, *J* = 7.1 Hz, 2H), 3.89 – 3.77 (m, 4H), 3.50 (s, 2H), 2.47 – 2.38 (m, 4H), 1.27 (t, *J* = 7.1 Hz, 3H).

2-(4-Benzylpiperazin-1-yl)pyrimidine-5-carbohydrazide (100): This compound was synthesized through the same procedure of ***93a*** to ***94a*** starting from ***99*** and it was a white solid in quantitative yield.

2-(4-Benzylpiperazin-1-yl)-N'-propylpyrimidine-5-carbohydrazide (101a) and 2-(4-benzylpiperazin-1-yl)-N'-hexylpyrimidine-5-carbohydrazide (101b) were synthesized through the same procedure of ***84*** to ***85b*** starting from ***100*** and propionaldehyde or hexanal. ***101a*** was white solid in 86% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (d, J = 5.4 Hz, 1H), 8.70 (s, 2H), 7.35 – 7.21 (m, 5H), 5.01 (d, J = 5.5 Hz, 1H), 3.84 – 3.76 (m, 4H), 3.50 (s, 2H), 2.71 (dd, J = 11.8, 6.7 Hz, 2H), 2.44 – 2.37 (m, 4H), 1.42 (dt, J = 14.5, 7.2 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H); ***101b*** was white solid in 36% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (d, J = 5.9 Hz, 1H), 8.70 (s, 2H), 7.35 – 7.22 (m, 5H), 4.98 (d, J = 5.8 Hz, 1H), 3.85 – 3.73 (m, 4H), 3.50 (s, 2H), 2.73 (dd, J = 12.3, 6.5 Hz, 2H), 2.43 – 2.36 (m, 4H), 1.41 (dt, J = 14.0, 6.9 Hz, 2H),

1.35 – 1.23 (m, 6H), 0.84 (t, J = 6.8 Hz, 3H).

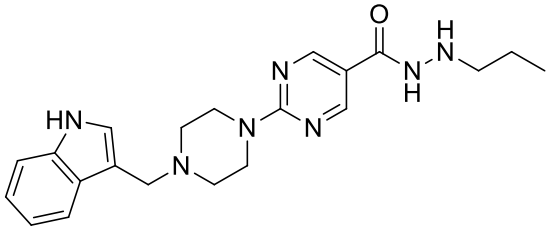
Tert-butyl 2-(2-(4-benzylpiperazin-1-yl)pyrimidine-5-carbonyl)-1-propylhydrazinecarboxylate (102a) and tert-butyl 2-(2-(4-benzylpiperazin-1-yl)pyrimidine-5-carbonyl)-1-hexylhydrazinecarboxylate (102b): A mixture of $(Boc)_2O$ (1.1 mmol), ***101a*** or ***101b*** (1.0 mmol) and TEA (2.5 mmol) in DCM (7 mL) was stirred at room temperature overnight. The mixture was concentrated and the residue was purified by column chromatography (EtOAc : heptane = 1 : 3 or DCM : MeOH = 50 : 1 to 30 : 1) to give ***102a*** or ***102b***. ***102a*** was white solid in 96% yield; ***102b*** was white solid in 87% yield, m/z ($APCI^+$) 497.2 $(M+H)^+$.

2-(Piperazin-1-yl)-N'-propylpyrimidine-5-carbohydrazide (103a) and N'-hexyl-2-(piperazin-1-yl)pyrimidine-5-carbohydrazide (103b): A stirred mixture of ***102a*** or ***102b*** (1.0 mmol), 10% wt Pd/C (40 mg) and ammonium formate (4.0 mmol) in MeOH (5 mL) was heated to about 60 ^oC for 4 h. The reaction was filtered. The resulting filtrate was evaporated and the residue was extracted with EtOAc and brine three times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give ***103a*** or ***103b*** as colorless oil. ***103a*** (yield: 80%), m/z ($APCI^+$) 365.4 $(M+H)^+$; MS of ***103b*** was not determined.

Procedure for the synthesis of *105a (PSP49)*, *105b (PSP73)*, *105c (PSP50)* and *105d (PSP74)*: A mixture of amine (***103a*** or ***103b***) (1.0 mmol), indole-3-carbaldehyde or 1-methyl-indole-3-carbaldehyde (1.0 mmol) and $NaBH(AcO)_3$ (2.0 mmol) in DCM (4 mL) was stirred at room temperature overnight. The reaction was then concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 1 : 1 and then DCM : MeOH = 30 : 1, 25 : 1) to respectively give ***104a***, ***104b***, ***104c*** or ***104d.***

The following procedure was same as ***80a*** using corresponding *1**04a***, ***104b***, ***104c*** or *1**04d*** to give final compounds ***105a-105d*** respectively.

2-(4-((1*H*-indol-3-yl)methyl)piperazin-1-yl)-*N*'-propylpyrimidine-5-carbohydrazi -de 105a (PSP49):



Light pink solid (yield: 56%).

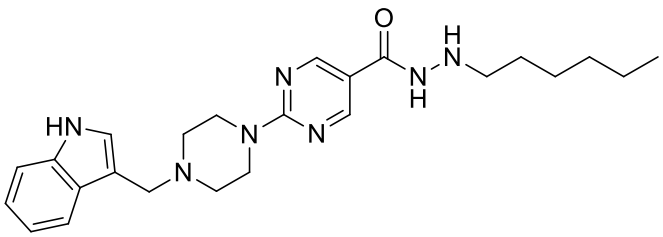
^{1}H NMR (400 MHz, DMSO-d_6). δ 10.92 (d, *J* = 1.4 Hz, 1H), 9.84 (s, 1H), 8.71 (s, 2H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.22 (d, *J* = 2.3 Hz, 1H), 7.08 – 7.01 (m, 1H), 7.00 – 6.93 (m, 1H), 5.02 (s, 1H), 3.82 – 3.72 (m, 4H), 3.64 (s, 2H), 2.72 (t, *J* = 7.1 Hz, 2H), 2.46 – 2.34 (m, 4H), 1.48 – 1.38 (m, 2H), 0.88 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 162.98, 161.35, 157.27, 136.35, 127.58, 124.68, 120.93, 119.03, 118.43, 114.86, 111.34, 110.44, 53.18, 53.13, 52.25, 43.57, 20.86, 11.63.

HRMS calculated for $C_{21}H_{28}N_7O^+$ (M+H): 394.2350, found: 394.2343.

HPLC: rt = 9.04 min (90.60 %).

2-(4-((1*H*-indol-3-yl)methyl)piperazin-1-yl)-*N*'-hexylpyrimidine-5-carbohydrazid -e 105b (PSP73):



White solid (yield: 34%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (d, *J* = 1.7 Hz, 1H), 9.84 (s, 1H), 8.72 (d, *J* = 8.1 Hz, 2H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.22 (d, *J* = 2.3 Hz,

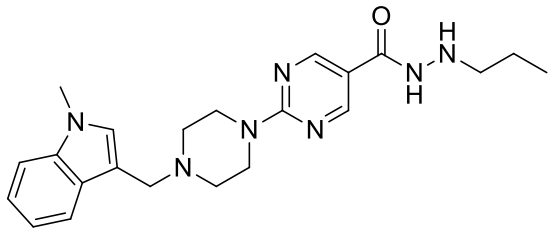
1H), 7.01 (dtd, *J* = 15.9, 7.1, 1.1 Hz, 2H), 5.00 (s, 1H), 3.82 – 3.72 (m, 4H), 3.64 (s, 2H), 2.74 (t, *J* = 7.1 Hz, 2H), 2.46 – 2.38 (m, 4H), 1.40 (dd, *J* = 14.4, 7.3 Hz, 2H), 1.33 – 1.19 (m, 6H), 0.83 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.40, 161.79, 157.71, 136.80, 128.03, 125.12, 121.37, 119.47, 118.86, 115.30, 111.78, 110.89, 53.60, 52.70, 51.76, 44.02, 31.66, 28.06, 26.79, 22.53, 14.35.

HRMS calculated for $C_{24}H_{34}N_7O^+$ (M+H): 436.2819, found: 436.282.

HPLC: rt = 11.90 min (98.59 %).

2-(4-((1-Methyl-1*H*-indol-3-yl)methyl)piperazin-1-yl)-*N*'-propylpyrimidine-5-carbohydrazide 105c (PSP50):



White solid (yield: 56%).

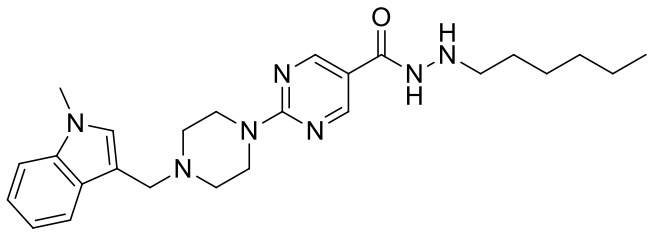
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.85 (s, 1H), 8.72 (s, 2H), 7.65 (d, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 8.2 Hz, 1H), 7.20 (s, 1H), 7.15 – 7.11 (m, 1H), 7.03 – 6.99 (m, 1H), 5.04 (s, 1H), 3.82 – 3.75 (m, 4H), 3.74 (s, 3H), 3.64 (s, 2H), 2.72 (t, *J* = 7.1 Hz, 2H), 2.46 – 2.40 (m, 4H), 1.48 – 1.40 (m, 2H), 0.89 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 163.41, 161.79, 157.72, 137.19, 129.43, 128.38, 121.52, 119.68, 119.02, 115.32, 110.17, 109.97, 53.63, 53.34, 52.67, 44.02, 32.71, 21.32, 12.09.

HRMS calculated for $C_{22}H_{30}N_7O^+$ (M+H): 408.2506, found: 408.2501.

HPLC: rt = 9.92 min (98.04 %).

***N*'-hexyl-2-(4-((1-methyl-1*H*-indol-3-yl)methyl)piperazin-1-yl)pyrimidine-5-carbohydrazide 105d (PSP74)**:



White solid (yield: 36%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H), 8.71 (s, 2H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.19 (s, 1H), 7.14 – 7.09 (m, 1H), 7.03 – 6.98 (m, 1H), 5.00 (s, 1H), 3.81 – 3.75 (m, 4H), 3.72 (s, 3H), 3.62 (s, 2H), 2.74 (t, *J* = 7.1 Hz, 2H), 2.46 – 2.36 (m, 4H), 1.41 (dt, *J* = 14.2, 7.0 Hz, 2H), 1.32 – 1.19 (m, 6H), 0.83 (t, *J* = 6.8 Hz, 3H).

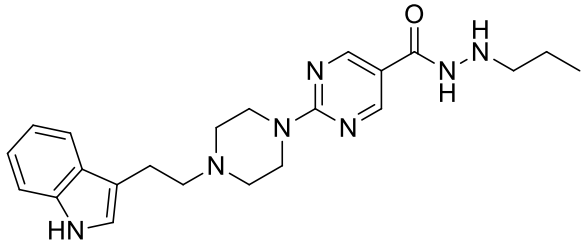
^{13}C NMR (101 MHz, DMSO-d_6) δ 163.39, 161.7, 157.71, 137.18, 129.41, 128.37, 121.50, 119.66, 119.00, 115.31, 110.14, 109.95, 53.33, 52.65, 51.76, 44.00, 32.70, 31.66, 28.07, 26.79, 22.53, 14.35. HRMS calculated for $C_{25}H_{36}N_7O^+$ (M+H): 450.2976, found: 450.297.

HPLC: rt = 12.61 min (98.37 %).

Procedure for the synthesis of *105e (PSP51), 105f (PSP72), 105g (PSP52)* and *105h (PSP71)*: A stirred mixture of amine (***103a*** or ***103b***) (1.0 mmol), 3-(2-bromoethyl)-indole or 3-(2-bromoethyl)-1-methyl-indole (1.2 mmol) and K_2CO_3 (2.5 mmol) in DMF (4 mL) was heated to about 80 ^{o}C overnight. The mixture was extracted with EtOAc and water two times. The combined organic layers were washed with brine two times, dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 1 : 1 and then DCM : MeOH = 30 : 1, 25 : 1) to give ***104e***, ***104f***, ***104g*** or ***104h*** respectively**.**

The following procedure was same as ***80a*** using corresponding *1**04e***, ***104e***, ***104g*** or *1**04h*** to give final compounds ***105e-105h*** respectively.

2-(4-(2-(1*H*-indol-3-yl)ethyl)piperazin-1-yl)-*N*'-propylpyrimidine-5-carbohydrazi-de 105e (PSP51):



White solid (yield: 55%).

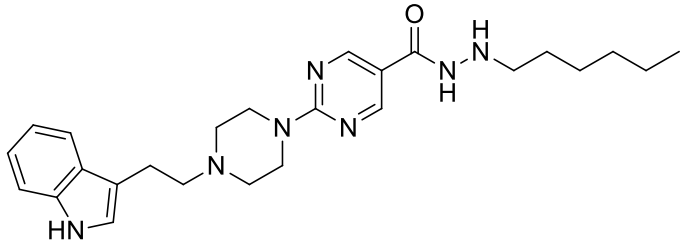
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 9.88 (s, 1H), 8.75 (s, 2H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.16 (d, *J* = 2.2 Hz, 1H), 7.07 – 7.03 (m, 1H), 6.96 (ddd, *J* = 7.9, 7.1, 1.0 Hz, 1H), 5.07 (s, 1H), 3.82 (d, *J* = 26.9 Hz, 4H), 2.93 – 2.86 (m, 2H), 2.73 (t, *J* = 7.1 Hz, 2H), 2.66 (s, 2H), 2.57 (s, 4H), 1.49 – 1.41 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 163.39, 161.86, 157.76, 136.64, 127.66, 122.96, 121.28, 118.71, 118.59, 115.46, 112.74, 111.78 (s), 59.05, 53.62, 52.85, 43.87, 22.74, 21.33, 12.11.

HRMS calculated for $C_{22}H_{30}N_7O^+$ (M+H): 408.2506, found: 408.2505.

HPLC: rt = 8.99 min (97.90 %).

2-(4-(2-(1*H*-indol-3-yl)ethyl)piperazin-1-yl)-*N*'-hexylpyrimidine-5-carbohydrazid-e 105f (PSP72):



White solid (yield: 23%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H), 9.85 (s, 1H), 8.73 (s, 2H), 7.50 (d, J = 7.8 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.14 (s, 1H), 7.04 (t, J = 7.5 Hz, 1H), 6.95 (t, J = 7.4 Hz, 1H), 5.00 (s, 1H), 3.82 (s, 4H), 2.91 – 2.83 (m, 2H), 2.74 (t, J = 7.0 Hz, 2H), 2.66 – 2.59 (m, 2H), 2.52 (s, 4H), 1.45 – 1.37 (m, 2H), 1.34 – 1.20 (m, 6H), 0.84 (t, J = 6.8 Hz, 3H).

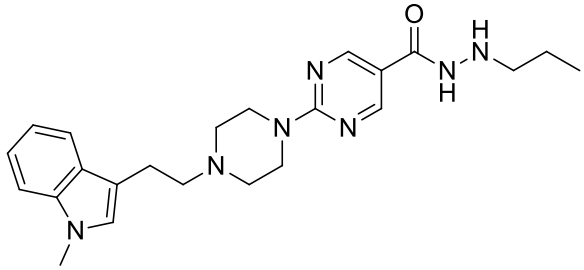
^{13}C NMR (101 MHz, DMSO-d_6) δ 163.38, 161.86, 157.73, 136.63, 127.67, 122.91, 121.24, 118.70, 118.56, 115.39, 112.87, 111.75, 59.16, 52.92, 51.74, 43.98, 31.66,

28.06, 26.78, 22.84, 22.53, 14.36.

HRMS calculated for $C_{25}H_{36}N_7O^+$ (M+H): 450.2976, found: 450.297.

HPLC: rt = 11.67 min (95.92 %).

2-(4-(2-(1-Methyl-1*H*-indol-3-yl)ethyl)piperazin-1-yl)-*N*'-propylpyrimidine-5-carbohydrazide 105g (PSP52):



White solid (yield: 52%).

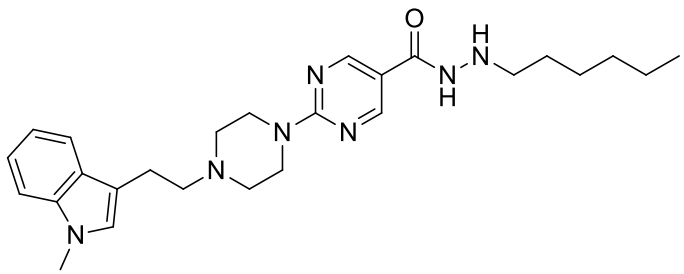
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.90 (s, 1H), 8.78 (s, 2H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 1H), 7.11 (dd, *J* = 13.8, 5.7 Hz, 2H), 7.00 (t, *J* = 7.4 Hz, 1H), 5.06 (s, 1H), 3.87 – 3.78 (m, 4H), 3.69 (s, 3H), 2.89 – 2.81 (m, 2H), 2.75 (t, *J* = 7.0 Hz, 2H), 2.62 – 2.56 (m, 2H), 2.50 (d, *J* = 4.6 Hz, 4H), 1.50 – 1.41 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 163.46, 161.88, 157.76, 137.03, 128.02, 127.31, 121.40, 118.95, 118.67, 115.39, 112.32, 109.87, 59.20, 53.68, 52.93, 43.98, 32.61, 22.70, 21.35, 12.10.

HRMS calculated for $C_{23}H_{32}N_7O^+$ (M+H): 422.2663, found: 422.2665.

HPLC: rt = 10.21 min (97.23 %).

***N*'-hexyl-2-(4-(2-(1-methyl-1*H*-indol-3-yl)ethyl)piperazin-1-yl)pyrimidine-5-carbohydrazide 105h (PSP71)**:



White solid (yield: 44%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.73 (s, 2H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 1H), 7.17 – 7.04 (m, 2H), 6.98 (dd, *J* = 11.0, 3.9 Hz, 1H), 5.01 (s, 1H), 3.90 – 3.78 (m, 4H), 3.70 (s, 3H), 2.91 – 2.81 (m, 2H), 2.74 (t, *J* = 7.0 Hz, 2H), 2.65 – 2.56 (m, 2H), 2.55 – 2.48 (m, 4H), 1.46 – 1.37 (m, 2H), 1.33 – 1.18 (m, 6H), 0.84 (t, *J* = 6.8 Hz, 3H).

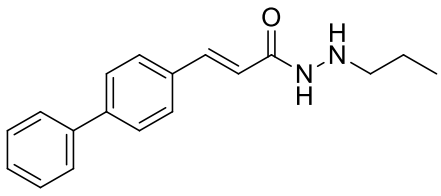
^{13}C NMR (101 MHz, DMSO-d_6) δ 163.38, 161.86, 157.73, 137.01, 127.99, 127.35, 121.39, 118.94, 118.66, 115.38, 112.28, 109.89, 59.18, 52.92, 51.76, 43.97, 32.63, 31.66, 28.07, 26.79, 22.67, 22.53, 14.36.

HRMS calculated for $C_{26}H_{38}N_7O^+$ (M+H): 464.3132, found: 464.3126.

HPLC: rt = 12.40 min (92.40 %).

General procedure for the synthesis of *(E)-3-([1,1'-biphenyl]-4-yl)-N'-propylacrylohydrazide 110a (NI-105)* and *(E)-3-([1,1'-biphenyl]-4-yl)-N'-hexylacrylohydrazide 110b (PSP85)*: T**o** a stirred mixture of (*E*)-3-([1,1'-biphenyl]-4-yl)acrylic acid (***108***) (200 mg, 0.89 mmol, 1.0 eq), HATU (340 mmg, 0.89 mmol, 1.0 eq) and DIPEA (345 mg, 2.67 mmol, 3.0 eq) in DMF (2 ml) was added hydrazine monohydrate (90 mg, 1.78 mmol, 2.0 eq). The resulting reaction was stirred for an additional 5 min at room temperature then diluted with water until a precipitate appeared. The precipitate was filtered and washed with cold water, dried to give compound ***109*** as light yellow solid (230 mg) in a quantitative yield, m/z (APCI$^+$) 239.12 (M+H)$^+$. Through the same procedure of ***84*** to ***85b*** using ***109*** and propionaldehyde or hexanal to give ***110a*** or ***110b***.

(*E*)-3-([1,1'-biphenyl]-4-yl)-*N*'-propylacrylohydrazide 110a (NI-105):



White solid (yield: 75%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.59 (s, 1H), 7.73 – 7.61 (m, 6H), 7.51 – 7.43 (m, 3H), 7.40 – 7.34 (m, 1H), 6.57 (d, *J* = 15.8 Hz, 1H), 5.05 (s, 1H), 2.69 (t, *J* = 6.9 Hz,

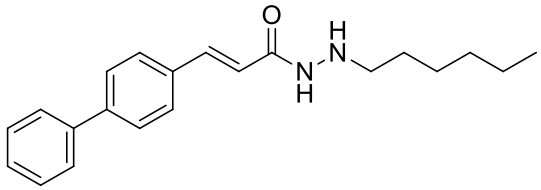
2H), 1.48 – 1.37 (m, 2H), 0.88 (dd, *J* = 8.8, 6.1 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 164.24, 141.41, 139.78, 138.40, 134.46, 129.44, 128.53, 128.23, 127.56, 127.04, 120.70, 53.55, 21.24, 12.01.

HRMS calculated for $C_{18}H_{21}N_2O^+$ (M+H): 281.1648, found: 281.1646.

HPLC: rt = 13.55 min (93.31 %).

(*E*)-3-([1,1'-biphenyl]-4-yl)-*N*'-hexylacrylohydrazide 110b (PSP85):



White solid (yield: 74%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (s, 1H), 7.70 (t, *J* = 8.2 Hz, 4H), 7.64 (d, *J* = 8.3 Hz, 2H), 7.54 – 7.42 (m, 3H), 7.37 (t, *J* = 7.3 Hz, 1H), 6.59 (d, *J* = 15.8 Hz, 1H), 5.03 (s, 1H), 2.72 (s, 2H), 1.51 – 1.37 (m, 2H), 1.29 (dt, *J* = 14.9, 6.5 Hz, 6H), 0.85 (t, *J* = 6.7 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 164.26, 141.41, 139.77, 138.42, 134.46, 129.43, 128.54, 128.22, 127.55, 127.03, 120.69, 51.72, 31.67, 28.02, 26.72, 22.53, 14.37.

HRMS calculated for $C_{21}H_{27}N_2O^+$ (M+H): 323.2118, found: 323.212.

HPLC: rt = 15.31 min (94.51 %).

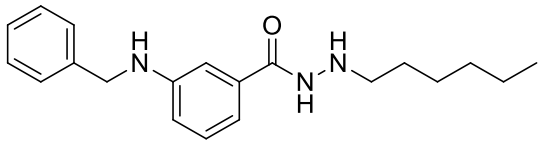
General procedure for the synthesis of *3-(benzylamino)-N'-hexylbenzohydrazide 114a (NI-82)* and *3-(benzylamino)-N'-hexyl-4-methylbenzohydrazide 114b (NI-85)*:

Ethyl 3-(benzylamino)benzoate (112a)* and *ethyl 3-(benzylamino)-4-methylbenzoate (112b): A mixture of aniline (***111a*** or ***111b***) (1.1 mmol), benzyl chloride (1.0 mmol) and K_2CO_3 (2.5 mmol) in DMF (2 mL) was heated to 100 ^{o}C in a microwave reactor for 2 h. The reaction was extracted with water and EtOAc twice. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (chloroform : MeOH) to give ***112a*** or ***112b*** as colorless oil in about 22% yield.

3-(Benzylamino)-4-methylbenzohydrazide (113a) and ***3-(benzylamino)benzohydrazide (113b)***: ***113a*** and ***113b*** were synthesized according to the procedure of method 2 used for ***94a*** using ***112a*** or ***112b*** and hydrazine monohydrate.

114a and ***114b*** were finally synthesized through the same procedure of ***84*** to ***85b*** using ***113a*** or ***113b*** and hexanal.

3-(Benzylamino)-*N*'-hexylbenzohydrazide 114a (NI-82):



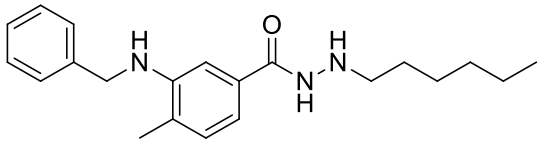
Colorless oil (60 mg, yield: 30%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 5H), 7.19 (t, J = 7.9 Hz, 1H), 7.09 – 7.05 (m, 1H), 6.98 (d, J = 7.6 Hz, 1H), 6.73 (dd, J = 8.1, 2.4 Hz, 1H), 4.36 (s, 2H), 2.92 (t, J = 7.3 Hz, 2H), 1.57 – 1.47 (m, 2H), 1.40 – 1.24 (m, 6H), 0.89 (t, J = 6.9 Hz, 3H).

HRMS calculated for $C_{20}H_{28}N_3O^+$ (M+H): 326.2227, found: 326.223.

HPLC: rt = 13.58 min (97.18 %).

3-(Benzylamino)-*N*'-hexyl-4-methylbenzohydrazide 114b (NI-85):



White solid (70 mg, yield: 29%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.24 (m, 5H), 7.10 (d, J = 7.6 Hz, 1H), 7.05 (d, J = 1.6 Hz, 1H), 6.96 (dd, J = 7.6, 1.6 Hz, 1H), 4.41 (s, 2H), 2.94 – 2.85 (m, 2H), 2.18 (s, 3H), 1.56 – 1.46 (m, 2H), 1.41 – 1.23 (m, 6H), 0.89 (t, J = 6.9 Hz, 3H).

HRMS calculated for $C_{21}H_{30}N_3O^+$ (M+H): 340.2383, found: 340.238.

HPLC: rt = 14.04 min (97.50 %).

Procedure for the synthesis of *N-((4'-(2-hexylhydrazinecarbonothioyl)-[1,1'-biphenyl]-4-yl)methyl)acetamide 120 (PSP81)*:

Tert-butyl 1-hexyl-2-(4'-((tritylamino)methyl)-[1,1'-biphenyl]-4-carbonyl)hydrazinecarboxylate (115): A mixture of tert-butyl 2-(4'-(aminomethyl)-[1,1'-biphenyl]-4-carbonyl)-1-hexylhydrazinecarboxylate (***191***) (1.2 g, 2.82 mmol, 1.0 eq), triphenylmethyl chloride (TrtCl) (0.83 g, 2.96 mmol, 1.05 eq) and TEA (0.71 g, 7.05 mmol, 2.5 eq) in DCM (15 mL) was stirred at room temperature overnight. The reaction was then evaporated directly. Water was added to the residue and the resulting mixture was stirred for several minutes. The precipitate was filtered and dried to give intermediate ***115*** as white solid (1.6 g, yield: 85%).

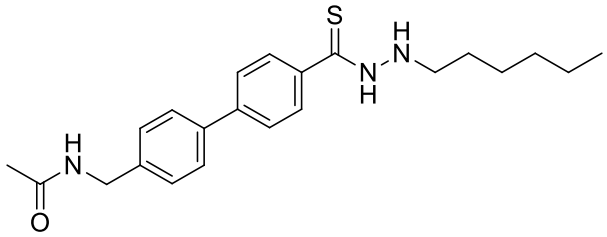
Tert-butyl 1-hexyl-2-(4'-((tritylamino)methyl)-[1,1'-biphenyl]-4-carbonothioyl)hydrazinecarboxylate (116): A stirred mixture of compound ***115*** (560 mg, 0.84 mmol, 1.0 eq) and Lawesson's reagent (204 mg, 0.503 mmol, 0.6 eq) in toluene (6 mL) was kept under argon atmosphere. The reaction was heated to reflux for 25 min. The reaction was concentrated and the resulting residue was purified by column chromatography (EtOAc: heptane = 0 : 1, 1 : 10, 1 : 8 gradually) to give ***116*** as yellow solid (160 mg, yield: 28%). m/z ($APCI^+$) 684.6 $(M+H)^+$.

Tert-butyl 2-(4'-(aminomethyl)-[1,1'-biphenyl]-4-carbonothioyl)-1-hexylhydrazinecarboxylate (117): A mixture of above compound ***116*** in AcOH (4 mL) and water (1 mL) was stirred at room temperature overnight. The reaction was evaporated and the resulting residue was added saturated Na_2CO_3 solution until pH ≈ 10. The mixture was extracted with EtOAc and brine. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated to give ***117*** as sticky oil (120 mg) that was

directly used without further purification. m/z (APCI$^+$) 442.5 (M+H)$^+$.

Tert-butyl 2-(4'-(acetamidomethyl)-[1,1'-biphenyl]-4-carbonothioyl)-1-hexylhydrazinecarboxylate 118: Acetyl chloride (22 mg, 0.27 mmol, 1.0 eq) was added to a stirred mixture of ***117*** (120 mg, 0.27 mmol, 1.0 eq) and TEA (83 mg, 0.81 mmol, 3.0 eq) in DCM (5 mL) at room temperature. The resulting mixture was stirred for 10 min and concentrated. The residue was purified by column chromatography (EtOAc: heptane = 0 : 1, 1 : 3 and then DCM : MeOH = 1 : 0, 40 : 1 gradually) to give 118 as yellow solid (100 mg, yield: 77%). m/z (APCI$^+$) 484.6 (M+H)$^+$.

***N*-((4'-(2-hexylhydrazinecarbonothioyl)-[1,1'-biphenyl]-4-yl)methyl)acetamide 120 (PSP81)**:



120 was synthesized through the same procedure of ***79a*** to ***80a*** using ***118***.

120 was yellow solid (20 mg, yield: 25%).

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 12.08 (s, 1H), 8.36 (t, *J* = 5.8 Hz, 1H), 7.85 – 7.64 (m, 6H), 7.35 (d, *J* = 8.3 Hz, 2H), 6.68 (s, 1H), 4.28 (d, *J* = 5.9 Hz, 2H), 3.03 (s, 2H), 1.88 (s, 3H), 1.37 – 1.20 (m, 8H), 0.86 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d$_6$) δ 169.58, 139.95, 128.40, 127.08, 126.47, 46.15, 42.27, 31.45, 30.87, 26.61, 23.03, 22.44, 14.34.

HRMS calculated for $C_{22}H_{30}N_3OS^+$ (M+H): 384.2104, found: 384.210.

HPLC: rt = 14.41 min (93.60 %).

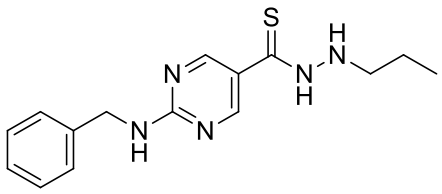
General procedure for the synthesis of *2-(benzylamino)-N'-propylpyrimidine-5-carbothiohydrazide 123a (PSP82)* and *2-(benzylamino)-N'-hexylpyrimidine-5-carbothiohydrazide 123b (PSP83)*:

Tert-butyl 2-(2-(benzylamino)pyrimidine-5-carbonyl)-1-propylhydrazinecarboxylate (121a) **and** ***tert-butyl 2-(2-(benzylamino)pyrimidine-5-carbonyl)-1-hexylhydrazinecarboxylate (121b)***: These two intermediate were synthesized through the same procedure of ***101a*** to ***102a*** starting from ***95a*** and ***95l***. Both of them were white solid in 69% yield and 70% yield respectively.

Tert-butyl 2-(2-(benzylamino)pyrimidine-5-carbonothioyl)-1-propylhydrazinecarboxylate (122a) **and** ***tert-butyl 2-(2-(benzylamino)pyrimidine-5-carbonothioyl)-1-hexylhydrazinecarboxylate (122b):*** These two compounds were synthesized through the same procedure of ***115*** to ***116*** starting from ***121a*** and ***121b***. Both of them were gray solid. ***122a***: m/z (APCI$^+$) 402.3 $(M+H)^+$, 803.6 $(2M+H)^+$.

122a and ***122b*** were converted to ***123a*** and ***123b*** through the same procedure of ***79a*** to ***80a***.

2-(Benzylamino)-*N*'-propylpyrimidine-5-carbothiohydrazide 123a (PSP82):



Light yellow solid (yield: 32% over two steps).

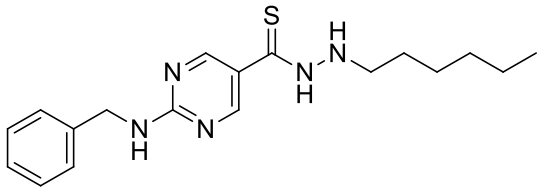
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.56 (s, 1H), 8.69 (s, 2H), 8.25 (s, 1H), 7.32 – 7.18 (m, 5H), 4.55 (d, *J* = 6.4 Hz, 2H), 2.95 (t, *J* = 7.0 Hz, 2H), 1.60 – 1.47 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.01, 157.85, 157.09, 140.25, 128.65, 127.46, 127.07, 125.34, 51.48, 44.51, 30.87, 11.94.

HRMS calculated for $C_{15}H_{20}N_5S^+$ (M+H): 302.1434, found: 302.143.

HPLC: rt = 12.60 min (92.69 %).

2-(Benzylamino)-*N*'-hexylpyrimidine-5-carbothiohydrazide 123b (PSP83):



Light yellow solid (yield: 37% over two steps).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (s, 1H), 8.68 (s, 2H), 8.25 (s, 1H), 7.33 – 7.17 (m, 5H), 4.55 (d, *J* = 6.4 Hz, 2H), 2.96 (t, *J* = 7.0 Hz, 2H), 1.50 (dd, *J* = 14.0, 7.0 Hz, 2H), 1.35 – 1.22 (m, 6H), 0.85 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.01, 157.84, 157.03, 140.25, 128.65, 127.46, 127.07, 125.33, 49.70, 44.50, 31.43, 30.86, 26.60, 22.44, 14.32.

HRMS calculated for $C_{18}H_{26}N_5S^+$ (M+H): 344.1903, found: 344.190.

HPLC: rt = 14.84 min (93.08 %).

Procedure for the synthesis of *2-(4-((1H-indol-3-yl)methyl)piperazin-1-yl)-N'-hexylpyrimidine-5-carbothiohydrazide* 128 (PSP84):

Tert-butyl 1-hexyl-2-(2-(4-tritylpiperazin-1-yl)pyrimidine-5-carbonyl)hydrazinecarboxylate (124): This compound was synthesized through the same procedure of ***191*** to ***115*** starting from tert-butyl 1-hexyl-2-(2-(piperazin-1-yl)pyrimidine-5-carbonyl)hydrazinecarboxylate (***103b***). This compound was white solid in 94% yield.

Tert-butyl 1-hexyl-2-(2-(4-tritylpiperazin-1-yl)pyrimidine-5-carbonothioyl)hydrazinecarboxylate (125): This compound was synthesized through the same procedure of ***115*** to ***116*** (refluxed within 20 min) using ***124*** (yield: 21%). m/z (APCI$^+$) 665.6 $(M+H)^+$.

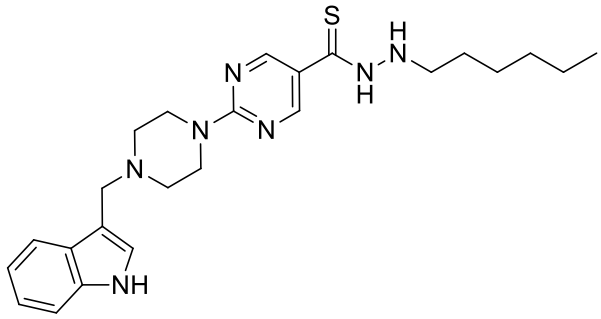
Tert-butyl

1-hexyl-2-(2-(piperazin-1-yl)pyrimidine-5-carbonothioyl)hydrazinecarboxylate

(126): This compound was synthesized through the same procedure of ***116*** to ***117*** using ***125***. m/z (APCI$^+$) 423.5 $(M+H)^+$, 845.9 $(2M+H)^+$.

The final compound ***128*** was synthesized through the same procedure of ***103a*** to ***105a*** starting from intermediate ***126***.

2-(4-((1*H*-indol-3-yl)methyl)piperazin-1-yl)-*N*'-hexylpyrimidine-5-carbothiohydr -azide 128 (PSP84):



Yellow solid (yield: 11% over two steps).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H), 8.71 (s, 2H), 7.65 (d, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.24 (d, *J* = 2.3 Hz, 1H), 7.08 – 7.03 (m, 1H), 7.00 – 6.95 (m, 1H), 3.85 – 3.73 (m, 4H), 3.67 (s, 2H), 2.97 (t, *J* = 7.1 Hz, 2H), 2.48 – 2.41 (m, 4H), 1.56 – 1.46 (m, 2H), 1.34 – 1.19 (m, 6H), 0.85 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 161.51, 157.22, 136.77, 128.02, 125.21, 121.49, 121.40, 119.48, 118.89, 111.79, 110.77, 53.55, 52.68, 49.66, 44.09, 31.41, 27.19, 26.57, 22.42, 14.32.

HRMS calculated for $C_{24}H_{34}N_7S^+$ (M+H): 452.2591, found: 452.259.

HPLC: rt = 13.89 min (94.20 %).

5.3.3 Synthesis procedure and compound characterization for part 4

3-Aminopiperidine-2,6-dione TFA salt (136a) or HCl salt (136b): To a stirred mixture of CDI (3.29 g, 20.3 mmol, 1.0 eq) and Boc-L-glutamin (5 g, 20.3 mmol, 1.0 eq) in THF (15 mL) was added 4-Dimethylaminopyridine (DMAP) (0.0125 g, 0.102mmol, 0.005eq). The resulting mixture was heated to reflux and stirred overnight. The mixture was then cooled to room temperature and stirred at 0 ^{o}C for about 2 h. The white precipitate was filtered, washed with cold THF and dried to give tert-butyl (2,6-dioxopiperidin-3-yl)carbamate (***135***) as white solid (3.6 g, yield: 73%).

The intermediate ***135*** (1.0 g) was added to a solution of TFA (2 mL) and DCM (8 mL) or a solution of HCl/dioxane (4M) (2 mL) and DCM (6 mL). The mixture was stirred at room temperature overnight. Then, the mixture was evaporated directly to give ***136a*** or ***136b*** as white solid in a quantitative yield. ***136b***: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 1H), 8.54 (s, 3H), 4.18 (dd, *J* = 13.1, 5.3 Hz, 1H), 2.74 – 2.65 (m, 1H), 2.58 (ddd, *J* = 17.7, 4.8, 2.2 Hz, 1H), 2.17 (dtd, *J* = 7.4, 5.2, 2.1 Hz, 1H), 2.05 – 1.93 (m, 1H).

Procedure for the synthesis of *4-amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (139)*: To a stirred mixture of DIPEA (40.0 mmol) and ***136a*** (10.0 mmol) in THF (20 mL) was added 3-Nitrophthalic acid anhydride (***137***) (10.0 mmol). The resulting mixture was stirred for 15 min at room temperature. $SOCl_2$ (30.0 mmol) was added dropwise to the reaction. The resulting reaction was stirred at room temperature overnight. Then, cold water was added to the mixture and stirring was continued for 30 min. The precipitate was filtered and dried to give an intermediate 2-(2,6-dioxopiperidin-3-yl)-4-nitroisoindoline-1,3-dione (***138***) as dark blue solid in a 78% yield. m/z (APCI^{+}) 304.1 $(M+H)^{+}$, 705.1 $(2M+H)^{+}$. ^{1}H NMR (400 MHz,

DMSO-d_6) δ 11.00 (s, 1H), 8.46 (dd, *J* = 8.2, 0.8 Hz, 1H), 8.17 (d, *J* = 6.9 Hz, 1H), 7.83 (t, *J* = 7.8 Hz, 1H), 5.16 (dd, *J* = 13.2, 5.2 Hz, 1H), 2.94 – 2.84 (m, 1H), 2.62 – 2.51 (m, 2H), 2.05 – 1.97 (m, 1H).

$SnCl_2 \cdot H_2O$ (5.0 mmol) was added to a stirred mixture of intermediate ***138*** (1.0 mmol) in EtOH (3 mL). The reaction was heated to reflux for 2 h and then cooled to room temperature. The precipitate was directly filtered and dried to give ***139*** as light yellow solid (yield: 50%), m/z ($APCI^+$) 274.3 $(M+H)^+$.

Procedure for the synthesis of *2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (262)*: A stirred mixture of 4-fluoroisobenzofuran-1,3-dione (***261***) (1.1 g, 6.62 mmol, 1.1 eq), ***135*** (1.38 g, 6.02 mmol, 1.0 eq) and NaOAc (sodium acetate) (0.98 g, 7.02 mmol, 1.2 eq) in AcOH (20 mL) was heated to reflux overnight. Then, water (150 mL) was added to the cooled reaction until precipitate appeared. The precipitate was filtered, washed with water and heptane and dried to give ***262*** as gray solid (1.48 g, yield: 87%).

Procedure for the synthesis of *3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione (144)*:

Methyl 2-methyl-3-nitrobenzoate (141): To a stirred mixture of 2-methyl-3-nitrobenzoic acid (***140***) (10 g, 55.2 mmol, 1.0 eq) in acetone (100 mL) was added CH_3I (14 mL, 220.8 mmol, 4.0 eq) and followed by K_2CO_3 (9.2 g, 66.24 mmol, 1.2 eq). The resulting mixture was refluxed overnight. Then, the solvent was removed by evaporation. The residue was extracted with EtOAc and water. The collected organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give ***141*** as white solid (10.8 g) in a quantitative yield. m/z ($APCI^+$) 196.2 $(M+H)^+$.

Methyl 2-(bromomethyl)-3-nitrobenzoate (142): Benzoyl peroxide (373 mg, 1.54 mmol, 0.1 eq) was added to a stirred mixture of ***141*** (3.0 g, 15.4 mmol, 1.0 eq) and

NBS (3.0 g, 16.9 mmol, 1.1 eq) in carbon tetrachloride (30 mL). The reaction was refluxed for 7 h. The reaction was then cooled to room temperature and filtered. The resulting filtrate was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give ***142*** as light brown solid (4.8 g) in a quantitative yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.15 (d, *J* = 8.1 Hz, 1H), 8.10 (d, *J* = 7.9 Hz, 1H), 7.72 (td, *J* = 8.0, 1.4 Hz, 1H), 5.01 (s, 2H), 3.91 (s, 3H).

3-(4-Amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione (144): A stirred mixture of ***136b*** (1.8 g, 10.9 mmol, 1.2 eq), ***142*** (2.5 g, 9.12 mmol, 1.0 eq) and K_2CO_3 (3.16 g, 22.9 mmol, 2.5 eq) in DMF (13 mL) was heated to 40-45 ^{o}C overnight. Then, the mixture was cooled to room temperature. Cold water (20 mL) was added to the mixture and the stirring was continued for 30 min. The resulting precipitate was filtered, washed with water and heptane and dried to give 3-(4-nitro-1-oxoisoindolin-2-yl)piperidine-2,6-dione (***143***) as gray solid (1.51 g, yield: 64%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 1H), 8.46 (dd, J = 8.2, 0.8 Hz, 1H), 8.17 (d, J = 6.9 Hz, 1H), 7.83 (t, J = 7.8 Hz, 1H), 5.16 (dd, J = 13.2, 5.2 Hz, 1H), 4.84 (dd, J = 45.6, 19.3 Hz, 2H), 2.94 – 2.85 (m, 1H), 2.63 – 2.51 (m, 2H), 2.04 – 1.97 (m, 1H). A degassed mixture of Pd/C (10%) (150 mg) and ***143*** (1.5 g) in MeOH (15 mL) was kept under hydrogen atmosphere. The reaction was stirred at room temperature overnight. Then, the mixture was filtered and resulting residue was washed with THF. The filtrate was concentrated to give ***144*** as white solid (0.69 g, yield: 51%). m/z (APCI$^+$) 260.0 $(M+H)^+$, 519.1 $(2M+H)^+$.

Procedure for the synthesis of *3-(4-Bromo-1-oxoisoindolin-2-yl)piperidine-2,6-dione (147)*: Through the same procedure used for ***142*** using methyl 3-bromo-2-methylbenzoate (***145***) as a starting material to give methyl 3-bromo-2-(bromomethyl)benzoate (***146***) as orange oil in a quantitative yield. A stirred mixture of intermediate ***146*** (3.12g, 1.0 eq), intermediate ***136b*** (2.0g, 1.2 eq) and DIPEA (3.9g, 3.0 eq) in MeCN (30 mL) was heated to about 80 ^{o}C overnight. The mixture was cooled to room temperature. Water was added to

the mixture until precipitate appeared. The mixture was stirred for an additional 1 h and then filtered. The resulting residue was washed with cold water and dried to give ***147*** as blue solid (2.29 g, yield: 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.98 (s, 1H), 7.85 (d, *J* = 7.9 Hz, 1H), 7.76 (d, *J* = 7.4 Hz, 1H), 7.50 (t, *J* = 7.7 Hz, 1H), 5.13 (dd, *J* = 13.3, 5.2 Hz, 1H), 4.33 (dd, *J* = 63.5, 17.6 Hz, 2H), 2.94 – 2.84 (m, 1H), 2.62 – 2.54 (m, 1H), 2.42 (dd, *J* = 13.5, 4.4 Hz, 1H), 2.00 (dtd, *J* = 12.6, 5.4, 2.1 Hz, 1H).

Procedure for the synthesis of *4-(prop-2-yn-1-yloxy)benzoyl chloride (161)*:

Methyl 4-(prop-2-yn-1-yloxy)benzoate (159): A mixture of methyl 4-hydroxybenzoate (***158***) (10.0 g, 65.74 mmol, 1.0 eq), propargyl bromide (80% in toluene) (8.15 mL, 75.6 mmol, 1.15 eq) and K_2CO_3 (11.81 g, 85.46 mmol, 1.3 eq) in DMF (50 mL) was stirred at room temperature overnight. Then, another portion of propargyl bromide (80% in toluene) (6 mL, 0.85 eq) was added to the reaction and the stirring was continued for 6h. The reaction was extracted with EtOAc and water three times. The combined organic layers were washed with brine three times, dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 1 : 8 to 1 : 4 gradually) to give ***159*** as white solid (12.5 g) in a quantitative yield. m/z (APCI$^+$) 190.3 $(M+H)^+$.

4-(Prop-2-yn-1-yloxy)benzoic acid (160): To a stirred mixture of ***159*** (2.0 g, 10.51 mmol, 1.0 eq) in MeOH (90 mL) and water (30 mL) was added NaOH (2.1 g, 52.5 mmol, 5.0 eq). The reaction was stirred at room temperature for 24 h. Then, part of solvent was removed by evaporation. HCl (10%) solution was added dropwise to the residue until pH ≈ 2 and precipitate appeared. The precipitate was filtered, washed with cold water and dried to give ***160*** as white solid (1.8 g, yield: 97%).

4-(Prop-2-yn-1-yloxy)benzoyl chloride (161): A stirred mixture of ***160*** (1.0 eq) and $SOCl_2$ (4.0 eq) was heated to reflux for 1 h until precipitate was dissolved. Then, the reaction was concentrated and the residue was directly used without further purification.

Procedure for the synthesis of *tert-butyl (2-(4-(prop-2-yn-1-yloxy)benzamido)-4-(thiophen-2-yl)phenyl)carbamate (166a)* and *tert-butyl (4'-fluoro-3-(4-(prop-2-yn-1-yloxy)benzamido)-[1,1'-biphenyl]-4-yl)carbamate (166b)*:

Tert-butyl (4-bromo-2-nitrophenyl)carbamate (163): A mixture of 4-bromo-2-nitroaniline (***162***) (5.0 g, 23 mmol, 1.0 eq), $(Boc)_2O$ (10.03 g, 46 mmol, 2.0 eq), TEA (7.0 g, 69 mmol, 3.0 eq) and DMAP (28 mg, 0.23 mmol, 0.01 eq) in DCM (50 mL) was stirred at room temperature for 5 h. The reaction was concentrated and the resulting residue was purified by column chromatography (EtOAc : heptane = 0 : 1, 1 : 40) to give ***163*** as a yellow solid (6.04 g, yield: 83%).

Tert-butyl (2-nitro-4-(thiophen-2-yl)phenyl)carbamate (164a): A mixture of compound ***163*** (1.0 g, 31.5 mmol, 1.0 eq), thiophen-2-ylboronic acid (0.45 g, 34.7 mmol, 1.1 eq), K_2CO_3 (0.96 g, 69.5 mmol, 2.2 eq) and $Pd(PPh_3)_4$ (182 mg, 1.6 mmol, 0.05 eq) in THF (15 mL) and water (3 mL) was kept under argon atmosphere and refluxed for 1 h. The reaction was extracted with EtOAc and water. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (EtOAc : heptane) to give ***164a*** as yellow solid (0.8 g, yield: 79%). m/z ($APCI^+$) 321.4 $(M+H)^+$, 265.4 $(M-tBu+2H)^+$.

Tert-butyl (4'-fluoro-3-nitro-[1,1'-biphenyl]-4-yl)carbamate (164b): This compound was synthesized according to the procedure used for ***164a*** using (4-fluorophenyl)boronic acid and ***163***. ***164b*** was white solid (0.7 g, yield: 68%).

Tert-butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate (165a)* and tert-butyl *(3-amino-4'-fluoro-[1,1'-biphenyl]-4-yl)carbamate (165b): A mixture of ***164a*** (0.8 g) or ***164b*** (0.7 g) (1.0 eq) and Zn powder (4.0 eq) in AcOH (1 mL) and MeOH (15 mL) was stirred at room temperature for about 1 h. The mixture was filtered. Saturated

Na_2CO_3 solution was added drpwise to the resulting filtrate until pH ≈ 10. The mixture was extracted with EtOAc and water. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 0 : 1 to 1 : 1 gradually) to give ***165a*** or ***165b***. ***165a*** was in a 45% yield about. m/z (APCI$^+$) 291.7 $(M+H)^+$, 235.5 $(M\text{-}tBu+2H)^+$; ***165b*** was in a 77% yield. m/z (APCI$^+$) 303.7$(M+H)^+$, 247.5 $(M\text{-}tBu+2H)^+$.

Tert-butyl (2-(4-(prop-2-yn-1-yloxy)benzamido)-4-(thiophen-2-yl)phenyl)carbamate (166a) **and** ***tert-butyl (4'-fluoro-3-(4-(prop-2-yn-1-yloxy)benzamido)-[1,1'-biphenyl]-4-yl)carbamate (166b)***: A solution of ***161*** **(**1.2 mmol) in anhydrous THF (2 mL) was added to a mixture of ***165a*** or ***165b*** (1.0 mmol) and DIPEA (5.0 mmol) in anhydrous THF (5 mL). The resulting mixture was stirred at room temperature for 30 min. Then, the reaction was concentrated and the resulting residue was purified by column chromatography (EtOAc : heptane) to give ***166a*** or ***166b*** both in a 90% yield. ***166b*** was white solid. m/z (APCI$^+$) 405.8 $(M\text{-}tBu+2H)^+$, 361.8 (M-Boc+2H); ***166a*** was light pink solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.03 (s, 1H), 7.85 – 7.81 (m, 3H), 7.27 (d, *J* = 11.1 Hz, 2H), 7.18 (dd, *J* = 5.1, 0.9 Hz, 1H), 7.15 (dd, *J* = 3.5, 0.9 Hz, 1H), 6.98 (dd, *J* = 5.0, 3.7 Hz, 1H), 6.60 (d, *J* = 8.7 Hz, 2H), 4.46 (s, 1H), 3.93 (d, *J* = 2.3 Hz, 2H), 2.25 (t, *J* = 2.4 Hz, 1H), 1.47 (s, 9H).

Procedure for the synthesis of ***N-(2-amino-4-fluorophenyl)-4-(prop-2-yn-1-yloxy)benzamide (169a)*** **and** ***N-(2-amino-4-chlorophenyl)-4-(prop-2-yn-1-yloxy)benzamide (169b)***: A mixture of 5-fluoro-2-nitroaniline (***167a***) (1.0 g, 1.0 eq), Pd/C (10%) (100 mg) and ammonium formate (1.61g, 4.0 eq) in EtOH (10 mL) was stirred at room temperature for about 1 h. The mixture was then filtered. The filtrate was concentrated and the resulting residue was extracted with EtOAc and water. The collected organic layer was washed with water three times, dried over anhydrous Na_2SO_4 and concentrated to give 4-fluorobenzene-1,2-diamine (***168a***) as gray solid in a 75% yield. m/z (APCI$^+$) 127.2

$(M+H)^+$. The intermediate 4-chlorobenzene-1,2-diamine (***168b***) was commercial available.

A mixture of ***168a*** or ***168b*** (1.0 mmol), intermediate ***160*** (1.0 mmol), HATU (1.2 mmol) and DIPEA (3.0 mmol) in DMF (1.5 mL) was stirred at room temperature for about 10 min. Water was added to the reaction and the resulting mixture was extracted with EtOAc three times. The combined organic layers were washed with brine two times, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (EtOAc : heptane) to give ***169a*** or ***169b***. ***169a*** was white solid in a 98% yield. m/z ($APCI^+$) 285.4 $(M+H)^+$, ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 – 7.86 (m, 2H), 7.57 (s, 1H), 7.16 (dd, *J* = 8.5, 5.8 Hz, 1H), 7.09 – 7.04 (m, 2H), 6.59 – 6.46 (m, 2H), 4.77 (d, *J* = 2.4 Hz, 2H), 3.99 (s, 2H), 2.56 (t, *J* = 2.4 Hz, 1H); ***169b*** was yellow solid in a 83% yield. m/z ($APCI^+$) 301.5 $(M+H)^+$.

Procedure for the synthesis of *tert-butyl (4-fluoro-2-(4-(prop-2-yn-1-yloxy)benzamido)phenyl)carbamate (174a)* and *tert-butyl (2-(4-(prop-2-yn-1-yloxy)benzamido)phenyl)carbamate (174b)*:

Di Tert-butyl (4-fluoro-2-aminophenyl)carbamate (171a): To a stirred mixture of 4-fluoro-2-nitroaniline (***170a***) (1.0 g, 6.4 mmol, 1.0 eq), $(Boc)_2O$ (2.8 g, 12.8 mmol, 2.0 eq) and TEA (1.94 g, 19.2 mmol, 3.0 eq) in DCM (10 mL) was added DMAP (78.3 mg, 0.64 mmol, 0.1 eq) and the reaction was stirred at room temperature for an additional 2 h. The reaction was then concentrated directly and the resulting residue was purified by column chromatography (EtOAc : heptane = 0 : 1 to 1 : 10 gradually) to give ***171a*** as yellow solid (2.1 g, yield: 92%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.15 (dd, J = 9.1, 5.4 Hz, 1H), 7.19 (ddd, J = 9.1, 7.2, 2.8 Hz, 1H), 7.05 (dd, J = 8.3, 2.7 Hz, 1H), 1.41 (s, 18H).

Tert-butyl (4-fluoro-2-nitrophenyl)carbamate (172a): To a stirred mixture of ***171a*** (2.1 g, 5.89 mmol, 1.0 eq) in DCM (20 mL) was added TFA (0.46 mL, 5.9 mmol, 1.0 eq) over several portions. The resulting reaction was stirred at room temperature for

80 min. Then, saturated Na_2CO_3 solution was added dropwise to the reaction until no bubbles appeared. The reaction was extracted with DCM and brine. The collected organic layer was dried over Na_2SO_4 and evaporated to give ***172a*** as yellow solid (1.3 g, yield: 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 – 7.07 (m, 1H), 6.50 – 6.41 (m, 2H), 5.98 (s, 1H), 1.50 (s, 9H).

Tert-butyl (2-amino-4-fluorophenyl)carbamate (173a): This compound was synthesized through the same procedure used for ***168a*** using ***172a***. ***173a*** was gray solid in 95% yield.

Tert-butyl (4-fluoro-2-(4-(prop-2-yn-1-yloxy)benzamido)phenyl)carbamate (174a): This compound was synthesized through the same procedure used for ***166a*** using ***173a***. ***174a*** was a white solid in 96% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H), 7.95 – 7.90 (m, 2H), 7.74 (dd, *J* = 10.2, 2.7 Hz, 1H), 7.12 (dd, *J* = 8.8, 5.6 Hz, 1H), 7.07 – 7.02 (m, 2H), 6.86 – 6.80 (m, 1H), 6.58 (s, 1H), 4.77 (d, *J* = 2.4 Hz, 2H), 2.55 (t, *J* = 2.4 Hz, 1H), 1.52 (s, 9H).

Tert-butyl (2-(4-(prop-2-yn-1-yloxy)benzamido)phenyl)carbamate (174b): Using 4-chloro-2-nitroaniline (***170b***) through the same procedure of ***170a*** to ***173a*** and a last step of ***168a*** to ***169a*** to give ***174b*** as a yellow solid in 80% yield (last step)

Procedure for the synthesis of *tert-butyl (2-(4-(aminomethyl)benzamido)-4-(thiophen-2-yl)phenyl)carbamate (178a)*, *N-(2-amino-4-fluorophenyl)-4-(aminomethyl)benzamide (178b)* and *4-(aminomethyl)-N-(2-aminophenyl)benzamide (178c)*:

4-((2,2,2-trifluoroacetamido)methyl)benzoic acid (176): A mixture of 4-(aminomethyl)benzoic acid (***175***) (3.0 g, 19.85 mmol, 1.0 eq) in trifluoroacetic anhydride (TFAA) (10.5 g, 7 mL, 2.5 eq) was stirred at room temperature overnight. The mixture was then poured to cold water and white precipitate appeared. The precipitate was filtered and the residue was dried to give ***176*** as white solid (4.5g,

92% yield).

Intermediate *Tert-butyl (4-(thiophen-2-yl)-2-(4-((2,2,2-trifluoroacetamido)methyl)benzamido)phenyl)carbam at* (***177a***), *N*-(2-amino-4-fluorophenyl)-4-((2,2,2-trifluoroacetamido)methyl)benzamide (***177b***) and N-(2-aminophenyl)-4-((2,2,2-trifluoroacetamido)methyl)benzamide (***177c***) were synthesized through the same procedure of ***168a*** to ***169a*** using substituted aniline (***165a, 168a*** and *o-phenylendiamine*) as described in Figure 83. ***177a*** was light yellow solid in 94% yield. m/z (APCI$^+$) 520.0 (M+H)$^+$, ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.07 (t, *J* = 6.0 Hz, 1H), 9.88 (s, 1H), 8.70 (s, 1H), 7.95 (d, *J* = 8.3 Hz, 2H), 7.81 (d, *J* = 2.1 Hz, 1H), 7.60 (d, *J* = 8.6 Hz, 1H), 7.52 – 7.48 (m, 2H), 7.43 (dd, *J* = 4.8, 3.6 Hz, 3H), 7.11 (dd, *J* = 5.1, 3.6 Hz, 1H), 4.47 (d, *J* = 6.0 Hz, 2H), 1.44 (s, 9H); ***177b*** was light yellow solid in a quantitative yield. m/z (APCI$^+$) 356.0 (M+H)$^+$; ***177c*** was yellow solid with a quantitative yield. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.06 (s, 1H), 9.61 (s, 1H), 7.94 (d, *J* = 8.0 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 7.5 Hz, 1H), 6.95 (t, *J* = 7.1 Hz, 1H), 6.76 (d, *J* = 7.7 Hz, 1H), 6.58 (t, *J* = 7.3 Hz, 1H), 4.88 (s, 2H), 4.45 (d, *J* = 5.7 Hz, 2H). m/z (APCI$^+$) 338.1 (M+H)$^+$.

Gneneral procedure for the synthesis of *178a-178c*: A mixture of (***177a-177c***) (1.0 mmol), K_2CO_3 (4.0 mmol) in MeOH (5mL) and H_2O (5mL) was stirred at room temperature overnight. Then the mixture was extracted with EtOAc and water three times. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to give compounds ***178a-178c. 178a*** was black oil in 59% yield. m/z (APCI$^+$) 424.0 (M+H)$^+$; ***178b*** was black semisolid in 83% yield. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.60 (s, 1H), 7.94 (d, *J* = 7.7 Hz, 2H), 7.44 (d, *J* = 7.8 Hz, 2H), 7.18 – 7.08 (m, 1H), 6.57 (dd, *J* = 11.2, 2.5 Hz, 1H), 6.36 (td, *J* = 8.4, 2.4 Hz, 1H), 5.22 (s, 2H), 3.79 (s, 2H), 2.67 (s, 2H). (APCI$^+$) 259.9 (M+H)$^+$; ***178c*** was black semisolid.

Procedure for the synthesis of *tert-butyl*

(2-(4-aminobenzamido)-4-(thiophen-2-yl)phenyl)carbamate (183):

4-(2,2,2-Trifluoroacetamido)benzoic acid (180): To a stirred solution of 4-aminobenzoic acid (***179***) (2.0 g, 14.58 mmol, 1.0 eq) in TFA (10 mL) was added dropwise TFAA (6.13 g, 4.15 mL, 29.16 mmol, 2.0 eq) under ice bath. The reaction was stirred at room temperature overnight. Then the reaction was poured to cold water and precipitate appeared. Then the precipitate was filtered and washed with cold water followed by heptane, and dried to give compound ***180*** as white solid (3.6 g) in quantitative yield. m/z (APCI$^+$) 234.2 $(M+H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.91 (s, 1H), 11.49 (s, 1H), 7.99 – 7.93 (m, 2H), 7.79 (d, *J* = 8.8 Hz, 2H).

Tert-butyl

(4-(thiophen-2-yl)-2-(4-(2,2,2-trifluoroacetamido)benzamido)phenyl)carbamate (182): The intermediate was synthesized through the same procedure of ***160*** to ***169a*** using ***180*** in 65% yield. m/z (APCI$^+$) 506.2 $(M+H)^+$.

Tert-butyl (2-(4-aminobenzamido)-4-(thiophen-2-yl)phenyl)carbamate (183): This compound was synthesized using the same procedure of ***177a*** to ***178a*** starting from ***182*** in 34% yield. ***183*** was as gray oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 7.92 (s, 1H), 7.80 (d, J = 8.4 Hz, 2H), 7.68 – 7.63 (m, 1H), 7.59 – 7.41 (m, 2H), 7.38 – 7.28 (m, 2H), 7.25 – 7.18 (m, 2H), 7.02 (dd, J = 5.0, 3.6 Hz, 1H), 6.73 (d, J = 7.9 Hz, 2H), 1.50 (s, 9H), m/z (APCI$^+$) 410.7 $(M+H)^+$, 354.7$(M-But+2H)^+$.

Procedure for the synthesis of *4-((2-amino-4-fluorophenyl)carbamoyl)benzoic acid (187)*: Intermediate methyl 4-((2-amino-4-fluorophenyl)carbamoyl)benzoate ***(186)*** was synthesized through the same procedure of ***168a*** to ***169a*** using 4-(methoxycarbonyl)benzoic acid (***185***) and ***168a*** in 69% yield. ***186*** was pink solid.

A stirred mixture of ***186*** (1.14g, 1.0 eq) and $LiOH{\cdot}H_2O$ (190 mg, 2.0 eq) in THF (10 mL) and H_2O (10 mL) was heated to reflux for 1 h. Part of solvent was removed by evaporation. Then, a HCl (10%) solution was added dropwise to the resulting residue

until pH ≈ 4 and precipitate appeared. The residue was filtered and dried to give ***187*** as gray solid (1.0g, yield: 92%). m/z (APCI$^+$) 275.1 $(M+H)^+$.

Procedure for the synthesis of *tert-butyl 2-(4-(aminomethyl)benzoyl)-1-propylhydrazinecarboxylate (87a)* and *tert-butyl 2-(4-(aminomethyl)benzoyl)hydrazinecarboxylate (87c)*:

Tert-butyl 2-(4-((((benzyloxy)carbonyl)amino)methyl)benzoyl)-1-propylhydrazinecarboxylate (86a)* and *tert-butyl 2-(4-((((benzyloxy)carbonyl)amino)methyl)benzoyl)hydrazinecarboxylate (86c): A mixture of benzyl 4-(2-propylhydrazinecarbonyl)benzylcarbamate (***85a***) (1.0 mmol), $(Boc)_2O$ (1.2 mmol) and DIPEA (2.0 mmol) in THF (3 mL) was stirred at room temperature overnight. Then, the reaction was evaporated directly and the resulting residue was purified by column chromatography (EtOAc : heptane = 1 : 4) to give ***86a*** as white solid in 96% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.74 (d, *J* = 7.7 Hz, 2H), 7.55 – 7.27 (m, 7H), 5.15 (s, 2H), 4.43 (d, *J* = 6.0 Hz, 2H), 3.58 – 3.52 (m, 2H), 1.63 (dt, *J* = 14.6, 7.3 Hz, 2H), 1.46 (s, 9H), 0.94 (t, *J* = 7.4 Hz, 3H). ***86c*** was synthesized using benzyl 4-(hydrazinecarbonyl)benzylcarbamate (***84***) through the same procedure while it was purified by (EtOAc : heptane = 0 : 1, 1:1 and then DCM : MeOH = 40 : 1) to give ***86c*** as white solid in 82% yield.

87a and ***87c*** were synthesized through the procedure of ***77a*** to ***78a*** using ***86a*** and ***86c***. ***87a*** was light green oil in 95% yield; ***87c*** was gray oil in 59% yield.

Procedure for the synthesis of *tert-butyl 2-(4'-(aminomethyl)-[1,1'-biphenyl]-4-carbonyl)-1-propylhydrazinecarboxylate (78a)* and *tert-butyl 2-(4'-(aminomethyl)-[1,1'-biphenyl]-4-carbonyl)-1-hexylhydrazinecarboxylate (191)*:

Benzyl 4-bromobenzylcarbamate (188): Chloroformate (3.23 g, 18.9 mmol, 1.05 eq) was added to a stirred mixture of (4-bromophenyl)methanamine hydrochloride1(***48***)

(4.0 g, 18.0 mmol, 1.0 eq) and NaOH (1.59 g, 39.8 mmol, 2.2 eq) in THF (20 mL) and H_2O (20 mL) under ice bath. The reaction was stirred at room temperature overnight. Cold water was then added to the mixture and precipitate appeared. The precipitate was filtered and dried to give ***188*** as white solid (5.8 g) in a quantitative yield.

Tert-butyl 2-(4'-((((benzyloxy)carbonyl)amino)methyl)-[1,1'-biphenyl]-4-carbonyl)-1-propylhydrazinecarboxylate (77a) and ***tert-butyl 2-(4'-((((benzyloxy)carbonyl)amino)methyl)-[1,1'-biphenyl]-4-carbonyl)-1-hexylhydrazinecarboxylate (190)*** :

The procedure for ***77a*** and ***190*** were same as the procedure of ***80d-3*** to ***81d*** using ***188*** and (***76a*** or ***76d***). ***190*** was white solid in 93% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (s, 1H), 7.98 – 7.83 (m, 3H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.41 – 7.20 (m, 7H), 5.04 (s, 2H), 4.25 (d, *J* = 6.2 Hz, 2H), 3.42 (s, 2H), 1.57 – 1.21 (m, 17H), 0.88 – 0.82 (m, 3H).

Tert-butyl 2-(4'-(aminomethyl)-[1,1'-biphenyl]-4-carbonyl)-1-hexylhydrazinecarboxylate (***191***) was synthesized through the same procedure used for ***78a*** using ***190*** giving ***191*** as a sticky gray solid in 62% yield, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H), 7.97 – 7.86 (m, 2H), 7.78 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 7.9 Hz, 2H), 7.44 (d, J = 8.1 Hz, 2H), 4.07 (s, 1H), 3.76 (s, 2H), 3.42 (s, 2H), 1.98 (s, 2H), 1.56 – 1.21 (m, 17H), 0.85 (d, J = 6.3 Hz, 3H).

Procedure for the synthesis of *tert-butyl 2-(2-((4-(aminomethyl)benzyl)amino)pyrimidine-5-carbonyl)-1-propylhydrazinecarboxylate (198a)* and *tert-butyl 2-(2-((4-(aminomethyl)benzyl)amino)pyrimidine-5-carbonyl)-1-hexylhydrazinecarboxylate (198b)*:

Benzyl 4-(aminomethyl)benzylcarbamate (193a): A solution of benzyl chloroformate

(2.1 mL, 14.68 mmol, 1.0 eq) in DCM (100 mL) was added dropwise over 40 min to a stirred mixture of 1,4-phenylenedimethanamine (***192***) (2.0 g, 14.68 mmol, 1.0 eq) and TEA (1.49 g, 14.68 mmol, 1.0 eq) in DCM (50 mL) in ice bath. The resulting mixture was stirred for 3 h. The reaction was then evaporated and the residue was purified by column chromatography (DCM : MeOH = 1 : 0 to 20 : 1 gradually) to give ***193a*** as white solid (1.32 g, yield: 33%). m/z (APCI$^+$) 271.0 (M+H)$^+$.

Benzyl 4-(((5-(2-propylhydrazinecarbonyl)pyrimidin-2-yl)amino)methyl)benzylcarbamate (196a): This compound was synthesized through the same procedure of ***92*** to ***95a*** using ***193a***. Ethyl 2-((4-((((benzyloxy)carbonyl)amino)methyl)benzyl)amino)pyrimidine-5-carboxylate ***(194a)*** was white solid in 63% yield; Benzyl 4-(((5-(hydrazinecarbonyl)pyrimidin-2-yl)amino)methyl)benzylcarbamate (***195a***) was white solid in 81% yield. m/z (APCI$^+$) 421.2 (M+H)$^+$, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.64 (s, 2H), 8.22 (t, *J* = 6.2 Hz, 1H), 7.75 (t, *J* = 6.1 Hz, 1H), 7.36 – 7.13 (m, 9H), 5.01 (s, 2H), 4.49 (d, *J* = 6.2 Hz, 2H), 4.37 (s, 2H), 4.14 (d, *J* = 6.1 Hz, 2H); ***196a*** was white solid in 58% yield. m/z (APCI$^+$) 449.1 (M+H)$^+$.

Tert-butyl 2-(2-((4-((((benzyloxy)carbonyl)amino)methyl)benzyl)amino)pyrimidine-5-carbonyl)-1-propylhydrazinecarboxylate (197a): This compound was synthesized according to the procedure used for ***102a*** using ***196a***. ***197a*** was white solid in 67% yield. m/z (APCI$^+$) 549.2 (M+H)$^+$.

Tert-butyl 2-(2-((4-(aminomethyl)benzyl)amino)pyrimidine-5-carbonyl)-1-propylhydrazinecarboxylate (***198a***) was synthesized through the same procedure of ***86a*** to ***87a*** using ***197a*** to give ***198a*** as black solid.

N-(4-(aminomethyl)benzyl)-1,1,1-triphenylmethanamine (193b): A solution of triphenylmethyl chloride (4.0 g, 14.67 mmol, 1.0 eq) in dioxane (40 mL) was added dropwise to a stirred mixture of 1,4-phenylenedimethanamine (***192***) (3.0 g, 22.0 mol, 1.5 eq) in dioxane (30 mL). The reaction was stirred at room temperature overnight. The reaction was then concentrated and the resulting residue was extracted with DCM and saturated Na_2CO_3 solution. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (DCM : MeOH = 1 : 0, 30 : 1, 15 : 1 gradually) to give ***193b*** as white solid (3.3 g, yield: 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.53 (m, 6H), 7.36 (d, J = 8.0 Hz, 2H), 7.33 – 7.26 (m, 8H), 7.20 (t, J = 7.3 Hz, 3H), 3.86 (s, 2H), 3.32 (s, 2H).

N'-hexyl-2-((4-((tritylamino)methyl)benzyl)amino)pyrimidine-5-carbohydrazide (196b): This compound was synthesized through the same procedure of ***92*** to ***95a*** using *N*-(4-(aminomethyl)benzyl)-1,1,1-triphenylmethanamine (***193b***). Ethyl 2-((4-((tritylamino)methyl)benzyl)amino)pyrimidine-5-carboxylate (***194b***) was white solid in 74% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (d, *J* = 37.8 Hz, 2H), 7.59 – 7.50 (m, 6H), 7.38 (d, *J* = 8.1 Hz, 2H), 7.29 (t, *J* = 7.5 Hz, 8H), 7.20 (t, *J* = 7.3 Hz, 3H), 5.91 (t, *J* = 5.9 Hz, 1H), 4.69 (d, *J* = 5.9 Hz, 2H), 4.34 (q, *J* = 7.1 Hz, 2H), 3.33 (s, 2H), 1.86 (s, 1H), 1.36 (t, *J* = 7.1 Hz, 3H); 2-((4-((Tritylamino)methyl)benzyl)amino)pyrimidine-5-carbohydrazide (***195b***) was white solid in a quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.65 (s, 2H), 8.24 (t, J = 6.4 Hz, 1H), 7.49 – 7.41 (m, 6H), 7.34 – 7.15 (m, 13H), 4.51 (d, J = 6.3 Hz, 2H), 4.38 (s, 2H), 3.15 – 3.09 (m, 2H), 3.02 (t, J = 8.2 Hz, 1H); 2-((4-((Tritylamino)methyl)benzyl)amino)pyrimidine-5-carbohydrazide (***196b***) was a white solid in 76% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.78 (d, *J* = 5.5 Hz, 1H), 8.64 (s, 2H), 8.26 (t, *J* = 6.5 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 6H), 7.36 – 7.20 (m, 10H), 7.17 (t, *J* = 7.3 Hz, 3H), 4.97 (d, *J* = 6.1 Hz, 1H), 4.51 (d, *J* = 6.4 Hz, 2H), 3.11 (d, *J* = 8.1 Hz, 2H), 3.05 – 3.01 (m, 1H), 2.72 (dd, *J* = 12.2, 6.8 Hz, 2H), 1.41 (dt, *J* = 13.9, 6.8 Hz, 2H), 1.32 – 1.22 (m, 6H), 0.84 (t, *J* = 6.6 Hz, 3H).

Tert-butyl 1-hexyl-2-(2-((4-((tritylamino)methyl)benzyl)amino)pyrimidine-5-carbonyl)hydrazinecarboxylate (197b): This compound was synthesized according to the procedure used for ***102a*** using ***196b***. ***197b*** was white solid in a quantitative yield.

Tert-butyl 2-(2-((4-(aminomethyl)benzyl)amino)pyrimidine-5-carbonyl)-1-hexylhydrazinecarboxylate (198b): A stirred mixture of ***197b*** **(**1.9 g) in AcOH (8 mL) and water (2 mL) was heated to 60 ^{o}C for 1.5 h. Then, most of solvent was removed by evaporation. Saturated Na_2CO_3 solution was added dropwise to the residue until no bubbles appeared. The mixture was extracted with EtOAc and brine. The collected organic layer was washed with brine twice, dried over anhydrous Na_2SO_4 and concentrated to give ***198b*** as yellow solid without further purification.

Procedure for the synthesis of *tert-butyl 2-(2-((4-(aminomethyl)phenyl)amino)pyrimidine-5-carbonyl)-1-propylhydrazinecarboxylate (206a)* and *tert-butyl 2-(2-((4-(aminomethyl)phenyl)amino)pyrimidine-5-carbonyl)-1-hexylhydrazinecarboxylate (206b)*:

N-(4-nitrobenzyl)-1,1,1-triphenylmethanamine (200): A mixture of (4-nitrophenyl)methanamine hydrochloride (2.0 g, 10.6 mmol, 1.0 eq), triphenylmethyl chloride (2.96 g, 10.6 mmol, 1.0 eq) and TEA (2.7 g, 26.17 mmol, 2.5 eq) in DCM (30 mL) was stirred at room temperature for 7 h. Then. Solvent was removed by evaporation. Water was added to the resulting residue and the mixture was stirred for 5 minutes. The precipitate was filtered, washed with water and heptane and dried to give compound ***200*** as white solid (4.2 g) in a quantitative yield.

4-((Tritylamino)methyl)aniline (201): Pd/C (10%) (380 mg) was added to a mixture of intermediate ***200*** (3.8 g, 9.6 mmol, 1.0 eq) and ammonium formate (2.42 g, 38.4 mmo, 4.0 eq) in EtOAc (40 mL) and MeOH (10 mL). The mixture was stirred and heated to 40-50 ^{o}C for 1 h. The reaction was then filtered. The filtrate was washed with brine three times, dried over anhydrous Na_2SO_4 and concentrated to give ***201*** as

a yellow oil (3.8 g) in quantitative yield.

Ethyl 2-((4-((tritylamino)methyl)phenyl)amino)pyrimidine-5-carboxylate (202): A stirred mixture of compound ***92*** (1.53 g, 8.2 mmol, 1.0 eq), ***201*** (3.0 g, 8.2 mmol, 1.0 eq) and K_2CO_3 (2.84 g, 20.55 mmol, 2.5 eq) in DMF (15 mL) was heated to 80-90 ^oC overnight. The reaction was extracted with EtOAc and water. The collected organic layer was washed with brine two times, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (EtOAc : heptane = 1 : 15 to 1 : 5 gradually and then DCM) to give ***202*** as a light yellow solid (2.74 g, yield: 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 8.87 (s, 2H), 7.67 (d, J = 8.5 Hz, 2H), 7.47 (d, J = 7.5 Hz, 6H), 7.38 – 7.25 (m, 8H), 7.18 (t, J = 7.3 Hz, 3H), 4.28 (q, J = 7.1 Hz, 2H), 3.13 (d, J = 8.1 Hz, 2H), 3.07 – 3.01 (m, 1H), 1.30 (t, J = 7.1 Hz, 3H).

2-((4-((Tritylamino)methyl)phenyl)amino)pyrimidine-5-carbohydrazide (203): A stirred mixture of hydrazine monohydrate (8.0 g, 16.0 mmol, 30 eq) and compound ***202*** (2.74 g, 5.32 mmol, 1.0 eq) in EtOH (20 mL) was refluxed for 3 h until ***202*** was dissolved clearly. The reaction was cooled to room temperature. Water (30 mL) was added to the reaction and stirring was continued for 1 h at room temperature. The precipitate was filtered and dried to give ***203*** as a white solid (2.3 g, yield: 86%). m/z (APCI$^+$) 501.3 $(M+H)^+$, 539.6 $(M+K)^+$.

N'-hexyl-2-((4-((tritylamino)methyl)phenyl)amino)pyrimidine-5-carbohydrazide (204b): This compound was synthesized through the same procedure of ***94a*** to ***95a*** using ***203*** and hexanal. ***204b*** was white solid in 80% yield. m/z (APCI$^+$) 585.3 $(M+H)^+$.

Tert-butyl1-hexyl-2-(2-((4-((tritylamino)methyl)phenyl)amino)pyrimidine-5-carbonyl)hydrazinecarboxylate (205b): A mixture of compound ***204b*** (2.15 g, 3.68 mmol, 1.0 eq), $(Boc)_2O$ (0.88 g, 0.40 mmol, 1.1 eq) and TEA (0.74 g, 7.35 mmol, 2.0 eq) in THF

(25 mL) was stirred at room temperature overnight. The reaction was then evaporated. The residue was extracted with EtOAc and aq HCl (10%). The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated to give ***205b*** as colorless oil (2.74 g) in a quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 1H), 10.10 (s, 1H), 8.84 (s, 2H), 7.66 (d, *J* = 8.5 Hz, 2H), 7.47 (d, *J* = 7.5 Hz, 6H), 7.39 – 7.26 (m, 7H), 7.19 (t, *J* = 7.3 Hz, 4H), 3.40 (s, 2H), 3.13 (d, *J* = 8.1 Hz, 2H), 3.05 – 2.99 (m, 1H), 1.48 – 1.21 (m, 17H), 0.85 (t, *J* = 6.5 Hz, 3H).

Tert-butyl 2-(2-((4-(aminomethyl)phenyl)amino)pyrimidine-5-carbonyl)-1-hexylhydrazinecarboxylate (206b): This compound was synthesized according to the procedure used for ***198b*** using ***205b***. ***205b*** was light yellow solid. m/z (APCI$^+$) 443.7 $(M+H)^+$.

Tert-butyl 2-(2-((4-(aminomethyl)phenyl)amino)pyrimidine-5-carbonyl)-1-propylhydrazinecarboxylate (206a): This compound was synthesized according to the procedure used for ***206b*** using ***203***. *N*'-propyl-2-((4-((tritylamino)methyl)phenyl)amino)pyrimidine-5-carbohydrazide (***204a***) was white solid in a 83% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H), 9.93 (d, J = 4.4 Hz, 1H), 8.81 (s, 2H), 7.66 (d, J = 8.6 Hz, 2H), 7.50 – 7.44 (m, 6H), 7.31 (dd, J = 15.0, 7.6 Hz, 8H), 7.19 (t, J = 7.3 Hz, 3H), 5.07 (d, J = 5.5 Hz, 1H), 3.12 (d, J = 8.2 Hz, 2H), 3.03 – 2.98 (m, 1H), 2.73 (dd, J = 11.0, 7.4 Hz, 2H), 1.45 (dd, J = 14.5, 7.2 Hz, 2H), 0.90 (t, J = 7.4 Hz, 3H); Tert-butyl 1-propyl-2-(2-((4-((tritylamino)methyl)phenyl)amino)pyrimidine-5-carbonyl)hydrazinecarboxylate (***205a***) was light yellow solid in a quantitative yield; ***206b*** was yellow solid.

General procedure for the synthesis of *212a (PSP4)* to *212f (PSP2)*:

6-Bromohexanoyl chloride (208): A stirred mixture of $SOCl_2$ (50.0 mmol) and 6-bromohexanoic acid (10.0 mmol) was heated to reflux for 2 h. The reaction was

then concentrated directly to give ***208*** as a colorless solid that was used without further purification.

6-Bromo-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)hexanamide (209): 4-Amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (***139)*** (3.3 mmol) was added to a solution of ***208*** in THF (9 mL). The resulting mixture was heated to reflux overnight. The reaction was then evaporated and ether was added to the residue. The precipitate was filtered, washed with ether and dried to give ***209*** as gray white solid in a 94% yield. m/z (APCI$^+$) 450.5 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12 (s, 1H), 9.70 (s, 1H), 8.45 (d, *J* = 8.3 Hz, 1H), 7.82 (dd, *J* = 8.3, 7.5 Hz, 1H), 7.60 (d, *J* = 7.3 Hz, 1H), 5.13 (dd, *J* = 12.7, 5.4 Hz, 1H), 3.70 – 3.50 (m, 4H), 2.94 – 2.83 (m, 1H), 2.63 – 2.51 (m, 2H), 2.10 – 2.00 (m, 1H), 1.89 – 1.58 (m, 4H), 1.51 – 1.38 (m, 2H).

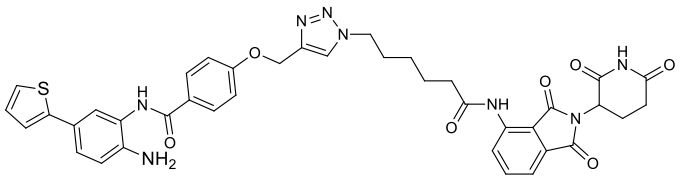
6-Azido-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)hexanamide (210): To a stirred mixture of ***209*** (1.0 g, 2.22 mmol, 1.0 eq) in DMF (10 mL) was added NaN_3 (0.29 g, 4.44 mmol, 2.0 eq). The reaction was heated to about 80 ^{o}C for 16 h. The reaction was extracted with EtOAc and water. The collected organic layer was washed with brine twice, dried over anhydrous Na_2SO_4 and concentrated to give ***210*** as a yellow solid (0.7 g, yield: 76%) that was directly used without further purification.

General procedure for the synthesis of *211a-211d, 212e* and *212f*: A stirred mixture of the corresponding intermediate (***166a***, ***166b***, ***169a***, ***169b***, ***174a***, ***174b***) (0.75 mmol), compound ***210*** (1.0 mmol), $CuSO_4{\cdot}H_2O$ (0.015mmol) and L-Sodium ascorbate (0.075 mmol) in a solution of t-BuOH (4 mL), H_2O (2 mL) and DMF (1 mL) was heated to about 60 ^{o}C for 8h. The reaction was then extracted with EtOAc and water three times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 0 : 1 to 2 : 1 gradually and then DCM : MeOH = 30 : 1, 20 : 1, 15 : 1 gradually) to give ***211a-211d, 212e*** and ***212f*** respectively. ***212e*** and ***212f*** were purified again by

preparative HPLC.

A mixture of compound (***211a-211d***) in a solution of HCl/dioxane (4M) and DCM (1 : 2) or TFA/DCM (1 : 5) was stirred for 3 h at room temperature. Saturated Na_2CO_3 solution was then added dropwise to the mixture until no bubbles appeared. The mixture was extracted with EtOAc and water three times. The combined organic layers were concentrated and the resulting residue was purified by column chromatography (DCM : MeOH = 1 : 0 to 20 : 1 gradually with several drops of TEA) to give final compounds ***212a-212d*** respectively.

_N_-(2-amino-5-(thiophen-2-yl)phenyl)-4-((1-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-6-oxohexyl)-1_*H*_-1,2,3-triazol-4-yl)methoxy)benzamide 212a (PSP4):



White solid (60 mg, yield: 41%).

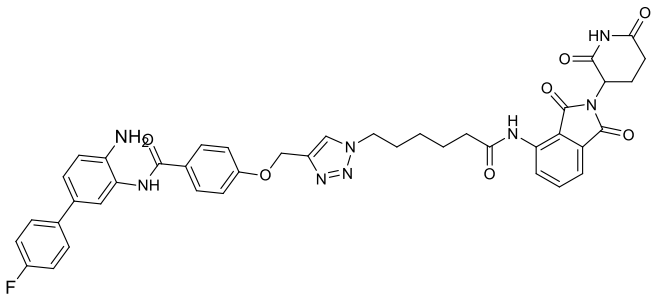
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12 (s, 1H), 9.68 (s, 1H), 9.59 (s, 1H), 8.44 (d, *J* = 8.0 Hz, 1H), 8.25 (s, 1H), 7.97 (d, *J* = 8.9 Hz, 2H), 7.80 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.62 – 7.57 (m, 1H), 7.45 (d, *J* = 2.1 Hz, 1H), 7.33 (dd, *J* = 5.1, 1.1 Hz, 1H), 7.27 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.22 (dd, *J* = 3.6, 1.1 Hz, 1H), 7.13 (d, *J* = 8.9 Hz, 2H), 7.03 (dd, *J* = 5.1, 3.6 Hz, 1H), 6.79 (d, *J* = 8.4 Hz, 1H), 5.28 – 5.06 (m, 5H), 4.37 (t, *J* = 7.1 Hz, 2H), 2.88 (ddd, *J* = 16.5, 13.6, 5.1 Hz, 1H), 2.63 – 2.50 (m, 2H), 2.45 (d, *J* = 7.4 Hz, 2H), 2.09 – 2.01 (m, 1H), 1.86 (dt, *J* = 14.8, 7.3 Hz, 2H), 1.65 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.30 (dt, *J* = 15.3, 7.7 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.17, 172.30, 170.21, 168.10, 167.09, 165.29, 161.02, 144.71, 143.46, 142.69, 136.95, 136.51, 131.90, 130.15, 128.63, 127.34, 126.78, 124.97, 124.36, 124.23, 124.07, 123.63, 122.72, 121.43, 118.76, 117.47, 116.82, 114.70, 61.77, 49.71, 49.37, 36.64, 31.38, 29.87, 25.80, 24.56, 22.43.

HRMS calculated for $C_{39}H_{37}N_8O_7S^+$ (M+H): 761.2500, found: 761.2498.

HPLC: rt = 10.48 min (98.03 %).

***N*-(4-amino-4'-fluoro-[1,1'-biphenyl]-3-yl)-4-((1-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-6-oxohexyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzamide 212b (PSP3)**:



White solid (45 mg, yield: 31%).

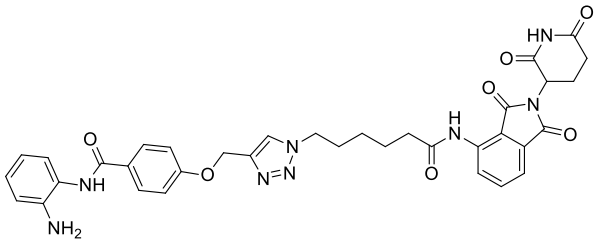
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12 (s, 1H), 9.68 (s, 1H), 9.59 (s, 1H), 8.45 (d, *J* = 8.1 Hz, 1H), 8.26 (s, 1H), 7.98 (d, *J* = 8.8 Hz, 2H), 7.81 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.61 – 7.53 (m, 3H), 7.47 (d, *J* = 2.1 Hz, 1H), 7.28 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.23 – 7.12 (m, 4H), 6.84 (d, *J* = 8.3 Hz, 1H), 5.21 (s, 2H), 5.13 (dd, *J* = 12.8, 5.4 Hz, 1H), 5.05 (s, 2H), 4.37 (t, *J* = 7.1 Hz, 2H), 2.93 – 2.83 (m, 1H), 2.63 – 2.50 (m, 2H), 2.45 (d, *J* = 7.4 Hz, 2H), 2.09 – 2.01 (m, 1H), 1.86 (dt, *J* = 14.8, 7.2 Hz, 2H), 1.65 (dt, *J* = 15.0, 7.4 Hz, 2H), 1.35 – 1.27 (m, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.18, 172.30, 170.21, 168.10, 167.10, 165.28, 161.00, 143.17, 142.70, 137.21, 137.18, 136.95, 136.52, 131.91, 130.12, 127.77, 127.70, 127.62, 127.44, 126.79, 125.19, 124.98, 124.26, 118.76, 117.49, 116.96, 116.03, 115.82, 114.70, 61.78, 49.71, 49.37, 36.64, 31.39, 29.88, 25.80, 24.57, 22.44.

HRMS calculated for $C_{41}H_{37}FN_8NaO_7^+$ (M+H): 795.2661, found: 795.267.

HPLC: rt = 10.76 min (97.84 %).

***N*-(2-aminophenyl)-4-((1-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-6-oxohexyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzamide 212c (PSP9)**:



Light yellow solid (60 mg, yield: 44%).

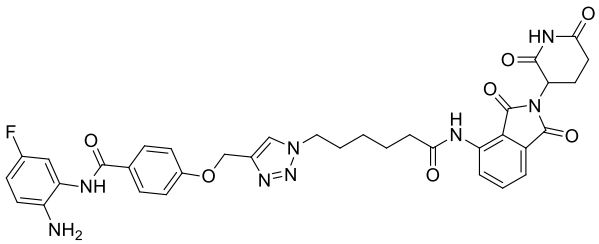
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 9.68 (s, 1H), 9.53 (s, 1H), 8.45 (d, *J* = 8.4 Hz, 1H), 8.25 (s, 1H), 7.95 (d, *J* = 8.8 Hz, 2H), 7.84 – 7.77 (m, 1H), 7.59 (d, *J* = 7.1 Hz, 1H), 7.13 (t, *J* = 7.1 Hz, 3H), 6.98 – 6.91 (m, 1H), 6.77 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.62 – 6.55 (m, 1H), 5.20 (s, 2H), 5.13 (dd, *J* = 12.7, 5.4 Hz, 1H), 4.85 (s, 2H), 4.37 (t, *J* = 7.0 Hz, 2H), 2.88 (ddd, *J* = 16.4, 13.6, 5.0 Hz, 1H), 2.64 – 2.51 (m, 2H), 2.45 (d, *J* = 7.4 Hz, 2H), 2.09 – 2.02 (m, 1H), 1.86 (dt, *J* = 14.7, 7.2 Hz, 2H), 1.65 (dt, *J* = 14.9, 7.4 Hz, 2H), 1.31 (dt, *J* = 15.1, 7.7 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.19, 172.31, 170.22, 168.11, 167.10, 165.14, 160.94, 143.57, 142.71, 136.95, 136.52, 131.89, 130.06, 127.47, 127.12, 126.77, 124.96, 124.00, 118.76, 117.46, 116.74, 116.59, 114.69, 61.76, 49.72, 49.37, 36.64, 31.38, 29.87, 25.80, 24.56, 22.44.

HRMS calculated for $C_{35}H_{35}N_8O_7^+$ (M+H): 679.2623, found: 679.2620.

HPLC: rt = 8.07 min (92.13 %).

_N_-(2-amino-5-fluorophenyl)-4-((1-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-6-oxohexyl)-1_H_-1,2,3-triazol-4-yl)methoxy)benzamide 212d (PSP1):



White solid (80 mg, yield: 29%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 9.68 (s, 1H), 9.52 (s, 1H), 8.45 (d, *J* = 8.3 Hz, 1H), 8.25 (s, 1H), 7.99 – 7.91 (m, 2H), 7.81 (dd, *J* = 8.3, 7.5 Hz, 1H), 7.60 (dd, *J* = 7.3, 0.5 Hz, 1H), 7.19 – 7.08 (m, 3H), 6.83 – 6.73 (m, 2H), 5.21 (s, 2H), 5.13

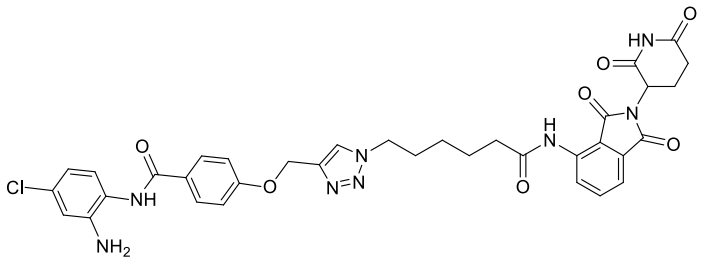
(dd, *J* = 12.8, 5.4 Hz, 1H), 4.80 (s, 2H), 4.37 (t, *J* = 7.1 Hz, 2H), 2.88 (ddd, *J* = 16.7, 13.7, 5.2 Hz, 1H), 2.64 – 2.51 (m, 2H), 2.45 (d, *J* = 7.4 Hz, 2H), 2.09 – 2.01 (m, 1H), 1.86 (dt, *J* = 14.8, 7.3 Hz, 2H), 1.70 – 1.60 (m, 2H), 1.35 – 1.25 (m, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.18, 172.31, 170.21, 168.11, 167.10, 165.18, 161.10, 155.43, 153.14, 142.67, 139.31 139.30, 136.95, 136.52, 131.90, 130.13, 127.23, 126.77, 124.98, 124.91, 124.81, 118.76, 117.46, 117.08, 117.00, 114.76, 112.86, 112.81, 112.64, 112.57, 61.78, 49.72, 49.37, 36.64, 31.38, 29.87, 25.80, 24.56, 22.44.

HRMS calculated for $C_{35}H_{34}FN_8O_7^+$ (M+H): 697.2529, found: 697.2528.

HPLC: rt = 9.07 min (99.18 %).

***N*-(2-amino-4-chlorophenyl)-4-((1-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-6-oxohexyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzamide 212e (PSP8)**:



Light yellow solid (90 mg, yield: 67%).

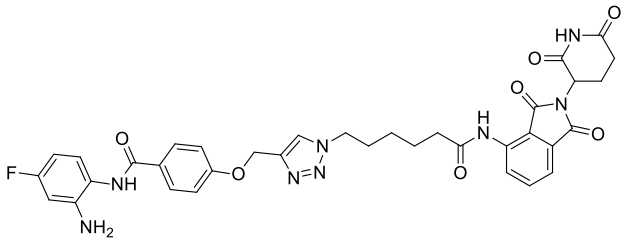
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 9.68 (s, 1H), 9.49 (s, 1H), 8.45 (d, *J* = 8.3 Hz, 1H), 8.25 (s, 1H), 7.94 (d, *J* = 8.8 Hz, 2H), 7.84 – 7.77 (m, 1H), 7.59 (d, *J* = 6.9 Hz, 1H), 7.21 – 7.04 (m, 3H), 6.79 (d, *J* = 2.4 Hz, 1H), 6.57 (dd, *J* = 8.4, 2.4 Hz, 1H), 5.27 – 4.99 (m, 5H), 4.37 (t, *J* = 7.1 Hz, 2H), 2.88 (ddd, *J* = 16.6, 13.6, 5.2 Hz, 1H), 2.64 – 2.51 (m, 2H), 2.45 (d, *J* = 7.4 Hz, 2H), 2.09 – 2.02 (m, 1H), 1.86 (dt, *J* = 14.7, 7.2 Hz, 2H), 1.65 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.35 – 1.26 (m, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.18, 172.30, 170.21, 168.10, 167.10, 165.34, 161.01, 145.26, 142.69, 136.94, 136.52, 131.90, 130.72, 130.13, 128.68, 127.29, 126.77, 124.97, 122.69, 118.76, 117.46, 115.86, 115.25, 114.68, 61.76, 49.71, 49.37, 36.64, 31.38, 29.87, 25.80, 24.56, 22.44.

HRMS calculated for $C_{35}H_{36}ClN_8O7^+$ (M+H): 713.2234, found: 713.2233.

HPLC: rt = 10.76 min (97.69 %).

***N*-(2-amino-4-fluorophenyl)-4-((1-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-6-oxohexyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzamide 212f (PSP2)**:



White solid (100 mg, yield: 85%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 9.68 (s, 1H), 9.46 (s, 1H), 8.45 (d, *J* = 8.3 Hz, 1H), 8.29 (s, 1H), 8.24 (d, *J* = 3.3 Hz, 1H), 7.95 (d, *J* = 8.8 Hz, 2H), 7.81 (dd, *J* = 8.3, 7.5 Hz, 1H), 7.59 (dd, *J* = 7.2, 0.4 Hz, 1H), 7.16 – 7.04 (m, 3H), 6.53 (dd, *J* = 11.2, 2.9 Hz, 1H), 6.34 (td, *J* = 8.5, 2.9 Hz, 1H), 5.31 – 5.01 (m, 5H), 4.37 (t, *J* = 7.1 Hz, 2H), 2.88 (ddd, *J* = 16.6, 13.7, 5.2 Hz, 1H), 2.65 – 2.50 (m, 2H), 2.45 (d, *J* = 7.4 Hz, 2H), 2.10 – 2.00 (m, 1H), 1.92 – 1.80 (m, 2H), 1.65 (dt, *J* = 15.0, 7.5 Hz, 2H), 1.31 (dt, *J* = 15.2, 7.8 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.18, 172.31, 170.21, 168.11, 167.10, 165.42, 162.59, 160.95, 160.21, 145.96, 145.84, 142.70, 136.95, 136.52, 131.90, 130.08, 129.02, 128.91, 127.36, 126.77, 124.96, 119.95, 118.76, 117.46, 114.65, 102.60, 102.37, 102.05, 101.80, 61.75, 49.72, 49.37, 36.64, 31.38, 29.87, 25.80, 24.56, 22.44.

HRMS calculated for $C_{35}H_{33}FN_8NaO_7^+$ (M+H): 719.2348, found: 719.2352.

HPLC: rt = 9.76 min (93.42 %).

General procedure for the synthesis of *218a* to *218l*:

Tert-butyl 5-aminopentanoate (214a), tert-butyl 6-aminohexanoate (214b), tert-butyl 7-aminoheptanoate (214c)* and *tert-butyl 8-aminooctanoate (214d)*:** A mixture of $SOCl_2$ (10.0 mmol) and substituted amino acid (213a-213d***) (1.0 mmol) was stirred at room temperature for 2 h. Then, the reaction was evaporated. t-BuOH

(2 mL) was added to the resulting residue followed by $NaHCO_3$ solid (2.2 mmol). The reaction was stirred overnight. The mixture was extracted with DCM and brine four times. The combined organic layers were dried over Na_2SO_4 and concentrated to give ***214a-214d*** respectively as yellow oil with a good smell. ***214a*** in a 66% yield; ***214b*** in a 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.70 (t, J = 7.0 Hz, 2H), 2.21 (t, J = 7.5 Hz, 2H), 1.66 (s, 2H), 1.59 (dt, J = 15.1, 7.4 Hz, 2H), 1.52 – 1.40 (m, 11H), 1.39 – 1.30 (m, 2H); ***214c*** in a 87% yield; ***214d*** in a 95% yield.

Procedure for the synthesis of 5-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)pentanoic acid (216a), 6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexanoic acid (216b), 7-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)heptanoic acid (216c) **and** ***8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octanoic acid (216d)***:

Tert-butyl 5-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)pentanoate (215a), Tert-butyl 6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexanoate (215b), Tert-butyl 7-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)heptanoate (215c) and Tert-butyl 8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octanoate (215d): A mixture of the corresponding amine (***214a-214d***) (1.0 mmol), 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (***262***) (0.33 mmol) and DIPEA (1.0 mmol) in NMP (N-methyl-2-pyrrolidone) (1.5 mL) was heated to 110 ^{o}C in a microwave reactor for 2 h. The reaction was extracted with EtOAc and brine. The collected organic layer was washed with brine three times and concentrated. The resulting residue was purified by column chromatography (EtOAc: hepane = 1 : 5, 1 : 3, 1 : 1) to give ***215a-215d*** respectively. ***215a*** was green oil (yield: 30%); ***215b*** was yellow solid (yield: 21%). m/z (APCI$^+$) 388.0 $(M\text{-}tBu+2H)^+$, 444.0 $(M+H)^+$. ^{1}H NMR

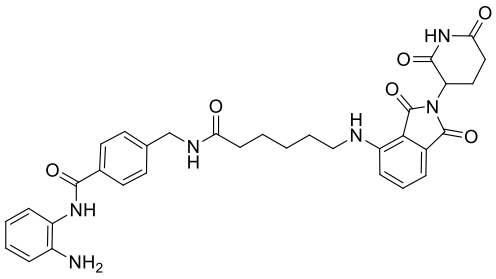
(400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 7.56 (dd, *J* = 8.4, 7.1 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.00 (d, *J* = 6.8 Hz, 1H), 6.51 (t, *J* = 5.9 Hz, 1H), 5.03 (dd, *J* = 12.8, 5.4 Hz, 1H), 3.26 (d, *J* = 6.8 Hz, 1H), 3.14 (dd, *J* = 11.8, 5.3 Hz, 1H), 2.86 (ddd, *J* = 17.1, 14.0, 5.5 Hz, 1H), 2.62 – 2.50 (m, 2H), 2.16 (ddd, *J* = 7.3, 6.1, 2.3 Hz, 2H), 2.05 – 1.96 (m, 1H), 1.60 – 1.44 (m, 4H), 1.41 – 1.25 (m, 11H); ***215c*** was yellow oil (39% yield); ***215d*** was yellow oil (yield: 35%).

A mixture of ***215a-215d*** in a solution of TFA/DCM (1 : 5) was stirred for 3 h at room temperature. The mixture was then directly concentrated to give ***216a-216d***. ***216a***: m/z (APCI$^+$) 374.0 $(M+H)^+$; ***216b***: m/z (APCI$^+$) 388.9 $(M+H)^+$; ***216c***: m/z (APCI$^+$) 402.0 $(M+H)^+$; ***216d***: m/z (APCI$^+$) 416.0 $(M-tBu+2H)^+$.

General procedure for the synthesis of 218a-218l: A mixture of corresponding carboxylic acid (***216a-216d***) (1.0 mmol), corresponding amine (***178a-178c***, ***191***, ***206a***, ***206b***) (0.83 mmol), HATU (1.25 mmol) and DIPEA (4.17 mmol) in DMF (4 mL) was stirred for 10 min at room temperature. The reaction was extracted with EtOAc and water. The collected organic layer was washed with brine three times, dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc: heptane = 1 : 2 and then DCM : MeOH = 30 : 1 gradually) to give (***217b-217e***, ***217j***, ***217k***, ***217l***) as yellow oil and (***218a***, ***218f***, ***218g***, ***218h***, ***218i***) as yellow solid.

A mixture of corresponding compound (***217b-217e***, ***217j***, ***217k***, ***217l***) in a solution of TFA/DCM (1 : 3) was stirred for 3 h at room temperature. Saturated Na_2CO_3 solution was then added dropwise to the mixture until no bubbles appeared. The mixture was extracted with EtOAc and water three times. The collected organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 1 : 2 and then DCM : MeOH = 1 : 0, 30 : 1, 25 : 1 gradually with several drops of TEA) to give products (***218b-218e***, ***218j***, ***218k***, ***218l***) as yellow solid.

***N*-(2-aminophenyl)-4-((6-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)hexanamido)methyl)benzamide 218a (PSP12)**:



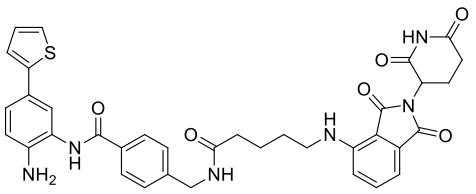
(20 mg, yield: 15%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.59 (s, 1H), 8.36 (t, *J* = 6.0 Hz, 1H), 8.00 – 7.84 (m, 2H), 7.56 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.34 (d, *J* = 8.2 Hz, 1H), 7.26 (dd, *J* = 5.8, 3.2 Hz, 1H), 7.15 (d, *J* = 6.9 Hz, 1H), 7.07 (dt, *J* = 5.8, 3.4 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 6.98 – 6.89 (m, 1H), 6.77 (dd, *J* = 8.0, 1.3 Hz, 1H), 6.66 – 6.55 (m, 1H), 6.51 (t, *J* = 6.0 Hz, 1H), 5.03 (dd, *J* = 12.9, 5.4 Hz, 1H), 4.32 (d, *J* = 5.9 Hz, 2H), 4.08 (s, 2H), 3.58 – 3.54 (m, 2H), 3.29 – 3.25 (m, 1H), 2.81 (s, 1H), 2.61 – 2.52 (m, 1H), 2.35 – 2.21 (m, 2H), 2.17 (t, *J* = 7.4 Hz, 1H), 1.63 – 1.48 (m, 4H), 1.38 – 1.30 (m, 2H).

HRMS calculated for $C_{33}H_{35}N_6O_6^+$ (M+H): 611.2613, found: 611.2609.

HPLC: rt = 8.23 min (93.29 %).

***N*-(2-amino-5-(thiophen-2-yl)phenyl)-4-((5-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)pentanamido)methyl)benzamide 218b (PSP15)**:



(30 mg, yield: 24%).

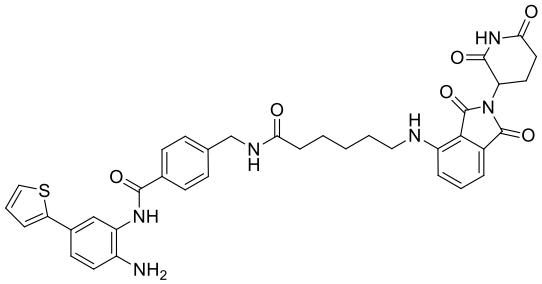
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.67 (s, 1H), 8.40 (t, J = 5.9 Hz, 1H), 7.93 (d, J = 8.2 Hz, 2H), 7.64 – 7.39 (m, 3H), 7.38 – 7.21 (m, 3H), 7.20 – 6.86 (m, 4H), 6.80 (d, J = 8.3 Hz, 1H), 6.53 (dt, J = 22.7, 5.9 Hz, 1H), 5.58 – 4.92 (m, 4H), 4.33 (d, J = 5.8 Hz, 2H), 2.90 – 2.80 (m, 1H), 2.63 – 2.49 (m, 2H), 2.37 – 2.11 (m,

4H), 2.05 – 1.96 (m, 1H), 1.66 – 1.51 (m, 4H).

HRMS calculated for $C_{36}H_{35}N_6O_6S^+$ (M+H): 679.2333, found: 679.2333.

HPLC: rt = 12.70 min (99.61 %). HPLC: rt = 9.80 min (90.97 %).

***N*-(2-amino-5-(thiophen-2-yl)phenyl)-4-((6-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)hexanamido)methyl)benzamide 218c (PSP10)**:



(50 mg, yield: 23%).

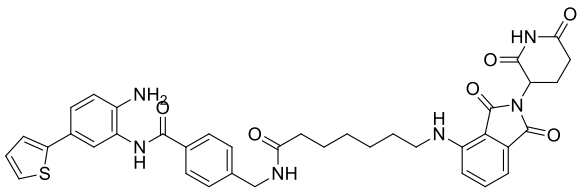
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.08 (s, 1H), 9.71 (s, 1H), 8.39 (t, J = 5.9 Hz, 1H), 7.95 (d, J = 8.1 Hz, 2H), 7.61 – 7.55 (m, 1H), 7.48 (d, J = 1.8 Hz, 1H), 7.40 – 7.33 (m, 3H), 7.30 (dd, J = 8.3, 2.2 Hz, 1H), 7.24 (d, J = 3.5 Hz, 1H), 7.09 (d, J = 8.6 Hz, 1H), 7.05 (dd, J = 5.0, 3.6 Hz, 1H), 7.02 (d, J = 7.0 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H), 6.53 (t, J = 5.9 Hz, 1H), 5.14 (s, 2H), 5.03 (dd, J = 12.8, 5.4 Hz, 1H), 4.34 (d, J = 6.0 Hz, 2H), 3.28 (d, J = 6.6 Hz, 1H), 2.86 (ddd, J = 17.4, 14.2, 5.4 Hz, 1H), 2.60 – 2.54 (m, 3H), 2.19 (t, J = 7.3 Hz, 2H), 2.05 – 1.98 (m, 1H), 1.63 – 1.56 (m, 4H), 1.36 (dt, J = 15.0, 7.7 Hz, 2H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 172.60, 170.71, 169.44, 167.77, 165.74 , 146.88, 144.70, 143.90, 143.44, 136.73, 133.40, 132.68, 128.66, 128.33, 127.34, 124.35, 123.91, 123.67, 122.72, 121.47, 117.63, 116.84, 110.84, 109.53, 49.06, 42.23, 40.92, 35.73, 31.51, 28.95, 26.46, 25.50, 22.67.

HRMS calculated for $C_{37}H_{37}N_6O_6S^+$ (M+H): 693.2490, found: 693.249.

HPLC: rt = 10.65 min (94.59 %).

***N*-(2-amino-5-(thiophen-2-yl)phenyl)-4-((7-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)heptanamido)methyl)benzamide 218d (PSP16)**:



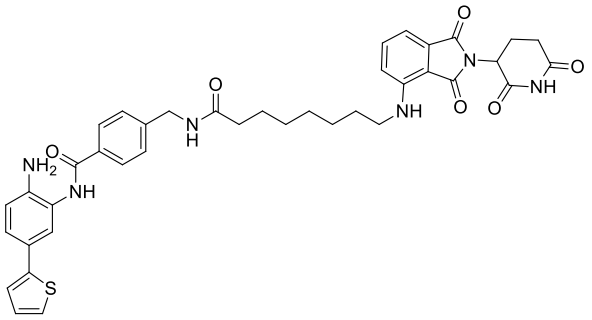
(30 mg, yield: 18%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.66 (s, 1H), 8.36 (t, J = 6.0 Hz, 1H), 7.93 (d, J = 8.2 Hz, 2H), 7.58 – 7.54 (m, 1H), 7.46 (d, J = 2.1 Hz, 1H), 7.41 – 7.18 (m, 5H), 7.03 (ddd, J = 16.3, 14.8, 7.8 Hz, 3H), 6.80 (d, J = 8.4 Hz, 1H), 6.51 (t, J = 5.9 Hz, 1H), 5.17 – 4.99 (m, 3H), 4.32 (d, J = 6.0 Hz, 2H), 3.26 (d, J = 6.6 Hz, 1H), 2.86 (ddd, J = 17.3, 13.9, 5.5 Hz, 1H), 2.61 – 2.50 (m, 3H), 2.15 (t, J = 7.4 Hz, 2H), 2.04 – 1.97 (m, 1H), 1.61 – 1.50 (m, 4H), 1.38 – 1.26 (m, 4H).

HRMS calculated for $C_{38}H_{39}N_6O_6S^+$ (M+H): 707.2646, found: 707.2647.

HPLC: rt = 10.59 min (99.37 %).

***N*-(2-amino-5-(thiophen-2-yl)phenyl)-4-((8-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)octanamido)methyl)benzamide 218e (PSP17)**:



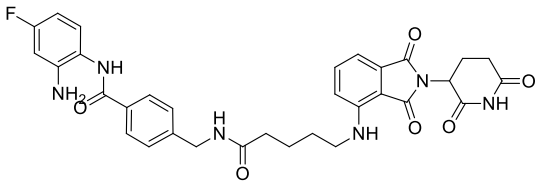
(30 mg, yield: 17%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.67 (s, 1H), 8.35 (t, *J* = 6.0 Hz, 1H), 7.93 (d, *J* = 8.2 Hz, 2H), 7.55 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.46 (d, *J* = 2.1 Hz, 1H), 7.38 – 7.14 (m, 5H), 7.03 (ddd, *J* = 16.5, 15.0, 7.8 Hz, 3H), 6.80 (d, *J* = 8.4 Hz, 1H), 6.51 (t, *J* = 5.8 Hz, 1H), 5.16 – 4.98 (m, 3H), 4.32 (d, *J* = 5.9 Hz, 2H), 3.26 (d, *J* = 6.7 Hz, 1H), 2.85 (ddd, *J* = 17.8, 14.0, 5.5 Hz, 1H), 2.63 – 2.49 (m, 3H), 2.14 (t, *J* = 7.4 Hz, 2H), 2.03 – 1.97 (m, 1H), 1.62 – 1.46 (m, 4H), 1.37 – 1.17 (m, 6H).

HRMS calculated for $C_{39}H_{41}N_6O_6S^+$ (M+H): 721.2803, found: 721.281.

HPLC: rt = 10.95 min (91.54 %).

***N*-(2-amino-4-fluorophenyl)-4-((5-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)pentanamido)methyl)benzamide 218f (PSP18)**:



(79 mg, yield: 75%).

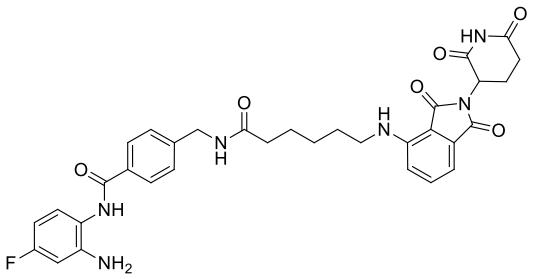
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.52 (s, 1H), 8.39 (t, *J* = 6.0 Hz, 1H), 7.90 (d, *J* = 8.1 Hz, 2H), 7.56 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.13 – 7.05 (m, 2H), 7.00 (d, *J* = 7.0 Hz, 1H), 6.58 – 6.49 (m, 2H), 6.34 (td, *J* = 8.5, 2.9 Hz, 1H), 5.18 (s, 2H), 5.03 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.31 (d, *J* = 5.9 Hz, 2H), 3.28 (d, *J* = 6.3 Hz, 1H), 2.85 (ddd, *J* = 17.4, 14.1, 5.5 Hz, 1H), 2.62 – 2.49 (m, 2H), 2.45 (d, *J* = 4.4 Hz, 1H), 2.20 (t, *J* = 6.9 Hz, 2H), 2.05 – 1.96 (m, 1H), 1.65 – 1.51 (m, 4H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.23, 172.50, 170.52, 169.36, 167.73, 165.82, 162.62, 160.25, 146.83, 145.91, 145.79, 143.76, , 136.70, 133.38, 132.65, 128.99, 128.88, 128.25, 127.28, 119.76, 117.61, 110.83, 109.50, 102.61, 102.38, 102.04, 101.79, 49.00, 42.21, 41.99, 35.43, 31.42, 28.82, 23.10, 22.60.

HRMS calculated for $C_{32}H_{32}FN_6O_6^+$ (M+H): 615.2362, found: 615.2364.

HPLC: rt = 8.57 min (95.27 %).

***N*-(2-amino-4-fluorophenyl)-4-((6-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)hexanamido)methyl)benzamide 218g (PSP11)**:



(80 mg, yield: 33%).

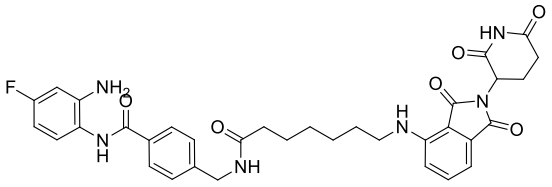
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.53 (s, 1H), 8.37 (t, *J* = 5.9 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.53 (dd, *J* = 10.6, 4.9 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 2H),

7.17 – 6.89 (m, 4H), 6.56 – 6.43 (m, 2H), 6.34 (td, *J* = 8.5, 2.9 Hz, 1H), 5.33 – 4.96 (m, 3H), 4.50 (dd, *J* = 10.6, 4.4 Hz, 1H), 4.31 (d, *J* = 5.9 Hz, 2H), 3.45 (s, 2H), 3.27 (d, *J* = 6.5 Hz, 1H), 2.63 – 2.50 (m, 1H), 2.26 (dd, *J* = 8.1, 4.5 Hz, 2H), 2.17 (t, *J* = 7.4 Hz, 1H), 1.66 – 1.50 (m, 4H), 1.34 (dt, *J* = 15.3, 7.6 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.99, 172.58, 170.42, 169.95, 168.20, 165.82, 162.62, 160.25, 146.86, 146.66, 145.92, 145.80, 143.80, 136.30, 133.37, 133.06, 128.25, 127.28, 117.05, 110.52, 110.22, 102.60, 102.37, 102.04, 101.79, 52.07, 51.66, 42.20, 35.71, 30.99, 28.97, 26.47, 25.49, 24.07. HRMS calculated for $C_{33}H_{34}FN_6O_6^+$ (M+H): 629.2518, found: 629.2523.

HPLC: rt = 9.39 min (93.06 %).

***N*-(2-amino-4-fluorophenyl)-4-((7-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)heptanamido)methyl)benzamide 218h (PSP19)**:



(60 mg, yield: 64%).

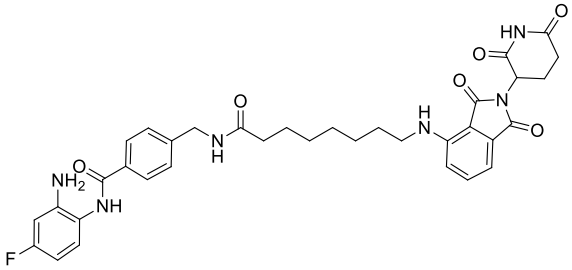
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.52 (s, 1H), 8.35 (t, *J* = 6.0 Hz, 1H), 7.91 (d, *J* = 8.1 Hz, 2H), 7.56 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.12 – 7.03 (m, 2H), 7.00 (d, *J* = 7.0 Hz, 1H), 6.52 (dt, *J* = 10.3, 5.1 Hz, 2H), 6.34 (td, *J* = 8.5, 2.9 Hz, 1H), 5.18 (s, 2H), 5.03 (dd, *J* = 12.9, 5.4 Hz, 1H), 4.31 (d, *J* = 5.9 Hz, 2H), 3.27 (dd, *J* = 13.4, 6.7 Hz, 2H), 2.90 – 2.80 (m, 1H), 2.55 (ddd, *J* = 17.2, 6.1, 2.6 Hz, 2H), 2.15 (t, *J* = 7.4 Hz, 2H), 2.04 – 1.97 (m, 1H), 1.60 – 1.49 (m, 4H), 1.37 – 1.25 (m, 4H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.23, 172.65, 170.52, 169.39, 167.73, 165.81, 162.62, 160.25, 146.87, 145.91, 145.80, 143.83, 136.72, 133.36, 132.64, 128.99, 128.88, 128.24, 127.26, 119.74, 117.60, 110.83, 109.48, 102.60, 102.38, 102.05, 101.80, 49.00, 42.27, 42.19, 35.75, 31.42, 29.03, 28.87, 26.53, 25.68, 22.60.

HRMS calculated for $C_{34}H_{36}FN_6O_6^+$ (M+H): 643.2675, found: 643.268.

HPLC: rt = 9.62 min (96.68 %).

***N*-(2-amino-4-fluorophenyl)-4-((8-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)octanamido)methyl)benzamide 218i (PSP20)**:



(40 mg, yield: 59%).

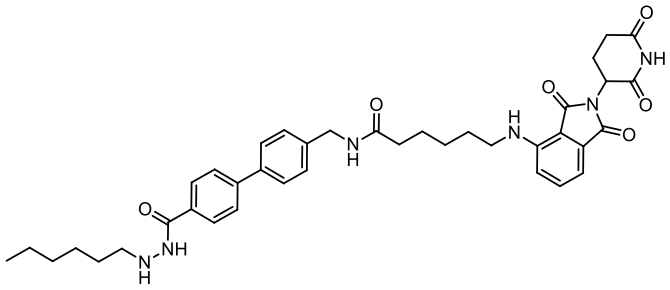
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.52 (s, 1H), 8.34 (t, *J* = 6.0 Hz, 1H), 7.90 (d, *J* = 8.1 Hz, 2H), 7.56 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.13 – 7.04 (m, 2H), 7.00 (d, *J* = 7.0 Hz, 1H), 6.55 – 6.45 (m, 2H), 6.34 (td, *J* = 8.5, 2.9 Hz, 1H), 5.18 (s, 2H), 5.03 (dd, *J* = 12.9, 5.4 Hz, 1H), 4.30 (d, *J* = 5.9 Hz, 2H), 3.29 – 3.21 (m, 2H), 2.86 (ddd, *J* = 17.3, 14.1, 5.4 Hz, 1H), 2.62 – 2.50 (m, 2H), 2.14 (t, *J* = 7.4 Hz, 2H), 2.04 – 1.97 (m, 1H), 1.53 (dt, *J* = 14.2, 6.6 Hz, 4H), 1.35 – 1.22 (m, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.22, 172.67, 170.51, 169.39, 167.73, 165.80, 162.62, 160.24, 146.87, 145.92, 145.80, 143.84, 136.71, 133.36, 132.63, 128.99, 128.87, 128.23, 127.26, 128.23, 127.26, 119.73, 117.61, 110.81, 109.46, 102.59, 102.37, 102.04, 101.79, 48.99, 42.27, 42.17, 35.77, 31.42, 29.11, 29.08, 28.93, 26.67, 25.69, 22.59.

HRMS calculated for $C_{35}H_{38}FN_6O_6^+$ (M+H): 657.2831, found: 657.283.

HPLC: rt = 10.14 min (95.77 %).

6-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-*N*-((4'-(2-hexylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl)methyl)hexanamide 218j (PSP64):



(80 mg, yield: 23%).

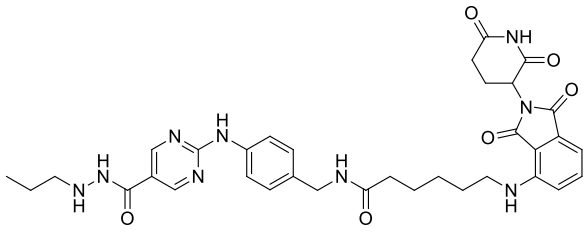
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 10.03 (s, 1H), 8.32 (t, *J* = 5.9 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 2H), 7.71 (d, *J* = 8.5 Hz, 2H), 7.64 (d, *J* = 8.3 Hz, 2H), 7.55 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.33 (d, *J* = 8.3 Hz, 2H), 7.03 (dd, *J* = 26.9, 7.8 Hz, 2H), 6.50 (t, *J* = 5.8 Hz, 1H), 5.02 (dd, *J* = 12.9, 5.4 Hz, 2H), 4.28 (d, *J* = 5.8 Hz, 2H), 3.29 – 3.24 (m, 2H), 2.92 – 2.75 (m, 3H), 2.60 – 2.51 (m, 2H), 2.16 (t, *J* = 7.4 Hz, 2H), 2.00 (ddd, *J* = 10.6, 7.1, 4.8 Hz, 1H), 1.57 (dt, *J* = 14.9, 7.5 Hz, 4H), 1.48 – 1.40 (m, 2H), 1.37 – 1.21 (m, 8H), 0.85 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.21, 172.50, 170.50, 169.39, 167.73, 165.33, 146.87, 142.93, 140.13, 138.03, 136.71, 132.63, 132.34, 128.31, 128.10, 127.15, 126.78, 117.61, 110.82, 109.47, 51.70, 48.99, 42.23, 42.15, 35.72, 31.67, 31.42, 28.92, 28.05, 26.79, 26.44, 25.49, 22.60, 22.53, 14.38.

HRMS calculated for $C_{39}H_{47}N_6O_6^+$ (M+H): 695.3552, found: 695.3552.

HPLC: rt = 14.06 min (96.89 %).

2-(4-((6-(2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)hexanamido) methyl)phenylamino)-*N*'-propylpyrimidine-5-carbohydrazide 218k (PSP77):



(180 mg, yield: 35%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.97 (d, *J* = 17.4 Hz, 2H), 8.81 (s, 2H), 8.22 (t, *J* = 5.8 Hz, 1H), 7.66 (t, *J* = 7.2 Hz, 2H), 7.54 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.17 (d, *J* = 8.6 Hz, 2H), 7.02 (dd, *J* = 22.7, 7.8 Hz, 2H), 6.49 (t, *J* = 5.8 Hz, 1H), 5.03

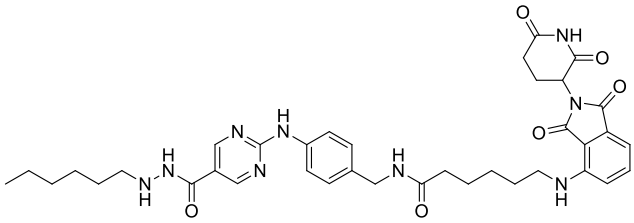
(dd, *J* = 12.8, 5.4 Hz, 1H), 4.19 (d, *J* = 5.7 Hz, 3H), 4.09 (s, 1H), 3.25 (dd, *J* = 13.2, 6.7 Hz, 2H), 2.91 – 2.81 (m, 1H), 2.73 (t, *J* = 7.1 Hz, 2H), 2.61 – 2.50 (m, 2H), 2.13 (t, *J* = 7.4 Hz, 2H), 2.04 – 1.97 (m, 1H), 1.56 (dt, *J* = 14.8, 7.4 Hz, 4H), 1.46 (dt, *J* = 14.5, 7.3 Hz, 2H), 1.32 (dt, *J* = 15.1, 7.6 Hz, 2H), 0.89 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.23, 172.42, 170.51, 169.38, 167.73, 163.12, 161.13, 157.72, 146.85, 138.67, 136.68, 134.10, 132.61, 127.97, 120.06, 117.68, 117.56, 110.81, 109.46, 53.57, 48.99, 42.21, 42.13, 35.72, 31.42, 28.92, 26.43, 25.50, 22.60, 21.28, 12.06.

HRMS calculated for $C_{34}H_{40}N_9O_6^+$ (M+H): 670.3096, found: 670.310.

HPLC: rt = 11.77 min (98.73 %).

2-(4-((6-(2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)hexanamido) methyl)phenylamino)-*N*'-hexylpyrimidine-5-carbohydrazide 218l (PSP75):



(120 mg, yield: 34%).

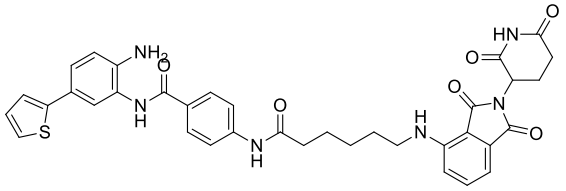
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.99 (s, 1H), 9.93 (s, 1H), 8.80 (s, 2H), 8.21 (t, *J* = 5.9 Hz, 1H), 7.65 (d, *J* = 8.5 Hz, 2H), 7.59 – 7.52 (m, 1H), 7.17 (d, *J* = 8.5 Hz, 2H), 7.06 (d, *J* = 8.6 Hz, 1H), 6.99 (d, *J* = 7.0 Hz, 1H), 6.50 (t, *J* = 5.8 Hz, 1H), 5.03 (dd, *J* = 12.7, 5.5 Hz, 2H), 4.19 (d, *J* = 5.8 Hz, 2H), 3.26 (dd, J = 13.4, 6.7 Hz, 2H), 2.86 (ddd, *J* = 16.8, 13.8, 5.3 Hz, 1H), 2.75 (t, *J* = 7.1 Hz, 2H), 2.60 – 2.49 (m, 2H), 2.13 (t, *J* = 7.4 Hz, 2H), 2.04 – 1.97 (m, 1H), 1.65 – 1.49 (m, 4H), 1.46 – 1.39 (m, 2H), 1.36 – 1.18 (m, 8H), 0.85 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 173.21, 172.34, 170.51, 169.38, 167.72, 163.08, 161.13, 157.71, 146.86, 138.67, 136.69, 134.11, 132.63, 127.98, 120.05, 117.69, 117.60, 110.80, 109.47, 51.69, 48.99, 42.21, 42.11, 35.72, 31.65, 31.42, 28.93, 28.05, 26.77, 26.44, 25.49, 22.60, 22.53, 14.37.

HRMS calculated for $C_{37}H_{46}N_9O_6^+$ (M+H): 712.3566, found: 712.357.

HPLC: rt = 13.57 min (96.88 %).

***N*-(2-amino-5-(thiophen-2-yl)phenyl)-4-(6-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)hexanamido)benzamide 218m (PSP5)**:



A mixture of ***183*** (100 mg, 0.244 mmol, 1.3 eq), 6-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)hexanoic acid (***216b***) (73 mg, 0.188 mmol, 1.0 eq), TCFH (64 mg, 0.23 mmol, 1.2 eq) and NMI (1-Methylimidazole) (54 mg, 0.66 mmol, 3.5 eq) in CH_3CN (2 mL) was stirred at room temperature overnight. The reaction was concentrated and the resulting residue was purified by column chromatography (EtOAc : heptane = 1 : 2 and then DCM : MeOH = 30 : 1 gradually) to give an Boc-protected ***218m*** which was added to a solution of TFA (2 mL) and DCM (6 mL). The reaction was stirred at room temperature for 3 h and treated with the same procedure used for ***218b*** to give ***218 m*** as yellow solid (69mg, yield: 54% over two steps).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 10.12 (s, 1H), 9.60 (s, 1H), 7.94 (d, J = 8.8 Hz, 2H), 7.70 (dd, J = 8.9, 1.9 Hz, 2H), 7.63 – 7.51 (m, 2H), 7.45 (d, J = 2.1 Hz, 1H), 7.35 – 7.31 (m, 1H), 7.29 – 7.21 (m, 2H), 7.04 (ddd, J = 16.3, 12.9, 7.8 Hz, 2H), 6.80 (d, J = 8.3 Hz, 1H), 6.53 (t, J = 5.9 Hz, 1H), 5.10 (s, 2H), 5.05 – 4.99 (m, 1H), 2.94 – 2.76 (m, 2H), 2.61 – 2.50 (m, 2H), 2.38 – 2.23 (m, 3H), 2.04 – 1.97 (m, 1H), 1.62 (ddt, J = 23.1, 15.5, 7.7 Hz, 4H), 1.44 – 1.34 (m, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.78, 171.60, 170.08, 168.92, 164.88, 146.42, 144.25, 142.97, 142.17, 132.19, 128.70, 128.56, 128.16, 127.52, 123.86, 123.58, 123.19, 122.29, 120.99, 118.09, 116.39, 114.42, 110.39, 109.02, 43.17, 41.73, 30.98, 28.53, 25.99, 24.74, 22.15.

HRMS calculated for $C_{36}H_{35}N_6O_6S^+$ (M+H): 679.2333, found: 679.234.

HPLC: rt = 10.62 min (92.47 %).

General procedure for the synthesis of *N-(2-amino-5-(thiophen-2-yl)phenyl)-4-((6-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-ylamino)hexanamido)methyl)benzamide 224a (PSP23)* and *6-{[2-(2,6-dioxopiperidin-3-yl)-1-oxo-2,3-dihydro-1H-isoindol-4-yl]amino}-N-{[4'-(N'-hexylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl]methyl}hexanamide 224b (PSP65)*:

Tert-butyl 6-bromohexanoate (220): This compound was synthesized according to the procedure used for ***214a*** using 6-bromohexanoic acid (***207***). ***220*** was light red oil (yield: 66%). m/z (APCI$^+$) 253.0 (M+H)$^+$.

T***ert-butyl 6-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-ylamino)hexanoate (221)***: A stirred mixture of 3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione (***144***) (400 mg, 1.54 mmol, 1.0 eq), ***220*** (465 mg, 1.85 mmol, 1.2 eq) and DIPEA (600 mg, 4.63 mmol, 3.0 eq) in NMP (5 mL) was heated to 110 ^{o}C overnight. Then, the resulting mixture was extracted with EtOAc and brine three times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 1 : 4 to 1 : 1 gradually) to give ***221*** as a light orange solid (0.41 g, yield: 62%). m/z (APCI$^+$) 430.2 (M+H)$^+$, ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.97 (s, 1H), 7.26 (t, *J* = 7.7 Hz, 1H), 6.90 (d, *J* = 6.9 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 1H), 5.52 (t, *J* = 5.4 Hz, 1H), 5.09 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.15 (dd, *J* = 40.6, 17.2 Hz, 2H), 3.09 (dd, *J* = 12.7, 6.9 Hz, 2H), 2.95 – 2.86 (m, 1H), 2.60 (d, *J* = 15.6 Hz, 1H), 2.28 – 2.21 (m, 1H), 2.17 (t, *J* = 7.3 Hz, 2H), 2.05 – 1.98 (m, 1H), 1.59 – 1.46 (m, 4H), 1.39 – 1.34 (m, 11H).

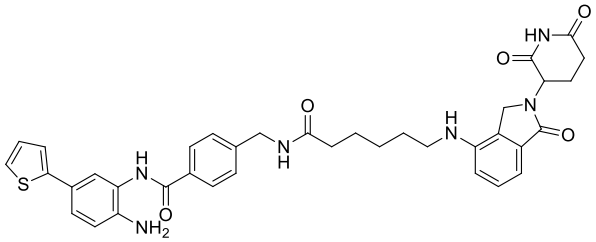
6-(2-(2,6-Dioxopiperidin-3-yl)-1-oxoisoindolin-4-ylamino)hexanoic acid (222): A mixture of intermediate ***221*** (0.40g) in TFA (2 mL) and DCM (6 mL) was stirred at room temperature for 3 h. The reaction was evaporated directly to give intermediate ***222*** in a quantitative yield. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.98 (s, 1H), 7.26 (t, *J* = 7.7 Hz, 1H), 6.91 (d, *J* = 6.9 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 1H), 5.52 (s, 1H), 5.09

(dd, *J* = 13.2, 5.1 Hz, 1H), 4.16 (dd, *J* = 40.5, 17.1 Hz, 2H), 3.09 (t, *J* = 6.9 Hz, 2H), 2.97 – 2.88 (m, 1H), 2.60 (dd, *J* = 14.3, 2.6 Hz, 1H), 2.29 (t, *J* = 7.4 Hz, 2H), 2.23 – 2.14 (m, 1H), 2.05 – 1.99 (m, 1H), 1.60 – 1.50 (m, 4H), 1.39 – 1.33 (m, 2H).

Compound ***224a*** was synthesized through the same procedure of ***216b*** to ***218b*** using ***222*** and ***178a. 224a*** was purified by preparative HPLC.

Compound ***224b*** was synthesized through the same procedure of ***216b*** to ***218b*** using ***222*** and ***191. 224b*** were purified by preparative HPLC.

***N*-(2-amino-5-(thiophen-2-yl)phenyl)-4-((6-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-ylamino)hexanamido)methyl)benzamide 224a (PSP23)**:



Gray solid (43 mg, yield: 11%).

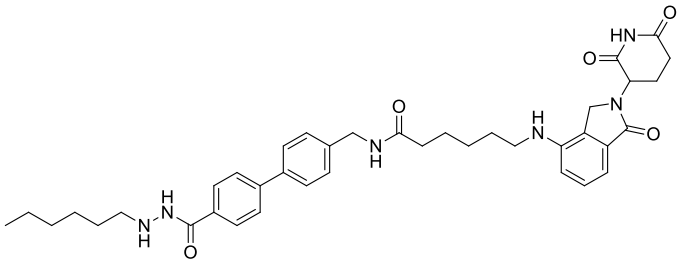
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.99 (s, 1H), 9.71 (s, 1H), 8.40 (s, 1H), 7.96 (d, *J* = 7.8 Hz, 2H), 7.49 (s, 1H), 7.31 (ddd, *J* = 24.7, 16.1, 5.5 Hz, 5H), 7.09 – 7.02 (m, 1H), 6.92 (t, *J* = 9.2 Hz, 1H), 6.83 (d, *J* = 8.2 Hz, 1H), 6.74 (t, *J* = 9.9 Hz, 1H), 5.57 (s, 1H), 5.11 (dd, *J* = 13.2, 4.8 Hz, 3H), 4.35 (d, *J* = 5.2 Hz, 2H), 4.19 (dd, *J* = 49.4, 17.1 Hz, 2H), 3.13 (d, *J* = 5.4 Hz, 2H), 2.97 – 2.88 (m, 1H), 2.62 (d, *J* = 17.0 Hz, 1H), 2.33 – 2.25 (m, 1H), 2.20 (t, *J* = 7.2 Hz, 2H), 2.07 – 2.01 (m, 1H), 1.67 – 1.54 (m, 4H), 1.43 – 1.35 (m, 2H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 173.34, 172.68, 171.70, 169.35, 165.75, 144.70, 144.23, 143.93, 143.44, 133.39, 132.52, 129.68, 128.67, 128.33, 127.33, 126.93, 124.36, 123.91, 123.68, 122.74 121.48, 116.86, 112.21, 110.39, 51.97, 46.21, 43.09, 42.23, 35.83, 31.72, 28.79, 26.81, 25.63, 23.31.

HRMS calculated for $C_{37}H_{39}N_6O_5S^+$ (M+H): 679.2697, found: 679.2698.

HPLC: rt = 9.61 min (96.25 %).

6-((2-(2,6-Dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)-*N*-((4'-(2-hexylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl)methyl)hexanamide 224b (PSP65):



White solid (80 mg, yield: 23%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.99 (s, 1H), 10.04 (s, 1H), 8.34 (t, *J* = 5.8 Hz, 1H), 7.90 (d, *J* = 8.1 Hz, 2H), 7.72 (d, *J* = 8.3 Hz, 2H), 7.66 (d, *J* = 7.9 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.27 (t, *J* = 7.7 Hz, 1H), 6.92 (d, *J* = 7.5 Hz, 1H), 6.74 (d, *J* = 8.0 Hz, 1H), 5.55 (t, *J* = 5.3 Hz, 1H), 5.10 (dd, *J* = 13.3, 5.2 Hz, 2H), 4.30 (d, *J* = 5.8 Hz, 2H), 4.18 (dd, *J* = 48.5, 17.1 Hz, 2H), 3.11 (dd, *J* = 12.6, 6.5 Hz, 2H), 2.95 – 2.87 (m, 1H), 2.79 (t, *J* = 7.0 Hz, 2H), 2.61 (d, *J* = 21.3 Hz, 1H), 2.31 – 2.24 (m, 1H), 2.17 (t, *J* = 7.3 Hz, 2H), 2.05 – 1.99 (m, 1H), 1.52 (ddt, *J* = 27.1, 14.4, 7.4 Hz, 6H), 1.42 – 1.14 (m, 8H), 0.87 (t, *J* = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 173.33, 172.59, 171.68, 169.34, 165.36, 144.23, 140.16, 132.50, 132.36, 129.67, 128.32, 128.12, 127.17, 126.91, 126.80, 112.18, 110.38, 51.95, 51.71, 46.19, 43.10, 42.16, 35.82, 31.69, 28.77, 28.10, 28.07, 26.81, 25.63, 23.28, 22.54, 14.40.

HRMS calculated for $C_{39}H_{49}N_6O_5^+$ (M+H): 681.3759, found: 681.376.

HPLC: rt = 13.75 min (92.33 %).

General procedure for the synthesis of *229a-229g*:

Method 1 for the synthesis of *227a-227i*:

Procedure for the synthesis of TFA-protected amino acids (*225b-225d*): A stirred mixture of amino acid (***213b-213d***) (5.0 mmol) in TFAA (10.0 mmol) was heated to 80 ^{o}C for 3 h. The reaction was then cooled to room temperature. Cold water (14 mL) was added to the reaction. The mixture was extracted with EtOAc three times. The

combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give intermediate ***225b-225d*** as white solid in an up 85% yield.

Procedure for the synthesis of intermediates *(226a-226g)*: A mixture of corresponding carboxylic acid (***225b-225d***) (1.0 mmol), corresponding amine (***178a/b***, ***87a/c***, ***78a***, ***198a***) (0.91 mmol), HATU (1.1 mmol) and DIPEA (2.73 mmol) in DMF (5 mL) was stirred at room temperature for about 10 min. Then, the reaction was extracted with EtOAc and water three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 1 : 1 and then DCM : MeOH = 1 : 0, 30 : 1) to give ***226a-226g*** respectively.

Procedure for the synthesis of intermediates *(227a-227g)*: A mixture of corresponding compound (***226a-226g***) (1.0 mmol) and K_2CO_3 (4.0 mmol) in MeOH (3 mL) and H_2O (3 mL) was stirred at room temperature overnight. The reaction was then extracted with EtOAc and water three times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give the corresponding intermediates (***227a-227g***) as gray or black solid in up 30% yield over two steps.

Method 2 for the synthesis of *227a-227i*:

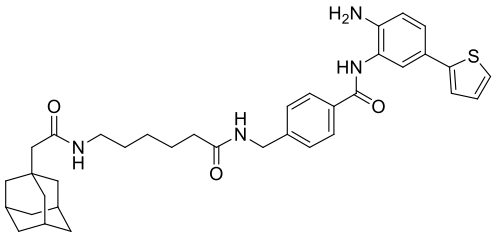
Procedure for the synthesis of Boc-protected amino acids (*230a* and *230c*): Amino acid (***213a*** or ***213c***) (1.0 mmol) was added to a stirred mixture of NaOH (1.0 mmol) in dioxane (3 mL) and H_2O (1.5 mL) followed by addition of $(Boc)_2O$ (1.1 mmol). The reaction was stirred at room temperature overnight. HCl (10%) solution was then added dropwise to the reaction until pH ≈ 4. The reaction was extracted with EtOAc three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give ***230a*** or ***230c*** as colorless oil in quantitative yield.

Procedure for the synthesis of intermediates *(227h* and *227i)*: Through the same

procedure used for ***226a-226g*** using ***178b*** and ***230a*** or ***230c*** to give intermediates ***226h*** and ***226i***. A mixture of ***226h*** or ***226i*** in a solution of HCl/dioxane (4M) and DCM (1 : 3) was stirred at room temperature for 3 h. The mixture was then evaporated directly to give ***227h*** or ***227i*** as HCl salt.

Final compounds ***229a-229g*** were synthesized according to the procedure used for ***218a*** and ***218b*** using ***227a-227i***.

4-((6-(2-(Adamantan-1-yl)acetamido)hexanamido)methyl)-*N*-(2-amino-5-(thioph en-2-yl)phenyl)benzamide 229a (PSP21):



White solid (45 mg, yield: 56%).

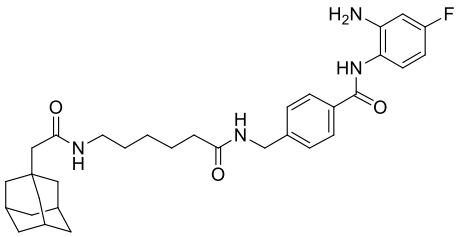
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.69 (s, 1H), 8.38 (t, *J* = 5.9 Hz, 1H), 7.96 (d, *J* = 8.1 Hz, 2H), 7.63 (t, *J* = 5.4 Hz, 1H), 7.48 (d, *J* = 1.5 Hz, 1H), 7.36 (t, *J* = 6.5 Hz, 3H), 7.30 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.24 (d, *J* = 3.4 Hz, 1H), 7.05 (dd, *J* = 5.0, 3.7 Hz, 1H), 6.83 (s, 1H), 5.14 (s, 2H), 4.34 (d, *J* = 5.8 Hz, 2H), 3.01 (dd, *J* = 12.7, 6.7 Hz, 2H), 2.16 (t, *J* = 7.5 Hz, 2H), 1.90 (s, 3H), 1.80 (s, 2H), 1.73 – 1.45 (m, 14H), 1.43 – 1.36 (m, 2H), 1.27 (dt, *J* = 14.9, 7.3 Hz, 2H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 172.63, 170.14, 144.71, 143.93, 143.43, 133.39, 128.65, 128.33, 127.31, 124.35, 123.91, 123.67, 122.74, 121.47, 116.85, 50.56, 42.61, 42.21, 38.67, 36.96, 35.80, 32.61, 29.48, 28.52, 26.68, 25.49.

HRMS calculated for $C_{36}H_{45}N_4O_3S^+$ (M+H): 613.3207, found: 613.321.

HPLC: rt = 11.46 min (95.92 %).

4-((6-[2-(Adamantan-1-yl)acetamido]hexanamido)methyl)-*N*-(2-amino-4-fluorop henyl)benzamide 229b (PSP22):



White solid (98 mg, yield: 50%).

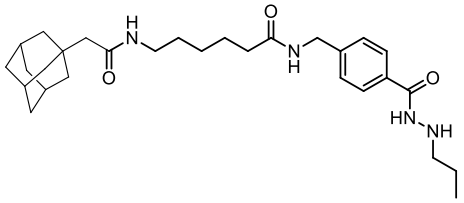
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.56 (s, 1H), 8.38 (t, *J* = 5.5 Hz, 1H), 7.94 (d, *J* = 7.7 Hz, 2H), 7.64 (s, 1H), 7.36 (d, *J* = 7.8 Hz, 2H), 7.15 – 7.07 (m, 1H), 6.56 (dd, *J* = 11.1, 1.9 Hz, 1H), 6.37 (t, *J* = 7.4 Hz, 1H), 5.22 (s, 2H), 4.34 (d, *J* = 5.6 Hz, 2H), 3.02 (dd, *J* = 12.3, 6.2 Hz, 2H), 2.16 (t, *J* = 7.3 Hz, 2H), 1.91 (s, 3H), 1.82 (s, 2H), 1.73 – 1.47 (m, 14H), 1.40 (dt, *J* = 14.1, 7.2 Hz, 2H), 1.28 (dd, *J* = 14.5, 7.6 Hz, 2H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 172.64, 170.17, 165.86, 162.42, 160.52, 145.94, 145.84, 143.85, 133.42, 129.00, 128.91, 128.28, 127.29, 119.81, 102.60, 102.42, 102.06, 101.86, 50.58, 42.63, 42.22, 38.68, 36.97, 35.81, 32.62, 29.49, 28.54, 26.69, 25.51.

HRMS calculated for $C_{32}H_{42}FN_4O_3^+$ (M+H): 549.3236, found: 549.324.

HPLC: rt = 10.58 min (97.33 %).

6-[2-(Adamantan-1-yl)acetamido]-*N*-([4-(*N*'-propylhydrazinecarbonyl)phenyl]methyl)hexanamide 229c (PSP33):



White solid (58 mg, yield: 45%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H), 8.35 (t, *J* = 5.7 Hz, 1H), 7.75 (d, *J* = 8.1 Hz, 2H), 7.63 (t, *J* = 5.1 Hz, 1H), 7.28 (d, *J* = 8.1 Hz, 2H), 4.28 (d, *J* = 5.9 Hz, 2H), 2.98 (dd, *J* = 12.6, 6.6 Hz, 2H), 2.75 (s, 1H), 2.12 (t, *J* = 7.4 Hz, 2H), 1.88 (s, 3H), 1.78 (s, 2H), 1.65 – 1.48 (m, 14H), 1.47 – 1.21 (m, 8H), 0.88 (t, *J* = 7.4 Hz, 3H).

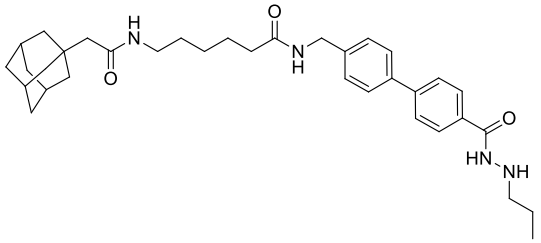
^{13}C NMR (101 MHz, DMSO-d_6) δ 172.60, 170.14, 165.49, 143.57, 129.78, 127.52, 127.32, 53.48, 50.53, 45.93, 42.58, 38.64, 36.93, 35.75, 32.58, 29.43, 28.51, 26.64,

25.45, 21.33, 12.08.

HRMS calculated for $C_{29}H_{45}N_4O_3^+$ (M+H): 497.3486, found: 497.3479.

HPLC: rt = 13.70 min (95.68 %).

6-[2-(Adamantan-1-yl)acetamido]-*N*-([4'-(*N*'-propylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl]methyl)hexanamide 229d (PSP34):



White solid (45 mg, yield: 49%).

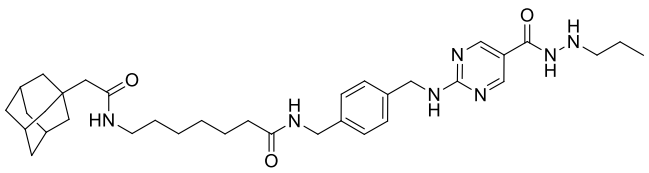
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.04 (s, 1H), 8.32 (t, *J* = 5.7 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 2H), 7.77 – 7.57 (m, 5H), 7.33 (d, *J* = 8.1 Hz, 2H), 5.09 (s, 1H), 4.29 (d, *J* = 5.7 Hz, 2H), 2.99 (dd, *J* = 12.6, 6.6 Hz, 2H), 2.75 (t, *J* = 6.9 Hz, 2H), 2.13 (t, *J* = 7.4 Hz, 2H), 1.88 (s, 3H), 1.78 (s, 2H), 1.67 – 1.19 (m, 20H), 0.90 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.52, 170.10, 165.34, 142.96, 140.16, 138.03, 132.34, 128.26, 128.11, 127.17, 126.78, 53.56, 50.53, 42.58, 42.12, 38.65, 36.93, 35.78, 32.58, 29.46, 28.49, 26.66, 25.49, 21.31, 12.12.

HRMS calculated for $C_{35}H_{48}N_4O_3Na^+$ (M+H): 595.3619, found: 595.3613.

HPLC: rt = 10.32 min (87.17 %).

7-[2-(Adamantan-1-yl)acetamido]-*N*-([4-(([5-(*N*'-propylhydrazinecarbonyl)pyrimidin-2-yl]amino)methyl)phenyl]methyl)heptanamide 229f (PSP58):



White solid (53 mg, yield: 27%).

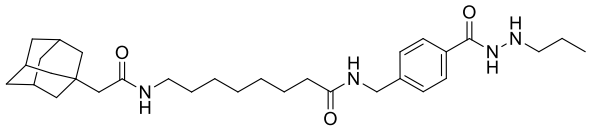
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.81 (s, 1H), 8.66 (s, 2H), 8.37 (s, 1H), 8.27 – 8.23 (m, 1H), 7.62 (t, *J* = 5.3 Hz, 1H), 7.23 (d, *J* = 7.9 Hz, 2H), 7.16 (d, *J* = 7.9 Hz, 2H),

4.51 (d, *J* = 6.3 Hz, 2H), 4.20 (d, *J* = 5.8 Hz, 2H), 2.99 (dd, *J* = 12.7, 6.6 Hz, 2H), 2.72 (t, *J* = 7.0 Hz, 2H), 2.10 (t, *J* = 7.3 Hz, 2H), 1.90 (s, 3H), 1.80 (s, 2H), 1.67 – 1.53 (m, 11H), 1.51 – 1.41 (m, 4H), 1.38 – 1.32 (m, 2H), 1.24 (s, 3H), 0.90 (t, *J* = 7.4 Hz, 3H).

HRMS calculated for $C_{35}H_{52}N_7O_3^+$ (M+H): 618.4126, found: 618.4127.

HPLC: rt = 14.42 min (100.00 %).

8-[2-(Adamantan-1-yl)acetamido]-*N*-([4-(*N*'-propylhydrazinecarbonyl)phenyl]methyl)octanamide 229g (PSP54):



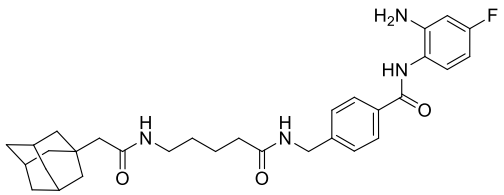
White solid (85 mg, yield: 40%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, 1H), 8.31 (t, *J* = 5.8 Hz, 1H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.60 (t, *J* = 5.2 Hz, 1H), 7.27 (d, *J* = 8.1 Hz, 2H), 5.07 (s, 1H), 4.27 (d, *J* = 5.9 Hz, 2H), 2.98 (dd, *J* = 12.5, 6.5 Hz, 2H), 2.73 (t, *J* = 7.0 Hz, 2H), 2.12 (t, *J* = 7.3 Hz, 2H), 1.88 (s, 3H), 1.78 (s, 2H), 1.63 (d, *J* = 11.8 Hz, 3H), 1.54 (d, *J* = 10.8 Hz, 10H), 1.40 (ddd, *J* = 27.4, 13.3, 6.5 Hz, 5H), 1.23 (s, 6H), 0.89 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.63, 170.10, 165.52, 143.56, 132.09, 127.46, 127.32, 53.56, 50.55, 42.60, 42.15, 38.67, 36.93, 35.76, 32.58, 29.62, 29.11, 28.90, 28.51, 26.80, 25.67, 21.30, 12.10. HRMS calculated for $C_{31}H_{49}N_4O_3^+$ (M+H): 525.3799, found: 525.3798.

HPLC: rt = 14.34 min (97.61 %).

4-((5-[2-(Adamantan-1-yl)acetamido]pentanamido)methyl)-*N*-(2-amino-4-fluorophenyl)benzamide 229h (PSP29):



White solid (70 mg, yield: 50%).

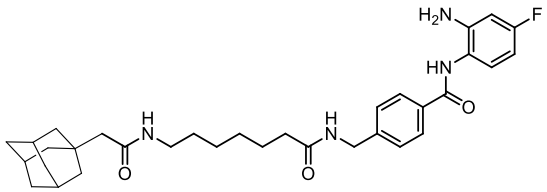
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.36 (t, *J* = 6.0 Hz, 1H), 7.91 (d, *J* = 8.1 Hz, 2H), 7.64 (t, *J* = 5.5 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.10 (dd, *J* = 8.5, 6.5 Hz, 1H), 6.53 (dd, *J* = 11.2, 2.9 Hz, 1H), 6.34 (td, *J* = 8.5, 2.9 Hz, 1H), 5.19 (s, 2H), 4.31 (d, *J* = 5.9 Hz, 2H), 3.00 (dd, *J* = 12.7, 6.8 Hz, 2H), 2.15 (t, *J* = 7.4 Hz, 2H), 1.88 (s, 3H), 1.79 (s, 2H), 1.73 – 1.43 (m, 14H), 1.37 (dt, *J* = 14.1, 7.1 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.56, 170.17, 165.83, 162.62, 160.25, 145.91, 145.79, 143.79, 133.37, 128.96, 128.86, 128.24, 127.25, 119.78, 102.60, 102.37, 102.06, 101.81, 50.54, 42.59, 42.19, 38.51, 36.92, 35.43, 32.59, 29.36, 28.50, 23.30.

HRMS calculated for $C_{31}H_{40}FN_4O_3^+$ (M+H): 535.3079, found: 535.3075.

HPLC: rt = 9.90 min (95.00 %).

4-((7-(2-(-Adamantan-1-yl)acetamido)heptanamido)methyl)-*N*-(2-amino-4-fluoro phenyl)benzamide 229i (PSP30):



White solid (55 mg, yield: 39%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.36 (t, *J* = 6.0 Hz, 1H), 7.91 (d, *J* = 8.1 Hz, 2H), 7.61 (t, *J* = 5.5 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.09 (dd, *J* = 8.5, 6.5 Hz, 1H), 6.53 (dd, *J* = 11.2, 2.9 Hz, 1H), 6.34 (td, *J* = 8.5, 2.9 Hz, 1H), 5.18 (s, 2H), 4.31 (d, *J* = 5.9 Hz, 2H), 2.99 (dd, *J* = 12.6, 6.6 Hz, 2H), 2.13 (t, *J* = 7.4 Hz, 2H), 1.88 (s, 3H), 1.78 (s, 2H), 1.63 (d, *J* = 11.9 Hz, 3H), 1.57 – 1.45 (m, 11H), 1.35 (dd, *J* = 13.2, 6.6 Hz, 2H), 1.26 – 1.23 (m, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.70, 170.17, 165.83, 162.63, 160.26, 145.92, 145.80, 143.83, 133.35, 129.00, 128.89, 128.23, 127.23, 119.76, 102.60, 102.37, 102.05, 101.80, 50.55, 42.59, 42.17, 38.68, 36.92, 35.79, 32.58, 29.52, 28.84, 28.50, 26.66, 25.76.

HRMS calculated for $C_{33}H_{44}FN_4O_3^+$ (M+H): 563.3392, found: 563.339.

HPLC: rt = 10.54 min (95.79 %).

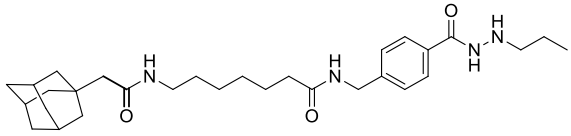
Procedure for the synthesis of *7-(2-(-adamantan-1-yl)acetamido)-N-(4-(2-propylhydrazinecarbonyl)benzyl)heptan amide 229j (PSP53)*:

7-(((Benzyloxy)carbonyl)amino)heptanoic acid (231): A mixture of 7-aminoheptanoic acid (***213c***) (1.0 g, 6.89 mmol, 1.0 eq), benzyl chloroformate (1.48 mL, 10.34 mmol, 1.5 eq) and K_2CO_3 (1.98 g, 13.8 mmol, 2.0 eq) in H_2O (20 mL) was stirred at room temperature overnight. A solution of HCl (10%) was then added dropwise to the mixture until pH ≈ 2 and precipitate appeared. The precipitate was filtered and dried to give (***231***) as white solid (1.73 g, yield: 95%).

Tert-butyl 2-(4-((7-(((benzyloxy)carbonyl)amino)heptanamido)methyl)benzoyl)-1-propylhydrazinecarboxylate (232): A mixture of acid (***231***) (150 mg, 0.537 mmol, 1.1 eq), tert-butyl 2-(4-(aminomethyl)benzoyl)-1-propylhydrazinecarboxylate (***87a***) (150 mg, 0.488 mmol, 1.0 eq), HATU (205 mg, 0.537 mmol, 1.1 eq) and DIPEA (190 mg, 1.464 mmol, 3.0 eq) in DMF (1.5 mL) was stirred at room temperature for 10 min. The reaction was then extracted with EtOAc and brine. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 0 : 1 to 3 : 2 gradually) to give ***232*** as white solid (280 mg) in a quantitative yield. m/z ($APCI^+$) 569.4 $(M+H)^+$.

The final product ***229i*** was synthesized through the same procedure used for ***229h*** using ***232***.

7-(2-(-Adamantan-1-yl)acetamido)-*N*-(4-(2-propylhydrazinecarbonyl)benzyl)heptanamide 229j (PSP53):



White solid (98 mg, yield: 39%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.32 (t, *J* = 5.9 Hz, 1H), 7.75 (d, *J* = 8.3 Hz, 2H), 7.60 (t, *J* = 5.4 Hz, 1H), 7.28 (d, *J* = 8.3 Hz, 2H), 5.10 (s, 1H), 4.28 (d, *J* = 5.9 Hz, 2H), 2.98 (dd, *J* = 12.6, 6.6 Hz, 2H), 2.73 (t, *J* = 7.1 Hz, 2H), 2.12 (t, *J* = 7.4 Hz, 2H), 1.88 (s, 3H), 1.79 (s, 2H), 1.65 – 1.42 (m, 15H), 1.34 (dd, *J* = 13.2, 6.6 Hz, 2H), 1.26 – 1.22 (m, 5H), 0.93 – 0.85 (m, 3H).

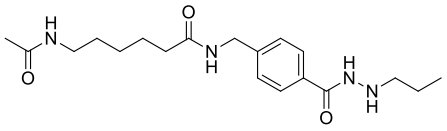
^{13}C NMR (101 MHz, DMSO-d_6) δ 172.64, 170.12, 165.54, 143.57, 132.09, 127.47, 127.31, 53.56 (s), 50.55, 42.60, 42.16, 38.67, 36.93, 35.77, 32.58, 29.53, 28.85, 28.52, 26.66, 25.75, 21.29, 18.52, 17.17, 12.09.

HRMS calculated for $C_{30}H_{47}N_4O_3^+$ (M+H): 511.3643, found: 511.3638.

HPLC: rt = 14.14 min (92.90 %).

Procedure for the synthesis of *6-acetamido-N-(4-(2-propylhydrazinecarbonyl)benzyl)hexanamide 229k (PSP56)*: This compound was synthesized using tert-butyl 2-(4-((6-aminohexanamido)methyl)benzoyl)-1-propylhydrazinecarboxylate (***227c***) through the same procedure of ***89*** to ***91***.

6-Acetamido-*N*-(4-(2-propylhydrazinecarbonyl)benzyl)hexanamide 229k (PSP56):



White solid (130 mg, yield: 36%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H), 8.34 (t, *J* = 5.6 Hz, 1H), 7.75 (d, *J* = 8.1 Hz, 3H), 7.28 (d, *J* = 8.1 Hz, 2H), 4.27 (d, *J* = 5.8 Hz, 2H), 2.98 (dd, *J* = 12.8, 6.7 Hz, 2H), 2.74 (s, 1H), 2.12 (t, *J* = 7.4 Hz, 2H), 1.76 (s, 3H), 1.55 – 1.31 (m, 6H), 1.29 – 1.18 (m, 4H), 0.89 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.60, 169.32, 165.52, 143.59, 132.07, 127.50, 123.33, 53.49, 42.15, 38.85, 35.72, 29.38, 26.60, 25.47, 23.05, 21.25, 12.08.

HRMS calculated for $C_{19}H_{31}N_4O_3^+$ (M+H): 363.2391, found: 363.2389.

HPLC: rt = 7.84 min (92.69 %).

Procedure for the synthesis of *6-(2-(adamantan-1-yl)-N-methylacetamido)-N-(4-(2-propylhydrazinecarbonyl)benzyl)hexanamide 229l (PSP60)*:

6-((Tert-butoxycarbonyl)amino)hexanoic acid (234): This compound was synthesized through the same procedure of ***213a*** to ***230c*** using 6-aminohexanoic acid (***213b***). m/z (APCI$^+$) 232.1 0 (M+H)$^+$.

Benzyl 6-((tert-butoxycarbonyl)amino)hexanoate (235): A stirred mixture of ***234*** (4.0 g, 17.3 mmol, 1.0 eq), benzyl chloride (2.19 g, 17.3 mmol, 1.0 eq) and K_2CO_3 (7.18 g, 51.9 mmol, 3.0 eq) in DMF (40 mL) was heated to about 80-90 ^oC overnight. The reaction was extracted with EtOAc and brine. The collected organic layer was washed with brine four time, dried over anhydrous Na_2SO_4 and concentrated to give ***235*** as colorless oil (4.9 g, yield: 88%). m/z (APCI$^+$) 322.1 (M+H)$^+$.

Benzyl 6-(methylamino)hexanoate (237): CH_3I (0.78 mL, 12.7 mmol, 4.0 eq) was added to a stirred mixture of ***235*** (1.0 g, 3.12 mmol, 1.0 eq) and NaH (60%) (0.19 g, 4.68 mmol, 1.5 q) in anhydrous THF (10 mL). The reaction was stirred at room temperature overnight. Brine was added to the reaction. The reaction was extracted with EtOAc. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 0 : 1 to 1 : 10 gradually) to give ***236*** as light yellow solid. m/z (APCI$^+$) 336.1 (M+H)$^+$. Compound ***236*** was added to a stirred solution of HCl/dioxane (4M) and DCM (1 : 3). The reaction was stirred for an additional 3 h at room temperature and evaporated directly. The resulting residue was washed with ether and dried to give ***237*** as a white solid (500 mg, yield: 68% over two steps) m/z (APCI$^+$) 236.0 (M+H)$^+$.

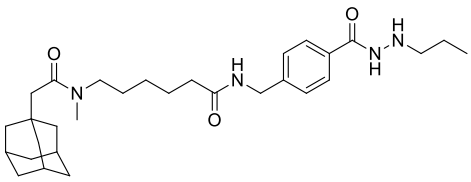
Benzyl 6-(2-(-adamantan-1-yl)-N-methylacetamido)hexanoate (238): Intermediate

237 was converted to ***238*** through the same procedure of ***225b*** to ***226a*** giving ***238*** as a colorless oil in quantitative yield. ($APCI^+$) 421.2 $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 7.51 – 7.15 (m, 5H), 5.06 (d, J = 1.6 Hz, 1.4H), 3.56 (d, J = 1.6 Hz, 0.7H), 3.26 – 3.18 (m, 2.0H), 2.91 (t, J = 5.1 Hz, 1.8H), 2.73 (t, J = 4.8 Hz, 1.2H), 2.35 – 2.26 (m, 2H), 2.01 (dd, J = 10.0, 3.8 Hz, 2H), 1.88 (s, 3H), 1.66 – 1.53 (m, 12H), 1.52 – 1.31 (m, 4H), 1.22 (t, J = 7.9 Hz, 2H).

6-(2-(Adamantan-1-yl)-N-methylacetamido)hexanoic acid (239): A stirred mixture of ***238*** (330 mg, 0.80 mmol, 1.0 eq) and $LiOH{\cdot}H_2O$ (68 mg, 1.6 mmol, 2.0 eq) in THF (3 mL) and H_2O (3 mL) was heated to reflux for 30 min. A solution of HCl (10%) was added dropwise to the reaction until pH ≈ 2. The reaction was then extracted with EtOAc. The collected organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give ***239*** as a white gray solid (250 mg) in quantitative yield.

Compound ***229l*** was prepared using the same procedure used for ***229h*** using ***239*** to give ***229l***.

6-(2-(Adamantan-1-yl)-*N*-methylacetamido)-*N*-(4-(2-propylhydrazinecarbonyl)benzyl)hexanamide 229l (PSP60):



White solid (153 mg, yield: 46%).

^1H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.34 (dd, *J* = 13.2, 6.2 Hz, 1H), 7.75 (d, *J* = 8.1 Hz, 2H), 7.28 (d, *J* = 8.1 Hz, 2H), 5.22 (s, 1H), 4.27 (d, *J* = 5.8 Hz, 2H), 3.23 (dt, *J* = 11.5, 7.5 Hz, 2H), 3.06 (td, *J* = 11.3, 7.1 Hz, 2H), 2.91 (d, *J* = 4.5 Hz, 2H), 2.73 (dd, *J* = 9.0, 5.1 Hz, 3H), 2.13 (dd, *J* = 15.1, 7.5 Hz, 2H), 2.02 (d, *J* = 2.4 Hz, 2H), 1.88 (s, 3H), 1.65 – 1.52 (m, 12H), 1.49 – 1.38 (m, 4H), 1.17 (t, *J* = 7.3 Hz,

2H), 0.89 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.54, 172.50, 170.19, 170.10, 165.52, 143.58, 143.56, 132.08, 132.06, 127.48, 127.33, 127.28, 53.54, 50.20, 46.98, 46.00, 45.82, 45.34, 42.53, 42.50, 42.16, 36.91, 36.55, 35.70, 35.62, 33.47, 33.34, 33.15, 28.54, 28.25, 26.98, 26.51, 26.15, 25.48, 25.45, 21.25, 12.08, 8.96.

HRMS calculated for $C_{30}H_{46}N_4O_3^+$ (M+H): 511.3643, found: 511.3644.

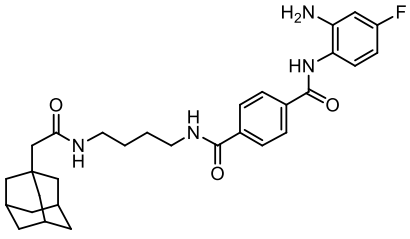
HPLC: rt = 14.31 min (95.52 %).

Procedure for the synthesis of *N^1-(4-(2-(adamantan-1-yl)acetamido)butyl)-N^4-(2-amino-4-fluorophenyl)terephthal amide 229m (PSP31)* and *N^1-(6-(2-(adamantan-1-yl)acetamido)hexyl)-N^4-(2-amino-4-fluorophenyl)terephthal amide 229n (PSP32)*:

Procedure for the synthesis of mono-Boc-protected diamine *(242)*: To stirred solution of diamine (5.0 mmol) in chloroform (6 mL) was added dropwise a solution of $(Boc)_2O$ (1.0 mmol) in chloroform (12 mL). The resulting reaction was stirred at room temperature overnight and then filtered. The filtrate was washed with brine three times. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated to give ***242*** as organge oil in a 90% yield.

229m and ***229n*** were synthesized through the same procedure of ***230a*** to ***229h*** using ***242***, intermediate ***187*** and intermediate ***157***.

N^1-(4-(2-(adamantan-1-yl)acetamido)butyl)-N^4-(2-amino-4-fluorophenyl)terepht halamide 229m (PSP31):



White solid (32 mg, yield: 47%).

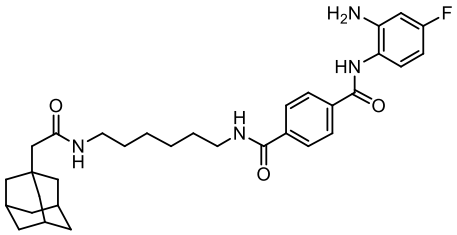
^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.66 (s, 1H), 8.58 (t, *J* = 5.5 Hz, 1H), 7.97 (dd, *J* = 41.2, 8.4 Hz, 4H), 7.65 (t, *J* = 5.6 Hz, 1H), 7.10 (dd, *J* = 8.6, 6.4 Hz, 1H), 6.53 (dd, *J* = 11.2, 2.9 Hz, 1H), 6.34 (td, *J* = 8.5, 2.9 Hz, 1H), 5.23 (s, 2H), 3.26 (dd, *J* = 12.6, 7.0 Hz, 2H), 3.03 (dd, *J* = 12.6, 6.7 Hz, 2H), 1.88 (s, 3H), 1.79 (s, 2H), 1.71 – 1.26 (m, 16H).

^{13}C NMR (101 MHz, DMSO-d$_6$) δ 170.19, 165.84, 165.44, 160.37, 146.06, 145.94, 137.44, 137.05, 128.16, 127.44, 119.42, 102.56, 102.34, 101.98, 101.73, 50.53, 42.59, 38.54, 36.92, 32.60, 28.49, 27.34, 27.14.

HRMS calculated for $C_{30}H_{38}FN_4O_3^+$ (M+H): 521.2923, found: 521.292.

HPLC: rt = 10.00 min (90.47 %).

N^1-(6-(2-(adamantan-1-yl)acetamido)hexyl)-N^4-(2-amino-4-fluorophenyl)terephthalamide 229n (PSP32):



White solid (78 mg, yield: 30%).

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.67 (s, 1H), 8.56 (t, *J* = 5.3 Hz, 1H), 7.98 (dd, *J* = 40.9, 8.2 Hz, 4H), 7.62 (t, *J* = 5.2 Hz, 1H), 7.11 (dd, *J* = 8.2, 6.7 Hz, 1H), 6.54 (dd, *J* = 11.2, 2.6 Hz, 1H), 6.35 (td, *J* = 8.5, 2.6 Hz, 1H), 5.24 (s, 2H), 3.26 (dd, *J* = 12.5, 6.4 Hz, 2H), 3.00 (dd, *J* = 12.3, 6.3 Hz, 2H), 1.88 (s, 3H), 1.79 (s, 2H), 1.69 – 1.45 (m, 14H), 1.42 – 1.25 (m, 6H).

^{13}C NMR (101 MHz, DMSO-d$_6$) δ 170.13, 165.82, 165.45, 162.75, 160.38, 146.06, 145.94, 137.48, 137.05, 129.12, 129.01, 128.16, 127.45, 119.46, 119.44, 102.56, 102.33, 102.00, 101.75, 50.55, 42.60, 38.64, 36.93, 32.59, 29.63, 29.53, 28.51, 26.65, 26.64.

HRMS calculated for $C_{32}H_{42}FN_4O_3^+$ (M+H): 549.3236, found: 549.3238.

HPLC: rt = 10.62 min (95.29 %).

Procedure for the synthesis of VHL ligand-based *(250b-250f, 255a* and *255b)*:

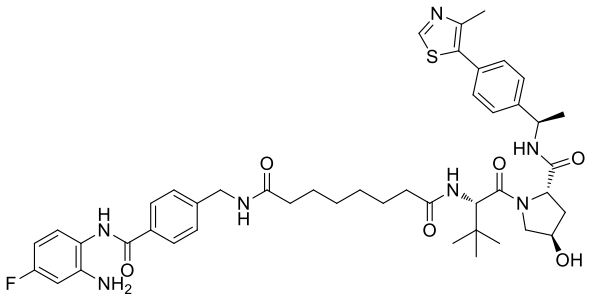
8-(Benzyloxy)-8-oxooctanoic acid (246a)* and *10-(benzyloxy)-10-oxodecanoic acid (246b): A stirred mixture of carboxylic acid (octanedioic acid (***245a***) or decanedioic acid (***245b***)) (2.5 mmol), benzyl bromide (1.0 mmol) and $NaHCO_3$ (4.0 mmol) in DMF (2 mL) and dioxane (2 mL) was heated to 90 ^oC for about 18 h. Then, the reaction was extracted with EtOAc and brine. The collected organic layer was dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 0 : 1, 1 : 2) to give ***246a*** or ***246b***. ***246a*** was colorless oil in 50% yield. m/z ($APCI^+$) 265.1 $(M+H)^+$; ***246b*** was colorless oil in 50% yield. m/z ($APCI^+$) 293.3 $(M+H)^+$.

The final compouns ***250b-250f*** were synthesized through the same procedure of ***237*** to ***229l*** using ***246a***.

The final compound ***255a*** was synthesized through the same procedure of ***237*** to ***229l*** using ***246b***.

The final compoun ***255b*** was synthesized through the same procedure of ***237*** to ***229l*** using ***251***.

N^1-(4-((2-amino-4-fluorophenyl)carbamoyl)benzyl)-N^8-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*R*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)octanediamide 250b (PSP26):



White solid (100 mg, yield: 90%).

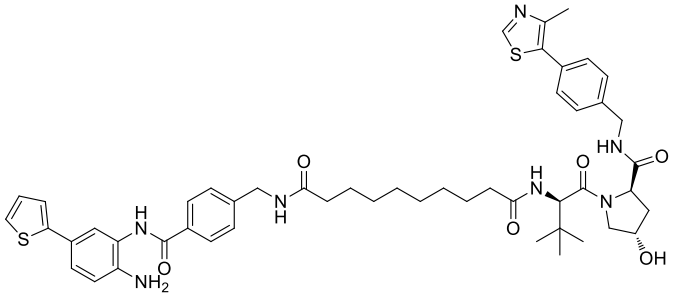
^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.64 (dd, *J* = 5.7, 2.9 Hz, 1H), 9.52 (s, 1H), 8.96 (s, 1H), 8.35 (t, *J* = 6.7 Hz, 2H), 7.91 (d, *J* = 8.1 Hz, 2H), 7.75 (d, *J* = 9.2 Hz, 1H), 7.37 (dt, *J* = 11.4, 8.3 Hz, 6H), 7.09 (dd, *J* = 8.6, 6.4 Hz, 1H), 6.53 (dd, *J* = 11.2, 2.9 Hz, 1H), 6.34 (td, *J* = 8.6, 2.9 Hz, 1H), 5.27 – 5.02 (m, 4H), 4.96 – 4.84 (m, 2H), 4.50 (d, *J* = 9.4 Hz, 1H), 4.41 (t, *J* = 8.0 Hz, 1H), 4.31 (d, *J* = 5.8 Hz, 1H), 4.26 (s, 1H), 3.58 (s, 1H), 2.44 (s, 3H), 2.23 – 2.12 (m, 3H), 2.00 – 1.96 (m, 1H), 1.81 – 1.75 (m, 1H), 1.53 – 1.43 (m, 4H), 1.35 (d, *J* = 7.0 Hz, 3H), 1.22 (s, 5H), 0.95 – 0.82 (m, 9H).

^{13}C NMR (101 MHz, DMSO-d$_6$) δ 172.72, 172.50, 171.06, 170.06, 151.90, 148.19, 145.08, 143.82, 143.15, 133.36, 131.55, 130.12, 129.25, 128.24, 127.25, 126.81, 102.60, 102.37, 102.04, 101.79, 72.77, 70.21, 69.20, 60.66, 58.99, 56.81, 56.69, 48.14, 42.18, 38.15, 35.80, 35.62, 35.35, 31.70, 29.44, 28.93, 26.89 25.80, 25.67, 22.85, 22.54, 21.49, 16.41 14.39.

HRMS calculated for $C_{45}H_{56}FN_7O_6SNa^+$ (M+Na): 864.3889, found: 864.389.

HPLC: rt = 10.07 min (96.50 %).

N^1-(4-((2-amino-5-(thiophen-2-yl)phenyl)carbamoyl)benzyl)-N^{10}-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)decanediamide 250c (PSP27):



White solid (93 mg, yield: 43%).

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.67 (s, 1H), 8.96 (s, 1H), 8.53 (t, *J* = 6.0 Hz, 1H), 8.36 (t, *J* = 5.9 Hz, 1H), 7.94 (d, *J* = 8.2 Hz, 2H), 7.80 (d, *J* = 9.3 Hz, 1H), 7.46 (d, *J* = 2.1 Hz, 1H), 7.44 – 7.30 (m, 6H), 7.28 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.22 (dd, *J* = 3.6, 1.1 Hz, 1H), 7.03 (dd, *J* = 5.1, 3.6 Hz, 1H), 6.80 (d, *J* = 8.3 Hz, 1H), 5.09 (d, *J* = 3.3 Hz, 4H), 4.52 (d, *J* = 9.4 Hz, 1H), 4.45 – 4.37 (m, 2H), 4.32 (d, *J* = 5.9 Hz, 3H), 4.20 (dd,

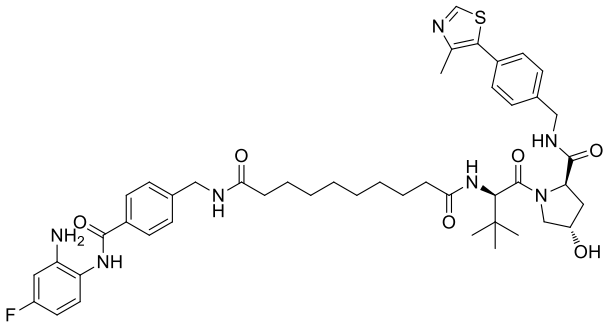
J = 15.9, 5.5 Hz, 1H), 3.64 (dt, *J* = 16.4, 8.0 Hz, 2H), 2.43 (s, 3H), 2.28 – 2.20 (m, 1H), 2.16 – 2.05 (m, 3H), 2.03 – 1.97 (m, 1H), 1.88 (ddd, *J* = 12.9, 8.5, 4.6 Hz, 1H), 1.57 – 1.42 (m, 4H), 1.24 (s, 7H), 0.92 (s, 9H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.72, 172.55, 172.37, 170.16, 151.87, 149.93, 148.15, 144.67, 143.92, 143.42, 139.94, 133.34, 131.60, 130.08, 129.07, 128.64, 128.29, 127.87, 127.29, 124.34, 123.87, 123.65, 122.72, 121.46, 116.83, 70.23, 69.30, 59.13, 56.74, 42.18, 42.10, 38.39, 35.82, 35.64, 35.33, 29.13, 26.83, 25.86, 25.75, 16.38.

HRMS calculated for $C_{50}H_{62}N_7O_6S_2^+$ (M+H): 920.4198, found: 920.420.

HPLC: rt = 10.68 min (95.12 %).

N^1-(4-((2-amino-4-fluorophenyl)carbamoyl)benzyl)-N^{10}-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)decanediamide 250d (PSP28):



White solid (230 mg, yield: 79%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.96 (s, 1H), 8.54 (t, *J* = 6.0 Hz, 1H), 8.35 (t, *J* = 6.0 Hz, 1H), 7.91 (d, *J* = 8.1 Hz, 2H), 7.81 (d, *J* = 9.4 Hz, 1H), 7.46 – 7.27 (m, 6H), 7.10 (dd, *J* = 8.4, 6.6 Hz, 1H), 6.53 (dd, *J* = 11.2, 2.9 Hz, 1H), 6.34 (td, *J* = 8.5, 2.8 Hz, 1H), 5.15 (d, *J* = 39.1 Hz, 4H), 4.53 (d, *J* = 9.4 Hz, 1H), 4.45 – 4.39 (m, 2H), 4.37 – 4.29 (m, 3H), 4.21 (dd, *J* = 15.8, 5.5 Hz, 1H), 3.68 – 3.61 (m, 2H), 2.44 (d, *J* = 3.9 Hz, 3H), 2.28 – 2.21 (m, 1H), 2.11 (dt, *J* = 14.3, 7.7 Hz, 3H), 2.02 (dd, *J* = 12.5, 7.8 Hz, 1H), 1.89 (ddd, *J* = 12.9, 8.5, 4.6 Hz, 1H), 1.55 – 1.43 (m, 4H), 1.24 (s, 7H), 0.96 – 0.88 (m, 9H).

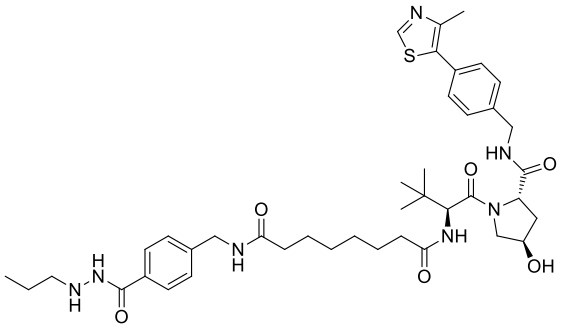
^{13}C NMR (126 MHz, DMSO-d_6) δ 172.73, 172.57, 172.39, 170.16, 165.83 162.40,

160.50, 151.86, 148.16, 145.91, 145.82, 143.83, 139.94, 133.36, 131.61, 130.09, 129.08, 128.24, 127.87, 127.26, 119.74, 102.59, 102.41, 102.03, 101.83, 69.32, 59.14, 56.75, 42.19, 42.11, 38.40, 35.82, 35.65, 35.33, 29.16, 29.13, 29.11, 26.83, 16.38.

HRMS calculated for $C_{46}H_{59}FN_7O_6S^+$ (M+H): 856.4226, found: 856.423.

HPLC: rt = 10.24 min (97.28 %).

N^1-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-N^8-(4-(2-propylhydrazinecarbonyl)benzyl)octanediamide 250e (PSP35):



White solid (110 mg, yield: 27%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, 1H), 8.96 (s, 1H), 8.53 (t, *J* = 6.0 Hz, 1H), 8.31 (t, *J* = 6.0 Hz, 1H), 7.78 (dd, *J* = 25.8, 8.8 Hz, 3H), 7.35 (dt, *J* = 35.1, 8.3 Hz, 6H), 5.10 (d, *J* = 3.2 Hz, 1H), 4.60 – 4.15 (m, 8H), 3.68 – 3.58 (m, 2H), 2.73 (t, *J* = 7.1 Hz, 2H), 2.43 (s, 3H), 2.27 – 2.19 (m, 1H), 2.18 – 2.07 (m, 3H), 2.00 (d, *J* = 8.0 Hz, 1H), 1.89 (ddd, *J* = 12.9, 8.5, 4.6 Hz, 1H), 1.44 (dt, *J* = 21.8, 7.3 Hz, 6H), 1.23 (s, 4H), 0.89 (dd, *J* = 14.5, 7.1 Hz, 12H).

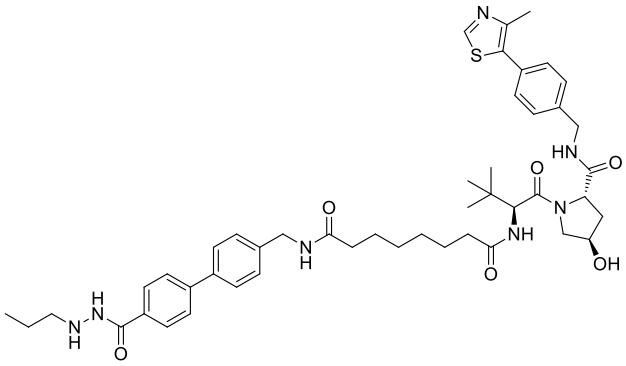
^{13}C NMR (101 MHz, DMSO-d_6) δ 172.68, 172.53, 172.38, 170.16, 165.55, 151.85, 148.15, 143.57, 139.93, 132.08, 131.60, 130.08, 129.07, 127.86, 127.48, 127.33, 69.32, 59.14, 56.74, 53.54, 42.16, 42.11, 38.39, 35.79, 35.65, 35.33, 28.93, 28.90, 26.83, 25.80, 25.66, 21.27, 16.38, 12.09.

HRMS calculated for $C_{41}H_{58}N_7O_6S^+$ (M+H): 776.4164, found: 776.4164.

HPLC: rt = 8.54 min (98.89 %).

N^1-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrr

olidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-*N*^{8}-((4'-(2-propylhydrazinecarbonyl)-[1,1'-biphenyl]-4-yl)methyl)octanediamide 250f (PSP36):



White solid (53 mg, yield: 33%).

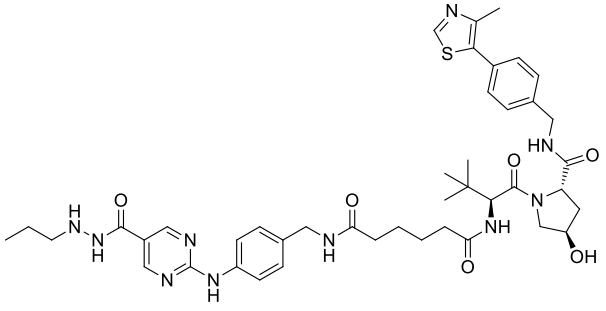
^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.07 (s, 1H), 8.96 (s, 1H), 8.55 (t, *J* = 5.7 Hz, 1H), 8.34 (t, *J* = 5.5 Hz, 1H), 8.00 – 7.62 (m, 7H), 7.36 (dt, *J* = 12.7, 8.1 Hz, 6H), 5.13 (d, *J* = 2.9 Hz, 1H), 4.64 – 4.08 (m, 8H), 3.64 (s, 2H), 2.84 – 2.66 (m, 2H), 2.42 (s, 3H), 2.27 – 2.19 (m, 1H), 2.10 (dt, *J* = 13.9, 6.1 Hz, 3H), 2.01 (d, *J* = 8.0 Hz, 1H), 1.93 – 1.85 (m, 1H), 1.46 (dd, *J* = 14.3, 7.1 Hz, 6H), 1.24 (s, 4H), 1.10 – 0.79 (m, 12H).

^{13}C NMR (101 MHz, DMSO-d$_6$) δ 172.64, 172.54, 172.39, 170.16, 165.34, 151.86, 148.15, 142.96, 140.17, 139.94, 138.02, 132.34, 131.60, 130.37, 130.07, 129.06, 128.27, 128.13, 127.86, 127.15, 126.78, 69.31, 65.34, 59.14, 56.74, 53.54, 42.13, 38.39, 35.82, 35.64, 35.34, 28.94, 26.83, 25.83, 25.71, 21.29, 16.38, 15.60, 12.10.

HRMS calculated for $C_{47}H_{62}N_7O_6S^+$ (M+H): 852.4477, found: 852.4473.

HPLC: rt = 13.38 min (93.54 %).

***N*^{1}-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-*N*^{6}-(4-((5-(2-propylhydrazinecarbonyl)pyrimidin-2-yl)amino)benzyl)adipamide 255a (PSP78)**:



White solid (57 mg, yield: 15%).

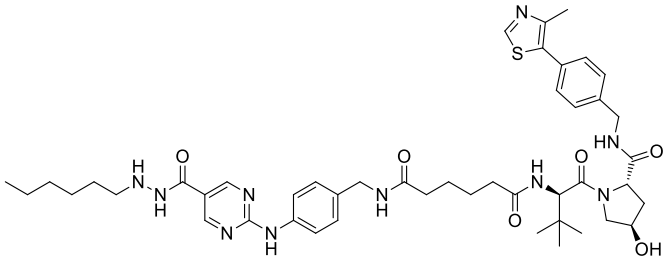
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (d, *J* = 22.7 Hz, 2H), 8.95 (s, 1H), 8.81 (s, 2H), 8.53 (t, *J* = 6.0 Hz, 1H), 8.20 (t, *J* = 5.8 Hz, 1H), 7.82 (d, *J* = 9.3 Hz, 1H), 7.66 (d, *J* = 8.5 Hz, 2H), 7.38 (q, *J* = 8.3 Hz, 4H), 7.16 (d, *J* = 8.5 Hz, 2H), 5.11 (d, *J* = 3.6 Hz, 1H), 4.53 (d, *J* = 9.4 Hz, 1H), 4.41 (dd, *J* = 14.9, 7.0 Hz, 2H), 4.23 (ddd, *J* = 29.3, 16.7, 6.4 Hz, 5H), 3.69 – 3.60 (m, 2H), 2.73 (t, *J* = 7.1 Hz, 2H), 2.42 (s, 3H), 2.25 (dd, *J* = 13.1, 7.2 Hz, 1H), 2.11 (t, *J* = 6.6 Hz, 3H), 2.05 – 1.99 (m, 1H), 1.89 (ddd, *J* = 12.8, 8.6, 4.5 Hz, 1H), 1.60 – 1.29 (m, 8H), 0.90 (dd, *J* = 14.4, 7.0 Hz, 12H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.39, 172.38, 172.36, 170.16, 163.10, 161.14, 157.72, 151.83, 148.14, 139.92, 138.68, 134.06, 131.59, 130.08, 129.06, 127.92, 127.86, 120.04, 117.69, 69.33, 59.15, 56.77, 55.32, 53.56, 42.11, 38.37, 35.65, 35.18, 26.83, 25.67, 25.53, 21.29, 16.36, 12.07.

HRMS calculated for $C_{43}H_{57}N_{10}O_6S^+$ (M+H): 841.4178, found: 841.418.

HPLC: rt = 12.53 min (97.84 %).

N^1-(4-((5-(2-hexylhydrazinecarbonyl)pyrimidin-2-yl)amino)benzyl)-N^6-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)adipamide 255b (PSP76):



White solid (120 mg, yield: 27%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (d, *J* = 25.6 Hz, 2H), 8.95 (s, 1H), 8.81 (s, 2H), 8.53 (t, *J* = 6.0 Hz, 1H), 8.20 (t, *J* = 5.8 Hz, 1H), 7.82 (d, *J* = 9.3 Hz, 1H), 7.68 – 7.62 (m, 2H), 7.42 – 7.34 (m, 4H), 7.16 (d, *J* = 8.6 Hz, 2H), 5.11 (d, *J* = 3.6 Hz, 1H), 4.54 (t, *J* = 6.3 Hz, 1H), 4.45 – 4.15 (m, 7H), 3.69 – 3.61 (m, 2H), 2.75 (t, *J* = 7.0 Hz, 2H), 2.42 (s, 3H), 2.25 (dd, *J* = 13.6, 6.8 Hz, 1H), 2.11 (t, *J* = 6.8 Hz, 3H), 2.05 – 1.99 (m, 1H), 1.89 (ddd, *J* = 12.9, 8.5, 4.6 Hz, 1H), 1.55 – 1.17 (m, 14H), 0.94 – 0.82 (m,

12H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.39, 172.37, 172.35, 170.15, 163.08, 161.14, 157.71, 151.84, 148.15, 139.93, 138.67, 134.07, 131.59, 130.08, 129.06, 127.92, 127.86, 120.04, 117.68, 69.32, 59.14, 56.76, 51.69, 42.11, 38.37, 35.66, 35.18, 31.65, 28.04, 26.83, 26.77, 25.67, 25.53, 22.53, 16.37, 14.37.

HRMS calculated for $C_{46}H_{63}N_{10}O_6S^+$ (M+H): 883.4647, found: 883.465.

HPLC: rt = 14.06 min (97.63 %).

General procedure for the synthesis of *6-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)-N-(4-(((5-(2-propylhydrazinecarbonyl)pyrimidin-2-yl)amino)methyl)benzyl)hex-5-ynamide 259a (PSP79)* and *6-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)-N-(4-(((5-(2-hexylhydrazinecarbonyl)pyrimidin-2-yl)amino)methyl)benzyl)hex-5-ynamide 259b (PSP80)*:

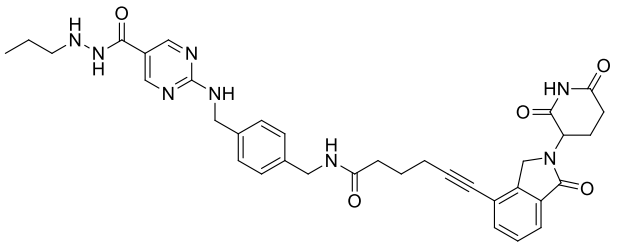
Tert-butyl hex-5-ynoate: TFAA (8.24 g, 5.5 mL, 39.25 mmol, 2.2 eq) was added dropwise to a stirred solution of hex-5-ynoic acid (***256***) (2.0 g, 17.84 mmol, 1.0 eq) in anhydrous THF under ice bath. The resulting reaction was stirred for 3.5 h. Then, tert-Butanol (4.63 g, 5.93 mL, 62.44 mmol, 3.5 eq) was added to the reaction and the reaction was increased to room temperature naturally and the stirring was continued overnight. Cold water was then poured to the reaction. The reaction was extracted with ether three times. The combined organic layer was dried over Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 0 : 1 to 1 : 40 gradually) to give tert-butyl hex-5-ynoate as light yellow oil (3.0 g, yield: 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (t, *J* = 7.4 Hz, 2H), 2.25 (td, *J* = 7.0, 2.7 Hz, 2H), 1.96 (t, *J* = 2.7 Hz, 1H), 1.82 (dd, *J* = 14.5, 7.2 Hz, 2H), 1.45 (s, 9H).

Tert-butyl 6-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)hex-5-ynoate (257): A stirred mixture of tert-butyl hex-5-ynoate (0.86 g, 5.11 mmol, 2.0 eq), intermediate ***147*** (0.83 g, 2.57 mmol, 1.0 eq), $Pd(PPh_3)_4Cl_2$ (0.18 g, 0.26 mmol, 0.1 eq), CuI (98 mg, 0.51 mmol, 0.2 eq) and TEA 6.8 mL in DMF (14 mL) was kept under argon

atmosphere and heated to 90 °C for 3 h. Then, the mixture was extracted with EtOAc and water twice. The combined organic layers were washed with brine twice, dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (EtOAc : heptane = 0 : 1, 1 : 1 and then DCM : MeOH = 1 : 0, 40 : 1, 30 : 1 gradually) to give intermediate ***257*** as gray solid (0.99 g yield: 94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.97 (s, 1H), 7.71 – 7.67 (m, 1H), 7.63 (dd, *J* = 7.6, 0.9 Hz, 1H), 7.50 (t, *J* = 7.6 Hz, 1H), 5.13 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.44 (d, *J* = 17.7 Hz, 1H), 4.29 (d, *J* = 17.7 Hz, 1H), 2.96 – 2.89 (m, 1H), 2.62 – 2.50 (m, 3H), 2.43 (dd, *J* = 12.9, 4.4 Hz, 1H), 2.35 (t, *J* = 7.4 Hz, 2H), 2.04 – 1.97 (m, 1H), 1.77 (p, *J* = 7.2 Hz, 2H), 1.38 (s, 9H).

The final compounds ***259a*** and ***259b*** were synthesized according to the procedure of ***221*** to ***224*** using ***257.***

6-(2-(2,6-Dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)-*N*-(4-(((5-(2-propylhydrazine carbonyl)pyrimidin-2-yl)amino)methyl)benzyl)hex-5-ynamide 259a (PSP79):



White solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 9.77 (s, 1H), 8.63 (s, 2H), 8.30 (t, *J* = 5.9 Hz, 1H), 8.23 (t, *J* = 6.3 Hz, 1H), 7.69 (dd, *J* = 7.6, 0.9 Hz, 1H), 7.62 (dd, *J* = 7.6, 1.0 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.18 (dd, *J* = 24.5, 8.2 Hz, 4H), 5.08 (dt, *J* = 35.2, 17.6 Hz, 2H), 4.47 (dd, *J* = 18.0, 12.1 Hz, 3H), 4.30 (d, *J* = 17.7 Hz, 1H), 4.20 (d, *J* = 5.8 Hz, 2H), 2.88 (ddd, *J* = 17.3, 13.6, 5.4 Hz, 1H), 2.68 (dd, *J* = 14.4, 7.3 Hz, 2H), 2.55 (ddd, *J* = 8.5, 4.3, 2.2 Hz, 1H), 2.47 – 2.34 (m, 3H), 2.29 (t, *J* = 7.4 Hz, 2H), 1.98 (dtd, *J* = 12.6, 5.2, 2.2 Hz, 1H), 1.80 (p, *J* = 7.1 Hz, 2H), 1.47 – 1.37 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H).

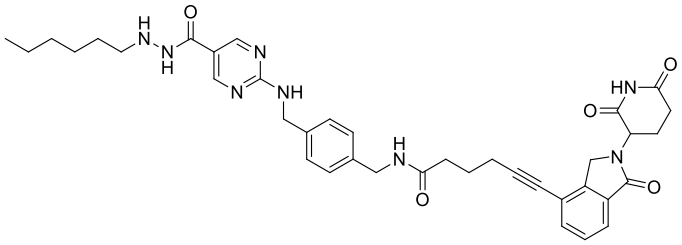
^{13}C NMR (101 MHz, DMSO-d_6) δ 173.26, 171.80, 171.37, 168.09, 163.50, 163.27,

144.24, 138.62, 138.43, 134.55, 132.41, 128.97, 127.58, 127.42, 123.06, 119.21, 115.96, 96.19, 77.15, 55.34, 52.09, 47.45, 44.17, 42.25, 34.58, 31.64, 24.74, 22.77, 21.28, 18.91, 12.08.

HRMS calculated for $C_{35}H_{39}N_8O_5^+$ (M+H): 651.3038, found: 651.304.

HPLC: rt = 11.26 min (95.92 %).

6-(2-(2,6-Dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)-*N*-(4-(((5-(2-hexylhydrazinecarbonyl)pyrimidin-2-yl)amino)methyl)benzyl)hex-5-ynamide 259b (PSP80):



White solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 9.77 (s, 1H), 8.63 (s, 2H), 8.27 (dt, *J* = 28.9, 6.1 Hz, 2H), 7.65 (dd, *J* = 25.4, 7.3 Hz, 2H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.18 (dd, *J* = 24.4, 8.1 Hz, 4H), 5.10 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.98 (s, 1H), 4.47 (dd, *J* = 18.1, 12.0 Hz, 3H), 4.30 (d, *J* = 17.8 Hz, 1H), 4.20 (d, *J* = 5.8 Hz, 2H), 2.93 – 2.83 (m, 1H), 2.72 (t, *J* = 6.9 Hz, 2H), 2.55 (d, *J* = 17.8 Hz, 1H), 2.47 – 2.36 (m, 3H), 2.29 (t, *J* = 7.3 Hz, 2H), 2.01 – 1.94 (m, 1H), 1.79 (dd, *J* = 14.8, 7.5 Hz, 2H), 1.40 (dt, *J* = 13.8, 6.9 Hz, 2H), 1.33 – 1.19 (m, 6H), 0.84 (t, *J* = 6.7 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.26, 171.79, 171.37, 168.08, 163.49, 163.27, 144.24, 138.62, 138.43, 134.55, 132.41, 128.97, 127.58, 127.42, 123.06, 119.21, 115.96, 96.19, 77.15, 55.34, 52.09, 51.71, 47.44, 44.17, 42.25, 34.58, 31.64, 28.03, 26.76, 24.74, 22.77, 22.52, 18.91, 14.37.

HRMS calculated for $C_{38}H_{45}N_8O_5^+$ (M+H): 693.3507, found: 693.351.

HPLC: rt = 13.11 min (95.03 %).

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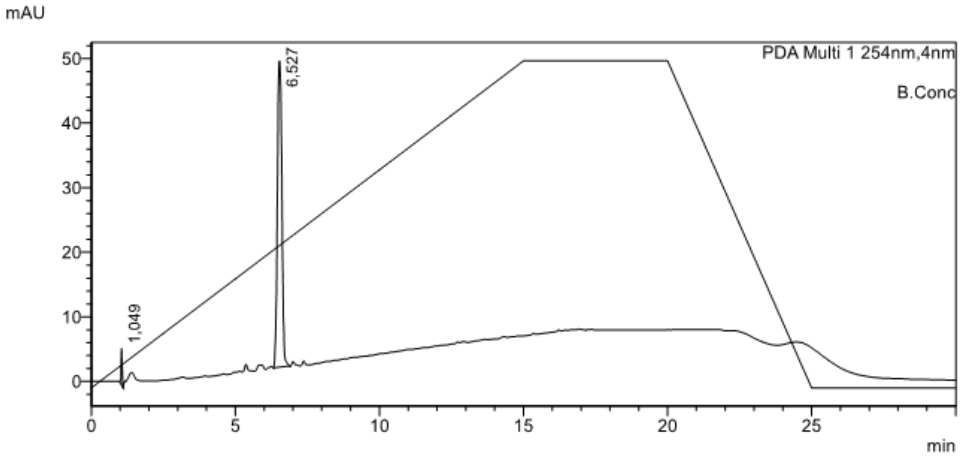
Part 7 Appendix: copies of representative relevant HPLC chromatograms and spectra

32b (PS25)

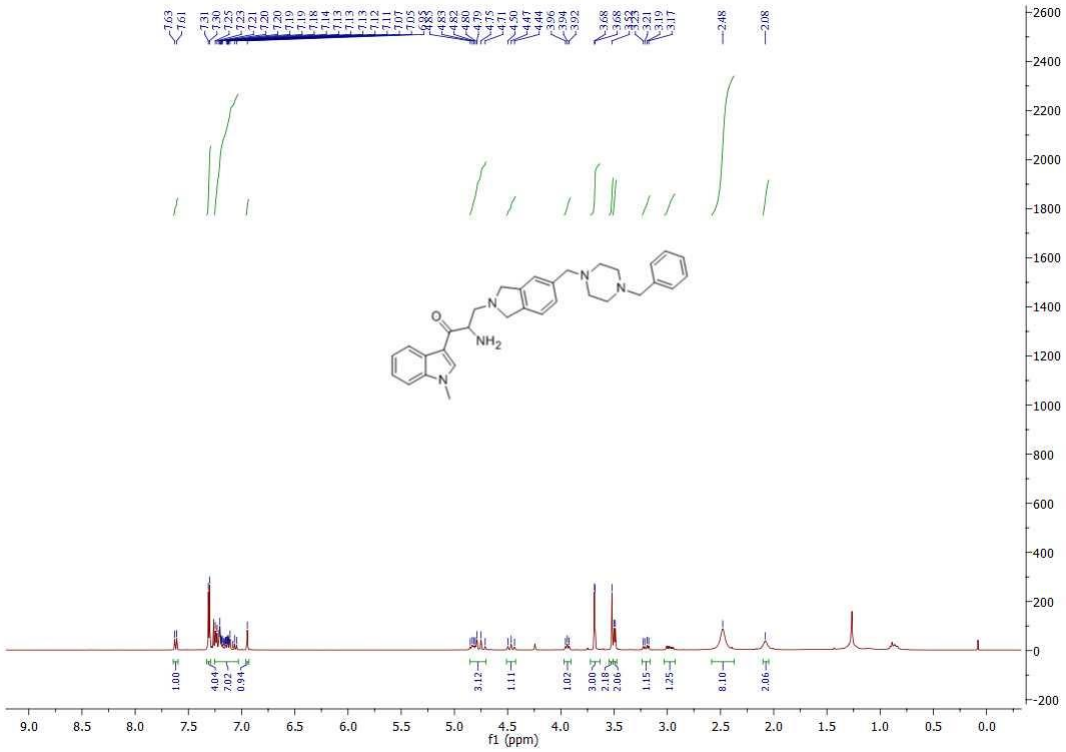
HPLC



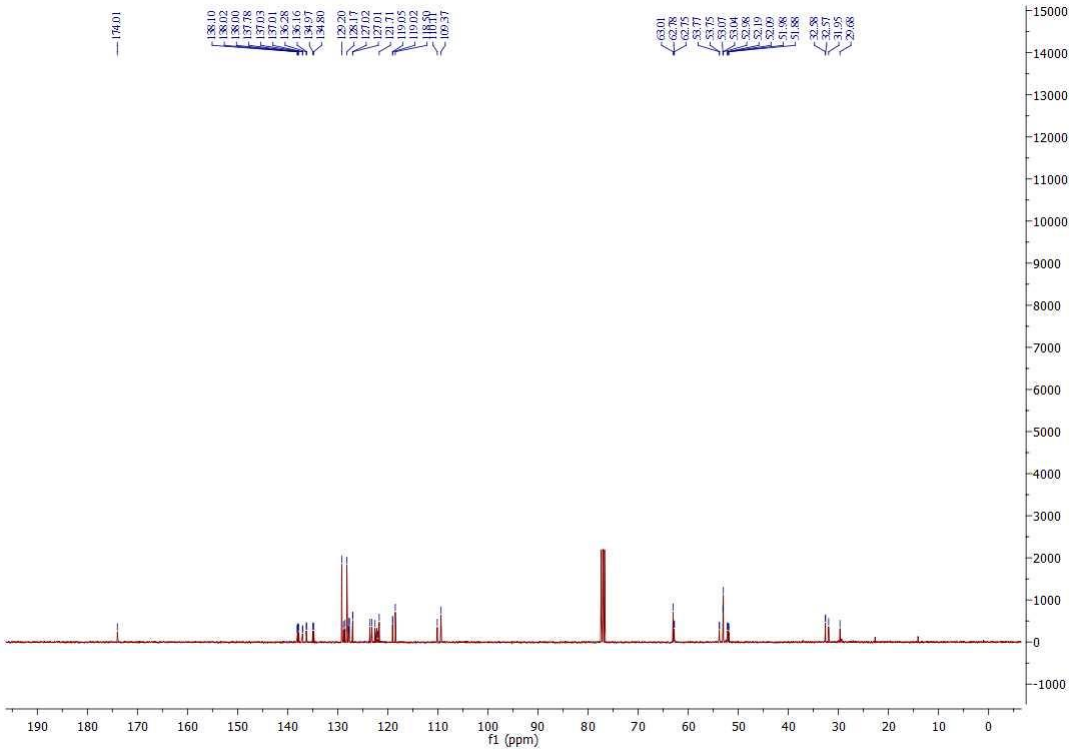
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1,049	13730	5729	2,602
2	6,527	513895	47410	97,398
Total		527625	53139	100,000

^{1}H-NMR

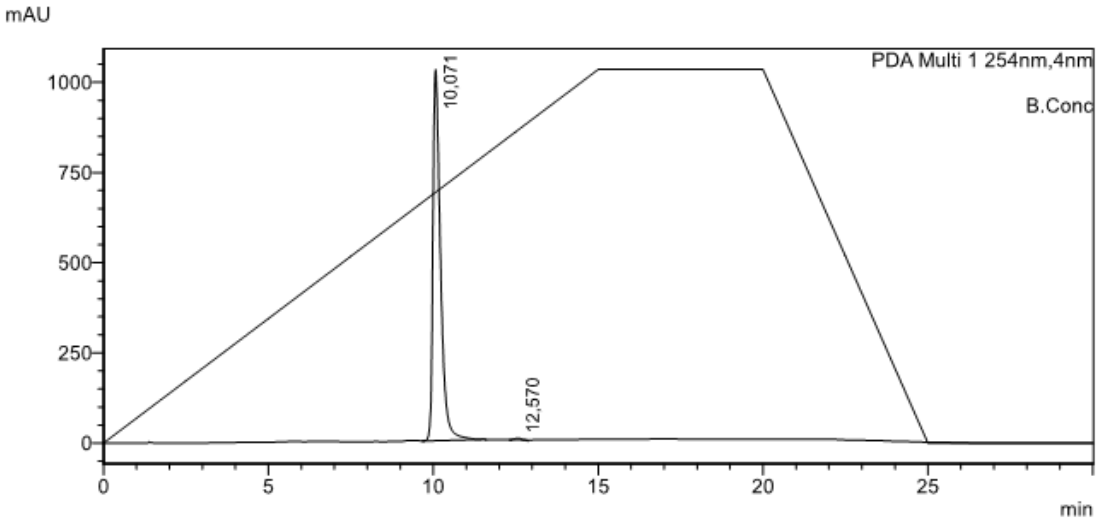


^{13}C-NMR



52j (PS57)

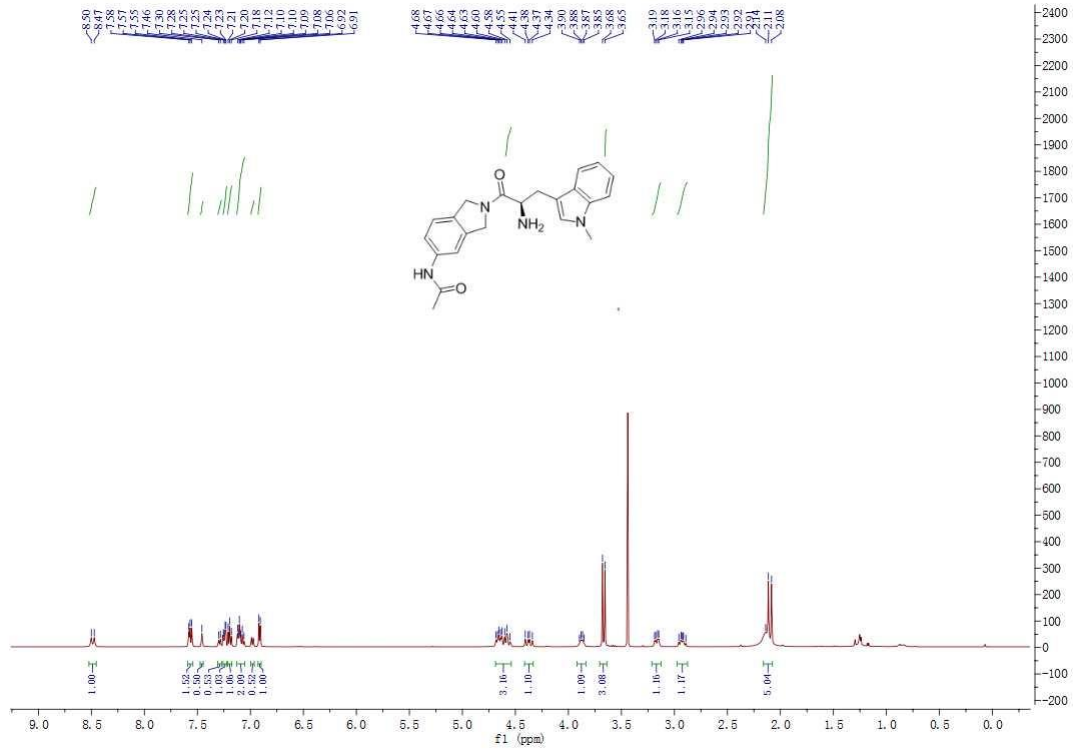
HPLC



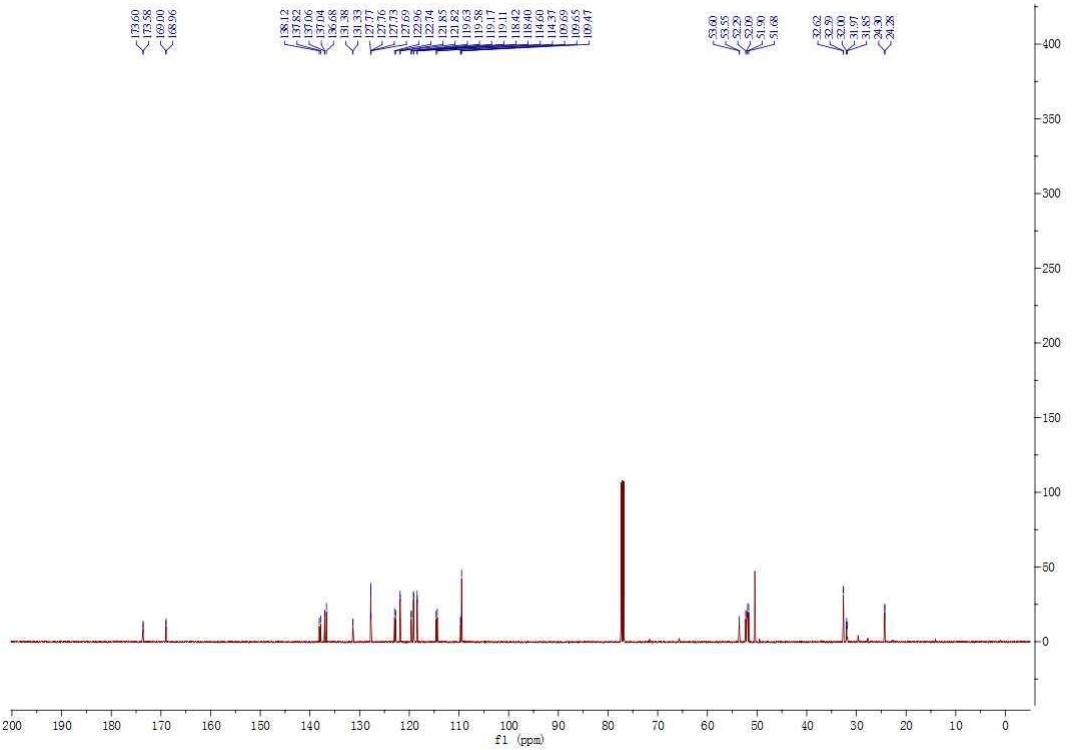
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	10,071	16485732	1028844	99,651
2	12,570	57746	4426	0,349
Total		16543478	1033270	100,000

^{1}H-NMR

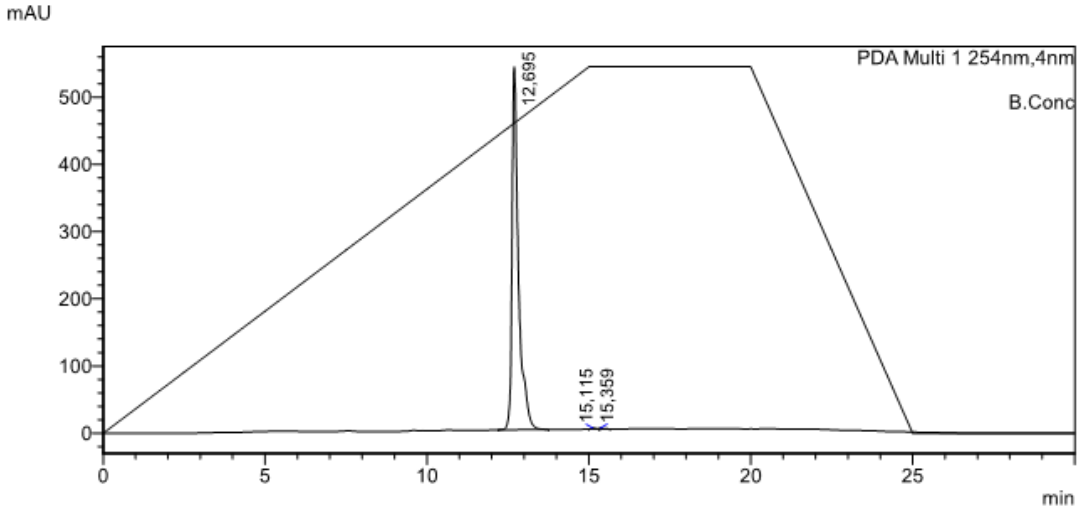


^{13}C-NMR



80d (PSP43)

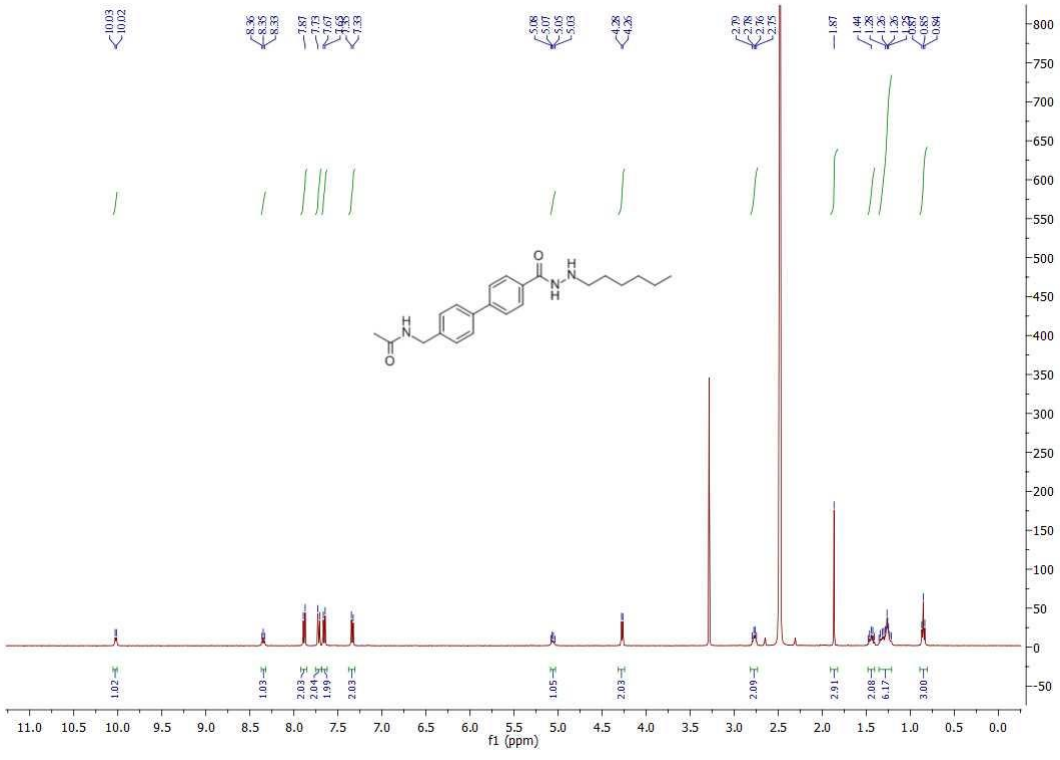
HPLC



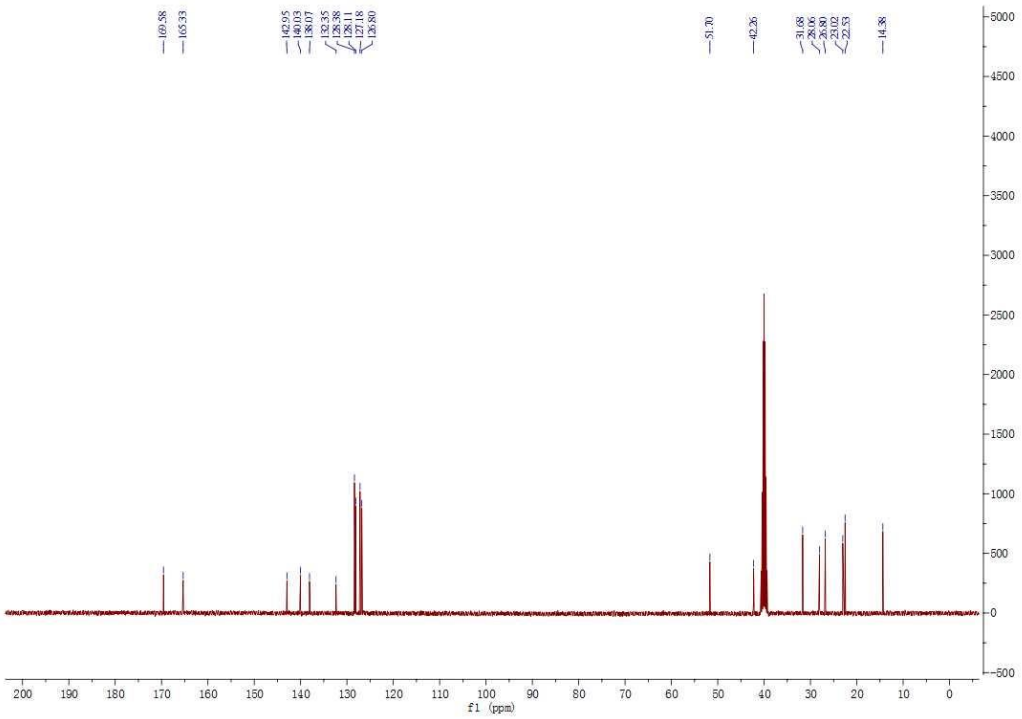
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	12,695	7537279	539820	99,608
2	15,115	16291	1447	0,215
3	15,359	13341	1444	0,176
Total		7566911	542711	100,000

^{1}H-NMR

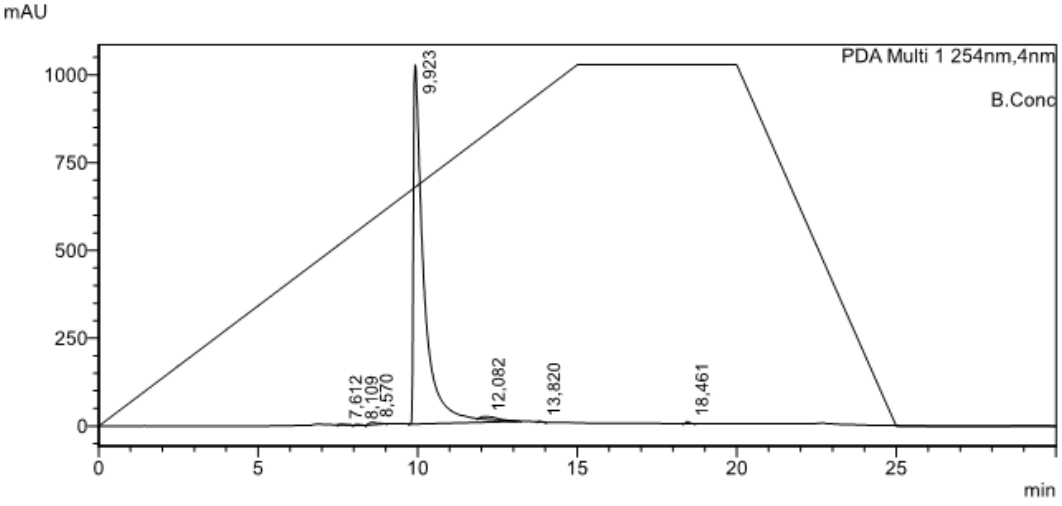


^{13}C-NMR



105c (PSP50)

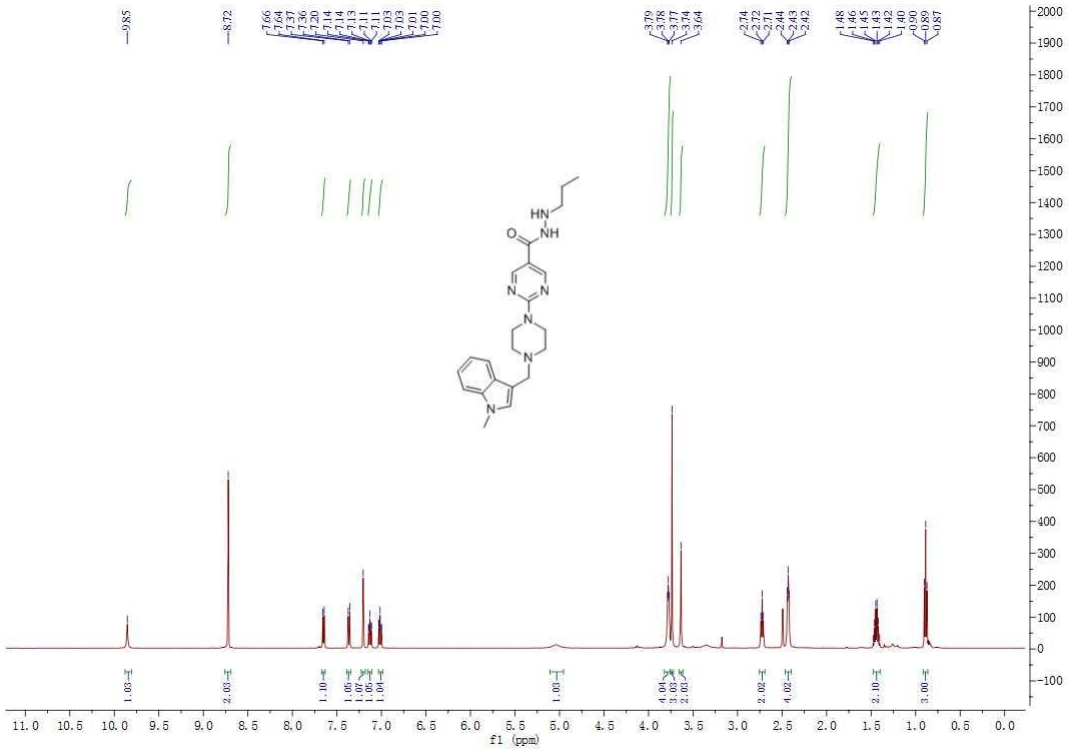
HPLC



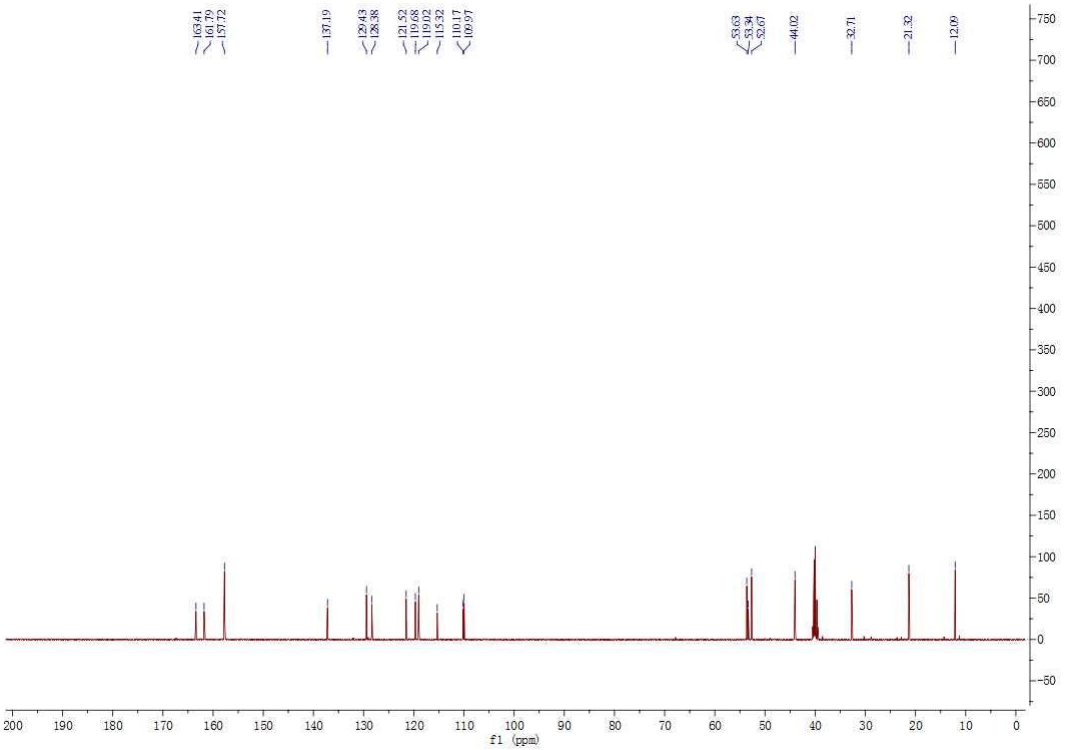
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7,612	24871	2408	0,104
2	8,109	15013	1502	0,063
3	8,570	116696	6198	0,489
4	9,923	23382862	1022472	98,038
5	12,082	256357	7461	1,075
6	13,820	14490	1930	0,061
7	18,461	40631	5760	0,170
Total		23850920	1047731	100,000

^{1}H-NMR

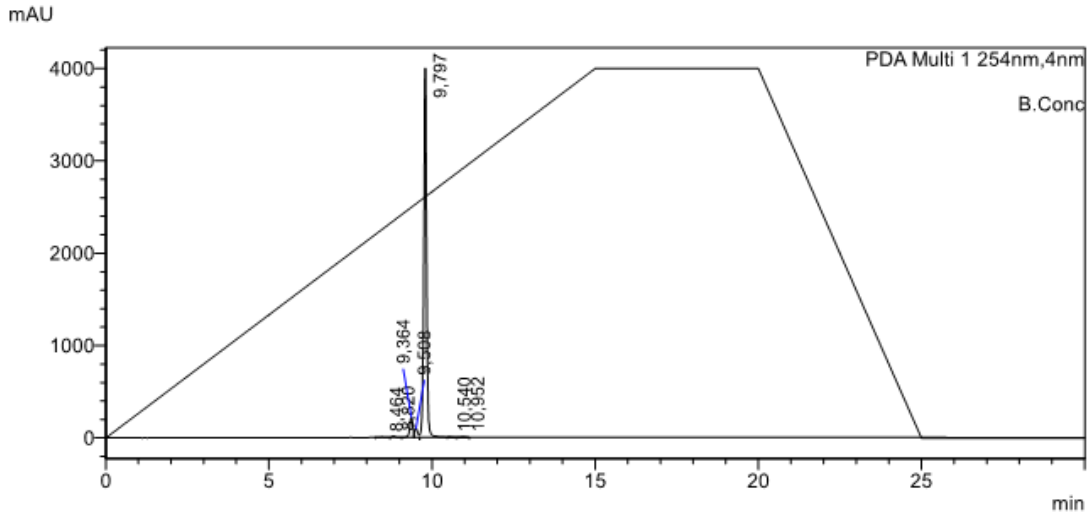


^{13}C-NMR



218b (PSP15)

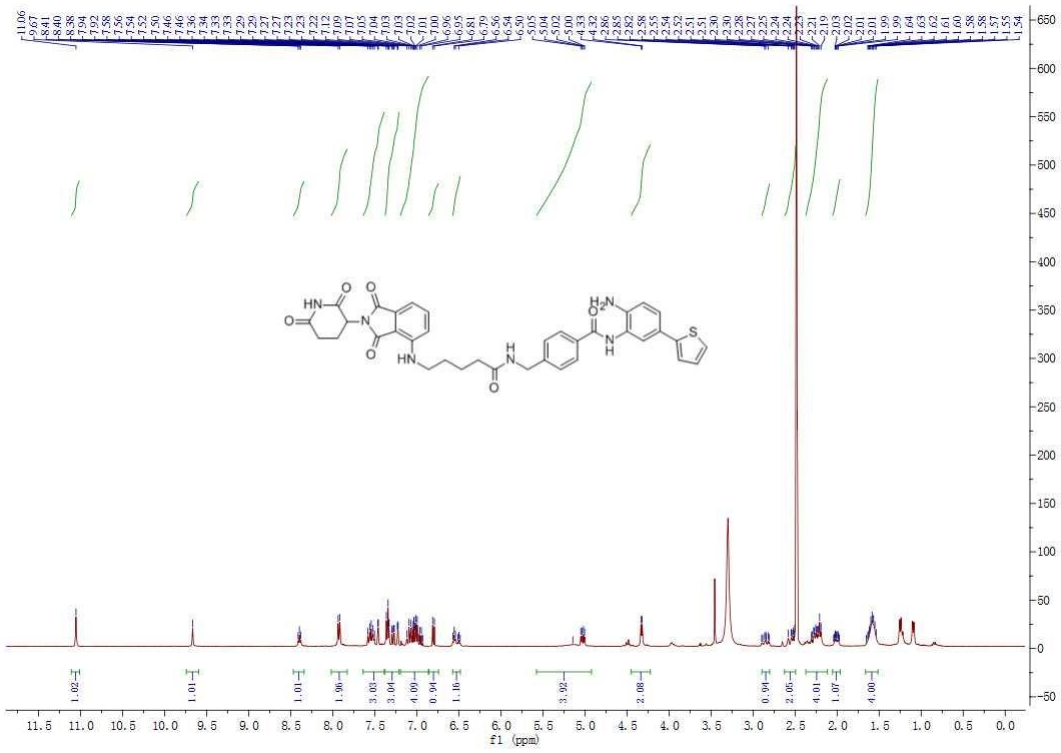
HPLC



PDA Ch1 254nm

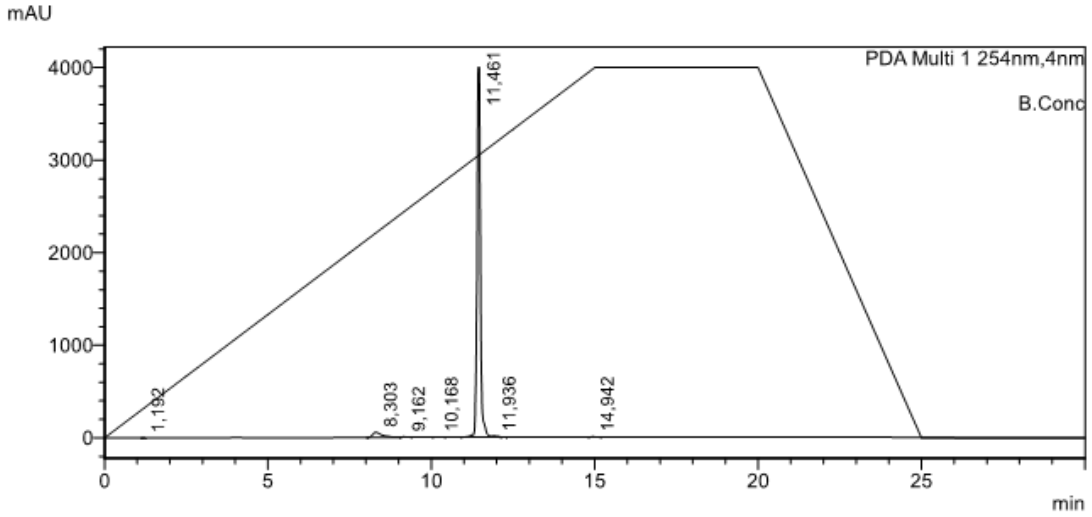
Peak#	Ret. Time	Area	Height	Area%
1	8,464	90601	5769	0,343
2	8,820	141654	18222	0,536
3	9,364	1500453	220280	5,675
4	9,508	599028	96993	2,266
5	9,797	24054114	3995544	90,974
6	10,540	13143	1916	0,050
7	10,952	41700	6530	0,158
Total		26440693	4345254	100,000

^{1}H-NMR



229a (PSP21)

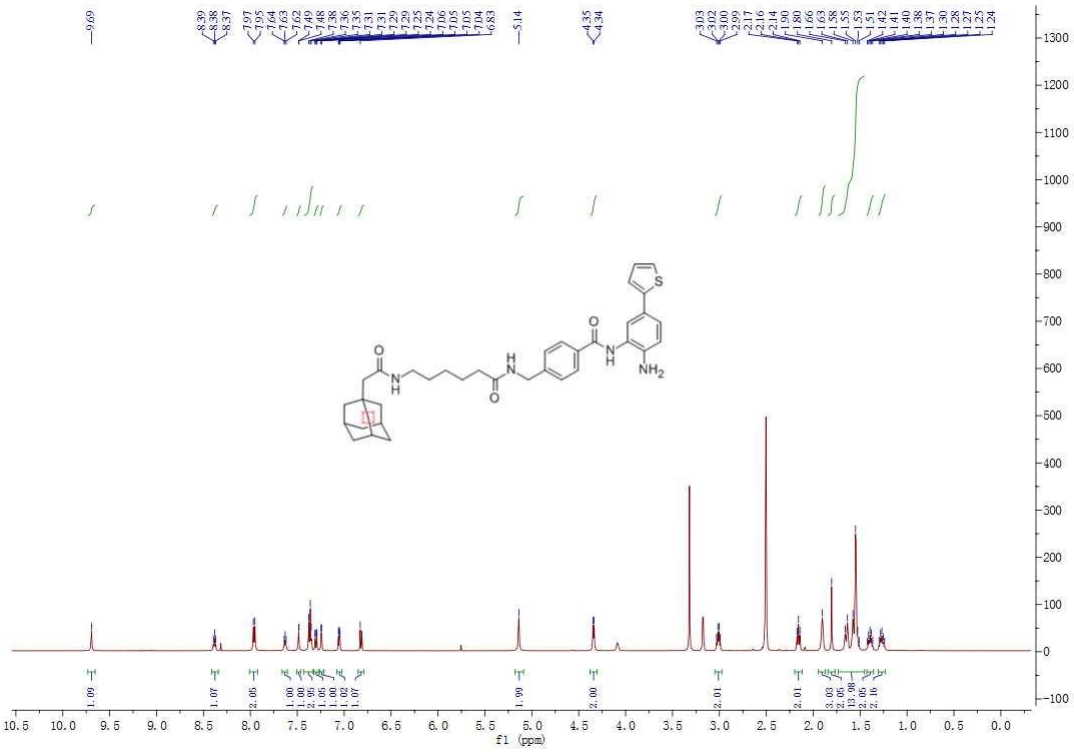
HPLC



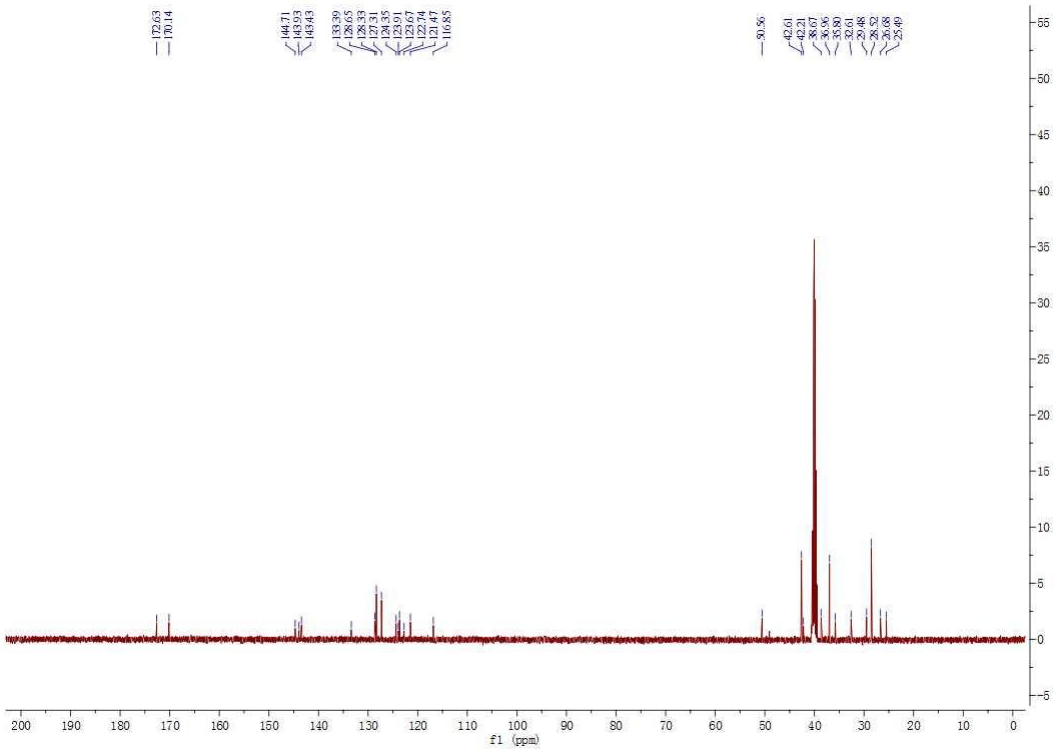
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1,192	6289	2369	0,021
2	8,303	1108591	55303	3,644
3	9,162	52089	6011	0,171
4	10,168	41379	3905	0,136
5	11,461	29184523	3991923	95,921
6	11,936	6158	865	0,020
7	14,942	26628	2895	0,088
Total		30425658	4063271	100,000

^{1}H-NMR

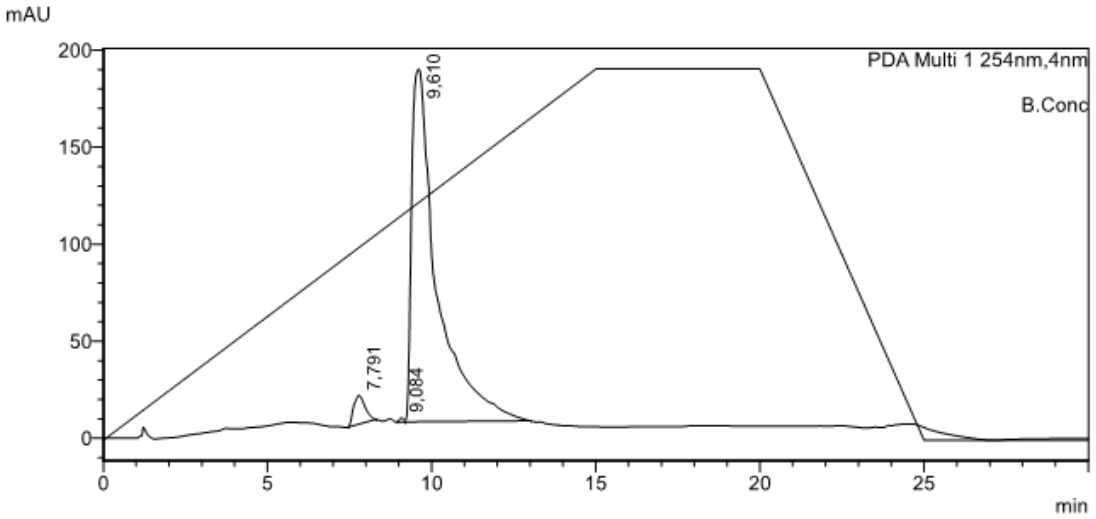


^{13}C-NMR



224a (PSP23)

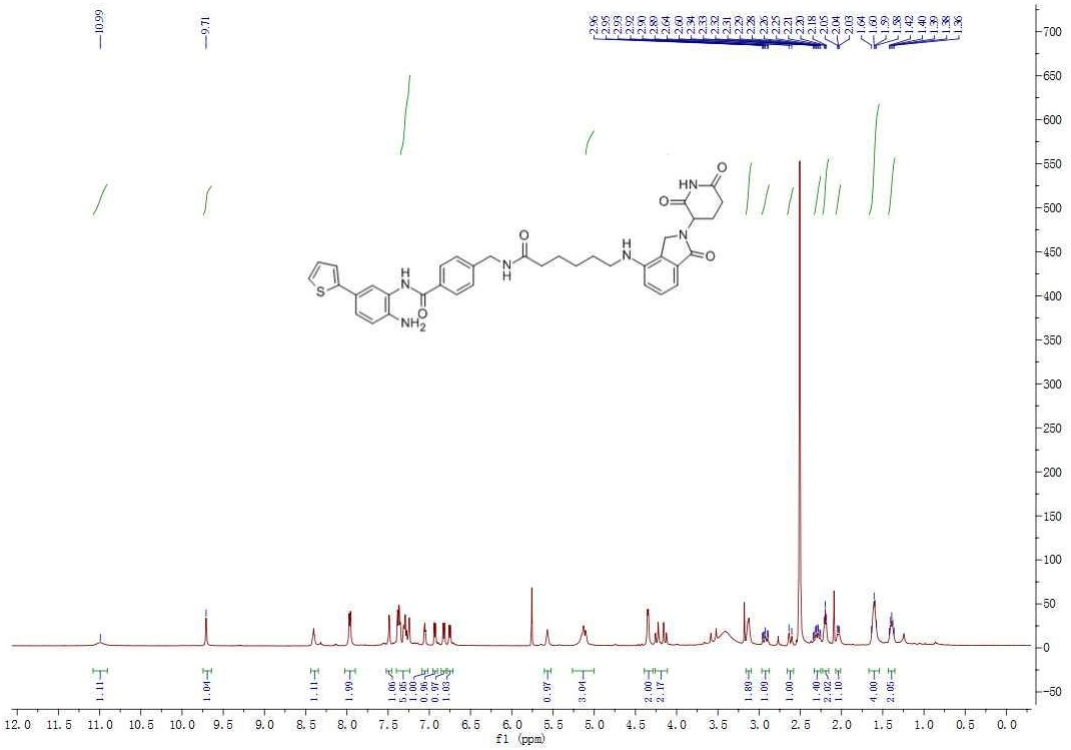
HPLC



PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7,791	360599	14693	3,580
2	9,084	17065	2082	0,169
3	9,610	9694032	181680	96,250
Total		10071696	198455	100,000

^{1}H-NMR



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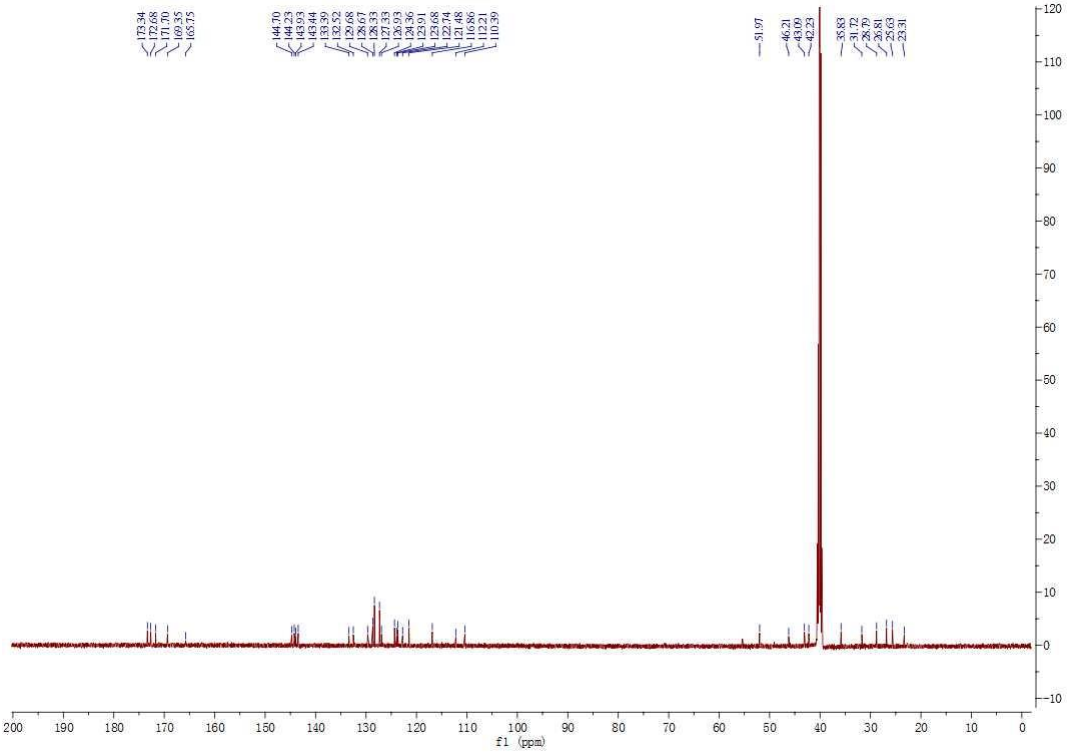
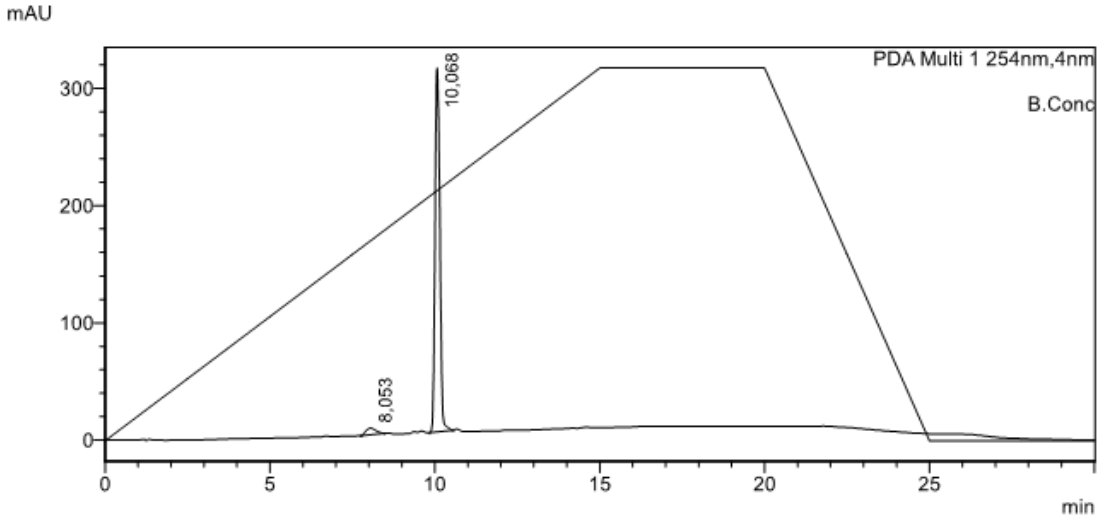


250b (PSP26)

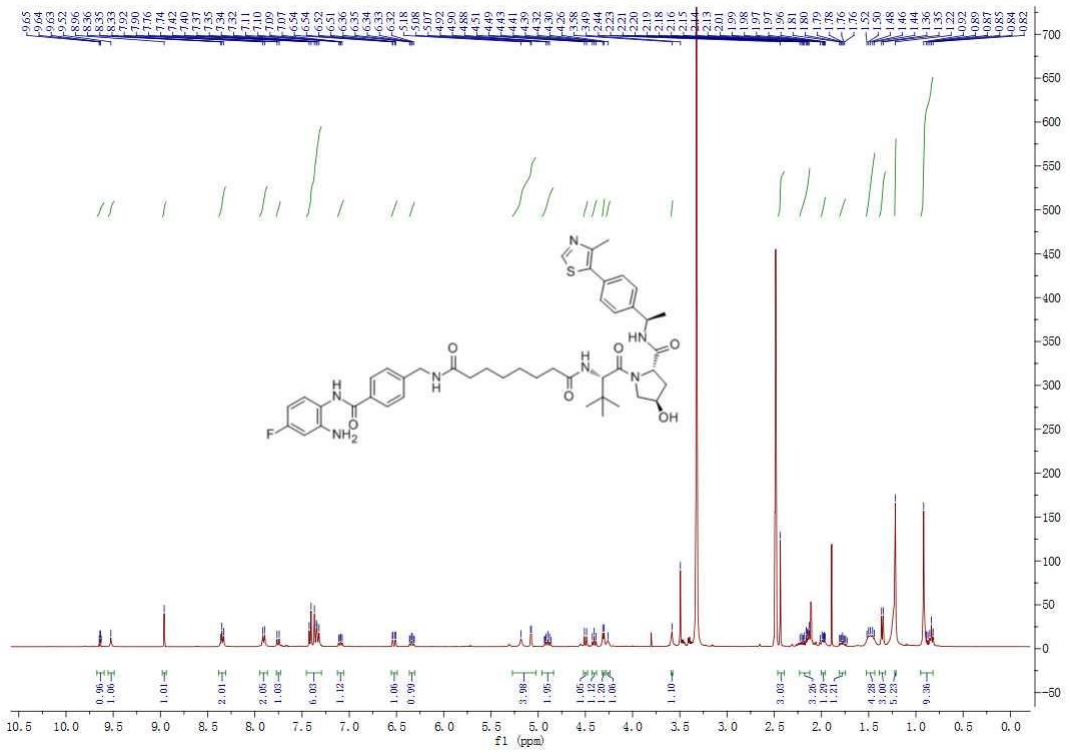
HPLC



PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	8,053	115584	5557	3,505
2	10,068	3181971	310337	96,495
Total		3297555	315894	100,000

^{1}H-NMR



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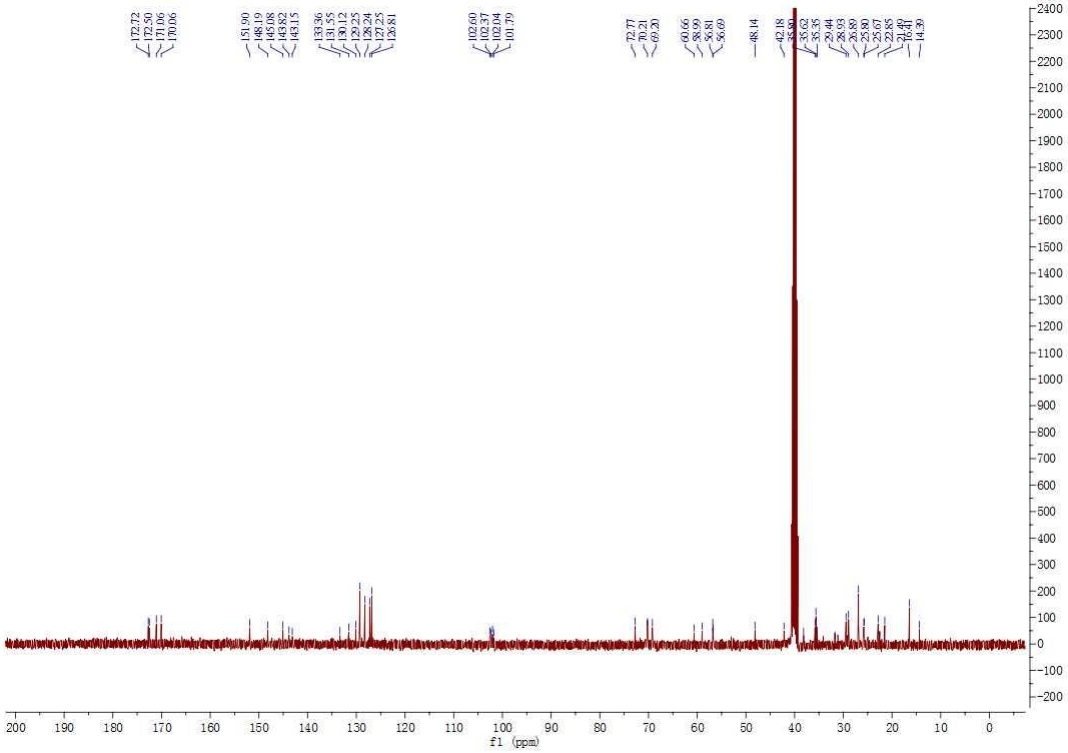
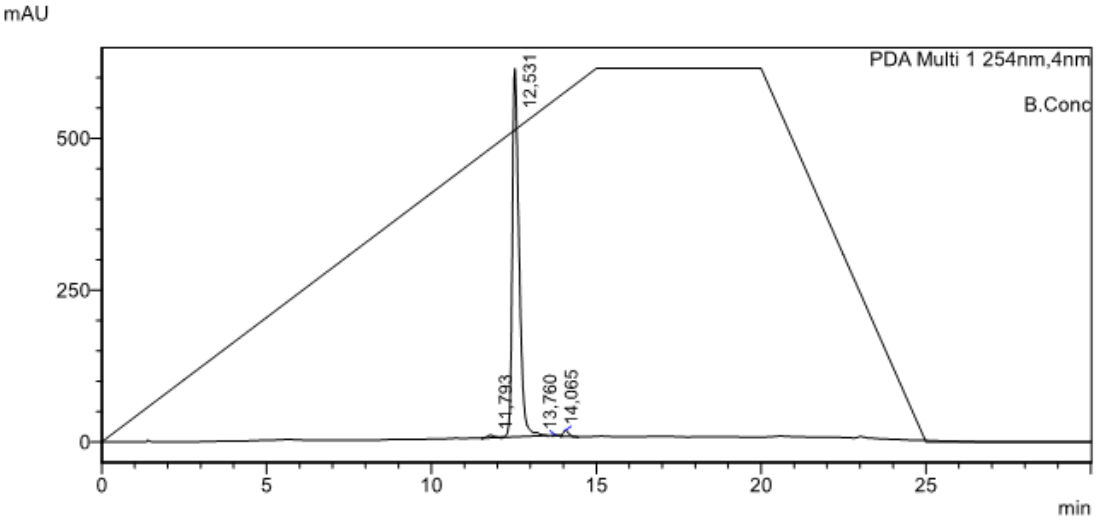


255a (PSP78)

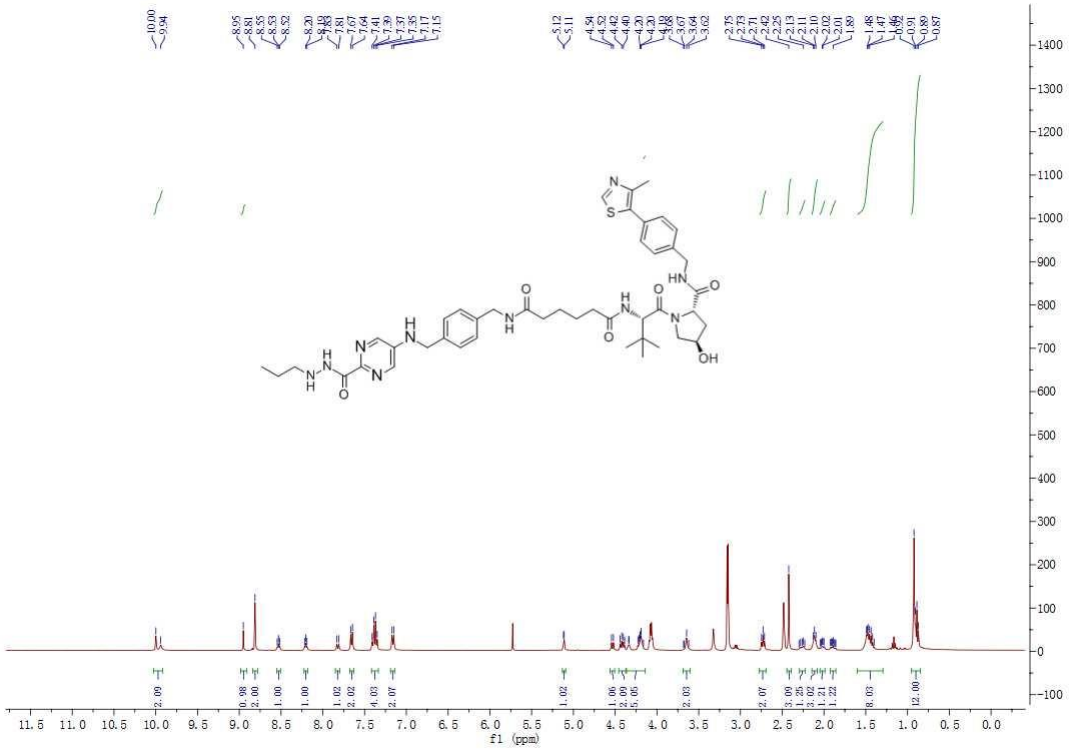
HPLC



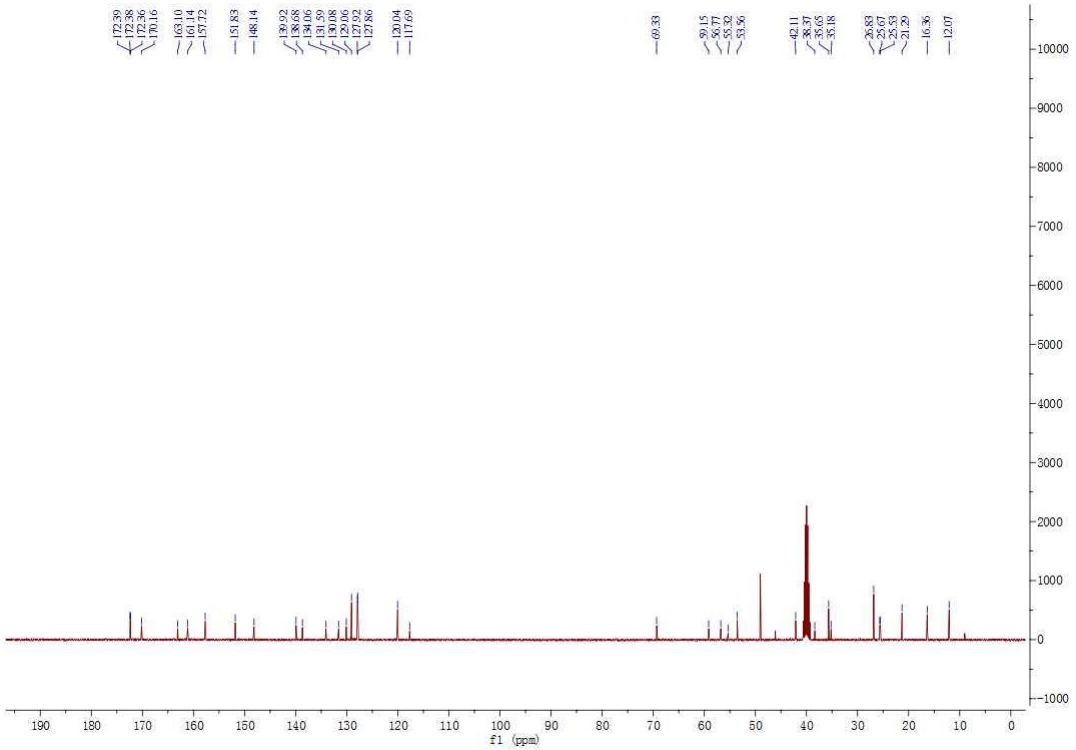
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	11,793	62129	4192	0,703
2	12,531	8648148	606600	97,840
3	13,760	15725	1276	0,178
4	14,065	113092	9943	1,279
Total		8839094	622011	100,000

^{1}H-NMR

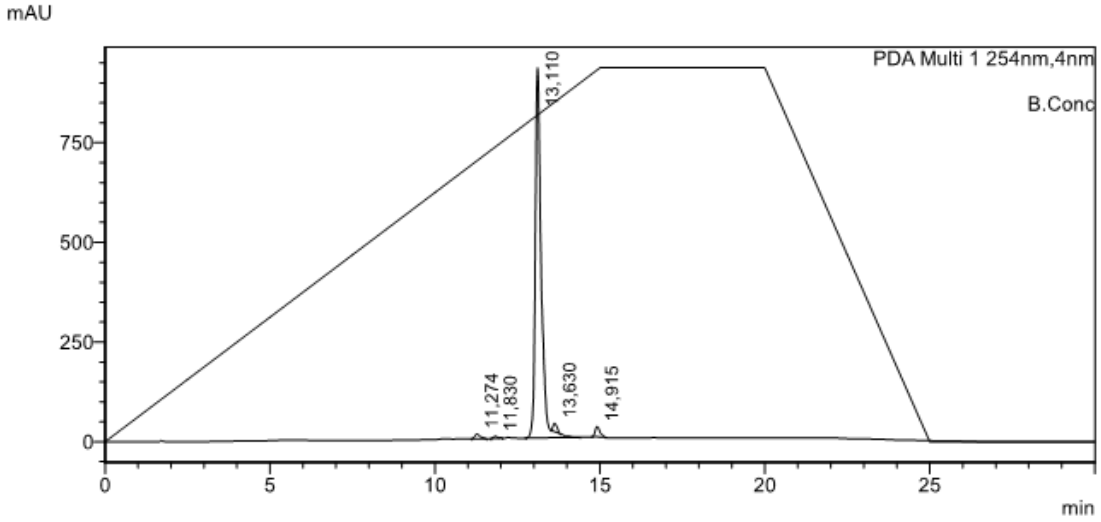


^{13}C-NMR



259b (PSP80)

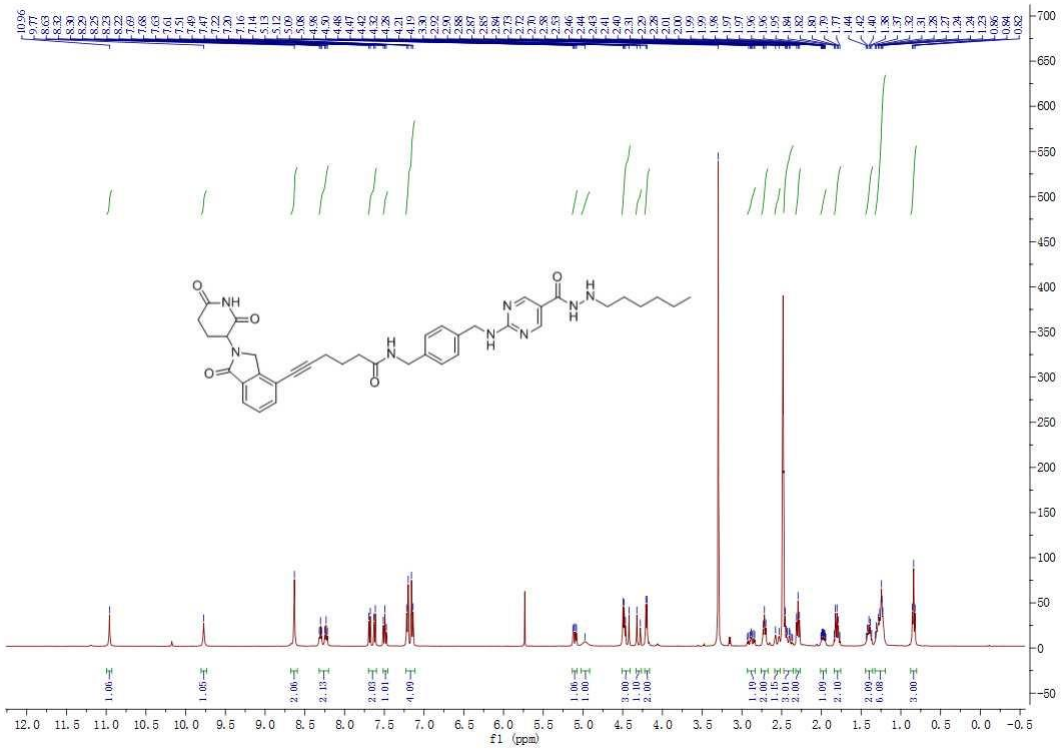
HPLC



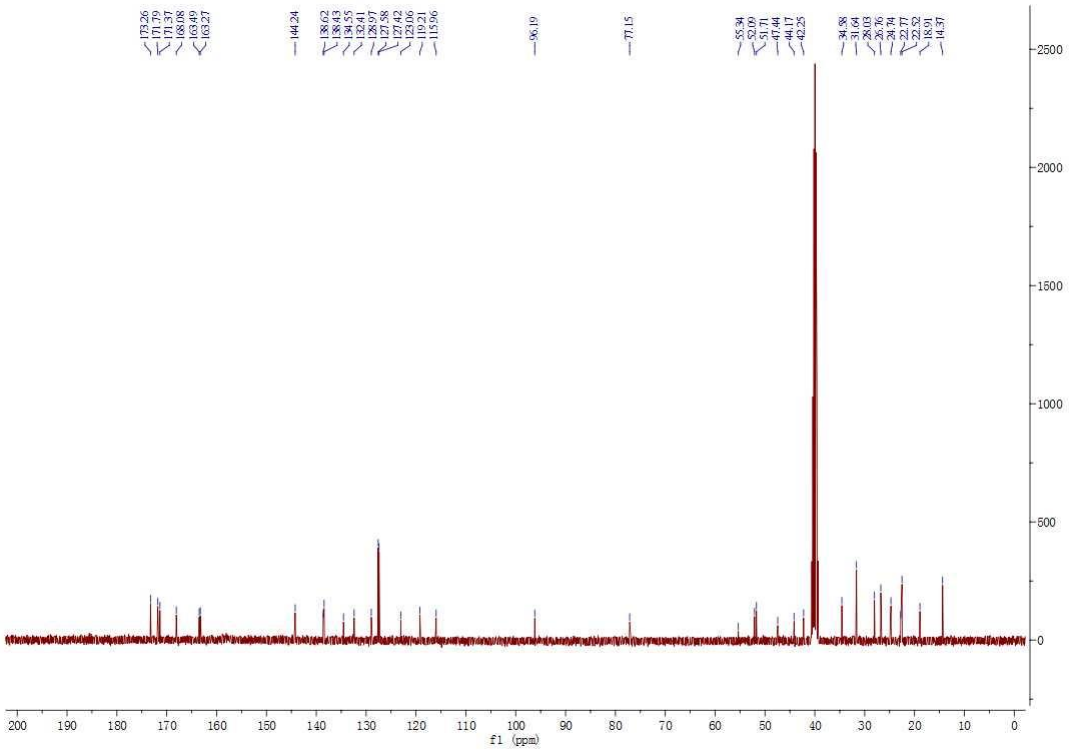
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	11,274	131702	12138	1,073
2	11,830	45264	4768	0,369
3	13,110	11661048	928139	95,027
4	13,630	176316	21064	1,437
5	14,915	256951	25756	2,094
Total		12271281	991866	100,000

^{1}H-NMR

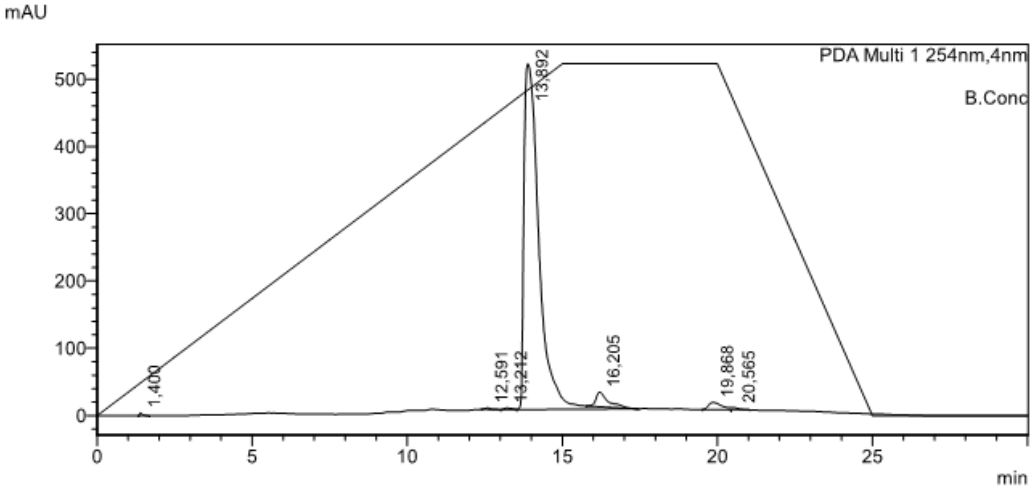


^{13}C-NMR



128 (PSP84)

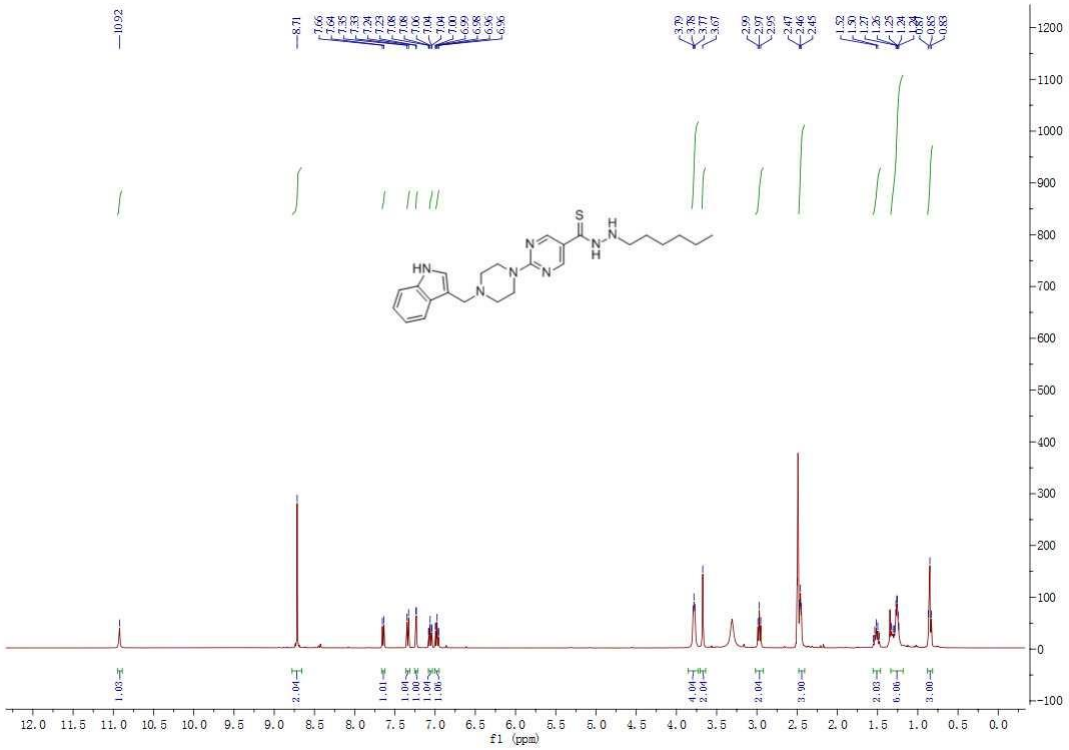
HPLC



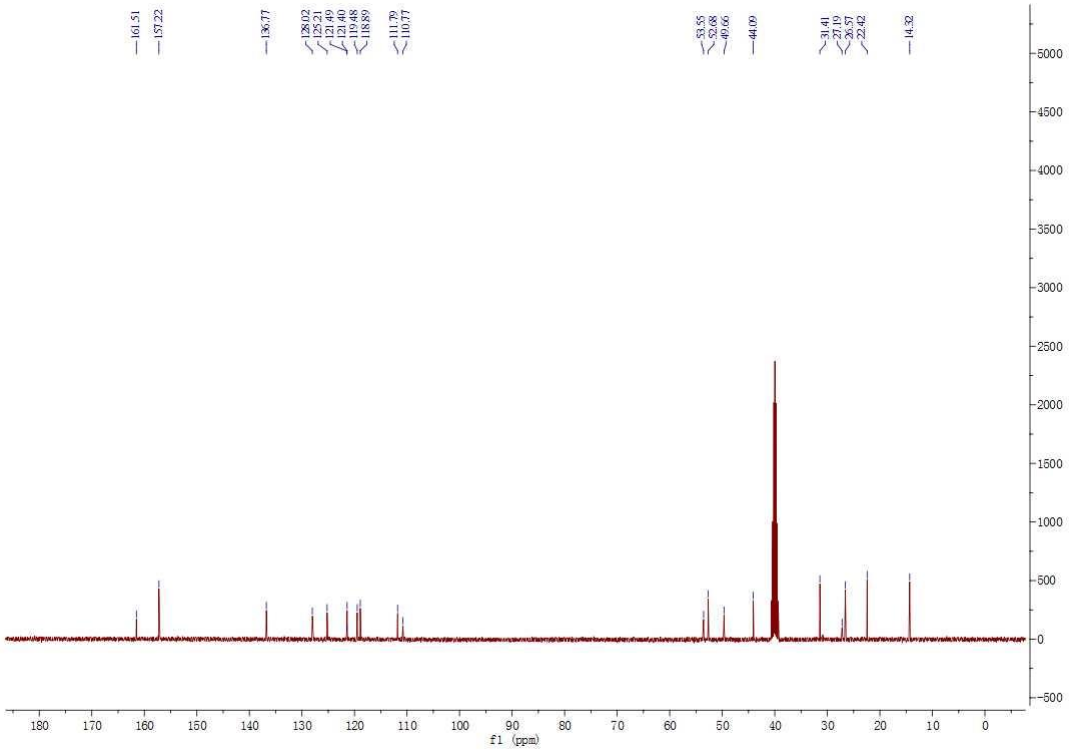
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1,400	20184	4131	0,109
2	12,591	32033	2352	0,174
3	13,212	40785	2339	0,221
4	13,892	17365747	513626	94,199
5	16,205	598154	21649	3,245
6	19,868	321685	10852	1,745
7	20,565	56557	3006	0,307
Total		18435145	557955	100,000

^{1}H-NMR

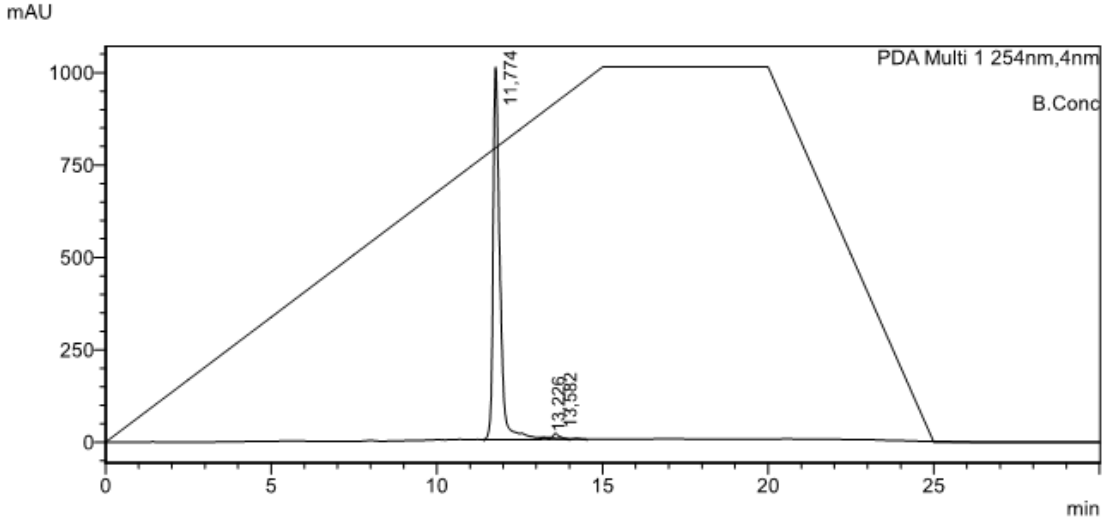


^{13}C-NMR



218k (PSP77)

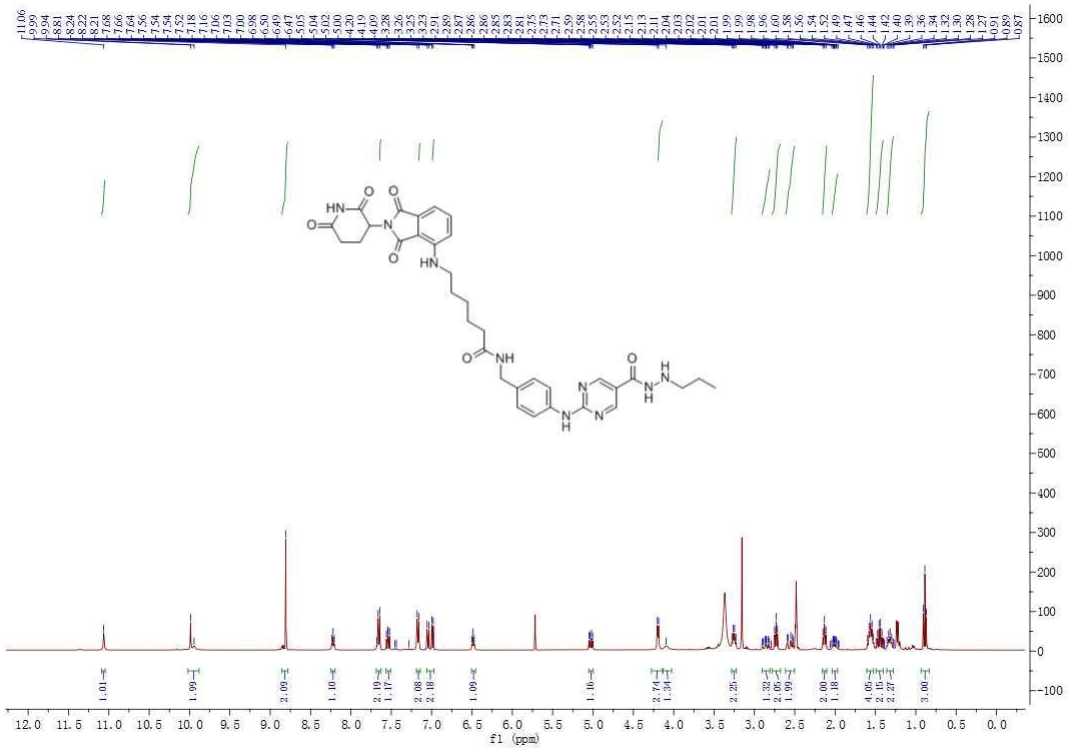
HPLC



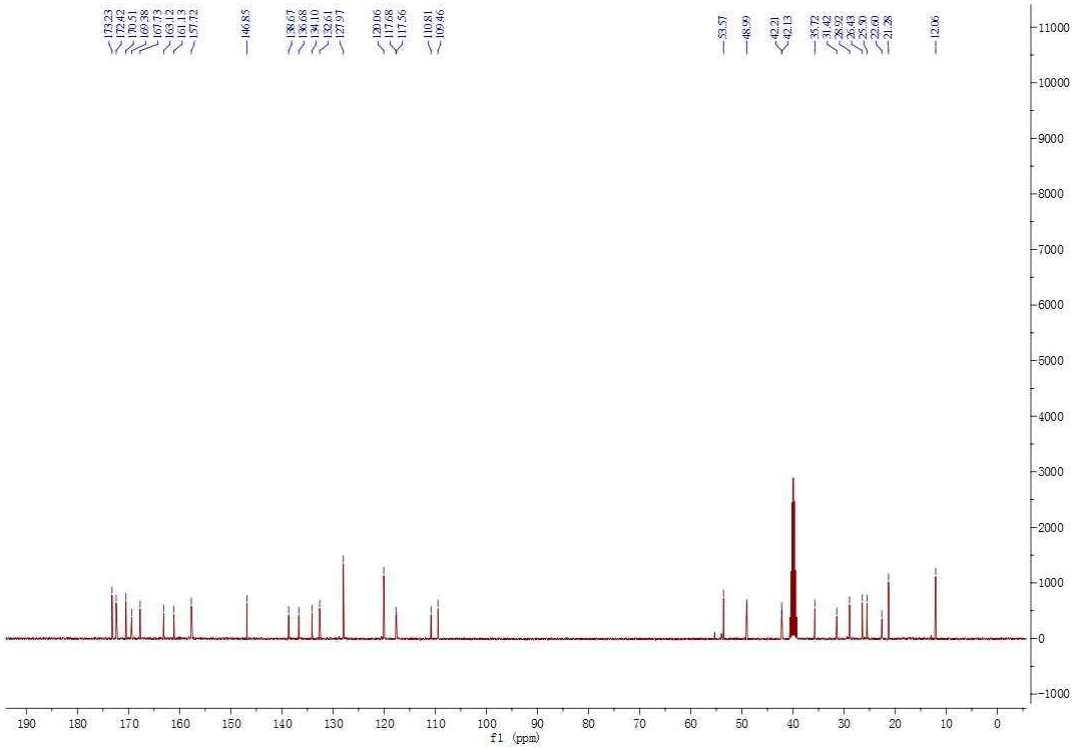
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	11,774	14602828	1008193	98,728
2	13,226	17741	1955	0,120
3	13,582	170339	13710	1,152
Total		14790908	1023858	100,000

^{1}H-NMR



^{13}C-NMR



All of the other related spectra were stored in the Department of Medicinal Chemistry, Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Halle, Germany.
ID: Z:\ag schmidt\Ping Sun\Dissertation

Acknowledgements

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Personal Declaration

Hereby, I declare that the presented thesis was composed independently by myself and without any other resources than the ones indicated. All thoughts taken directly or indirectly from external sources are properly denoted as such. This paper has neither been previously submitted to another authority nor has it been published yet.

Halle (Saale), 2022

Ping Sun

Curriculum Vitae

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09.2003-07.2007	Longxian High School. Shaanxi, China.
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10.2017-03.2022	Ph.D. student, Department of Medicinal Chemistry, Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Germany. Supervisor: Prof. Dr. Wolfgang Sippl

Work experience

10.2014-11.2015	Nanjing Huawe Medicine Group. Generic Drug Development Researcher, China.
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Scientific Publications

H. Ibrahim, M. Abdelsalam, Y. Zeyn, M. Zessin, E F. Bülbül, A. M. Mustafa, M. A. Fischer, P. Zeyen, A. Vecchio, P. Sun, F. Erdmann, M. Schmidt, D. Robaa, C. Romier, M. Schutkowski, O. H. Krämer, and W. Sippl
Synthesis and structure-activity relationship studies of pyrazine linked 2-aminobenzamides as new class I selective histone deacetylase (HDAC) inhibitors and their biological characterization in leukemic cells
Int. J. Mol. Sci. 23(1), 369, 2022. https://doi.org/10.3390/ijms23010369

P. Sun, J. Wang, K. Shahed Khan, W. Yang, W.-L. Ng, N. Ilment, M. Zessin, E. F. Bülbül, D. Robaa, F. Erdmann, M. Schmidt, C. Romier, M. Schutkowski, A. S.-L. Cheng, W. Sippl.
Development of alkylated hydrazides as selective HDAC8 inhibitors and analysis of antitumor immunity and efficacy of immune checkpoint blockade in hepatocellular carcinoma
To be submitted