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Article

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Design and Synthesis of Simplified Largazole Analogs as Isoform-Selective Human Lysine Deacetylase Inhibitors.

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Abstract

Selective inhibition of KDAC-isoforms while maintaining potency remains a challenge. Using the largazole macrocyclic depsipeptide structure as a starting point for developing new KDACIs with increased selectivity, a combination of four different simplified largazole analog (SLA) scaffolds with diverse zinc-binding groups (for a total of 60 compounds) were designed, synthesized and evaluated against class I KDACs 1, 3 and 8, and class II KDAC6. Experimental evidence as well as molecular docking poses converged to establish the cyclic tetrapeptides (CTPs) as the primary determinant of both potency and selectivity, by influencing the correct alignment of the zinc-binding group in the KDAC active site, providing a further basis for developing new KDACIs of higher isoform selectivity and potency.

Introduction

Lysine Deacetylases and Epigenetics.

Lysine deacetylases (KDACs)[1-3] are a class of enzymes found in bacteria, fungi, plants and animals that remove the acetyl group from the ε- amino groups of lysine residues in order to influence gene expression and modulate molecular recognition (epigenetics). Lysine acetyltransferase (KAT) catalyzes the acetylation of histone tails causing localized relaxation of chromatin and transcriptional activation of nearby genes and many other proteins modulating signaling, while KDACs catalyze the deacetylation of acetylated histones and other proteins leading to transcriptional repression and modulation of functional responses. There are 18 known human lysine deacetylases. KDACs 1-11 are zinc-based enzymes and hydrolyze an amide bond similar to a variety of zinc proteases. KDAC inhibitors (KDACIs) are an emerging class of antitumor drugs. Three FDA-approved KDAC inhibitors: Vorinostat (suberoylanilide hydroxamic acid, SAHA),[4] Romidepsin (FK228)[5] and Belinostat (PXD101)[6] are indicated for treatment of peripheral T-cell lymphomas, in particular, cutaneous T-cell lymphoma, and other types of non-Hodgkin's lymphoma. The role of KDACs in pathology is ubiquitous. Besides oncology, KDACIs have shown potential therapeutic roles in addiction, asthma, cardiovascular disease, immunosuppression, neurodegenerative diseases, sepsis, sickle-cell disease and termination of viral latency among others. It has become obvious that isoform-selective KDACIs are essential to help dissect the complex dynamics of epigenetic control of gene expression/signal transduction by lysine acetylation/deacetylation.

HIV-1 Latency.

As another example of potential therapeutic applications of lysine deacetylase inhibitors, KDACIs have been shown to reverse HIV-1 latency as a potential cure for patients infected with HIV to prevent subsequent development of AIDS.[7, 8] AIDS (acquired immunodeficiency syndrome) is a chronic, life-threatening condition due to infection by the human immunodeficiency virus (HIV-1). Highly active antiretroviral therapy (HAART) is an effective treatment to reduce HIV RNA in plasma to undetectable levels (generally defined as 50 copies/ml) which improves patients' lives, prevents subsequent development of AIDS, and reduces the risk of transmission to others. Even after 15 years of viral suppression by HAART, however, HIV can still be harvested from resting T-cells,[9] and other cells[10] with latent HIV infection[11], and viral rebound almost always occurs after HAART interruption.[12] Persistent proviral human immunodeficiency virus type 1 (HIV-1) infection, primarily within a small population of long-lived resting CD4+ T cells,[9] is a major obstacle to the eradication of HIV-1 infection. For this purpose, "shock and kill" strategies have been proposed by forcing HIV-infected T-cells to express their HIV genome,[13] so that infected cells can be identified and eradicated to eliminate HIV infection. Such efforts will be aided by further understanding of epigenetic mechanisms that regulate transcription from the HIV-1 long terminal repeat (LTR) promoter and the role of Tat/TAR interactions.[14, 15] Evidence suggests that dynamic acetylation and methylation can regulate HIV-1 proviral expression.[16] KDACs are recruited to the initiator and enhancer regions of the HIV-1 LTR by several transcription factors and co-repressor complexes. More recently it has been found that non-selective histone deacetylase inhibitors (pan KDACIs), such as SAHA,[4] Trichostatin A (TSA)[17] and FK228,[5] have been shown to disrupt HIV latency and provides *proof-of-principle*,[18, 19] but potential toxicity remains a significant concern with any epigenetic therapeutic.[20] Furthermore, no current KDACI is specific for any of the eleven zinc-based HDAC isoforms, and there is no clinical experience to estimate the length of treatment necessary to eradicate the HIV

reservoir. Recently, it has shown that KDAC inhibitors specific for a limited number of class I KDACs may offer a targeted approach to the disruption of persistent HIV-1 infection.[21, 22] Even if a vaccine were found to be effective, however, in preventing the spread of HIV, ~34 million of HIV-positive patients worldwide remain to be cured of their infections. To address this potential therapeutic opportunity, however, isoform-selective KDACIs are essential to determine the mechanism of overcoming HIV latency and to minimize adverse side effects in the clinic. Furthermore, development of isoform-specific inhibitors would enable pharmacological dissection of the role of KDAC isoforms in both physiology as well as pathology.

KDAC Inhibitors.

Many KDAC inhibitors have been reported, both naturally occurring such as TSA,[23] apicidin,[24] and trapoxin,[25] FK228,[5] azumamide analogs,[26-28] largazole[29], and a vast number of synthetic compounds, such as SAHA.[30] These KDAC inhibitors (**Figure 1)** can be divided into categories according to their structural chemistry, such as hydroxamates, carboxylates, benzamides, and cyclic peptides, though most are hydroxamic acid derivatives.[31] All classes of KDACIs contain three key structural elements: (i) a zinc-binding group (warhead) which coordinates the zinc ion at the bottom of the 12 Å narrow, active-site cavity, (ii) a capping or headgroup which interacts with the amino acids on the rim of the isoform's binding cavity as the substrate-protein recognition surface and (iii) a linker domain whose role is to ensure the correct positioning of the two former groups and to interact with the lipophilic binding tunnel. The structures of naturally occurring, potent inhibitors of human KDACs often contain a cyclic tetrapeptide "headgroup" linked to a pendant aliphatic spacer with a terminal "warhead" functional group that interacts with zinc in the active site. Despite an intensive search for over a decade in both academia and industry, and the discovery of selective KDAC1,[32] KDAC3,[33] KDAC6[34-36] inhibitors , the development of new KDACIs endowed with higher potency and selectivity is still necessary to clarify the therapeutic effect of a single KDAC isoform inhibition.

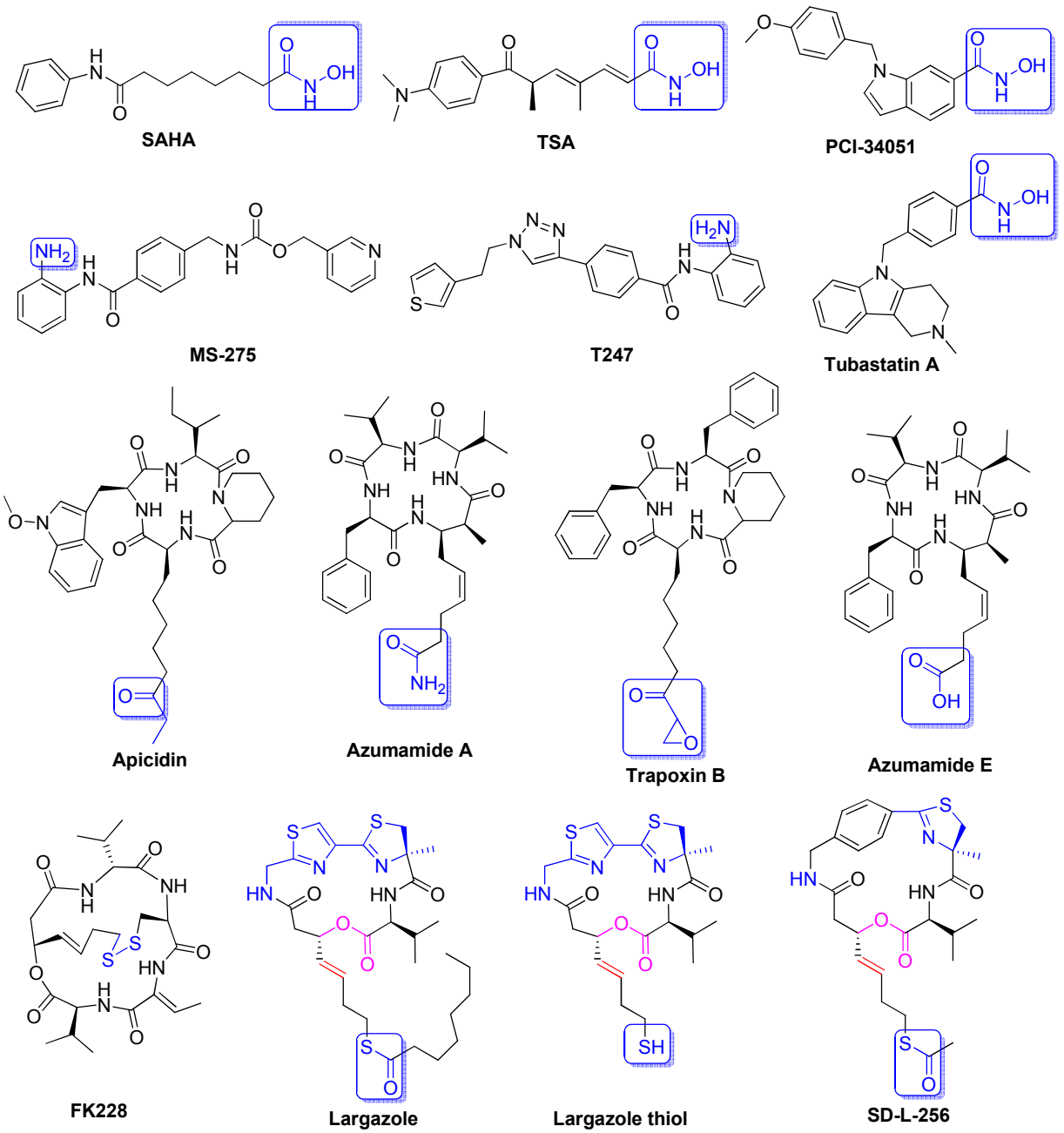


Figure 1: Examples of Potent KDAC inhibitors.

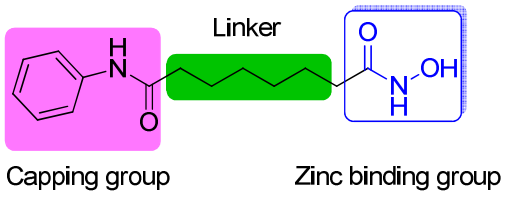


Figure 2. Schematic of KDAC inhibitors.

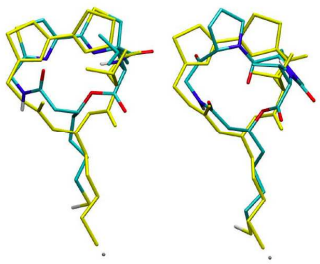
Design of Isoform-Selective KDAC Inhibitors.

Macrocyclic KDAC inhibitors (KDACIs) possess complex capping groups which interact with the KDAC enzyme's outer rim and show excellent KDACI potency with some isoform selectivity.[37] The structure of largazole in complex with human and *Schistosoma mansoni* KDAC8s have been solved.[38, 39] Comparison of the crystal structures of KDAC isoforms (2, 3, 4, 7, and 8) show that the length and conformation of the loops at the rim of the lysine-binding channel where the macrocyclic headgroups interact with the KDAC substrate are extremely variable, and a likely source of enzyme/protein-substrate specificity. Overemphasis on targeting the zinc in the essentially identical active site of KDAC isoforms may explain the limited progress in obtaining isoform selectivity. More recently, KDACIs have been developed without a zinc-binding group to examine this strategy.[40-42] Variability in the headgroup-binding region of KDAC isoforms provides one strategy for selectively targeting KDAC isoforms. KDACs target specific sites on substrate proteins; consequently, they must discriminate among the protein structures bearing the lysine residue to be modified. In naturally occurring KDACIs, macrocyclic cyclic tetrapeptides (CTPs) are located as capping groups at the recognition interface between the protein substrate and the KDAC. This strategy focuses on protein-substrate discrimination by different KDACs as seen in the example of the complex of largazole with human KDAC8[43] and its homolog from *Schistosoma mansoni*.[39]

Strategy for Isoform Selectivity in KDACIs.

In this work, we have chosen to explore four different simplified largazole analog (SLA) scaffolds with a wide variety of potential zinc-binding groups and directly compared their activities for a series of KDAC isoforms. Largazole thiol (**Figure 1**) is the most potent KDACI (picomolar against KDACs 1, 2 & 3) and selective to class I KDACs. Bowers et al. made one analog ~3-4-times as potent as largazole against KDAC1, 2 & 3 by substituing a pyridine group for one of the thiazole rings.[44] In our search of isoform-selective KDACIs, largazole has been modified to simplify the synthesis of analogs with amino-acid synthons as shown in **Figure 4.** Replacing the thiazole ring with heterochiral proline dipeptides (Pro-D-pro & D-pro-Pro) that stabilize reverse turns[45-47] generated two CTP scaffolds. Preliminary conformational analysis of the simplified largazole analogs (SLAs) gave promising overlaps with the conformation of largazole bound to KDAC8[43] (**Figure 3**). Further simplification replaced the unusual β-hydroxy acid bearing the thiol warhead with aspartic acid to which a variety of warheads could be linked by an amide bond when the *trans* olefin was replaced with an equivalent amide bond (**Figure 1**). Replacement of the depsipeptide ester link in the largazole ring with an amide bond by Bowers et al. showed little differences in isoform selectivity, and only a four-fold reduction in affinity to 4 nM.[48] The proline rings of the SLA provide a semi-rigid structure for potential replacement with substituted prolines to further enhance specificity for KDAC isoforms. Varying the stereogenic center (Asp vs. D-asp) where the linker and warhead were attached might further optimize the orientation of the zinc-

Figure 3. Original docking of largazole to KDAC8 (left) compared with crystal structure of largazole in complex; (right) docked structure of simplified largazole analog overlapped with crystal structure of largazole when complexed to KDAC8.



binding group. This proposed scaffold allowed combinatorial optimization of the CTP headgroup and the zinc-binding group by preassembly of the CTP headgroup followed by attachment of the linker-zinc-binding group through an amide bond. Optimization of both the linker and the zinc-binding group would hopefully generate maximal KDAC-isoform specificity. Unfortunately, the simplified largazole analogs did not bind to KDACs in the same orientation as largazole itself, based on the loss of inhibitory activity observed. A detectable KDAC6 inhibitory selectivity (among KDAC1, 3, 6 and 8) was measured for compound **25a**, to help improve ligand-KDAC isoform recognition. Due to the loses of biological responses, an comprehensive docking assessment protocol has been developed and applied to the human lysine deacetylases (KDACs),[49] determining the best docking protocol to be used in rationalizing SLAs-KDACs interactions.

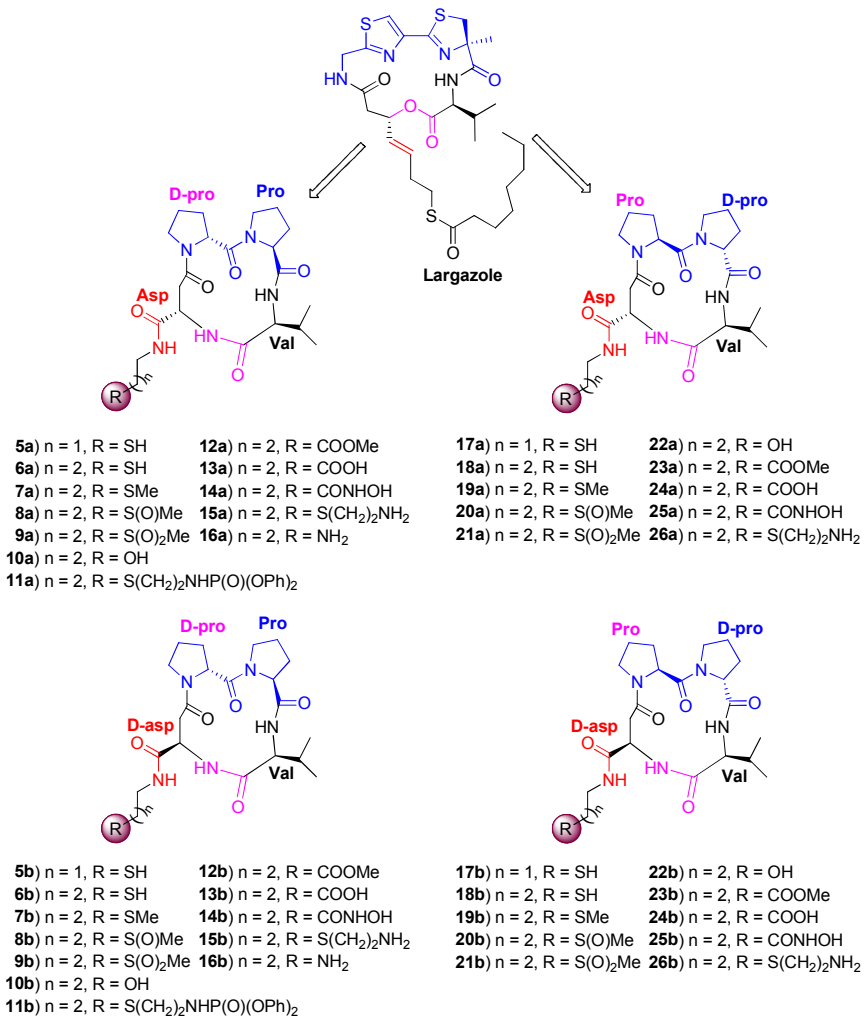


Figure 4: Designing of selective KDAC inhibitors.

The Yoshida group has shown significant isoform-selectivity enhancement against human KDACs with chlamydocin analogs by varying the chemical nature of the warhead interacting with the active-site zinc.[50] The choice of chemical functionality for binding to zinc at the active site is quite diverse.[51] In addition to optimizing the headgroup for isoform selectivity, we varied the chemical nature of the warhead interacting with the zinc in the active site from the sulfhydryl, present in FK228 and largazole, to thioether, sulfone, carboxyl ester, carboxylic acid, amine, hydroxyl and hydroxymate as well as methylsulphoxide functionality presents in sulforaphane, a naturally occurring HDAC inhibitor commonly found in cruciferous vegetables[52, 53]. Optimization of the

warhead interacting with the zinc in the active site by transition-state analogs (TSAs) of amide hydrolysis may further improves the selectivity and potency.[51] One can imagine extension of the zinc-binding headgroup to access the cavity associated with acetate release.[54] Thus, we plan to utilize experimental data from other classes of KDACIs to further optimize the isoform-selectivity advantages for KDAC as seen for each of the three components; CTP, linker and warhead.

Results and Discussions

First, we synthesized CTPs **1a, 1b, 2a and 2b** containing a pendant OBn-protected carboxylic acid on the alpha carbon of Asp, which was easily elongated with aminopropyl zinc-binding warheads. Synthesis of CTP shown in **Scheme 1**. Using a solution-phase strategy, we started with *O*-^{t}Butyl valine ester hydrochloride and coupled it with Fmoc-Pro/D-pro-OH using standard EDC coupling to give protected dipeptide **27** and **28** respectively. The Fmoc group at *N*-terminal of dipeptide was deprotected using 20% piperidine in dichlomethane (DCM) and coupled with Fmoc-D-pro/Pro-OH to yield the linear tripeptides **29** and **30,** these tripeptides aggregated to form an insoluble material once solidified that decomposed on silica gel. To overcome this difficulty, we proceeded further without purification and characterization of the tripeptides. The Fmoc groups at the *N*-terminals of the tripeptides **28** and **29** were deprotected and coupled with Boc-Asp(OH)-OBn or with Boc-D-asp(OH)-OBn to yield linear tetrapeptides **31a**, **31b**, **32a** and **32b** respectively**.** *N*-terminal Boc and *C*-terminal O^{t}Bu ester protection groups were then removed quantitatively using 30% trifluoroacetic acid in DCM to obtain the desired products. Then, macrolactamization was optimized at 2 mM in DMF using diphenylphosphoryl azide (DPPA) and diisopropylethylamine (DIPA); the CTPs were obtained in good yields as shown in **Scheme 1**. No epimerized or dimerized compound was observed during cyclization at 2 mM concentration in DMF. By synthesizing four CTP scaffolds with a pendant carboxyl group, a variety of warheads could be attached through an amide bond to determine

the relative affinities of different warheads in the same structural context against different KDAC isoforms.

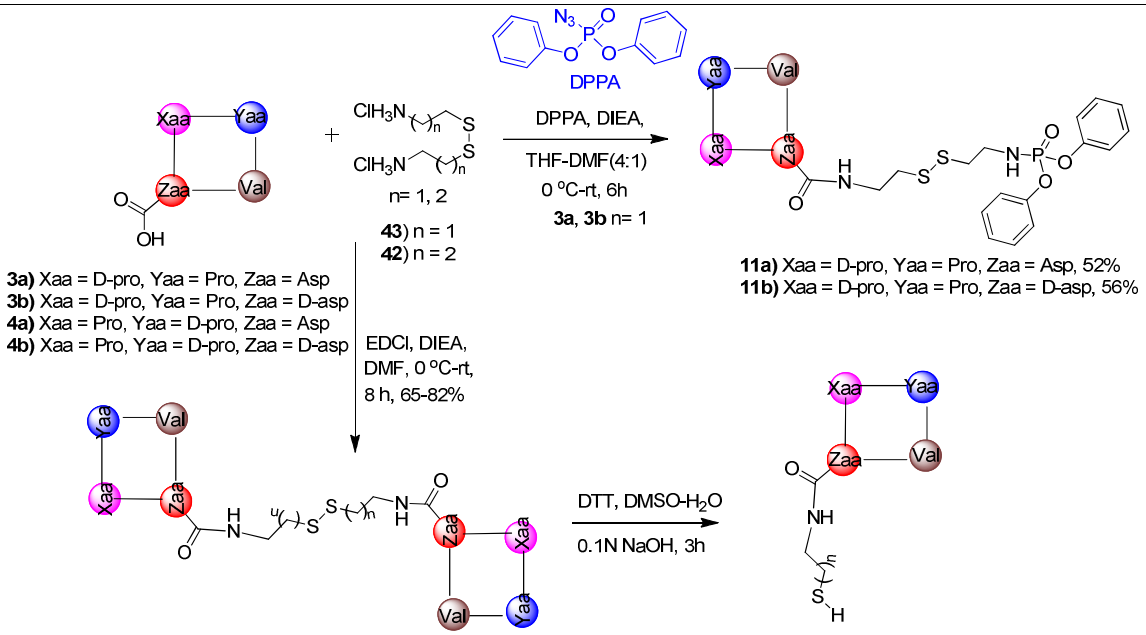
Scheme 1: Synthesis of CTP reverse-turn scaffolds.

Reagents and conditions: (i) EDCI, HOBt, DIEA, DCM, 9 h; (ii) 20% Piperidine/DCM, rt, 0.5 h, 100%; (iii) Fmoc-D-pro-OH, EDCI, HOBt, DIEA, DCM, 12 h; (iv) Boc-Zaa-OBn, EDCI, HOBt, DIEA, DCM, 24 h; (v) 30% TFA/DCM, 0 ^{o}C-rt, 3 h, 100%; (vi) DPPA, DIEA,DMF, 0^o C-rt, 72 h; (v) H_2, Pd/C, EtOH, rt, 5-6 h.

Diastereomerically pure CTPs **3a, 3b, 4a** and **4b** containing free pendant carboxyl groups were obtained quantitatively by hydrogenation of the OBn ester in the presence of Pd/C (see Supporting Information sections S11-S14). Epimerization at the Asp α-carbon stereocenter was observed, however, upon saponification of the OBn ester by lithium hydroxide (LiOH). The epimerized

compound was separated by HPLC and confirmed as **3b** by comparing LC-MS data, ^{1}H and ^{13}CNMR spectra of **3b** (see Supporting Information section S10)**,** which was authentically synthesized by Pd/C hydrogenation. CTP scaffolds with a pendant carboxyl group was coupled to 2,2′-diaminodiethyl disulfide and 3,3'-diaminodipropyl disulfide in presence of EDCL yielded the desired CTPs having disulfide bridges of two and three carbons, respectively; phosphamide warhead CTP analogs **11a** and **11b** where obtained in the presence of DPPA. Upon treatment of disulphide containing CTPs (**35a-38a** and **35b-38b**) with (2S,3S)-1,4-bis(sulfanyl)butane-2,3-diol (DTT), sulfhydryl-warhead SLA analogs (**5a, 6a, 5b, 6b, 17a, 17b, 18a** and **18b**) were obtained as shown in the **Scheme 2**.

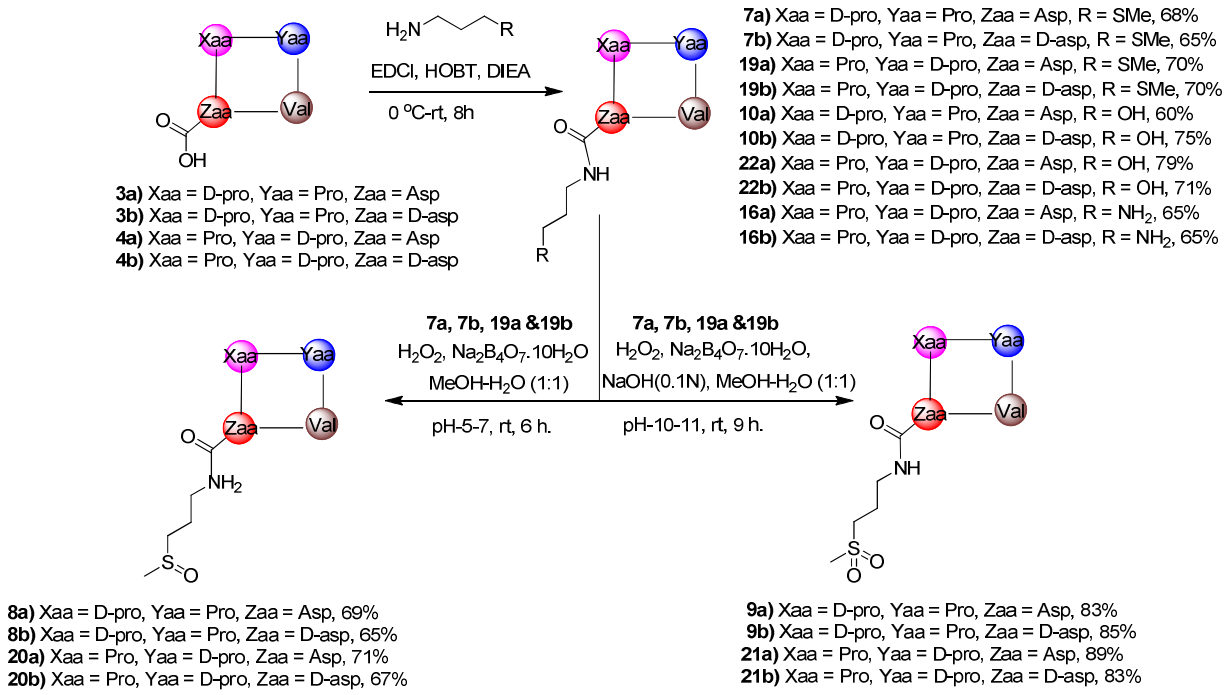
Scheme 2: Introduction of sulfhydryl zinc-binding functional group on CTP reverse-turn scaffolds.



Methylthioether, hydroxyl and amine zinc-binding, three-carbon warheads were introduced using standard EDCL coupling to give a wide variety of SLA analogs. Sulfoxides and sulfones were

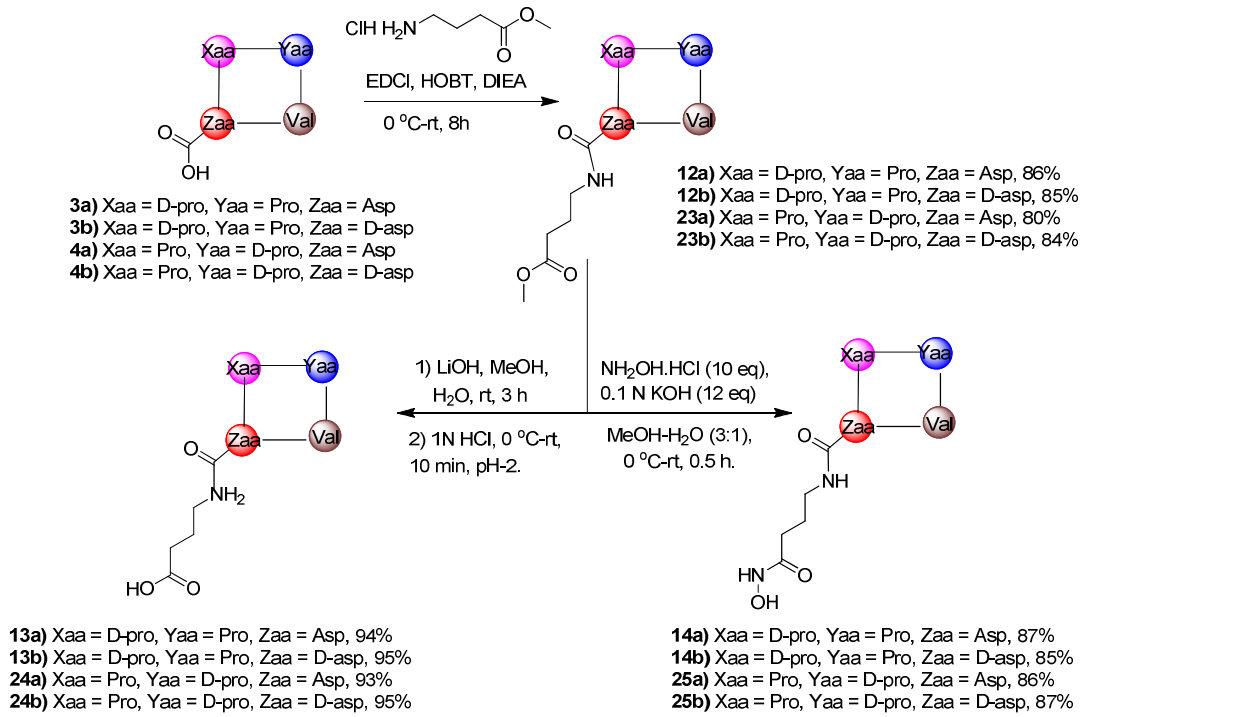
introduced by borax-catalyzed, pH-controlled selective oxidation of organic sulfides by hydrogen peroxide (H_2O_2) according to a reported procedure.[55] Methylthioether was treated with hydrogen peroxide in presence of borax in methanol (MeOH) and water mixture furnished the desired sulfoxide analogs (**8a**, **8b**, **20a** and **20b**) at acidic pH and sulfones (**9a**, **9b**, **21a** and **21b**) at basic pH (**Scheme 3**).

Scheme 3: Introduction of thiol, thioether, sulfoxide and sulfone zinc-binding groups.



Pendant carboxylic acids on CTPs were coupled with methyl 4-aminobutanoate to give methylester warheads (**12a**, **12b**, **23a** and **23b**), which upon treatment with hydroxylamine in presence of potassium hydroxide (KOH) furnished hydroxamic acid zinc-binding warheads (**14a**, **14b**, **25a** and **25b**). SLA analogs **13a**, **13b**, **24a** and **24b** having a carboxylic acid group were obtained quantitatively upon saponification of methyl esters by LiOH (**Scheme 4**).

Scheme 4: Introduction of carboxylic acid, ester, and hydroxamate zinc-binding groups



Bioactivity Against Isolated KDAC Isoforms.

A selection of synthetized SLA compounds (a total of 60, Supporting Information Section S18) was assayed for their inhibitory activity against four human KDACs (herein KDACs 1, 3, 6 and 8), to clarify how the different CTP headgroups determine isoform selectivity (see **Tables 1** and **2**). I*n vitro* assays were performed on recombinant human KDACs 1, 3 and 8 (Class I KDACs) and KDAC 6 (Class II KDACs) using the Caliper EZ Reader II system (Perkin-Elmer Caliper Life Sciences, USA).

Isoform selectivity.

Except for CTPs with hydroxymate groups (compounds **14a**, **14b**, **25a** and **25b**), the SAR results clearly demonstrate the limited ability of the simplified largazole scaffolds to determine strong or selective interactions with the 4 used KDAC isoforms, showing generally weak activities (**Table 1**

and Supporting Information **Tables 1-2**). Conversely, SLAs with hydroxamate groups (compounds **14a**, **14b**, **25a** and **25b**) show a general higher inhibitor potency compared to other SLAs (see Supporting Information **Tables 1-2**), confirming a prominent role of the hydroxamate group in driving the ligand-protein recognition by interacting with the zinc ion. Compounds **14a** and **25a** show IC_{50}s in a micromolar range with a detectable selectivity for KDAC6 (**Table 1** and Supporting Information **Tables 1-2**). These two compounds are D-pro-Pro and Pro-D-pro SLAs, and both are Asp derivatives. Thus, the presence of an Asp group instead of D-asp consistently justifies the general increased selectivity *vs* KDAC6 (compare inhibition percentages of **14a** with **14b** and **25a** with **25b** in Supporting Information **Tables 1-2**) which is worth of consideration in designing new KDAC6 selective SLAs. This is consistent with the hypothesis that the selected SLA scaffolds oriented the linker, the warhead and the capping group differently from largazole by binding in a different orientation, as confirmed by docking results (see molecular docking results of SLAs section and **Figure 10).** Nevertheless, some KDAC-isoform selectivity between KDACs 1, 3, 6 & 8 was observed for non-hydroxamate derivatives (**Figures 6-7** and Supporting Information **Tables 1-2**) that might be useful for future optimization. D-pro-Pro CTP analogs with thiomethyl, sulfoxide and sulfone warheads (compounds **8a-b** and **9a-b,** Supporting Information **Table 1**) showed some selectivity for KDAC8 compared with KDACs 1, 3 & 6. The data further suggest:

- The strong hydroxamic acid zinc-binding group generates, as reported above, the highest KDAC inhibitory activity (except in the case of the D-asp-Pro-D-pro scaffolds), In these cases, the strong zinc-binding group drives isoform recognition.
- Thiol zinc-binding groups (along with the disulfides which decompose to thiols) showed the highest KDAC6 inhibitory activity next to hydroxamates.
- Both the Asp α-carbon stereocenter and CTP type can modulate and change the activity profile and drive ligand-protein selectivity if a weaker zinc-binding groups are present: in the case of sulfoxides, sulfones, hydroxyls, thiomethyl and carboxylic acid zinc-binding groups,

the interaction seems to be dominated by the CTP moiety that favors interactions with the KDAC8 isoform.

- Asp-D-pro-Pro derivatives with sulfoxides, sulfones, hydroxyls zinc-binding groups and Asp-Pro-D-pro derivatives with thiomethyl, sulfoxides, sulfones and carboxylic acids zinc-binding group show the highest KDAC8 selectivity. About the D-pro-Pro derivatives, with the above zinc-binding groups, the replacement of L-Asp with D-asp doesn't appear to affect selectivity on KDAC8 (except for the thiomethyl case, in fact **7b** is completely selective on KDAC8, but not **7a**). For Pro-D-pro scaffolds with the above zinc-binding groups, the replacement of L-Asp with D-asp eliminates selectivity on KDAC8 (except for the thiomethyl case, in fact **19a** and **19b** have the same activity profile).

Cases with no high selectivity, but with significant activity changes.

- As shown in **Figures 6** and **7**, hydroxamic acid warheads (generally recognized as most potent, but with weak selectivity between different KDACs) provided, as in the case of the Asp-D-pro-Pro compound **14a** (**Figure 6A** and Supporting Information **Table 1**), the highest potency among the three different isoforms (KDACs 3, 6 and 8) with a detectable preference for KDACs 3 and 6 (2.16 and 2.65 times more active than with KDAC8, respectively) while the KDAC8 inhibitor activity was only average. The activity trend of compound **14a** was confirmed also for the D-asp-D-pro-Pro analog **14b** (**Figure 6B**), but interestingly without the large potency difference between KDAC3 and KDAC8 (1.29 times more active with respect to KDAC8, while the KDAC6 inhibitor potency was 2.52 times with respect to KDAC8). This clearly suggests a different KDAC3 conformational "availability and response" based on the Asp α-carbon stereocenter. Conversely, the Pro-D-pro hydroxamic acid derivatives showed a different activity potency and selectivity trend (compare compound

25a with **25b**, **Figures 7A-B**, Supporting Information **Table 2**), with a loss of activity for KDAC3 and KDAC6 in the case of the D-asp analog (compound **25b**). The effect on activity from the Asp α-carbon stereocenter dominates considering the Pro-D-pro derivative over the three KDAC isoforms even when a strong zinc-binding group (hydroxamic acid) was present.

- Another interesting case was represented by CTPs characterized with thiol warheads: among the 3-carbon linker-domain thiols, the Asp-D-pro-Pro and the D-asp-Pro-D-pro compounds **6a** and **18b**, respectively, showed the higher inhibition potency on KDAC6 (compounds **6b** and **18b**) suggesting, also in this case, a modulating role of the different CTPs. In fact, considering the D-pro-Pro derivatives, the Asp derivative (compound **6a**) was generally more active compared with the D-asp analog, whereas for Pro-D-pro derivatives, the Asp analog (compound **18a**) was less active (but not for KDAC8) compared with the D-asp derivative (compound **18b**).
- A third interesting case was the disulfide compounds with a 3-carbon linker (compounds **37a**, **37b**, **38a** and **38b**): among the D-pro-Pro analogs, it is appreciable how the D-asp derivative (compound **37b**) maintained detectable KDAC3 inhibitory activity that was totally lost with the Asp analog (compound **37a**).

Cases with high selectivity.

- Sulfoxides (compounds **8a**, **8b** and **20a**) derivatives showed a clear selectivity for KDAC8 regardless of the CTP type. Only in the case of the D-asp-Pro-D-pro analog (compound **20b**), no selectivity and potency was detected.
- Sulfones (compounds **9a**, **9b** and **21a**) derivatives showed clear selectivity for KDAC8 regardless the CTP type. Only in the case of the D-asp-Pro-D-pro analog (compound **21b**), no selectivity or potency was detected.
- Hydroxyls (compound **10a**, Asp-D-pro-Pro) showed selectivity for KDAC8, other analogs (**10b**, **22a** and **22b**) did not share that selectivity.

- Carboxylic Acids (only Pro-D-pro derivatives, compounds **24a** and **24b**) showed selectivity on KDAC8.
- Thiomethyl derivatives (**7b**, **19a** and **19b**) showed selectivity on KDAC8, the presence of Asp-D-pro-Pro (as in the case of **7a**) neutralized the selectivity and decreased the potency.

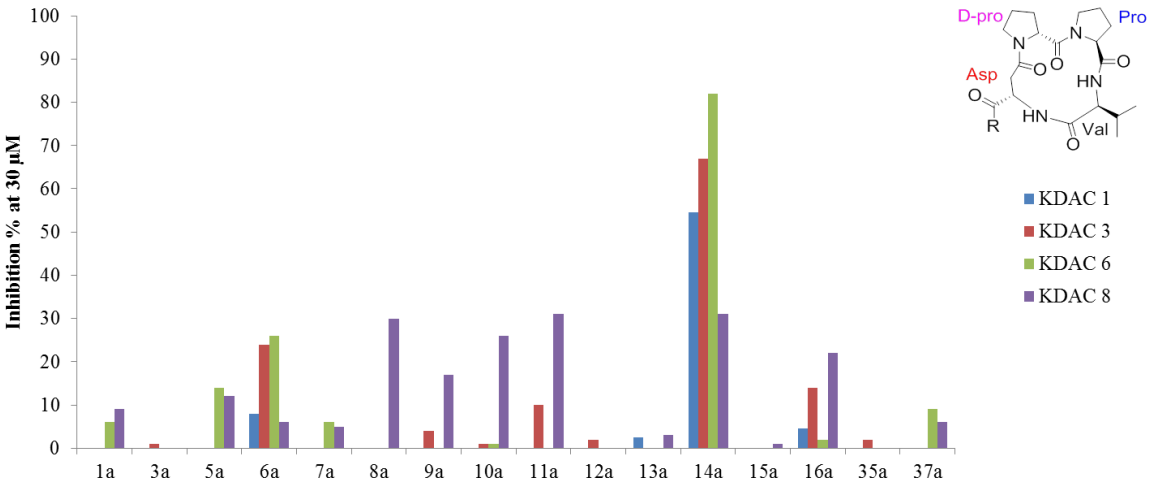
Our hypothesis that the warhead groups of the SLA scaffolds were not oriented correctly when bound to KDAC isoforms so that they did not interact optimally with the active-site zinc An intensive effort to find docking protocols that demonstrates such a difference by developing a protocol[49] capable to detect a reliable structure-based procedure and applied to the lysine deacetylase (KDAC) inhibitors,[49] providing a mechanism for optimizing novel CTP scaffolds. Molecular docking results confirmed this hypothesis (see Molecular Docking Results of SLAs paragraph and **Figure 10**).

Table 1: Most active SLAs and standard compounds (positive controls) IC_{50} (μM) values.

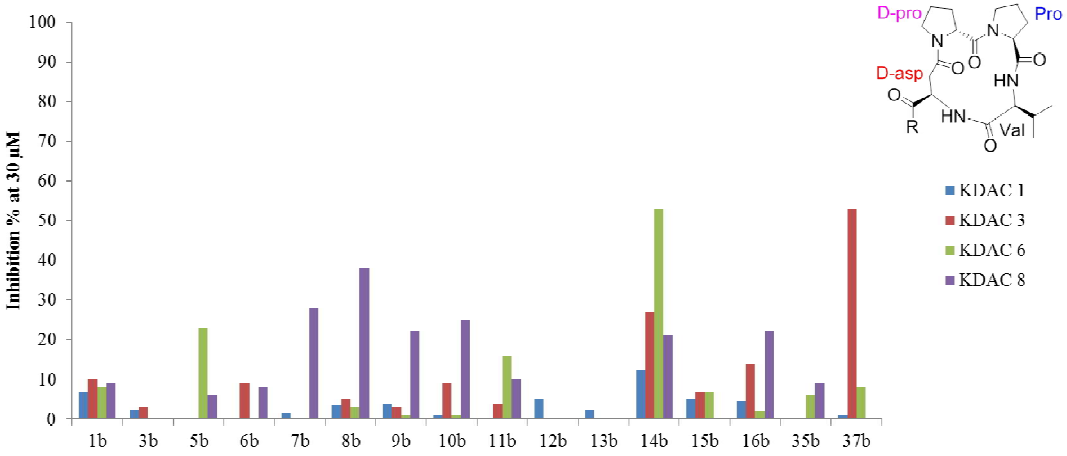
#	ID	IC_{50} μM KDAC isoform 1	3	6	8
1 (12)[a]	**14a**	25.1	13.7	5.07	>30
2 (43)[a]	**25a**	>30	>30	7.66	>30
3	**MS-275**[56]	1.48	0.79	>30	>30
4	**Largazole**[b][44]	2.33	1.36	9.29	>30
5	**PCI-34051**[32]	14.41	>30	4.57	0.49
6	**SAHA**[4]	0.0070	0.0014	0.0014	0.50
7	**SD-L-256**[57]	3.48	0.47	1.61	>30
8	**T247**[33]	1.11	3.94	>30	>30
9	**TSA**[17]	0.015	0.020	0.038	4.55
10	**Tubastatin A**[34]	2.87	0.77	0.014	2.34

[a]Number in the complete list of compounds (see Supporting Information section S18)

[b]The thioester form of largazole[44] (as depicted in **Figure 1**) was assayed.

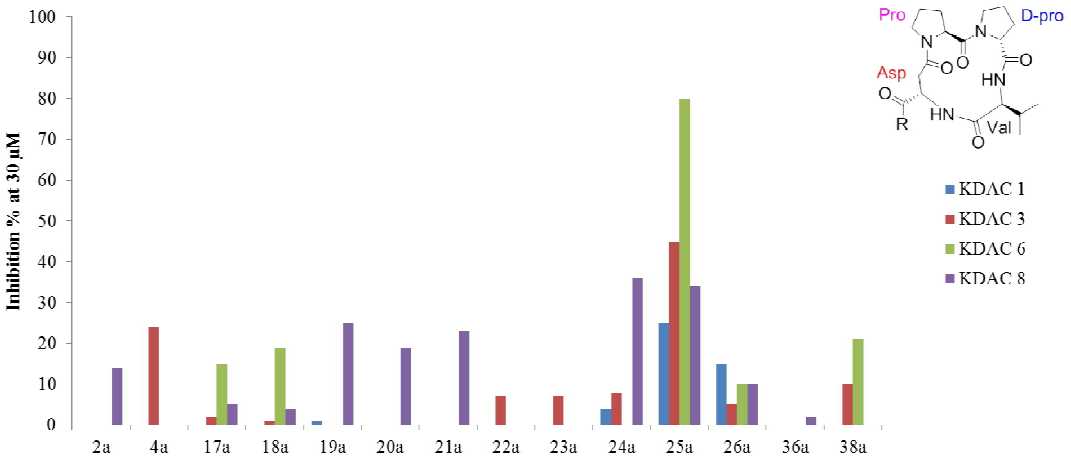


A

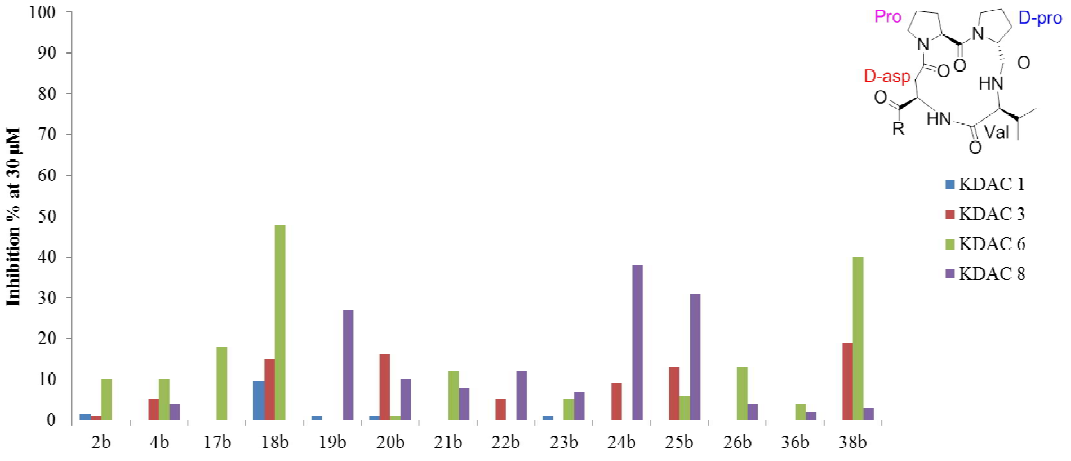


B

Figure 6. Simplified Largazole (D-pro-Pro) analogs inhibition profiles plots. A: Asp derivatives; B: D-asp derivatives.



A



B

Figure 7. Simplified Largazole (Pro-D-pro) analogs inhibition profiles plots. A: Asp derivatives; B: D-asp derivatives.

Molecular Modeling

Molecular docking was used to rationalize the SLA binding poses and help clarify any correlation with the obtained KDAC1, 3, 6 and 8 isoforms' biological activities. A comprehensive molecular

docking investigation on lysine deacetylases was performed by means of the recently released Clusterizer and DockAccessor software,[49] with the purpose of detecting a best docking protocol to be applied, and used also to develop predictive COMBINEr[58] or 3-D QSAR[59] models. As reported,[49] a comprehensive docking assessment proposed PLANTS[60] (using the PLP scoring function and considering the best docked poses, BD)[49] eligible for predicting reliable SLAs binding poses. Due to the loss in activity of SLAs, a reliable binding pose analysis was not possible among the different KDAC isoforms, except for comparing their predicted binding poses with respect to the largazole thiol experimental one characterizing the KDAC8 co-crystal structure (PDB code 3RQD).

KDAC8 complex structure preparation.

KDAC8 co-crystal structures (PDB code 3RQD)[43] was prepared as previously reported,[49] Subsequently, compounds **14a** and **25a** were docked as described.

Docking Assessment.

As previously reported,[49] an extensive docking assessment was applied to detect the best docking strategy using 9 different docking programs: AutoDock4,[61] AutoDock4(Zn),[62] AutoDock Vina,[63] DOCK6,[64] MpSDockZn,[65] PLANTS,[60] and Surflex-Dock.[66]). $PLANTS_{PLP}$[60] has been detected (using the Hungarian symmetry-corrected heavy-atom RMSD, HA_RMSD*h* and the obtained best docked poses, BD)[49] as the preferable docking program (showing the best compromise between performance and speed) to be used to dock largazole and its analogs, confirming its capability in predicting the binding pose of largazole thiol in the native protein (3RQD, **Figure 8**), thus validating it for docking SLAs.

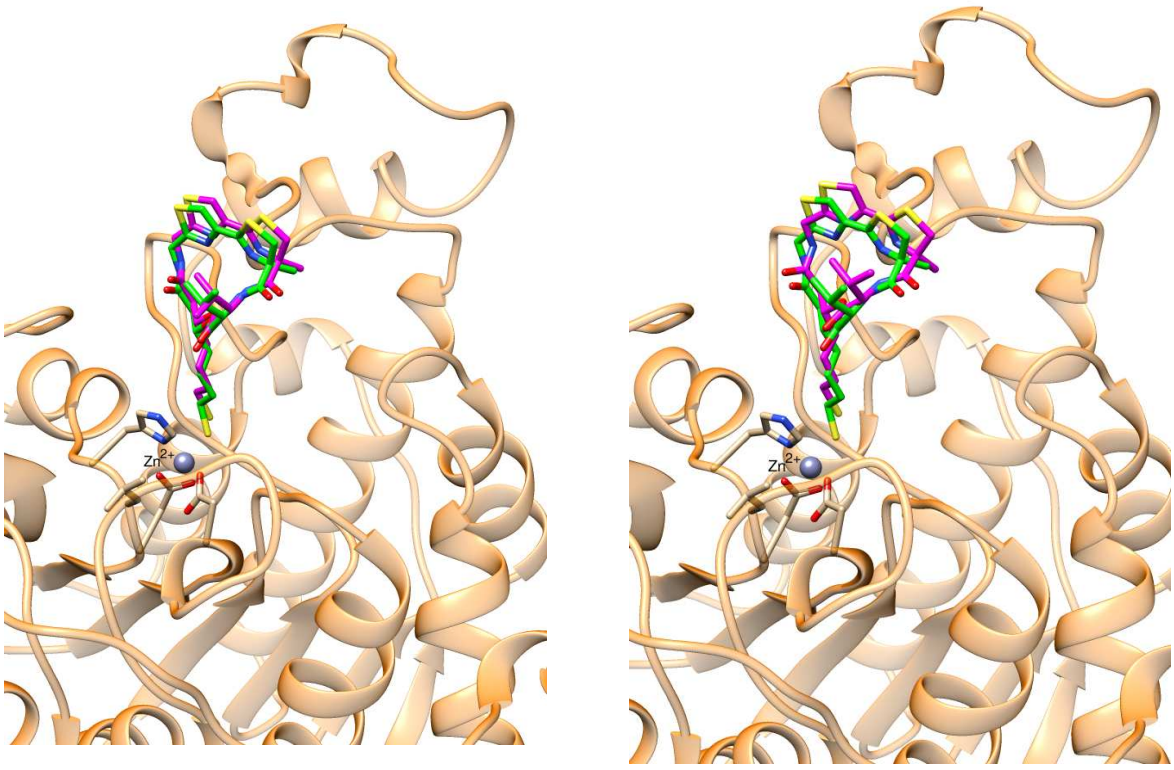
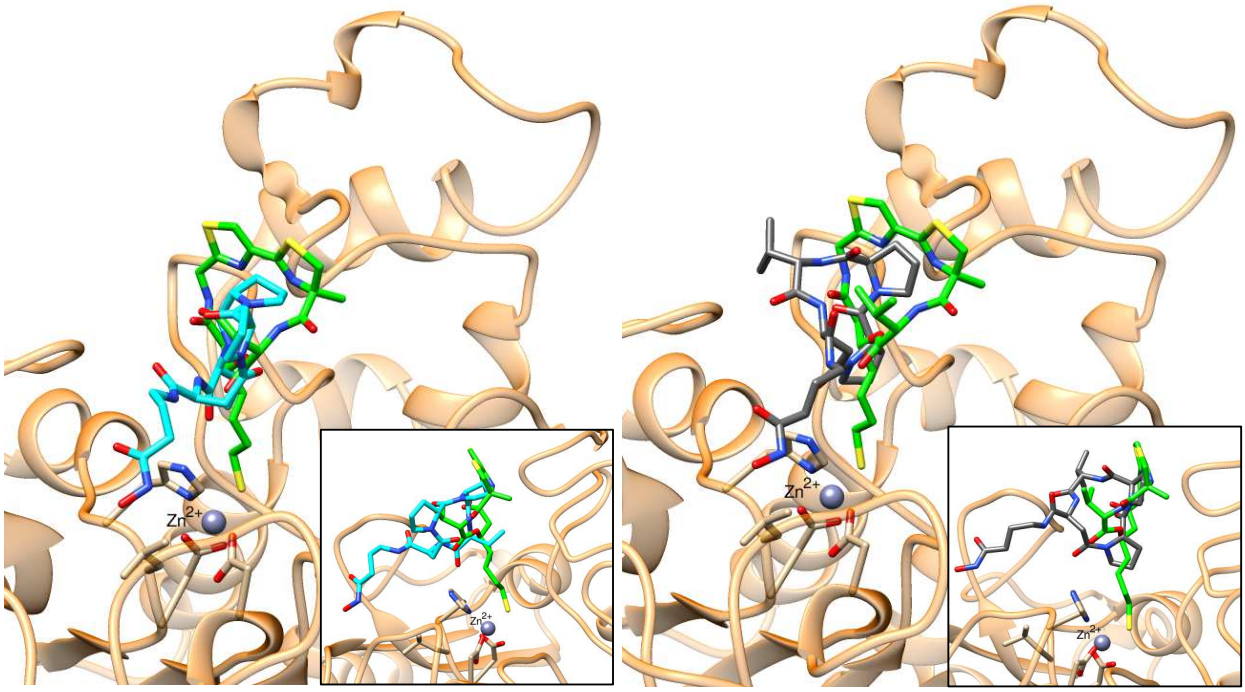


Figure 8. Docking of Largazole in 3RQD[43] chain - A. Best Docked poses (BD); A: Experimental Conformation Re-Docking (ECRD); B: Random Conformation Re-Docking (RCRD). Experimental binding pose is green colored, BD predicted binding pose is magenta colored.

*Molecular Docking Results of compounds **14a** and **25a**.*

$PLANTS_{PLP}$[60] was used to dock compounds **14a** and **25a** (the most active ones) in KDAC8 using a same strategy as previously reported.[49] A first comparison between the predicted binding poses of **14a** and **25a** and the experimental crystal structure of largazole thiol in KDAC8 (PDB code:3RQD) clearly show how the heteroproline dipeptide reverse-turn mimetics bind in a different manner compared to the largazole thiol (**Figure 9**), suggesting that the loss of planarity between the two heteroproline determines negative interactions and, as a consequence, low inhibition potency against KDAC8 (**Table 1**). The presence of a dihedral angle between the two heteroprolines leading to non-

planarity has a negative effect also on the inhibitor potency against other KDACs preventing the necessary interaction with the zinc ion.



A **B**

Figure 9. Comparison between largazole thiol (green) experimental binding pose and SLAs **14a** and **25a** BD poses in KDAC8 (PDB code: 3RQD). A: Predicted BD poses of compound **14a** (Asp-D-pro-Pro, cyan); B: Predicted BD poses of compound **25a** (Asp-Pro-D-pro, grey).

Conclusions

In conclusion, we have explored four different simplified largazole analog (SLA) scaffolds by replacing the thiazole ring in largazole with heterochiral proline dipeptides (Pro-D-pro & D-pro-Pro). We have synthesized totally 52 SLA analogs by introducing a wide variety of potential zinc-binding groups on pendant carboxylic acids in CTPs. The effort to reduce the synthetic complexity of largazole by replacement of the bis-thiazole and unusual amino acid with readily accessible amino

acid synthons did not give the anticipated results. Preliminary docking studies with KDAC8 of the simplified largazole analog (SLA) indicated a plausible overlay that encouraged the synthetic effort described herein. The experimental results obtained, however, clearly:

- indicate that binding of the SLA analogs reorients the linker and warhead in different positions that precluded their anticipated interaction with the zinc in the active site;
- suggest the detrimental effect of a dihedral angle between the two heteroproline;
- confirm the hydroxamate group capable of establishing stronger interactions with the zinc ion, partly reversing the depressing effect of the used CTP headgroups.
- Reveal the effect of the Asp group (where the linker and warhead are attached) which directs to a higher KDAC6 selectivity, suggesting its use when design new compounds with increase potency and selectivity against KDAC6.

A recent docking protocol has been developed and assessed based on these results and will be used, together with the above reported experimental evidencies, in the design of new SLA headgroups capable to properly interact with the zinc and endowed with higher activity and selectivity.

Experimental Section

Chemistry General. All the reactions were performed in oven-dried apparatus and were stirred using magnetic stirbars. Starting materials, reagents, and solvents were purchased from commercial vendors unless otherwise noted. Chromatography grade ethylacetate, dichloromethane, acetonitrile, and hexanes were obtained from Sigma-Aldrich. Column chromatography was performed on silica gel (100-200 mess) purchased from Sorbent Technologies. TLC was carried out on Analtech 200 microns silica-gel coated plastic-fiber sheets. All reactions were monitored by thin layer chromatography (TLC) carried out on Merck silica-gel plates (0.25 mm thick, 60F254), visualized by using UV (254 nm) or dyes such as ninhydrin, KMnO4, *p*-anisaldehyde or CAM (ceric ammonium molybdate). High-performance liquid chromatography (HPLC) was carried out on GILSON GX-281

using Waters C_{18} 5μM, 4.6*50mm and Waters Prep C18 5μM, 19*150mm reverse phase columns. The mobile phases used were A: H_2O with 0.05% TFA, B: CH_3CN with 0.05% TFA using a solvent gradient of A-B over 30 min with a flow rate of 14.8 mL/min, with detection at 220 and 254 nm UV detectors. Purity assessment and mass spectra (MS) data were obtained using a Hewlett-Packard HPLC/MSD using electrospray ionization (ESI) for detection. ^1H and ^{13}C NMR spectra were measured on a Varian 400 MHz NMR instrument. Chemical shifts are expressed in parts per million (ppm) from the residual of nondeuterated solvents as internal standard. (1H NMR: TMS $\delta = 0.00$ ppm, $CDCl_3$ $\delta = 7.26$ ppm, DMSO-d_6 $\delta = 2.50$ ppm, D_2O: $\delta = 4.79$ ppm; ^{13}C NMR (APT): TMS $\delta =$ 0.00 ppm, $CDCl_3$ $\delta = 77.16$ ppm, DMSO-d_6 $\delta = 39.52$ ppm). Coupling constants (J) are given in hertz (Hz). The following abbreviations were used to express the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; p = pentet; quin = quintet; sep = septet; hept = heptet; m = multiplet; dd = doublet of doublets; dt = doublet of triplet; td = triplet of doublet; m = multiplet; bs = broad singlet. All compounds used for biological assays are >95% pure based on NMR and LC-MS by UV absorbance at 210 nm and 254 nm wavelengths.

General procedure for synthesis of CTPs by using macrolactamization (1a, 1b, 2a and 2b) protocol:

To a cold (0° C) stirred solution of N- and C-terminal deprotected tetrapeptide (1 mmol) in dry DMF (500 mL) was added *N,N*-diisopropylethylamine (6 mmol) dropwise over 1 min followed by Diphenyl phosphorazidate (DPPA) (4 mmol) then slowly bring it to room temperature and stirred until the complete consumption of starting material, then DMF was removed under reduced pressure. The resulting viscous solution was diluted with water (5 mL) and thoroughly extracted with ethyl acetate (15 mL). The combined organic extracts were washed with 1 N HCl (5 mL), saturated aqueous sodium bicarbonate ($NaHCO_3$) (5 mL) and dried over anhydrous sodium sulphate (Na_2SO_4)

and concentrated to give a residue, which was purified by silica gel (100-200 mesh) flash column chromatograph.

General procedure for catalytic hydrogenation of benzyl ester (OBn) protecting group (3a, 3b, 4a and 4b):

A stirred solution of the benzyl ester (1 mmol) and 10% Pd/C (0.1 mmol) in EtOH (10 mL) at 25 °C was placed under an atmosphere of hydrogen. After 4 h, the mixture was filtered through Celite using EtOH as eluent, and concentrated under reduced pressure and proceeded further without purification.

General procedure for introducing warhead on pendent carboxylic acid by using EDCl peptide coupling (7a, 7b, 10a, 10b, 12a, 12b, 16a, 16b, 19a, 19b, 22a, 22b, 23a and 23b) protocol:

To a solution (0° C) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.3 mmol), 1-hydroxybenzotriazole (1.3 mmol), *cyclo*-Zaa(Xaa-Yaa-Val)-OH (1 mmol) in *N,N*-dimethylformamide (10 mL) added *N,N*-diisopropylethylamine (3 mmol) was stirred for 15 min. Then added solution of amino propyl linker (1 mmol) in *N,N*-dimethylformamide (2 mL) flask and the reaction mixture was stirred at room temperature till the starting material was consumed completely. *N,N*-dimethylformamide and dichloromethane were removed under reduced pressure and the resulting viscous solution was diluted with water (5 mL) and thoroughly extracted with ethyl acetate (15 mL). The combined organic extracts were washed with 1 N HCl (5 mL), saturated aqueous sodium bicarbonate ($NaHCO_3$) (5 mL) and dried over anhydrous sodium sulphate (Na_2SO_4) and concentrated to give a residue, which was purified by purified by HPLC.

General procedure for LiOH base-mediated hydrolysis of methyl ester (13a, 13b, 24a and 24b):

To a stirred solution of lithium hydroxide (1.5 mmol) in of distill water (1 mL) was added to the methyl ester (1 mmol) in MeOH (3 mL) and then stirred at room temperature for 3.5 h, then MeOH was removed under reduced pressure, acidified with 1N HCl until pH was between 2-3 at 0^{o} C. Then extracted with EtOAc, dried over anhydrous sodium sulphate (Na_2SO_4) and concentrated to give the desired acid, which was used further without purification.

General procedure for synthesis of hydroxamic acids from corresponding methyl esters (14a, 14b, 25a and 25b):

To a stirred solution of methyl ester (1 mmol) in MeOH (3 mL), hydroxylamine hydrochloride (10 mmol) was added followed by 0.1 N potassium hydroxide (12 mmol) in distilled water (1 mL) and then stirred at room temperature for 30 minutes, then MeOH was removed under reduced pressure, acidified with 1N HCl until pH was between 2-3 at 0^{o} C. The solution was extracted with EtOAc, dried over anhydrous sodium sulphate (Na_2SO_4) and concentrated to give the desired hydroxamic acid in high yield, which was further purified by HPLC.

General Procedure for Sulfoxide synthesis from Organic Sulfides by H_2O_2/Borax (8a, 8b, 20a ans 20b):

In a typical experiment to a 25 mL flask equipped with a magnetic stirrer and 30% H_2O_2 (6.0 mmol) in water was added borax (0.2 mmol) and MeOH (5 mL) followed by methyl thioether containing peptide (1 mmol). The reaction was monitored by TLC. After complete disappearance of the reactant added $Na_2S_2O_5$ to destroy the excess amount of H_2O_2 and then removed MeOH, the product was extracted with EtOAc, dried over anhydrous sodium sulphate (Na_2SO_4) and concentrated to give a desired sulfoxide, which was purified by reverse phase HPLC.

General Procedure for Sulfones synthesis from Organic Sulfides by H_2O_2/Borax (9a, 9b, 21a ans 21b):

In a typical experiment to a 25-mL flask equipped with a magnetic stirrer and 30% H_2O_2 (6.0 mmol) was added borax (0.2 mmol) and MeOH (5 mL) followed by methyl thioether containing peptide (1 mmol). To the resulting solution was added 0.1 N NaOH to maintain the pH of the solution at 10, and the mixture was stirred at room temperature for 12 -15 h and monitored by LC-MS. After complete disappearance of the reactant added $Na_2S_2O_5$ to destroy the excess amount of H_2O_2 and then removed MeOH, the product was extracted with EtOAc, dried over anhydrous sodium sulphate (Na_2SO_4) and concentrated to give a desired sulfone, which was purified by reverse phase HPLC.

General procedure for synthesis of sulfhydryl from corresponding homodimer (5a, 5b, 6a, 6b, 17a, 17a, 18a and 18b):

To a stirred solution of disulfide containg homodimer of SLA analog (1 mmol) in DMSO-water (1:1) (4 mL), Dithiothreitol (DTT) (20 mmol) was added followed by 0.1 N sodium hydroxide (0.01 mL) in distilled water and then stirred at room temperature for 2 hours. Then which was further purified by HPLC using 10-40 acetonitrile gradient over 30 minutes.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-OBn (1a):**

Cyclic tetrapeptide **1a** was synthesized by following the above general procedure for DPPA macrolactamisation and purified by silical gel column chromatography (EtOAc) as a white solid (yield: 79%, purity by LC-MS: 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.50 - 7.41 (m, 2 H), 7.41 - 7.28 (m, 3 H), 7.03 (d, *J* = 9.4 Hz, 1 H), 6.48 (d, *J* = 9.8 Hz, 1 H), 5.28 (d, *J* = 11.7 Hz, 1 H), 5.19 (d, *J* = 12.1 Hz, 1 H), 5.12 (t, *J* = 9.6 Hz, 1 H), 4.58 (dd, *J* = 3.6, 9.0 Hz, 1 H), 4.56 (t, *J* = 7.0 Hz, 1 H), 4.45 (dd, *J* = 3.9, 9.0 Hz, 1 H), 4.18 - 4.09 (m, 1 H), 3.63 - 3.54 (m, 1 H), 3.31 - 3.22 (m, 1 H), 3.12 (d, *J* = 12.9 Hz, 1 H), 3.01 (dt, *J* = 5.7, 9.7 Hz, 1 H), 2.79 (dd, *J* = 9.8, 12.5 Hz, 1 H), 2.53 (dt, *J* = 3.3, 6.7 Hz, 1 H), 2.41 - 2.29 (m, 1 H), 2.17 - 2.02 (m, 4 H), 1.93 - 1.79 (m, 2 H), 1.76 - 1.63 (m, 1

H), 0.93 (d, *J* = 7.0 Hz, 3 H), 0.89 (d, *J* = 6.7 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.7, 171.3, 171.1, 170.7, 169.9, 134.9, 129.2, 128.6, 128.5, 67.8, 62.6, 58.2, 56.0, 49.5, 47.8, 47.2, 35.8, 29.9, 29.1, 28.0, 26.1, 24.9, 20.2, 17.0. MS (ESI): found: $[M + H]^+$, 499.4.

***Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-OBn (1b):**

Cyclic tetrapeptide **1b** was synthesized by following the above general procedure for DPPA macrolactamisation and purified by silical gel column chromatography (EtOAc) as a white solid (yield: 78%, purity by LC-MS: 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.42 - 7.28 (m, 5 H), 7.09 (d, *J* = 9.0 Hz, 1 H), 6.51 (d, *J* = 6.3 Hz, 1 H), 5.24 (d, *J* = 12.1 Hz, 1 H), 5.14 (d, *J* = 12.5 Hz, 1 H), 4.69 (t, *J* = 6.5 Hz, 1 H), 4.52 (dd, *J* = 4.1, 9.6 Hz, 1 H), 4.48 (dd, *J* = 4.3, 8.6 Hz, 1 H), 4.28 - 4.14 (m, 2 H), 3.99 (td, *J* = 5.7, 11.6 Hz, 1 H), 3.66 - 3.62 (m, 2 H), 3.07 (t, *J* = 12.3 Hz, 1 H), 2.95 (dd, *J* = 5.3, 12.7 Hz, 1 H), 2.45 - 2.36 (m, 2 H), 2.29 - 2.20 (m, 2 H), 2.14 - 2.02 (m, 5 H), 0.92 (d, *J* = 5.1 Hz, 3 H), 0.91 (d, *J* = 5.1 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.2, 172.4, 171.7, 171.5, 169.5, 135.4, 128.5, 128.4, 128.3, 67.5, 62.5, 58.2, 56.0, 50.4, 47.7, 47.5, 35.2, 29.7, 28.5, 28.0, 25.9, 24.9, 20.1, 17.0; MS (ESI): found: $[M + H]^+$, 499.4.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-OH (3a):**

Cyclic tetrapeptide pendent carboxylic acid **3a** was synthesized from **1a** by following the above general procedure for catalytic hydrogenation of benzyl ester, obtained the desired pendent carboxylic acid as a white solid (yield: 96%, purity by LC-MS: >99%). ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 7.34 (bs, 1 H), 7.21 (d, *J* = 8.6 Hz, 1 H), 6.53 (d, *J* = 9.0 Hz, 1 H), 5.03 (t, *J* = 8.0 Hz, 1 H), 4.63 (t, *J* = 7.4 Hz, 1 H), 4.48 (dd, *J* = 4.7, 8.6 Hz, 1 H), 4.44 (dd, *J* = 4.7, 8.6 Hz, 1 H), 4.24 - 4.15 (m, 1 H), 3.71 - 3.52 (m, 3 H), 3.22 (dd, *J* = 2.1, 13.2 Hz, 1 H), 2.90 (dd, *J* = 9.0, 13.3 Hz, 1 H), 2.51 - 2.33 (m, 2 H), 2.24 - 1.96 (m, 6 H), 1.89 (d, *J* = 10.6 Hz, 1 H), 0.94 (d, *J* = 6.7 Hz, 3 H), 0.90 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.0, 172.7, 172.5, 170.7, 170.3, 62.6, 58.4,

56.7, 49.5, 48.2, 47.4, 35.5, 29.7, 29.1, 27.9, 26.2, 24.9, 20.0, 17.2; MS (ESI): found: $[M + H]^+$, 409.4.

***Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-OH (3b):**

Cyclic tetrapeptide pendent carboxylic acid **3b** was synthesized from **1b** by following the above general procedure for catalytic hydrogenation of benzyl ester, obtained the desired pendent carboxylic acid as a white solid (yield: 94%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.13 (d, *J* = 9.4 Hz, 1 H), 6.64 (d, *J* = 5.9 Hz, 1 H), 6.35 (bs, 1 H), 4.69 (t, *J* = 6.5 Hz, 1 H), 4.56 (dd, *J* = 3.9, 9.0 Hz, 1 H), 4.48 (dd, *J* = 4.7, 8.2 Hz, 1 H), 4.24 - 4.08 (m, 2 H), 3.76 - 3.68 (m, 2 H), 3.67 - 3.60 (m, 1 H), 2.46 - 2.34 (m, 2 H), 2.34 - 2.18 (m, 2 H), 2.17 - 2.10 (m, 2 H), 2.10 - 1.99 (m, 3 H), 0.93 (d, *J* = 6.7 Hz, 3 H), 0.92 (d, *J* = 6.5 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.8, 174.1, 172.3, 170.9, 62.5, 58.4, 56.2, 53.2, 47.8, 47.5, 35.3, 29.7, 28.4, 28.0, 25.9, 25.0, 20.1, 17.0; MS (ESI): found: $[M + H]^+$, 409.4.

Homo dimer – [*Cyclo*-Asp(^{D}Pro-Pro-Val)-$NHCH_2CH_2S]_2$ (35a)

The homodimer prodrug thioether **35a** was synthesized from **3a** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-47% acetonitrile gradient, obtained the desired homodimer as a white solid (yield: 82%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.24 (t, *J* = 5.9 Hz, 1 H), 7.18 (d, *J* = 9.4 Hz, 1 H), 6.50 (d, *J* = 10.2 Hz, 1 H), 5.04 (t, *J* = 9.0 Hz, 1 H), 4.62 (t, *J* = 6.8 Hz, 1 H), 4.60 (dd, *J* = 3.7, 9.0 Hz, 1 H), 4.44 (dd, *J* = 5.5, 8.6 Hz, 1 H), 4.14 (td, *J* = 6.7, 9.8 Hz, 1 H), 3.70 - 3.56 (m, 4 H), 3.49 (dd, *J* = 2.0, 12.9 Hz, 1 H), 2.81 (dt, *J* = 1.6, 6.8 Hz, 2 H), 2.63 (dd, *J* = 9.4, 12.9 Hz, 1 H), 2.57 (dd, *J* = 3.7, 6.8 Hz, 1 H), 2.48 - 2.35 (m, 1 H), 2.22 - 2.09 (m, 4 H), 2.09 - 2.00 (m, 1 H), 2.00 - 1.83 (m, 2 H), 0.92 (d, *J* = 7.0 Hz, 3 H), 0.88 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: ^{13}C NMR (101MHz ,$cdcl_3$) δ = 174.6, 172.8, 172.2, 171.1, 170.8, 77.3,

77.0, 76.7, 62.7, 58.5, 56.8, 50.8, 48.3, 47.4, 39.3, 37.0, 34.9, 29.8, 29.0, 28.0, 26.0, 25.0, 19.8, 16.8; MS (ESI): found: $[M + Na]^+$, 955.7.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$SH (5a)**

Thiol warhead having SLA analog **5a** was synthesized from **35a** by following the above general procedure for synthesis of sulfhydryl from corresponding homodimer, obtained the desired pendent thiol as a white solid (yield: 87%, purity by LC-MS: >99%). ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 7.31 (d, *J* = 9.4 Hz, 1 H), 7.08 (t, *J* = 5.7 Hz, 1 H), 6.46 (d, *J* = 10.6 Hz, 1 H), 5.04 (t, *J* = 9.8 Hz, 1 H), 4.67 (dd, *J* = 3.1, 9.8 Hz, 1 H), 4.63 (t, *J* = 7.2 Hz, 1 H), 4.48 (dd, *J* = 5.3, 8.8 Hz, 1 H), 4.15 (td, *J* = 6.7, 9.8 Hz, 1 H), 3.74 - 3.56 (m, 5 H), 3.35 (qd, *J* = 6.6, 13.1 Hz, 1 H), 2.75 - 2.61 (m, 2 H), 2.61 - 2.54 (m, 1 H), 2.44 (td, *J* = 7.9, 12.8 Hz, 1 H), 2.24 - 2.01 (m, 6 H), 1.97 - 1.87 (m, 2 H), 1.84 (t, *J* = 8.8 Hz, 1 H), 0.93 (d, *J* = 7.0 Hz, 3 H), 0.89 (d, *J* = 7.0 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 468.4.

Homo dimer – [*Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$S]$_2$ (35b)

The homodimer prodrug thioether **35b** was synthesized from **3b** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-47% acetonitrile gradient, obtained the desired homodimer as a white solid (yield: 78%, purity by LC-MS: 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.41 (t, *J* = 4.9 Hz, 1 H), 7.11 (d, *J* = 10.2 Hz, 1 H), 6.53 (d, *J* = 6.3 Hz, 1 H), 4.65 (t, *J* = 6.8 Hz, 1 H), 4.45 (t, *J* = 5.2 Hz, 1 H), 4.42 (t, *J* = 4.6 Hz, 1 H), 4.17 (td, *J* = 6.3, 9.7 Hz, 1 H), 4.10 - 4.01 (m, 1 H), 3.68 (t, *J* = 6.5 Hz, 2 H), 3.65 - 3.44 (m, 3 H), 3.05 (dd, *J* = 3.9, 12.5 Hz, 1 H), 2.92 (t, *J* = 12.5 Hz, 1 H), 2.85 - 2.65 (m, 2 H), 2.42 - 2.19 (m, 3 H), 2.19 - 1.90 (m, 6 H), 0.93 (d, *J* = 6.7 Hz, 3 H), 0.91 (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.8, 172.7, 172.5, 172.1, 170.0, 62.5, 58.2, 57.0, 52.5,

47.8, 47.4, 38.6, 37.5, 34.5, 29.7, 28.7, 28.1, 25.9, 24.9, 20.1, 17.4; MS (ESI): found: $[M + Na]^+$, 955.7.

***Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$SH (5b)**

Thiol warhead having SLA analog **5b** was synthesized from **35b** by following the above general procedure for synthesis of sulfhydryl from corresponding homodimer, obtained the desired pendent thiol as a white solid (yield: 80%, purity by LC-MS: >99%). ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 7.26 (bs, 1 H), 7.17 (d, *J* = 9.4 Hz, 1 H), 6.43 (d, *J* = 6.3 Hz, 1 H), 4.73 - 4.64 (m, 1 H), 4.54 (dd, *J* = 4.1, 9.6 Hz, 1 H), 4.46 (dd, *J* = 4.1, 8.4 Hz, 1 H), 4.15 (t, *J* = 6.5 Hz, 1 H), 4.02-3.95 (m, 1 H), 3.74 - 3.50 (m, 5 H), 3.32 (dd, *J* = 6.8, 13.1 Hz, 1 H), 3.08 (dd, *J* = 4.3, 12.5 Hz, 1 H), 2.95 (t, *J* = 12.3 Hz, 1 H), 2.74 - 2.60 (m, 2 H), 2.38 (dt, *J* = 3.5, 7.0 Hz, 2 H), 2.22 - 2.15 (m, 2 H), 2.14-2.05 (m 4 H), 1.50 (t, *J* = 8.6 Hz, 1 H), 0.94 (d, *J* = 3.1 Hz, 3 H), 0.92 (d, *J* = 3.1 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 468.4.

Homo dimer – [*Cyclo*-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$S]$_2$ prodrug (37a)

The homodimer prodrug thioether **37a** was synthesized **3a** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-47% acetonitrile gradient, obtained the desired homodimer as a white solid (yield: 65%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.09 (bs, 1 H), 7.00 (d, *J* = 8.6 Hz, 1 H), 6.38 (d, *J* = 5.0 Hz, 1 H), 4.58 (t, *J* = 6.7 Hz, 1 H), 4.37 (dd, *J* = 5.1, 8.2 Hz, 1 H), 4.34 (dd, *J* = 4.7, 8.2 Hz, 1 H), 4.14 - 4.05 (m,1 H), 4.03 - 3.91 (m, 1 H), 3.66 - 3.50 (m, 3 H), 3.39 - 3.20 (m, 2 H), 2.97 (dd, *J* = 3.9, 12.5 Hz, 1 H), 2.81 (t, *J* = 12.5 Hz, 1 H), 2.63 (t, *J* = 6.8 Hz, 2 H), 2.35 - 2.21 (m, 2 H), 2.17 (dd, *J* = 5.3, 10.8 Hz, 1 H), 2.13 - 2.06 (m, 1 H), 2.06 - 1.99 (m, 1 H), 1.99 - 1.87 (m, 2 H), 1.85 - 1.81 (m, 2 H), 1.83 (td, *J* = 6.6, 13.0 Hz, 2 H), 0.85 (d, *J* = 3.9 Hz, 3 H), 0.84 (d, *J* = 3.9 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.2, 173.2, 172.5, 172.2, 170.9, 65.5, 62.6, 58.4,

56.7, 53.0, 47.8, 47.5, 36.6, 34.4, 31.6, 29.7, 28.5, 28.1, 25.9, 25.0, 20.1, 17; MS (ESI): found: $[M + H]^+$, 961.8.

Cyclo-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$SH (6a)

Thiol warhead having SLA analog **6a** was synthesized from **37a** by following the above general procedure for synthesis of sulfhydryl from corresponding homodimer, obtained the desired pendent thiol as a white solid (yield: 79%, purity by LC-MS: >99%). ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 7.31 (d, *J* = 9.0 Hz, 1 H), 7.15 (bs, 1 H), 6.58 (d, *J* = 10.2 Hz, 1 H), 5.11 - 5.00 (m, 1 H), 4.64 (t, *J* = 7.2 Hz, 1 H), 4.56 (dd, *J* = 3.7, 9.2 Hz, 1 H), 4.48 (dd, *J* = 5.7, 8.4 Hz, 1 H), 4.14 (td, *J* = 7.0, 9.9 Hz, 1 H), 3.73 - 3.55 (m, 3 H), 3.54 - 3.41 (m, 2 H), 3.36 (td, *J* = 6.5, 13.2 Hz, 1 H), 2.67 (dd, *J* = 8.8, 13.1 Hz, 1 H), 2.57 (q, *J* = 7.2 Hz, 3 H), 2.50 - 2.40 (m, 1 H), 2.25 - 2.12 (m, 4 H), 2.07 (td, *J* = 6.2, 12.7 Hz, 1 H), 1.99 - 1.92 (m, 2 H), 1.89 - 1.79 (m, 2 H), 1.43 (t, *J* = 8.0 Hz, 1 H), 0.92 (d, *J* = 6.7 Hz, 3 H), 0.90 - 0.86 (d, *J* = 6.7 Hz, 3 H). MS (ESI): found: $[M + H]^+$, 482.4.

Homo dimer – [_Cyclo_-^{D}Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$S]$_2$ prodrug (37b)

The homodimer prodrug thioether **37b** was synthesized from **3b** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-47% acetonitrile gradient, obtained the desired homodimer as a white solid (yield: 67%, purity by LC-MS: 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.07 (d, *J* = 9.4 Hz, 1 H), 6.72 (t, *J* = 5.3 Hz, 1 H), 6.34 (d, *J* = 11.2 Hz, 1 H), 4.91 (t, *J* = 9.6 Hz, 1 H), 4.58 (dd, *J* = 2.7, 9.8 Hz, 1 H), 4.53 (t, *J* = 6.8 Hz, 1 H), 4.34 (dd, *J* = 4.9, 8.8 Hz, 1 H), 4.11 - 4.02 (m, 1 H), 3.62 - 3.38 (m, 6 H), 3.26 - 3.14 (m, 1 H), 2.64 (dt, *J* = 2.3, 7.0 Hz, 2 H), 2.54 (dt, *J* = 2.9, 6.6 Hz, 1 H), 2.46 (dd, *J* = 9.4, 12.5 Hz, 1 H), 2.37 - 2.28 (m, 1 H), 2.08 - 2.02 (m, 4 H), 2.02 - 1.93 (m, 2 H), 1.84 (p, *J* = 6.8 Hz, 12 H), 1.82 - 1.75 (m, 1 H), 0.84 (d, *J* = 6.7 Hz, 3 H), 0.80 (d, *J* = 6.7 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 961.8.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$SH (6b)**

Thiol warhead having SLA analog **6b** was synthesized from **37b** by following the above general procedure for synthesis of sulfhydryl from corresponding homodimer, obtained the desired pendent thiol as a white solid (yield: 85%, purity by LC-MS: >99%). ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 7.28 (d, *J* = 7.4 Hz, 1 H), 6.62 (bs, 1 H), 6.51 (d, *J* = 6.3 Hz, 1 H), 4.72 - 4.65 (m, 1 H), 4.51 (dd, *J* = 4.3, 8.2 Hz, 1 H), 4.47 (dd, *J* = 5.0, 9.2 Hz, 1 H), 4.17 (td, *J* = 6.6, 9.9 Hz, 2 H), 4.04 - 3.96 (m, 2 H), 3.72 - 3.61 (m, 3 H), 3.45 (td, *J* = 6.7, 13.3 Hz, 1 H), 3.37 (td, *J* = 6.6, 13.0 Hz, 1 H), 3.07 (dd, *J* = 4.7, 12.5 Hz, 1 H), 2.96 (t, *J* = 12.5 Hz, 1 H), 2.55 (d, *J* = 7.8 Hz, 1 H), 2.59 - 2.52 (m, 1 H), 2.47 - 2.18 (m, 4 H), 2.17 - 2.02 (m, 4 H), 1.87 - 1.78 (m, 2 H), 1.44 (t, *J* = 8.0 Hz, 1 H), 0.94 (d, *J* = 6.9 Hz, 3 H), 0.92 (d, *J* = 6.7 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 482.4.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$SCH$_3$ (7a)**

The cyclic peptide **7a** having the methylthioether warhead on pendent carboxylic acid was synthesized from **3a** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with 3-(methylthio)propan-1-amine and purified by HPLC using 5-55% acetonitrile gradient, obtained the desired peptide as a white solid (yield: 68%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.17 (d, *J* = 9.4 Hz, 1 H), 6.78 (t, *J* = 6.4 Hz, 1 H), 6.41 (d, *J* = 10.6 Hz, 1 H), 5.00 (t, *J* = 10.0 Hz, 1 H), 4.66 (dd, *J* = 3.1, 9.4 Hz, 1 H), 4.61 (t, *J* = 6.7 Hz, 1 H), 4.44 (dd, *J* = 4.9, 8.4 Hz, 1 H), 4.20 - 4.11 (m, 1 H), 3.70 - 3.60 (m, 3 H), 3.57 (d, *J* = 12.5 Hz, 1 H), 3.48 (dt, *J* = 6.7, 13.3 Hz, 1 H), 3.33 (dt, *J* = 6.7, 12.9 Hz, 1 H), 2.63 (dt, *J* = 2.9, 6.9 Hz, 1 H), 2.60 - 2.49 (m, 2 H), 2.47 - 2.35 (m, 1 H), 2.21 - 2.11 (m, 3 H), 2.08 (s, 3 H), 2.07 - 1.99 (m, 1 H), 1.98 - 1.88 (m, 2 H), 1.83 (quin, *J* = 7.0 Hz, 2 H), 0.92 (d, *J* = 6.7 Hz, 3 H), 0.89 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.8, 172.5, 172.2, 171.3, 170.3, 62.8, 58.6, 56.5, 51.1, 48.4, 47.4, 39.1, 34.8, 31.2, 29.9, 29.0, 28.3, 28.1, 26.1, 25.0, 20.0, 16.8, 15.3; MS (ESI): found: $[M + Na]^+$, 518.8.

***Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$SCH$_3$ (7b)**

The cyclic peptide **7b** having the methylthioether warhead on pendent carboxylic acid was synthesized from **3b** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with 3-(methylthio)propan-1-amine and purified by HPLC using 5-55% acetonitrile gradient, obtained the desired peptide as a white solid (yield: 65%, purity by LC-MS: 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.15 (d, *J* = 9.4 Hz, 1 H), 7.08 (t, *J* = 6.4 Hz, 1 H), 6.41 (d, *J* = 6.3 Hz, 1 H), 4.67 (t, *J* = 9.8 Hz, 1 H), 4.49 (dd, *J* = 3.2, 9.2 Hz, 1 H), 4.45 (dd, *J* = 4.8, 8.7 Hz, 1 H), 4.17 (td, *J* = 6.3, 9.8 Hz, 1 H), 3.97 (td, *J* = 5.5, 11.2 Hz, 1 H), 3.68 (t, *J* = 6.4 Hz, 2 H), 3.67 - 3.59 (m, 1 H), 3.45 - 3.28 (m, 2 H), 3.06 (dd, *J* = 4.1, 12.7 Hz, 1 H), 2.92 (t, *J* = 12.5 Hz, 1 H), 2.51 (t, *J* = 7.2 Hz, 2 H), 2.43 - 2.30 (m, 2 H), 2.29 - 2.17 (m, 2 H), 2.16 - 2.10 (m, 2 H), 2.09 (s, 3 H), 2.06 - 1.95 (m, 3 H), 1.81 (dp, *J* = 2.2, 7.0 Hz, 2 H), 0.94 (d, *J* = 3.5 Hz, 3 H), 0.92 (d, *J* = 3.1 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.1, 173.2, 172.3, 172.1, 169.6, 62.6, 58.3, 56.6, 53.3, 47.8, 47.4, 38.7, 34.4, 31.4, 29.7, 28.6, 28.3, 28.1, 25.9, 24.9, 20.1, 17.2, 15.4; MS (ESI): found: $[M + H]^+$, 496.4.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$S(=O)CH$_3$ (8a)**

The cyclic peptide **8a** having the methylsulfoxide warhead on pendent carboxylic acid was synthesized from **7a** by following the above general procedure for sulfoxide synthesis from organic sulfides by H_2O_2/Borax and purified by HPLC using 5-50% acetonitrile gradient, obtained the desired sulfoxide as a white solid (yield: 69%, purity by LC-MS: >99%). Non-separable diastereomeric mixture = 1.1:1; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.20 (d, *J* = 7.0 Hz, 1 H), 7.18 (d, *J* = 7.2 Hz, 1 H), 6.97 (t, *J* = 5.9 Hz, 1 H), 6.95 (t, *J* = 5.6 Hz, 1 H), 6.37 (d, *J* = 10.6 Hz, 1 H), 6.31 (d, *J* = 10.6 Hz, 1 H), 4.98 (t, *J* = 9.8 Hz, 2 H), 4.65 (dd, *J* = 3.1, 9.8 Hz, 2 H), 4.61 (t, *J* = 7.0 Hz, 2 H), 4.42 (dd, *J* = 4.7, 8.2 Hz, 1 H), 4.39 (dd, *J* = 4.5, 7.9 Hz, 1 H), 4.20 - 4.09 (m, 2 H), 3.73 -

3.54 (m, 8 H), 3.38 - 3.21 (m, 2 H), 2.91 (t, *J* = 8.1 Hz, 2 H), 2.81 (t, *J* = 8.1 Hz, 2 H), 2.67 (s, 3 H), 2.65 (s, 3 H), 2.64 - 2.59 (m, 2 H), 2.53 (ddd, *J* = 2.3, 9.6, 12.3 Hz, 2 H), 2.47 - 2.35 (m, 2 H), 2.20 - 2.09 (m, 9 H), 2.09 - 2.00 (m, 7 H), 1.98 - 1.83 (m, 4 H), 0.92 (d, *J* = 7.0 Hz, 6 H), 0.89 (d, *J* = 6.9 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 175.0, 175.0, 172.4, 172.3, 172.3, 171.1, 170.6, 170.5, 62.8, 62.8, 58.6, 56.5, 53.4, 51.0, 50.7, 50.3, 48.5, 47.4, 38.4, 37.6, 34.4, 34.3, 31.6, 29.9, 28.8, 28.1, 28.0, 26.1, 25.0, 22.8, 22.6, 19.9, 16.7; MS (ESI): found: $[M + H]^+$, 512.4.

***Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$S(=O)CH$_3$ (8b)**

The cyclic peptide **8b** having the methylsulfoxide warhead on pendent carboxylic acid was synthesized from **7b** by following the above general procedure for sulfoxide synthesis from organic sulfides by H_2O_2/Borax and purified by HPLC using 5-50% acetonitrile gradient, obtained the desired sulfoxide as a white solid (yield: 65%, purity by LC-MS: 98%). Non-separable diastereomeric mixture = 1:1. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 7.12 (d, *J* = 7.0 Hz, 1 H), 6.87 (bs, 1 H), 6.41 (bs, 1 H), 4.69 (t, *J* = 6.7 Hz, 1 H), 4.53 (td, *J* = 3.2, 9.3 Hz, 1 H), 4.44 (dd, *J* = 4.5, 8.8 Hz, 1 H), 4.16 (td, *J* = 6.4, 9.6 Hz, 1 H), 3.93 - 3.84 (m, 1 H), 3.72 - 3.52 (m, 4 H), 3.38 - 3.22 (m, 1 H), 3.06 (dd, *J* = 3.9, 12.5 Hz, 1 H), 2.91 (t, *J* = 11.9 Hz, 1 H), 2.86 - 2.70 (m, 2 H), 2.62 (s, 1.5 H), 2.61 (s, 1.5 H), 2.46 - 2.30 (m, 2 H), 2.29 - 2.17 (m, 1 H), 2.16 - 1.91 (m, 6 H), 1.87 - 1.70 (m, 2 H), 0.93 (d, *J* = 6.7 Hz, 3 H), 0.92 (d, *J* = 6.6 Hz, 3 H); MS (ESI): found: $[M + Na]^+$, 512.4.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$S(=O)$_2$CH$_3$ (9a)**

The cyclic peptide **9a** having the methylsulfone warhead on pendent carboxylic acid was synthesized from **7a** by following the above general procedure for sulfones synthesis from organic sulfides by H_2O_2/Borax and purified by HPLC using 5-48% acetonitrile gradient over 30 minutes, obtained the desired sulfone as a white solid (yield: 83%, purity by LC-MS: >99%). ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 7.19 (d, *J* = 9.4 Hz, 1 H), 6.89 (t, *J* = 9.4 Hz, 1 H), 6.35 (d, *J* = 10.4 Hz, 1 H), 4.98 (t, *J* =

10.0 Hz, 1 H), 4.66 (dd, *J* = 2.9, 9.6 Hz, 1 H), 4.61 (t, *J* = 7.4 Hz, 1 H), 4.39 (dd, *J* = 5.1, 8.6 Hz, 1 H), 4.15 (td, *J* = 6.7, 10.0 Hz, 1 H), 3.76 (dd, *J* = 5.5, 13.7 Hz, 1 H), 3.69 - 3.58 (m, 2 H), 3.34 - 3.23 (m, 1 H), 3.23 - 3.11 (m, 2 H), 3.11 - 2.93 (m, 2 H), 2.90 (s, 3 H), 2.63 (dt, *J* = 3.1, 6.8 Hz, 1 H), 2.52 (dd, *J* = 9.6, 12.3 Hz, 1 H), 2.46 - 2.35 (m, 1 H), 2.21 - 2.10 (m, 3H), 2.10 - 1.95 (m, 2 H), 1.95 - 1.83 (m, 1 H), 0.92 (d, *J* = 7.0 Hz, 3 H), 0.89 (d, *J* = 6.7 Hz, 3 H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 175.2, 172.4, 172.2, 171.1, 170.5, 62.8, 58.7, 56.5, 51.6, 51.0, 48.6, 47.4, 40.4, 37.9, 34.2, 29.9, 28.8, 28.0, 26.1, 25.0, 22.5, 19.9, 16.7; MS (ESI): found: $[M + H]^+$, 528.4.

***Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-$NHCH_2CH_2CH_2S(=O)_2CH_3$ (9b)**

The cyclic peptide **9b** having the methylsulfone warhead on pendent carboxylic acid was synthesized from **7b** by following the above general procedure for sulfones synthesis from organic sulfides by H_2O_2/Borax and purified by HPLC using 5-48% acetonitrile gradient over 30 minutes, obtained the desired sulfone as a white solid (yield: 85%, purity by LC-MS: 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.11 (d, *J* = 9.4 Hz, 1 H), 6.78 (t, *J* = 4.7 Hz, 1 H), 6.44 (d, *J* = 6.3 Hz, 1 H), 4.69 (t, *J* = 11.2 Hz, 1 H), 4.54 (dd, *J* = 3.7, 9.6 Hz, 1 H), 4.43 (dd, *J* = 4.5, 8.8 Hz, 1 H), 4.15 (dd, *J* = 6.8, 10.0 Hz, 1 H), 3.93 - 3.83 (m, 1 H), 3.71 - 3.54 (m, 4 H), 3.31 - 3.19 (m, 2 H), 3.10 - 3.01 (m, 2 H), 2.93 (s, 3 H), 2.88 (d, *J* = 4.7 Hz, 1 H), 2.44 - 2.30 (m, 2 H), 2.26 - 1.97 (m, 6 H), 1.91 (d, *J* = 7.0 Hz, 1 H), 0.93 (d, *J* = 4.3 Hz, 3 H), 0.91 (d, *J* = 3.9 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.5, 173.7, 172.6, 171.5, 169.7, 62.7, 58.4, 56.0, 53.0, 51.5, 47.8, 47.4, 40.8, 37.6, 34.3, 29.7, 28.5, 28.1, 26.0, 25.0, 22.5, 20.3, 17.0; MS (ESI): found: $[M + Na]^+$, 528.4.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-$NHCH_2CH_2CH_2OH$ (10a)**

The cyclic peptide **10a** having the *n*-propylalcohol warhead on pendent carboxylic acid was synthesized from **3a** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with 3-aminopropan-1-ol and purified by HPLC using 11-40% acetonitrile

gradient over 30 minutes, obtained the desired alcohol as viscous oil (yield: 60%, purity by LC-MS: >97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.18 (t, *J* = 5.7 Hz, 1 H), 7.04 (d, *J* = 9.6 Hz, 1 H), 6.28 (d, *J* = 10.4 Hz, 1 H), 4.98 (t, *J* = 10.0 Hz, 1 H), 4.68 (dd, *J* = 2.9, 9.8 Hz, 1 H), 4.63 (t, *J* = 6.7 Hz, 1 H), 4.42 (dd, *J* = 4.8, 8.7 Hz, 1 H), 4.16 (td, *J* = 6.6, 9.9 Hz, 1 H), 3.79 - 3.69 (m, 3 H), 3.67 - 3.56 (m, 4 H), 3.38 - 3.27 (m, 1 H), 2.69 - 2.59 (m, 1 H), 2.52 (dd, *J* = 9.5, 12.4 Hz, 1 H), 2.46 - 2.34 (m, 1 H), 2.17 - 2.02 (m, 4 H), 1.97 - 1.83 (m, 4 H), 1.73-1.71 (m, 1 H), 0.92 (d, *J* = 6.8 Hz, 3 H), 0.89 (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 175.0, 172.5, 172.0, 171.1, 170.9, 62.8, 60.5, 58.6, 56.6, 50.7, 48.3, 47.4, 38.0, 34.5, 31.1, 29.8, 28.8, 28.1, 26.0, 25.0, 19.9, 16.7; MS (ESI): found: $[M + H]^+$, 466.4.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$OH (10b)**

The cyclic peptide **10b** having the *n*-propylalcohol warhead on pendent carboxylic acid was synthesized from **3b** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with 3-aminopropan-1-ol and purified by HPLC using 11-40% acetonitrile gradient over 30 minutes, obtained the desired alcohol as viscous oil (yield: 75%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.38 - 7.17 (m, 2 H), 6.51 (bs, 1H), 4.78 - 4.67 (m, 1 H), 4.62 - 4.39 (m, 2 H), 4.32 - 4.15 (m, 1 H), 4.15 - 3.96 (m, 1 H), 3.84 - 3.67 (m, 3 H), 3.67 - 3.47 (m, 1 H), 3.44 - 3.24 (m, 1 H), 3.24 - 3.05 (m, 1 H), 3.05 - 2.92 (m, 1 H), 2.54 - 2.35 (m, 2 H), 2.35 - 2.23 (m, 2 H), 2.20 - 2.13 (m, 2 H), 2.13 - 1.92 (m, 3 H), 1.91 - 1.64 (m, 2 H), 1.22 - 0.95 (m, 6 H); ^{13}C NMR (100 MHz ,$CDCl_3$) δ ppm: 174.2, 173.2, 172.5, 172.1, 170.9, 170.0, 65.4, 62.5, 58.4, 56.6, 53.0, 47.8, 47.4, 34.4, 31.6, 29.7, 28.4, 28.1, 28.0, 25.9, 24.9, 20.1, 17.1; MS (ESI): found: $[M + H]^+$, 466.4.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$S-SCH$_2$CH$_2$NHP(O)(OPh) (11a)**

The cyclic peptide **11a** having the phosphate warhead on pendent carboxylic acid was synthesized by following from **3a** the above general procedure for DPPA peptide coupling of coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired peptide as a white solid (yield: 52%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.39 - 7.30 (m, 4 H), 7.28 - 7.22 (m, 8 H), 7.22 - 7.14 (m, 2 H), 7.09 (d, *J* = 9.8 Hz, 1 H), 7.03 (t, *J* = 5.3 Hz, 1 H), 6.46 (d, *J* = 10.4 Hz, 1 H), 5.00 (t, *J* = 10.0 Hz, 1 H), 4.64 (dd, *J* = 3.1, 9.4 Hz, 1 H), 4.60 (t, *J* = 7.0 Hz, 1 H), 4.38 (dd, *J* = 5.1, 8.2 Hz, 1 H), 4.15 - 4.06 (m, 1 H), 3.70 - 3.48 (m, 5 H), 3.47 - 3.35 (m, 2 H), 2.83 - 2.72 (m, 4 H), 2.61 (dt, *J* = 3.5, 6.8 Hz, 1 H), 2.55 (dd, *J* = 9.6, 12.7 Hz, 1 H), 2.39 - 2.26 (m, 1 H), 2.17 - 2.03 (m, 4 H), 2.03 - 1.91 (m, 2 H), 1.87 (dd, *J* = 8.0, 12.3 Hz, 1 H), 0.91 (d, *J* = 7.0 Hz, 3 H), 0.88 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.9, 172.5, 172.1, 171.0, 170.6, 150.6, 129.8, 125.2, 120.8, 62.8, 58.5, 56.5, 50.9, 48.3, 47.5, 40.2, 39.9, 37.3, 34.7, 29.9, 29.7, 28.9, 28.0, 26.0, 25.0, 19.9, 16.8; MS (ESI): found: $[M + Na]^+$, 775.5.

***Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$S-SCH$_2$CH$_2$NHP(O)(OPh) (11b)**

The cyclic peptide **11b** having the phosphate warhead on pendent carboxylic acid was synthesized from **3b** by following the above general procedure for DPPA peptide coupling of coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired peptide as a white solid (yield: 56%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.35 (t, *J* = 8.0 Hz, 4 H), 7.28 - 7.23 (m, 4 H), 7.18 (t, *J* = 7.2 Hz, 3 H), 7.12 (d, *J* = 9.4 Hz, 1 H), 6.47 (d, *J* = 6.3 Hz, 1 H), 4.66 (t, *J* = 6.7 Hz, 1 H), 4.49 (dd, *J* = 4.7, 9.4 Hz, 1 H), 4.44 (dd, *J* = 4.7, 8.6 Hz, 1 H), 4.21 - 4.14 (m, 1 H), 4.14 - 4.05 (m, 1 H), 4.04 - 3.95 (m, 1 H), 3.64 (t, *J* = 6.8 Hz, 2 H), 3.62 - 3.56 (m, 1 H), 3.55 - 3.44 (m, 1 H), 3.39 (dd, *J*

= 5.9, 12.1 Hz, 2 H), 3.03 (dd, *J* = 4.3, 12.5 Hz, 1 H), 2.94 - 2.85 (m, 1 H), 2.79 - 2.72 (m, 2 H), 2.71 (t, *J* = 6.6 Hz, 2 H), 2.43 - 2.26 (m, 2 H), 2.26 - 2.14 (m, 2 H), 2.14 - 2.04 (m, 3 H), 2.04 - 1.91 (m, 2 H), 0.91 (d, *J* = 6.7 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm : 174.1, 173.3, 172.2, 169.8, 167.0, 150.5, 129.8, 125.2, 120.2, 120.2, 62.6, 58.3, 56.6, 53.0, 47.7, 47.4, 40.4, 39.7, 38.4, 37.2, 34.4, 29.7, 28.5, 28.1, 25.9, 24.9, 20.1, 17.1; MS (ESI): found: $[M + Na]^+$, 775.5.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$COOMe (12a)**

The cyclic peptide **12a** having the methylcarboxylate warhead on pendent carboxylic acid was synthesized from **3a** by following the above general procedure for EDCl peptide coupling of coupling of pendent carboxylic acid with methyl 4-aminobutanoate and purified by HPLC using 11-45% acetonitrile gradient over 30 minutes, obtained the desired peptide as a white solid (yield: 86%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 7.18 (d, *J* = 9.4 Hz, 1 H), 6.85 (t, *J* = 4.7 Hz, 1 H), 6.41 (d, *J* = 10.2 Hz, 1 H), 5.00 (t, *J* = 9.8 Hz, 1 H), 4.67 (dd, *J* = 2.3, 9.4 Hz, 1 H), 4.61 (t, *J* = 6.7 Hz, 1 H), 4.45 (dd, *J* = 5.1, 8.2 Hz, 1 H), 4.20 - 4.11 (m, 1 H), 3.67 (s, 3 H), 3.63 (t, *J* = 7.1 Hz, 2 H), 3.56 (d, *J* = 12.9 Hz, 1 H), 3.44 - 3.34 (m, 1 H), 3.34 - 3.23 (m, 1 H), 2.68 - 2.60 (m, 1 H), 2.56 (dd, *J* = 10.0, 12.3 Hz, 1 H), 2.47 - 2.36 (m, 3 H), 2.21 - 2.02 (m, 5 H), 1.99 - 1.81 (m, 4 H), 0.93 (d, *J* = 7.0 Hz, 3 H), 0.89 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.8, 173.6, 172.5, 172.3, 171.2, 170.4, 62.7, 58.6, 56.5, 51.7, 51.0, 48.3, 47.4, 39.4, 34.8, 31.2, 29.9, 28.9, 28.1, 26.0, 25.0, 24.4, 19.9, 16.8; MS (ESI): found: $[M + H]^+$, 508.5.

***Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$COOMe (12b)**

The cyclic peptide **12b** having the methylcarboxylate warhead on pendent carboxylic acid was synthesized from **3b** by following the above general procedure for EDCl peptide coupling of coupling of pendent carboxylic acid with methyl 4-aminobutanoate and purified by HPLC using 11-45% acetonitrile gradient over 30 minutes, obtained the desired peptide as a white solid (yield: 85%,

purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.11 (d, *J* = 9.4 Hz, 1 H), 6.92 (t, *J* = 5.3 Hz, 1 H), 6.37 (d, *J* = 6.3 Hz, 1 H), 4.68 (t, *J* = 6.7 Hz, 1 H), 4.52 (dd, *J* = 4.3, 9.4 Hz, 1 H), 4.44 (dd, *J* = 4.3, 8.6 Hz, 1 H), 4.22 - 4.11 (m, 1 H), 3.94 - 3.85 (m, 1 H), 3.72 – 3.68 (m, 1 H), 3.67 (s, 3 H), 3.66 - 3.59 (m, 2 H), 3.39 - 3.22 (m, 2 H), 3.07 (dd, *J* = 4.1, 12.7 Hz, 1 H), 2.93 (t, *J* = 12.5 Hz, 1 H), 2.43 - 2.33 (m, 1 H), 2.36 (t, *J* = 6.9 Hz, 2 H), 2.29 - 2.19 (m, 1 H), 2.16 - 2.06 (m, 3 H), 2.06 - 1.98 (m, 2 H), 1.83 (p, *J* = 7.6 Hz, 2 H), 1.82 - 1.71 (m, 2 H), 0.93 (d, *J* = 6.9 Hz, 3 H), 0.93 (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.3, 173.7, 173.5, 172.5, 171.6, 169.4, 62.7, 58.3, 56.2, 53.4, 51.7, 47.8, 47.4, 39.0, 34.3, 31.2, 29.8, 28.5, 28.1, 25.9, 25.0, 24.6, 20.2, 17.1; MS (ESI): found: $[M + H]^+$, 508.5.

Cyclo-Asp(^D^Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$COOH (13a)

The cyclic peptide **13a** having the carboxylic acid warhead on pendent carboxylic acid was synthesized from **12a** by following the above general procedure for general procedure for LiOH base mediated hydrolysis of methyl ester and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired peptide as a white solid (yield: 94%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.68 (t, *J* = 4.9 Hz, 1 H), 7.16 (d, *J* = 9.4 Hz, 1 H), 6.21 (d, *J* = 10.2 Hz, 1 H), 5.08 (bs, 1 H), 4.98 (t, *J* = 10.0 Hz, 1 H), 4.76 (dd, *J* = 3.1, 9.4 Hz, 1 H), 4.60 (t, *J* = 6.8 Hz, 1 H), 4.40 (dd, *J* = 5.1, 8.6 Hz, 1 H), 4.21 - 4.05 (m, 1 H), 3.78 - 3.69 (m, 1 H), 3.68 - 3.57 (m, 2 H), 3.42 - 3.25 (m, 2 H), 2.67 - 2.58 (m, 1 H), 2.58 - 2.49 (m, 2 H), 2.42 (dd, *J* = 6.5, 13.1 Hz, 2 H), 2.22 - 1.99 (m, 5 H), 1.99 - 1.78 (m, 4 H), 0.93 (d, *J* = 6.7 Hz, 3 H), 0.89 (d, *J* = 7.0 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 177.0, 174.8, 173.6, 171.5, 171.2, 170.4, 63.0, 58.4, 56.7, 50.5, 48.4, 47.4, 40.9, 34.1, 31.8, 29.9, 28.8, 28.1, 26.0, 25.0, 22.5, 19.9, 16.8; MS (ESI): found: $[M + H]^+$, 494.4.

***Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$COOH (13b)**

The cyclic peptide **13b** having the carboxylic acid warhead on pendent carboxylic acid was synthesized from **12b** by following the above general procedure for general procedure for LiOH base mediated hydrolysis of methyl ester and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired peptide as a white solid (yield: 95%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.20 (bs, 1 H), 7.12 (d, *J* = 9.0 Hz, 1 H), 6.50 (d, *J* = 5.9 Hz, 1 H), 4.92 (bs, 1 H), 4.67 (t, *J* = 6.5 Hz, 1 H), 4.48 (dd, *J* = 4.9, 9.6 Hz, 1 H), 4.42 (dd, *J* = 4.5, 8.0 Hz, 1 H), 4.19 (q, *J* = 9.4 Hz, 1 H), 4.07 - 3.98 (m, 1 H), 3.69 (t, *J* = 6.1 Hz, 2 H), 3.64 (q, *J* = 8.5 Hz, 1 H), 3.39 (dd, *J* = 6.3, 13.3 Hz, 1 H), 3.30 (dd, *J* = 6.3, 12.9 Hz, 1 H), 3.06 (dd, *J* = 3.7, 12.3 Hz, 1 H), 2.92 (t, *J* = 12.4 Hz, 1 H), 2.40 (t, *J* = 6.7 Hz, 2 H), 2.38 - 2.29 (m, 2 H), 2.27 (d, *J* = 5.1 Hz, 1 H), 2.15 - 2.08 (m, 4 H), 2.04 - 1.95 (m, 4 H), 1.85 (quin, *J* = 6.6 Hz, 2 H), 0.94 (br. d, *J* = 6.3 Hz, 3 H), 0.92 (d, *J* = 6.3 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 176.1, 174.0, 172.9, 172.3, 172.2, 170.0, 62.6, 58.2, 56.9, 52.6, 47.8, 47.5, 39.2, 34.5, 31.4, 29.7, 28.6, 28.1, 25.9, 25.0, 24.4, 20.1, 17.3; MS (ESI): found: $[M + H]^+$, 494.4.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$CONHOH (14a)**

The cyclic peptide **14a** having the hydroxamic acid warhead on pendent carboxylic acid was synthesized from **12a** by following the above general procedure for general procedure for hydroxamic acid synthesis from the methyl ester and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired hydroxamic acid peptide as a white solid (yield: 87%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.30 (bs, 1 H), 8.00 (t, *J* = 4.7 Hz, 1 H), 6.91 (d, *J* = 8.2 Hz, 1 H), 6.50 (d, *J* = 9.8 Hz, 1 H), 4.68 (t, *J* = 7.0 Hz, 1 H), 4.59 (t, *J* = 6.3 Hz, 1 H), 4.24 (d, *J* = 3.5 Hz, 1 H), 4.16 (dd, *J* = 5.3, 8.4 Hz, 1 H), 3.96 (d, *J* = 8.6 Hz, 1 H), 3.74 - 3.64 (m, 1 H), 3.63 - 3.53 (m, 4 H), 3.16 - 3.06 (m, 2 H), 3.06 - 2.97 (m, 1 H), 2.51 - 2.40 (m, 1 H),

2.36-2.30 (m, 1 H), 2.29-2.25 (m, 1 H), 2.19-2.08 (m, 1 H), 2.06-1.99 (m, 2 H), 1.95 (t, *J* = 7.0 Hz, 2 H), 1.85 - 1.71 (m, 3 H), 1.67 - 1.59 (p, *J* = 6.8 Hz, 2 H), 0.84 (d, *J* = 6.7 Hz, 3 H), 0.79 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 173.7, 171.5, 171.0, 170.3, 170.2, 169.3, 62.5, 58.0, 56.4, 50.2, 47.9, 47.3, 39.0, 36.1, 30.2, 29.9, 29.1, 28.2, 25.8, 25.5, 24.9, 20.1, 17.6; MS (ESI): found: $[M + H]^+$, 509.5.

***Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$CONHOH (14b)**

The cyclic peptide **14b** having the hydroxamic acid warhead on pendent carboxylic acid was synthesized from **12b** by following the above general procedure for general procedure for hydroxamic acid synthesis from the methyl ester and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired hydroxamic acid peptide as a white solid (yield: 85%, purity by LC-MS: 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.29 (bs, 1 H), 7.73 (t, *J* = 4.9 Hz, 1 H), 6.89 (d, *J* = 5.5 Hz, 1 H), 6.37 (d, *J* = 9.8 Hz, 1 H), 4.68 - 4.62 (m, 1 H), 4.27 - 4.14 (m, 3 H), 4.01 - 3.86 (m, 2 H), 3.75 - 3.66 (m, 3 H), 3.61-3.58 (m, 2 H), 3.05 - 2.89 (m, 3 H), 2.30 - 2.04 (m, 6 H), 2.03 - 1.88 (m, 6 H), 1.88 - 1.75 (m, 4 H), 1.63 - 1.54 (m, 2 H), 0.84 (d, *J* = 6.8 Hz, 3 H), 0.78 (d, *J* = 6.9 Hz, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 173.9, 172.0, 171.6, 171.5, 169.4, 169.4, 62.5, 58.0, 56.3, 51.5, 47.6, 47.5, 38.8, 34.9, 30.2, 29.9, 28.6, 28.3, 25.9, 25.6, 24.9, 20.6, 17.6; MS (ESI): found: $[M + Na]^+$, 509.5.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$S-SCH$_2$CH$_2$NH$_2$ (15a)**

The amine-containing peptide **15a** was synthesized from **3a** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired homodimer as a viscous oil (yield: 65%, purity by LC-MS: >99%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.24 (br. s., 2 H), 7.76 (t, *J* = 6.1 Hz, 1 H), 7.19 (d, *J* = 9.0 Hz, 1 H), 6.30 (d, *J* = 10.6 Hz, 1 H),

5.04 (t, *J* = 10.0 Hz, 1 H), 4.59 (t, *J* = 7.2 Hz, 1 H), 4.50 (dd, *J* = 3.3, 9.6 Hz, 1 H), 4.43 (dd, *J* = 6.1, 8.0 Hz, 1 H), 4.20 - 4.12 (m, 1 H), 3.80 - 3.72 (m, 1 H), 3.70 - 3.52 (m, 4 H), 3.38 - 3.26 (m, 2 H), 3.19 - 3.10 (m, 2 H), 3.02 - 2.91 (m, 2 H), 2.62 - 2.53 (m, 2 H), 2.45 - 2.36 (m, 3 H), 2.20 - 2.09 (m, 4 H), 2.03 - 1.89 (m, 2 H), 0.86 (d, *J* = 6.9 Hz, 3 H), 0.85 (d, *J* = 7.0 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 543.5.

***Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$S-SCH$_2$CH$_2$NH$_2$ (15b)**

The amine-containing peptide **15b** was synthesized from **3b** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired homodimer as a viscous oil (yield: 67%, purity by LC-MS: 97%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.11 (t, *J* = 6.5 Hz, 1 H), 8.06 (bs, 2 H), 6.76 (d, *J* = 9.8 Hz, 1 H), 6.54 (d, *J* = 7.0 Hz, 1 H), 4.61 (dd, *J* = 6.1, 7.2 Hz, 1 H), 4.47 - 4.39 (m, 1 H), 4.35 - 4.29 (m, 1 H), 4.20 (dd, *J* = 7.0, 16.4 Hz, 1 H), 3.75 - 3.59 (m, 3 H), 3.39 - 3.28 (m, 3 H), 3.10 - 2.90 (m, 3 H), 2.76 (t, *J* = 12.7 Hz, 1 H), 2.41 - 2.31 (m, 1 H), 2.31 - 2.21 (m, 1 H), 2.20 - 2.08 (m, 3 H), 2.06 - 1.92 (m, 3 H), 1.82 - 1.72 (m, 3 H), 0.97 (d, *J* = 6.3 Hz, 3 H), 0.92 (d, *J* = 6.7 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 543.5.

***Cyclo*-Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$NH$_2$ (16a) and *Cyclo*-^{D}Asp(^{D}Pro-Pro-Val)-NHCH$_2$CH$_2$CH$_2$NH$_2$ (16b).**

The amine-containing unseparable peptides **16a** and **16b** were synthesized by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with 1,3-diaminopropane and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired diastereomeric mixture (3:1) of amino propyl amide as a viscous oil (yield: 81%, purity by LC-MS: 99%); Diastereomer **16a** : ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 7.04 (d, *J* = 9.0 Hz, 1 H), 6.57 (d, *J* = 10.2 Hz, 1 H), 5.07 (dt, *J* = 3.2, 9.8 Hz, 1 H), 4.63 (t, *J* = 7.0 Hz, 1 H), 4.48 (dd, *J* = 3.9, 8.6 Hz, 1

H), 4.36 (dd, *J* = 4.7, 8.6 Hz, 1 H), 4.21 - 4.11 (m, 1 H), 3.77 (dt, *J* = 6.7, 9.4 Hz, 1 H), 3.72 - 3.62 (m, 2 H), 3.62 - 3.54 (m, 1 H), 3.38 - 3.31 (m, 3 H), 2.66 (dd, *J* = 9.6, 13.5 Hz, 1 H), 2.55 - 2.44 (m, 1 H), 2.36 (qd, *J* = 8.0, 12.3 Hz, 1 H), 2.22 - 2.07 (m, 5 H), 2.07 - 1.82 (m, 6 H), 0.92 (d, *J* = 6.7 Hz, 3 H), 0.88 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.2, 170.8, 170.7, 169.3, 168.3, 167.9, 61.9, 57.5, 55.5, 48.4, 47.4, 46.4, 34.8, 29.1, 28.4, 27.8, 27.3, 25.2, 24.2, 20.0, 19.2, 16.4; Diastereomer **16b** : ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 6.99 (d, *J* = 9.4 Hz, 1 H), 6.50 (d, *J* = 6.3 Hz, 1 H), 5.02 - 4.95 (m, 1 H), 4.69 (dd, *J* = 6.3, 7.4 Hz, 1 H), 4.52 (dd, *J* = 3.5, 8.6 Hz, 1 H), 4.42 (dd, *J* = 4.7, 9.4 Hz, 1 H), 4.12 - 4.07 (m,1 H), 3.72 - 3.62 (m, 2 H), 3.62 - 3.54 (m, 1 H), 3.38 - 3.31 (m, 2 H), 3.01 (dd, *J* = 4.9, 12.3 Hz, 17 H), 2.89 (t, *J* = 12.0 Hz, 16 H) 2.55 - 2.36 (m, 2 H), 2.22 - 2.07 (m, 5 H), 2.07 - 1.82 (m, 6 H), 0.91 (d, *J* = 6.7 Hz, 3 H), 0.88 (d, *J* = 6.7 Hz, 3 H); MS (ESI): found: $[M + Na]^+$, 465.4.

***Cyclo*-Asp(Pro-^{D}Pro-Val)-OBn (2a):**

Cyclic tetrapeptide **2b** was synthesized by following the above general procedure for DPPA macrolactamisation and purified by silical gel column chromatography (EtOAc) as a white solid (yield: 67%, purity by LC-MS: >97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.74 (d, *J* = 10.0 Hz, 1 H), 7.41 - 7.27 (m, 5 H), 6.70 (d, *J* = 7.6 Hz, 1 H), 5.31 (d, *J* = 12.3 Hz, 1 H), 5.13 (d, *J* = 12.5 Hz, 1 H), 4.78 (dd, *J* = 4.7, 7.8 Hz, 1 H), 4.75 (d, *J* = 7.4 Hz, 3 H), 4.71 (dt, *J* = 2.7, 5.3 Hz, 3 H), 4.39 - 4.24 (m, 2 H), 3.71 - 3.55 (m, 1 H), 3.55 - 3.39 (m, 2 H), 3.12 (dd, *J* = 5.7, 15.5 Hz, 1 H), 3.01 (dd, *J* = 3.3, 15.5 Hz, 1 H), 2.90 - 2.84 (m, 1 H), 2.56 (dd, *J* = 6.8, 12.3 Hz, 1 H), 2.37 - 2.18 (m, 3 H), 2.18 - 2.05 (m, 2 H), 2.03 - 1.90 (m, 2 H), 1.83 - 1.73 (m, 1 H), 0.92 (d, *J* = 6.7 Hz, 3 H), 0.89 (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm : 172.5, 170.6, 170.2, 170.1, 168.9, 135.4, 128.5, 128.2, 128.0, 67.4, 59.0, 57.5, 57.3, 49.3, 47.3, 47.2, 36.2, 28.3, 27.3, 25.8, 25.5, 25.0, 19.8, 17.5; MS (ESI): found: $[M + H]^+$, 499.4.

***Cyclo*-^{D}Asp(Pro-^{D}Pro-Val)-OBn (2b):**

Cyclic tetrapeptide **2b** was synthesized by following the above general procedure for DPPA macrolactamisation and purified by silical gel column chromatography (EtOAc) as a white solid (yield: 73%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.41 - 7.29 (m, 5 H), 7.01 (d, *J* = 8.6 Hz, 1 H), 6.77 (d, *J* = 8.6 Hz, 1 H), 5.21 (s, 2 H), 4.73 - 4.66 (m, 1 H), 4.61 (dd, *J* = 2.2, 8.8 Hz, 1 H), 4.56 (dd, *J* = 2.3, 8.2 Hz, 1 H), 4.08 (dd, *J* = 6.3, 9.0 Hz, 1 H), 3.86 - 3.76 (m, 1 H), 3.76 - 3.68 (m, 1 H), 3.51 (td, *J* = 7.6, 11.8 Hz, 2 H), 3.04 (dd, *J* = 5.1, 14.9 Hz, 1 H), 2.83 (dd, *J* = 0.8, 5.1 Hz,1 H), 2.57 - 2.44 (m, 1 H), 2.42 - 2.37 (m, 1 H), 2.32 (dd, *J* = 3.3, 15.1 Hz, 1 H), 2.16 - 2.00 (m, 4 H), 1.99 - 1.91 (m, 2 H), 1.02 (d, *J* = 7.0 Hz, 3 H), 1.00 (d, *J* = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.1, 171.2, 170.4, 170.2, 168.8, 135.8, 128.4, 128.3, 128.0, 67.1, 61.6, 60.4, 60.1, 49.2, 46.9, 46.9, 36.0, 30.2, 29.5, 26.6, 25.7, 22.4, 19.3, 18.3; MS (ESI): found: $[M + H]^{+}$, 499.4.

***Cyclo*-Asp(Pro-^{D}Pro-Val)-OH (4a):**

Cyclic tetrapeptide pendent carboxylic acid **4a** was synthesized from **2a** by following the above general procedure for catalytic hydrogenation of benzyl ester, obtained the desired pendent carboxylic acid as a white solid (yield: 94%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.78 (d, *J* = 10.2 Hz, 1 H), 7.10 (d, *J* = 6.7 Hz, 1 H), 5.06 (bs, 1 H), 4.73 (t, *J* = 7.0 Hz, 2 H), 4.66 (q, *J* = 5.6 Hz, 1 H), 4.25 (dd, *J* = 8.8, 9.3 Hz, 1 H), 4.19 (dd, *J* = 2.9, 10.0 Hz, 1 H), 3.66 (q, *J* = 8.5 Hz, 1 H), 3.58 - 3.50 (m, 1 H), 3.46 (q, *J* = 8.5 Hz, 1 H), 3.26 (dd, *J* = 5.9, 15.7 Hz, 1 H), 2.95 (dd, *J* = 4.5, 15.5 Hz, 1 H), 2.48 (dd, *J* = 7.2, 12.3 Hz, 1 H), 2.39 - 2.24 (m, 2 H), 2.20 (q, *J* = 7.2 Hz, 1 H), 2.10 (q, *J* = 7.0 Hz, 2 H), 2.04 - 1.90 (m, 2 H), 1.87 - 1.74 (m, 1 H), 0.92 (d, *J* = 6.7 Hz, 3 H), 0.89 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm : 172.5, 172.2, 170.9, 170.4, 169.2,

59.1, 58.6, 57.7, 49.3, 47.5, 47.2, 35.7, 28.7, 27.4, 25.7, 25.5, 24.9, 19.5, 17.9; MS (ESI): found: $[M + H]^+$, 409.4.

***Cyclo*-^{D}Asp(Pro-^{D}Pro-Val)-OH (4b):**

Cyclic tetrapeptide pendent carboxylic acid **4b** was synthesized from **2b** by following the above general procedure for catalytic hydrogenation of benzyl ester, obtained the desired pendent carboxylic acid as a white solid (yield: 91%, purity by LC-MS: 97%);^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.20 (d, *J* = 8.2 Hz, 1 H), 6.94 (d, *J* = 8.2 Hz, 1 H), 6.55 (bs, 1 H), 4.74 (dd, *J* = 4.5, 7.4 Hz, 1 H), 4.65 (dd, *J* = 2.6, 8.2 Hz, 1 H), 4.60 (dd, *J* = 2.7, 8.0 Hz, 1 H), 4.22 - 4.14 (m, 1 H), 4.08 (dd, *J* = 6.7, 8.2 Hz, 1 H), 3.77 – 3.70 (m, 1 H), 3.57 - 3.45 (m, 2 H), 3.10 (dd, *J* = 4.9, 15.1 Hz, 1 H), 2.54 - 2.33 (m, 2 H), 2.35 (dd, *J* = 3.2, 15.2 Hz, 1 H), 2.19 - 1.71 (m, 7 H), 1.05 (d, *J* = 6.7 Hz, 3 H), 1.03 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm : 173.2, 173.1, 173.1, 173.0, 169.7, 61.8, 60.7, 60.3, 51.2, 47.1, 47.0, 39.8, 34.5, 30.1, 29.4, 27.6, 26.7, 25.7, 22.4, 19.3, 17.9; MS (ESI): found: $[M + H]^+$, 409.4.

Homo dimer – [*Cyclo*-Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$S]$_2$ (36a)

The homodimer prodrug thioether **36a** was synthesized **4a** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-47% acetonitrile gradient, obtained the desired homodimer as a white solid (yield: 77%, purity by LC-MS: >99%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.77 (d, *J* = 9.0 Hz, 1 H), 7.25 (bs, 1 H), 7.22 (bs, 1 H), 4.82 - 4.74 (m, 1 H), 4.73 - 4.66 (m, 2 H), 4.27 (t, *J* = 8.2 Hz, 1 H), 4.19 (t, *J* = 7.6 Hz, 1 H), 4.13 - 4.00 (m, 1 H), 3.72 - 3.60 (m, 2 H), 3.59 - 3.44 (m, 2 H), 3.01 (dd, *J* = 8.0, 14.0 Hz, 1 H), 2.92 (dd, *J* = 6.2, 14.4 Hz, 1 H), 2.78 (t, *J* = 6.5 Hz, 2 H), 2.50 - 2.39 (m, 1 H), 2.37 - 2.18 (m, 3 H), 2.12 (q, *J* = 6.8 Hz, 2 H), 2.06 - 1.90 (m, 2 H), 1.89 - 1.76 (m, 1 H), 0.92 (d, *J* = 6.7 Hz, 3 H), 0.89 (d, *J* = 7.0 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm : 172.5, 171.7, 170.8, 169.7,

59.3, 58.9, 57.9, 49.8, 47.5, 47.3, 47.2, 38.6, 37.6, 36.7, 29.3, 27.6, 25.9, 25.8, 25.0, 19.7, 17.6; MS (ESI): found: $[M + H]^+$, 933.7.

***Cyclo*-Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$SH (17a)**

Thiol warhead having SLA analog **17a** was synthesized by following the above general procedure for synthesis of sulfhydryl from corresponding homodimer (**36a**), obtained the desired pendent thiol as a white solid (yield: 74%, purity by LC-MS: >99%). ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 8.02 (bs, 1 H), 7.75 (d, *J* = 9.4 Hz, 1 H), 7.08 (t, *J* = 5.0 Hz, 1 H), 4.78 (dt, *J* = 5.1, 8.2 Hz, 1 H), 4.73 - 4.64 (m, 2 H), 4.25 - 4.07 (m, 2 H), 3.75 - 3.60 (m, 2 H), 3.57 - 3.46 (m, 2 H), 3.42 (quin, *J* = 6.5 Hz, 1 H), 3.04 (dd, *J* = 5.5, 14.5 Hz, 1 H), 2.89 (dd, *J* = 8.4, 14.7 Hz, 1 H), 2.68 - 2.59 (m, 2 H), 2.49 - 2.20 (m, 3 H), 2.20 - 2.11 (m, 2 H), 2.11 - 1.84 (m, 4 H), 1.52 (t, *J* = 8.6 Hz, 1 H), 0.95 (d, *J* = 7.0 Hz, 3 H), 0.91 (d, *J* = 6.7 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 468.4.

Homo dimer – [*Cyclo*-^{D}Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$S]$_2$ (36b)

The homodimer prodrug thioether **36b** was synthesized from **4b** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-47% acetonitrile gradient, obtained the desired homodimer as a white solid (yield: 73%, purity by LC-MS: >99%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.65 (d, *J* = 8.5 Hz, 2 H), 6.85 (bs, 1 H), 4.74 - 4.67 (m, 3 H), 4.59 – 4.41 (m, 1 H), 4.14 - 4.04 (m, 1 H), 3.78 - 3.65 (m, 2 H), 3.57 - 3.49 (m, 3 H), 3.21 – 3.15 (m, 1 H), 3.09 - 2.90 (m, 1 H), 2.79 (t, *J* = 6.3 Hz, 2 H), 2.48 - 2.40 (m, 1 H), 2.33 – 2.25 (m, 2 H), 2.20 - 2.14 (m, 1 H), 2.06 - 1.98 (m, 4 H), 1.91 - 1.78 (m, 1 H), 0.97 (d, *J* = 7.2 Hz, 3 H), 0.94 (d, *J* = 7.1 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 933.7.

***Cyclo*-^{D}Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$SH (17b)**

Thiol warhead having SLA analog **17b** was synthesized by following the above general procedure for synthesis of sulfhydryl from corresponding homodimer (**36b**), obtained the desired pendent thiol as a white solid (yield: 71%, purity by LC-MS: >99%). ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 7.64 (d, *J* = 9.8 Hz, 1 H), 7.45 (t, *J* = 5.3 Hz, 1 H), 6.87 (d, *J* = 6.7 Hz, 1 H), 4.76 - 4.69 (m, 2 H), 4.68 - 4.57 (m, 1 H), 4.17 - 4.09 (m, 2 H), 3.85 - 3.65 (m, 1 H), 3.61 - 3.47 (m, 4 H), 3.27 (dd, *J* = 11.3, 16.0 Hz, 1 H), 2.87 (dd, *J* = 5.1, 16.0 Hz, 1 H), 2.74 - 2.68 (m, 1 H), 2.67 - 2.61 (m, 1 H), 2.50 - 2.42 (m, 1 H), 2.35 - 2.29 (m, 1 H), 2.22 - 1.99 (m, 7 H), 1.45 (t, *J* = 8.6 Hz, 1 H), 0.96 (d, *J* = 6.9 Hz, 3 H), 0.94 (d, *J* = 6.9 Hz, 6 H); MS (ESI): found: $[M + H]^+$, 468.4.

Homo dimer – [*Cyclo*-Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$S]$_2$ prodrug (38a)

The homodimer prodrug thioether **38a** was synthesized from **4a** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-47% acetonitrile gradient, obtained the desired homodimer as a white solid (yield: 67%, purity by LC-MS: >99%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.60 (d, *J* = 9.0 Hz, 1 H), 7.53 (t, *J* = 7.2 Hz, 1 H), 6.57 (bs, 1 H), 4.74 - 4.61 (m, 2 H), 4.68 (dd, *J* = 7.6, 14.7 Hz, 1 H), 4.12 - 4.08 (m, 1 H), 4.07 (t, *J* = 7.2 Hz, 1 H), 3.80 - 3.65 (m, 1 H), 3.60 – 3.39 (m, 2 H), 3.29 - 3.19 (m, 1 H), 3.15 - 3.06 (m, 1 H), 2.80 (dd, *J* = 5.0, 16.0 Hz, 1 H), 2.72 (t, *J* = 7.0 Hz, 2 H), 2.41 - 2.28 (m, 4 H), 2.15 - 1.89 (m, 5 H), 1.88 (quin, *J* = 6.8 Hz, 2 H), 0.96 (t, *J* = 6.7 Hz, 6 H); MS (ESI): found: $[M + H]^+$, 961.7.

***Cyclo*-Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$SH (18a)**

Thiol warhead having SLA analog **18a** was synthesized by following the above general procedure for synthesis of sulfhydryl from corresponding homodimer (**38a**), obtained the desired pendent thiol as a white solid (yield: 88%, purity by LC-MS: >99%). ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 7.62 (d,

J = 9.8 Hz, 1 H), 7.21 (t, *J* = 5.5 Hz, 1 H), 6.61 (d, *J* = 6.7 Hz, 1 H), 4.80 - 4.62 (m, 3 H), 4.62 - 4.48 (m, 1 H), 4.16 (t, *J* = 9.4 Hz, 1 H), 3.69 (q, *J* = 8.1 Hz, 1 H), 3.61 - 3.44 (m, 2 H), 3.38 (tdd, *J* = 6.8, 13.2, 19.8 Hz, 2 H), 3.27 (dd, *J* = 11.0, 16.0 Hz, 1 H), 2.81 (dd, *J* = 5.3, 15.8 Hz, 1 H), 2.54 (q, *J* = 7.2 Hz, 2 H), 2.47 (dd, *J* = 7.0, 12.5 Hz, 1 H), 2.32 (td, *J* = 7.8, 11.3 Hz, 2 H), 2.16 - 2.14 (m, 1 H), 2.13 - 1.84 (m, 7 H), 1.80 (quin, *J* = 6.8 Hz, 2 H), 1.45 (t, *J* = 8.0 Hz, 1 H), 0.95 (d, *J* = 7.1 Hz, 3 H), 0.93 (d, *J* = 7.1 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 482.4.

Homo dimer – [*Cyclo*-^{D}Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$S]$_2$ prodrug (38b)

The homodimer prodrug thioether **38b** was synthesized from **4b** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-47% acetonitrile gradient, obtained the desired homodimer as a white solid (yield: 63%, purity by LC-MS: >99%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.79 (d, *J* = 9.0 Hz, 1 H), 6.83 (bs, 1 H), 6.68 (bs, 1 H), 4.74 - 4.64 (m, 3 H), 4.54 - 4.48 (m, 1 H), 4.32 - 4.25 (m, 1 H), 4.24 - 4.17 (m, 1 H), 3.78 - 3.38 (m, 5 H), 3.34 (dd, *J* = 6.5, 13.5 Hz, 1 H), 2.94 - 2.68 (m, 3 H), 2.49 (d, *J* = 7.8 Hz, 1 H), 2.42 - 2.28 (m, 2H), 2.16 - 1.88 (m, 8 H), 0.94 (d, *J* = 6.9 Hz, 3 H), 0.89 (d, *J* = 7.0 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 961.7.

***Cyclo*-^{D}Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$SH (18b)**

Thiol warhead having SLA analog **18b** was synthesized by following the above general procedure for synthesis of sulfhydryl from corresponding homodimer **(38b)**, obtained the desired pendent thioether as a white solid (yield: 87%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.79 (d, *J* = 9.4 Hz, 1 H), 6.89 (d, *J* = 8.2 Hz, 1 H), 6.67 (t, *J* = 5.3 Hz, 1 H), 4.75 - 4.65 (m, 3 H), 4.62 - 4.55 (m, 1 H), 4.26 (dd, *J* = 5.9, 9.4 Hz, 1 H), 3.67 - 3.56 (m, 2 H), 3.56 - 3.41 (m, 2 H), 3.38 - 3.27 (m, 1 H), 3.09 (dd, *J* = 5.5, 14.9 Hz, 1 H), 2.84 (dd, *J* = 5.7, 15.1 Hz, 1 H), 2.57 (q, *J* = 7.0 Hz, 2 H), 2.54 - 2.45 (m, 1 H), 2.43 - 2.27 (m, 3 H), 2.18 - 2.09 (m, 3 H), 2.09 - 1.96 (m, 2 H),

1.86 - 1.79 (m, 2 H), 1.49 (t, *J* = 8.0 Hz, 1 H), 0.95 (d, *J* = 7.0 Hz, 3 H), 0.90 (d, *J* = 7.1 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 482.4.

***Cyclo*-Asp(Pro-^{D}Pro-Val)-$NHCH_2CH_2CH_2SCH_3$ (19a)**

The cyclic peptide **19a** having the methylthioether warhead on pendent carboxylic acid was synthesized from **4a** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with 3-(methylthio)propan-1-amine and purified by HPLC using 5-55% acetonitrile gradient, obtained the desired peptide as a white solid (yield: 70%, purity by LC-MS: 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.72 (d, *J* = 9.4 Hz, 1 H), 7.01 (d, *J* = 8.6 Hz, 1 H), 6.82 (t, *J* = 4.8 Hz, 1 H), 4.74 - 4.62 (m, 2 H), 4.23 (dd, *J* = 6.8, 9.0 Hz, 2 H), 3.69 - 3.55 (m, 2 H), 3.54 - 3.46 (m, 2 H), 3.43 - 3.28 (m, 2 H), 2.98 (dd, *J* = 6.9, 14.8 Hz, 1 H), 2.89 (dd, *J* = 5.3, 14.5 Hz, 1 H), 2.52 (t, *J* = 7.1 Hz, 2 H), 2.39 - 2.23 (m, 3 H), 2.17 - 2.06 (m, 2 H), 2.09(s, 3H), 2.06 - 1.83 (m, 4 H), 1.81 (p, *J* = 7.2 Hz, 2 H), 0.93 (d, *J* = 6.8 Hz, 3 H), 0.90 (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 172.5, 171.6, 170.7, 170.4, 169.9, 59.6, 59.5, 58.8, 58.1, 49.9, 47.6, 47.3, 38.8, 36.1, 31.3, 28.9, 28.3, 27.5, 26.1, 25.7, 25.0, 19.5, 17.4; MS (ESI): found: $[M + Na]^+$, 518.4.

***Cyclo*-^{D}Asp(Pro-^{D}Pro-Val)-$NHCH_2CH_2CH_2SCH_3$ (19b).**

The cyclic peptide **19b** having the methylthioether warhead on pendent carboxylic acid was synthesized from **4b** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with 3-(methylthio)propan-1-amine and purified by HPLC using 5-55% acetonitrile gradient, obtained the desired peptide as a white solid (yield: 70%, purity by LC-MS: 98%). ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 7.63 (d, *J* = 10.2 Hz, 1 H), 7.16 (t, *J* = 5.7 Hz, 2 H), 6.54 (d, *J* = 6.7 Hz, 2 H), 4.79 - 4.71 (m, 1 H), 4.72 (t, *J* = 6.7 Hz, 2 H), 4.58 - 4.48 (m, 2 H), 4.20 - 4.09 (m, 1 H), 3.90 - 3.77 (m, 1 H), 3.73 - 3.61 (m, 1 H), 3.59 - 3.50 (m, 2 H), 3.49 - 3.42 (m, 1 H), 3.41 - 3.31 (m, 2 H), 3.27 (dd, *J* = 10.6, 16.0 Hz, 1 H), 2.81 (dd, *J* = 5.1, 16.0 Hz, 1 H), 2.64 - 2.41 (m, 1 H), 2.51 (t,

J = 6.7 Hz, 2 H), 2.31 (tdd, *J* = 4.3, 7.8, 11.5 Hz, 2 H), 2.16 – 2.07 (m, 2 H), 2.08 (s, 3 H), 2.05 – 1.95 (m, 2 H), 1.87 – 1.80 (m, 2 H), 1.79 (p, *J* = 2.7 Hz, 2 H), 0.95 (d, *J* = 2.7 Hz, 3 H), 0.93 (d, *J* = 2.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.4, 172.4, 171.0, 169.7, 169.3, 59.9, 59.2, 58.2, 52.5, 47.6, 47.1, 38.7, 34.3, 31.3, 29.7, 28.4, 27.6, 26.0, 25.6, 24.8, 22.8, 19.3, 18.5; MS (ESI): found: $[M + H]^+$, 496.4.

***Cyclo*-Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$S(=O)CH$_3$ (20a).**

The cyclic peptide **20a** having the methylsulfoxide warhead on pendent carboxylic acid was synthesized from **19a** by following the above general procedure for sulfoxide synthesis from organic sulfides by H_2O_2/Borax and purified by HPLC using 5-50% acetonitrile gradient, obtained the desired sulfoxide as a white solid (yield: 71%, purity by LC-MS: 98%). Non-separable diastereomeric mixture = 1.1:1; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.87 (d, *J* = 9.0 Hz, 1 H), 7.50 (t, *J* = 7.0 Hz, 1 H), 7.45 (d, *J* = 6.0 Hz, 1 H), 4.74 - 4.61 (m, 3 H), 4.22 - 4.16 (m, 1 H), 3.74 - 3.64 (m, 1 H), 3.64 - 3.55 (m, 1 H), 3.55 - 3.43 (m, 1 H), 3.28 (dd, *J* = 6.1, 12.7 Hz, 1 H), 3.14 (t, *J* = 7.8 Hz, 2 H), 2.94 (dd, *J* = 1.8, 4.0 Hz, 1 H), 2.92 (s, 3 H), 2.90 - 2.86 (m, 2 H), 2.39 (d, *J* = 9.8 Hz, 1 H), 2.36 - 2.20 (m, 3 H), 2.17 - 2.08 (m, 2 H), 2.08 - 1.96 (m, 5 H), 0.93 (d, *J* = 6.8 Hz, 3 H), 0.91 (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 172.4, 171.0, 171.0, 170.7, 169.2, 59.3, 58.9, 57.7, 51.8, 50.1, 47.4, 47.1, 37.6, 37.0, 29.5, 28.8, 27.4, 26.1, 25.6, 25.0, 22.6, 19.5, 17.6; MS (ESI): found: $[M + H]^+$, 512.4.

***Cyclo*-^{D}Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$S(=O)CH$_3$ (20b)**

The cyclic peptide **20b** having the methylsulfoxide warhead on pendent carboxylic acid was synthesized from **19b** by following the above general procedure for sulfoxide synthesis from organic sulfides by H_2O_2/Borax and purified by HPLC using 5-50% acetonitrile gradient, obtained the desired sulfoxide as a white solid (yield: 67%, purity by LC-MS: >99%). Non-separable

diastereomeric mixture = 1.1:1; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.80 (t, *J* = 8.0 Hz, 1 H), 6.92 (bs, 1 H), 6.82 (t, *J* = 9.4 Hz, 1 H), 4.79 - 4.68 (m, 2 H), 4.68 - 4.61 (m, 1 H), 4.31 – 4.26 (m, 2 H), 3.65 - 3.47 (m, 2 H), 3.42 - 3.28 (m, 1 H), 3.25-3.11 (m, 2 H), 2.92 - 2.75 (m, 2 H), 2.63 (s, 3 H), 2.49 – 2.38 (m, 1 H), 2.40 (td, *J* = 6.8, 12.9 Hz, 1 H), 2.36 - 2.22 (m, 2 H), 2.18 - 2.10 (m, 2 H), 2.09 - 1.93 (m, 6 H), 1.91 - 1.82 (m, 1 H), 0.95 (d, *J* = 7.0 Hz, 3 H), 0.90 (d, *J* = 7.0 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 172.3, 171.1, 170.8, 170.8, 169.1, 59.3, 58.9, 57.7, 51.4, 50.0, 47.4, 47.0, 38.4, 38.1, 37.1, 28.9, 27.5, 26.1, 25.6, 25.0, 22.7, 19.5, 17.8; MS (ESI): found: $[M + H]^+$, 512.4.

***Cyclo*-Asp(Pro-^{D}Pro-Val)-$NHCH_2CH_2CH_2S(=O)_2CH_3$ (21a)**

The cyclic peptide **21a** having the methylsulfone warhead on pendent carboxylic acid was synthesized from **19a** by following the above general procedure for sulfones synthesis from organic sulfides by H_2O_2/Borax and purified by HPLC using 5-48% acetonitrile gradient over 30 minutes, obtained the desired sulfone as a white solid (yield: 89%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.81 (d, *J* = 9.2 Hz, 1 H), 6.70 (t, *J* = 6.0 Hz, 1 H), 6.57 (d, *J* = 8.8 Hz, 1 H), 4.79 (t, *J* = 6.7 Hz, 1 H), 4.72 (d, *J* = 7.4 Hz, 1 H), 4.61 (ddd, *J* = 3.3, 5.8, 8.9 Hz, 1 H), 4.37 - 4.33 (m, 1 H), 4.33 - 4.28 (m, 1 H), 3.68 - 3.47 (m, 4 H), 3.32 - 3.22 (m, 1 H), 3.22 - 3.11 (m, 2 H), 2.90 (s, 3 H), 2.73 (dd, *J* = 6.0, 15.0 Hz, 1 H), 2.55 - 2.43 (m, 2 H), 2.38 - 2.26 (m, 2 H), 2.17 - 2.13 (m, 1 H), 2.14 (p, *J* = 6.8 Hz, 2 H), 2.09 - 1.95 (m, 3 H), 1.92 - 1.82 (m, 1 H), 0.97 (d, *J* = 6.8 Hz, 3 H), 0.90 (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.0, 171.1, 170.9, 170.8, 170.0, 59.4, 58.6, 58.1, 51.8, 50.3, 47.7, 47.6, 40.5, 37.8, 35.8, 29.7, 28.5, 27.4, 26.1, 25.8, 25.3, 22.9, 19.8, 16.9; MS (ESI): found: $[M + H]^+$, 528.4.

***Cyclo*-^{D}Asp(Pro-^{D}Pro-Val)-$NHCH_2CH_2CH_2S(=O)_2CH_3$ (21b)**

The cyclic peptide **21b** having the methylsulfone warhead on pendent carboxylic acid was synthesized from **19b** by following the above general procedure for sulfones synthesis from organic

sulfides by H_2O_2/Borax at 60 °C for 24 h and purified by HPLC using 5-48% acetonitrile gradient over 30 minutes, obtained the desired sulfone as a white solid (yield: 83%, purity by LC-MS: 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.82 (d, *J* = 8.9 Hz, 1 H), 6.83 (t, *J* = 5.7 Hz, 1 H), 6.75 (d, *J* = 8.6 Hz, 1 H), 4.76 (t, *J* = 6.7 Hz, 1 H), 4.71 (dd, *J* = 1.8, 7.9 Hz, 1 H), 4.67 - 4.61 (m, 1 H), 4.33 - 4.26 (m, 2 H), 3.65 - 3.50 (m, 2 H), 3.36 - 3.26 (m, 1 H), 3.19 - 3.11 (m, 2 H), 2.94 (d, *J* = 3.9 Hz, 1 H), 2.91 (s, 3 H), 2.80 (dd, *J* = 5.9, 14.9 Hz, 1 H), 2.52 - 2.40 (m, 2 H), 2.37 - 2.30 (m, 2 H), 2.14 (t, *J* = 7.0 Hz, 2 H), 2.10 - 1.99 (m, 3 H), 1.89 – 1.75 (m, 2 H), 1.64 (p, *J* = 6.8 Hz, 2 H), 0.96 (d, *J* = 7.0 Hz, 3 H), 0.90 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 171.5, 171.3, 170.9, 170.9, 165.6, 59.4, 58.7, 58.1, 51.8, 50.2, 47.6, 40.5, 37.8, 36.0, 31.9, 29.7, 28.6, 27.4, 26.1, 25.8, 25.3, 22.7, 19.7, 17.0; MS (ESI): found: $[M + H]^+$, 528.4.

Cyclo-Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$OH (22a) and _Cyclo_-^{D}Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$OH (22b).

The cyclic peptide **22a and 22b** having the *n*-propylalcohol warhead on pendent carboxylic acid was synthesized by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with 3-aminopropan-1-ol and purified by HPLC using 11-40% acetonitrile gradient over 30 minutes, obtained the desired alcohol as viscous oil (yield: 75%, purity by LC-MS: >96%). Nonseparable Diastereomeric mixture (1 : 0.65); Diastereomer 22a: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.81 (d, *J* = 9.0 Hz, 1 H), 6.72 (d, *J* = 8.5 Hz, 1 H), 6.65 (t, *J* = 5.5 Hz, 1 H), 4.75 (t, *J* = 7.0 Hz, 2 H), 4.70 - 4.64 (m, 1 H), 4.42 (t, *J* = 6.5 Hz, 1 H), 4.39 - 4.28 (m, 1 H), 3.72 - 3.67 (m, 1 H), 3.65 - 3.39 (m, 5 H), 3.37 - 3.26 (m, 1 H), 3.19 (dd, *J* = 4.3, 14.9 Hz, 1 H), 2.75 (t, *J* = 6.5 Hz, 1 H), 2.57 - 2.40 (m, 2 H), 2.38 - 2.20 (m, 2 H), 2.18 - 2.10 (m, 2 H), 2.10 - 1.80 (m, 5 H), 0.96 (d, *J* = 6.9 Hz, 3 H), 0.94 (d, *J* = 7.0 Hz, 3 H); Diastereomer 22b: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.99 (d, *J* = 9.8 Hz, 1 H), 7.02 (t, *J* = 6.5 Hz, 1 H), 6.75 (d, *J* = 8.4 Hz, 1 H), 4.73 (t, *J* = 7.0 Hz, 2 H), 4.63 - 4.59 (m, 1 H), 4.39 - 4.28 (m, 2 H), 3.65 - 3.39 (m, 5 H), 3.37 - 3.26 (m, 2 H), 2.71 (t, *J* = 7.0 Hz, 1 H), 2.57 -

2.40 (m, 2 H), 2.38 - 2.20 (m, 2 H), 2.18 - 2.10 (m, 2 H), 2.10 - 1.80 (m, 3 H), 1.71 (quin, J = 5.7 Hz, 2 H), 0.90 (d, J = 6.9 Hz, 3 H), 0.88 (d, J = 7.0 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 466.4.

***Cyclo*-Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$COOMe(23a)**

The cyclic peptide **23a** having the methylcarboxylate warhead on pendent carboxylic acid was synthesized from **4a** by following the above general procedure for EDCl peptide coupling of coupling of pendent carboxylic acid with methyl 4-aminobutanoate and purified by HPLC using 11-45% acetonitrile gradient over 30 minutes, obtained the desired peptide as a white solid (yield: 86%, purity by LC-MS: 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.79 (d, J = 9.0 Hz, 1 H), 6.91 (bs, 1 H), 6.78 (bs, 1 H), 4.72 (t, J = 6.5 Hz, 2 H), 4.69 - 4.63 (m, 1 H), 4.36 - 4.22 (m, 2 H), 3.68 (s, 3 H), 3.65 - 3.55 (m, 2 H), 3.50 (q, J = 8.6 Hz, 1 H), 3.34 - 3.25 (m, 2 H), 2.83 (dd, J = 5.1, 14.9 Hz, 1 H), 2.50 (dd, J = 7.0, 11.0 Hz, 1 H), 2.44 - 2.35 (m, 4 H), 2.35 - 2.24 (m, 2 H), 2.14 (q, J = 6.8 Hz, 2 H), 2.09 - 1.91 (m, 2 H), 1.84 (p, J = 6.3 Hz, 2 H), 0.94 (d, J = 6.7 Hz, 3 H), 0.90 (d, J = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.7, 172.7, 171.6, 170.5, 170.4, 170.0, 59.4, 58.5, 58.1, 51.7, 49.8, 47.5, 47.3, 39.1, 35.9, 31.2, 28.8, 27.5, 26.0, 25.7, 25.0, 24.4, 19.6, 17.2; MS (ESI): found: $[M + H]^+$, 508.5.

***Cyclo*-Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$COOMe (23b)**

The cyclic peptide **23b** having the methylcarboxylate warhead on pendent carboxylic acid was synthesized from **4b** by following the above general procedure for EDCl peptide coupling of coupling of pendent carboxylic acid with methyl 4-aminobutanoate and purified by HPLC using 11-45% acetonitrile gradient over 30 minutes, obtained the desired peptide as a white solid (yield: 85%, purity by LC-MS: >98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.62 (d, J = 10.2 Hz, 1 H), 7.13 (t, J = 5.6 Hz, 1 H), 6.74 (d, J = 6.7 Hz, 1 H), 4.71 (t, J = 8.5 Hz, 1 H), 4.72 (dd, J = 3.1, 7.7 Hz, 1 H), 4.55 - 4.49 (m, 1 H), 4.23 - 4.12 (m, 2 H), 3.77 - 3.67 (m, 1 H), 3.67 (s, 3 H), 3.59 - 3.45 (m, 2 H),

3.31 - 3.25 (m, 4 H), 2.77 (dd, *J* = 5.1, 16.0 Hz, 1 H), 2.53 - 2.39 (m, 2 H), 2.35 (t, *J* = 7.2 Hz, 2 H), 2.33 - 2.22 (m, 2 H), 2.20 - 2.10 (m, 1 H), 2.09 - 1.90 (m, 6 H), 1.81 (quin, *J* = 7.2 Hz, 2 H), 0.94 (d, *J* = 4.7 Hz, 3 H), 0.93 (d, *J* = 4.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.6, 173.3, 172.3, 170.9, 169.7, 169.2, 77.3, 77.0, 76.7, 59.9, 59.1, 58.0, 52.4, 51.7, 47.5, 47.0, 38.8, 34.2, 31.2, 29.7, 27.7, 26.0, 25.6, 24.8, 24.5, 19.3, 18.5; MS (ESI): found: $[M + H]^+$, 508.5.

Cyclo-Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$COOH (24a)

The cyclic peptide **24a** having the carboxylic acid warhead on pendent carboxylic acid was synthesized from **23a** by following the above general procedure for general procedure for LiOH base mediated hydrolysis of methyl ester and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired peptide as a white solid (yield: 93%, purity by LC-MS: >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.28 (d, *J* = 10.2 Hz, 1 H), 7.01 (t, *J* = 5.2 Hz, 1 H), 6.84 (d, *J* = 9.0 Hz, 1 H), 4.82 (d, *J* = 7.8 Hz, 1 H), 4.73 (t, *J* = 6.8 Hz, 1 H), 4.68 (dd, *J* = 4.5, 10.0 Hz, 2 H), 4.35 (dt, *J* = 2.0, 9.4 Hz, 1 H), 3.65 - 3.57 (m, 1 H), 3.57 - 3.39 (m, 3 H), 3.32 (dd, *J* = 2.0, 16.0 Hz, 1 H), 3.29 - 3.20 (m, 1 H), 2.75 (dd, *J* = 6.3, 16.0 Hz, 1 H), 2.60 - 2.44 (m, 3 H), 2.40 (dd, *J* = 2.7, 10.2 Hz, 1 H), 2.33 - 2.21 (m, 2 H), 2.18 - 2.10 (m, 2 H), 2.10 - 1.91 (m, 3 H), 1.90 - 1.76 (m, 2 H), 0.95 (d, *J* = 6.7 Hz, 3 H), 0.88 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm : 174.1, 174.0, 171.1, 170.0, 168.3, 167.7, 58.1, 58.0, 56.5, 48.6, 46.4, 45.9, 37.8, 36.5, 30.4, 28.6, 26.6, 24.8, 24.7, 23.9, 23.5, 18.5, 17.1; MS (ESI): found: $[M + H]^+$, 494.4.

Cyclo-^{D}Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$COOH (24b)

The cyclic peptide **24b** having the carboxylic acid warhead on pendent carboxylic acid was synthesized from **23b** by following the above general procedure for general procedure for LiOH base mediated hydrolysis of methyl ester and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired peptide as a white solid (yield: 95%, purity by LC-MS: >99%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 7.49 (d, *J* = 10.2 Hz, 1 H), 6.95 (t, *J* = 5.7 Hz, 1 H), 6.70 (d, *J* = 7.4 Hz, 1 H), 5.57 (bs, 1 H), 4.76 (d, *J* = 7.0 Hz, 1 H), 4.73 - 4.60 (m, 2 H), 4.32 (dd, *J* = 8.4, 10.0 Hz, 1 H), 4.19 (dt, *J* = 3.0, 9.0 Hz, 1 H), 3.69 - 3.40 (m, 3 H), 3.32 (dd, *J* = 8.4, 15.1 Hz, 1 H), 3.27 - 3.17 (m, 1 H), 2.75 (dd, *J* = 6.5, 15.1 Hz, 1 H), 2.56 - 2.30 (m, 4 H), 2.25 (td, *J* = 3.9, 7.5 Hz, 1 H), 2.20 - 2.10 (m, 2 H), 2.08 - 1.90 (m, 4 H), 1.86 (quin, *J* = 7.0 Hz, 2 H), 0.95 (d, *J* = 7.0 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 176.3, 173.7, 172.1, 170.9, 169.4, 169.3, 59.8, 59.5, 58.2, 51.2, 47.6, 47.0, 39.3, 34.0, 31.8, 29.7, 27.7, 26.1, 25.8, 24.7, 24.7, 19.4, 18.5; MS (ESI): found: $[M + H]^+$, 494.4.

***Cyclo*-Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$CONHOH (25a)**

The cyclic peptide **25a** having the hydroxamic acid warhead on pendent carboxylic acid was synthesized from **23a** by following the above general procedure for general procedure for hydroxamic acid synthesis from the methyl ester and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired hydroxamic acid peptide as a white solid (yield: 86%, purity by LC-MS: 99%); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm : 10.08 (bs, 1 H), 8.27 (d, *J* = 8.2 Hz, 1 H), 7.51 (t, *J* = 5.5 Hz, 1 H), 6.94 (d, *J* = 5.5 Hz, 1 H), 4.83 (dd, *J* = 2.1, 8.1 Hz, 1 H), 4.63 (dd, *J* = 2.0, 7.7 Hz, 1H), 4.54 (t, *J* = 7.2 Hz, 1 H), 4.16 (t, *J* = 6.1 Hz, 1 H), 3.88 (t, *J* = 7.8 Hz, 1 H), 3.77 - 3.69 (m, 1 H), 3.53 - 3.43 (m, 3 H), 3.40 - 3.38 (m, 1 H), 3.26 (td, *J* = 7.6, 11.3 Hz, 1 H), 3.03 - 2.93 (m, 2 H), 2.89 (dd, *J* = 5.1, 14.5 Hz, 1 H), 2.41 - 2.05 (m, 4 H), 1.99 - 1.60 (m, 5 H), 1.54 (quin, *J* = 6.8 Hz, 2 H), 0.83 (d, *J* = 7.0 Hz, 3 H), 0.78 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 173.0, 172.0, 171.0, 170.4, 169.5, 168.0, 60.0, 59.7, 57.2, 52.2, 47.0, 46.8, 40.8, 38.7, 30.1, 29.7, 28.0, 25.6, 25.6, 24.8, 22.5, 19.9, 17.9MS (ESI): found: $[M + H]^+$, 509.5.

***Cyclo*-Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$CH$_2$CONHOH (25b)**

The cyclic peptide **25b** having the hydroxamic acid warhead on pendent carboxylic acid was synthesized from **23b** by following the above general procedure for general procedure for hydroxamic acid synthesis from the methyl ester and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired hydroxamic acid peptide as a white solid (yield: 87%, purity by LC-MS: >99%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.33 (s, 1 H), 7.79 (d, *J* = 9.8 Hz, 1 H), 7.77 (d, *J* = 7.4 Hz, 1 H), 7.72 (t, *J* = 5.3 Hz, 1 H), 4.73 - 4.60 (m, 2 H), 4.46 - 4.32 (m, 1 H), 3.92 - 3.87 (m, 1 H), 3.84 (t, *J* = 9.2 Hz, 1 H), 3.63 (t, *J* = 6.5 Hz, 1 H), 3.58 - 3.38 (m, 3 H), 3.16 - 2.89 (m, 3 H), 2.80 (dd, *J* = 4.0, 16.0 Hz, 1 H), 2.60 (dd, *J* = 4.3, 15.7 Hz, 1 H), 2.37 - 2.20 (m, 1 H), 2.16 (dd, *J* = 3.0, 16.0 Hz, 1 H), 2.08 - 1.55 (m, 9 H), 0.85 (d, *J* = 6.7 Hz, 3 H), 0.83 (d, *J* = 6.7 Hz, 3 H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm**:** 171.9, 171.1, 170.6, 170.3, 169.2, 169.2, 59.5, 59.1, 58.7, 49.3, 47.2, 46.6, 38.8, 38.3, 30.2, 29.7, 27.7, 26.3, 25.5, 24.8, 22.5, 19.7, 18.8; MS (ESI): found: $[M + H]^+$, 509.5.

***Cyclo*-Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$S-SCH$_2$CH$_2$NH$_2$ (26a)**

The amine-containing peptide **26a** was synthesized from **4a** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired homodimer as a viscous oil (yield: 69%, purity by LC-MS: 96%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.39 (bs, 2 H), 7.87 (d, *J* = 9.0 Hz, 1 H), 7.72 (t, *J* = 5.7 Hz, 1 H), 7.45 (d, *J* = 8.6 Hz, 1 H), 4.72 - 4.67 (m, 3 H), 4.31 - 4.21 (m, 1 H), 4.17 (t, *J* = 8.2 Hz, 1 H), 3.69 - 3.61 (m, 1 H), 3.55 - 3.49 (m, 3 H), 3.35 – 3.15 (m, 5 H), 3.01 - 2.90 (m, 2 H), 2.89 - 2.76 (m, 1 H), 2.46 - 2.36 (m, 1 H), 2.36 - 2.16 (m, 3 H), 2.16 - 1.91 (m, 4 H), 1.90 - 1.77 (m, 1 H), 0.91 (d, *J* = 4.3 Hz, 3 H), 0.89 (d, *J* = 4.3 Hz, 3 H); MS (ESI): found: $[M + H]^+$, 543.5.

***Cyclo*-^{D}Asp(Pro-^{D}Pro-Val)-NHCH$_2$CH$_2$S-SCH$_2$CH$_2$NH$_2$ (26b)**

The amine-containing peptide **26b** was synthesized from **4b** by following the above general procedure for EDCl peptide coupling of pendent carboxylic acid with cysteamine diamine and purified by HPLC using 11-35% acetonitrile gradient over 30 minutes, obtained the desired homodimer as a viscous oil (yield: 65%, purity by LC-MS: 97%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.27 (t, *J* = 4.7 Hz, 1 H), 7.77 (br. s., 2 H), 7.37 (d, *J* = 8.2 Hz, 1 H), 6.77 (d, *J* = 7.0 Hz, 1 H), 4.97 - 4.84 (m, 1 H), 4.70 - 4.53 (m, 2 H), 3.95 (t, *J* = 9.2 Hz, 1 H), 3.90 - 3.80 (m, 1 H), 3.79 - 3.69 (m, 1 H), 3.69 - 3.58 (m, 1 H), 3.58 - 3.45 (m, 2 H), 3.36 - 3.18 (m, 4 H), 3.14 - 3.04 (m, 1 H), 3.02 - 2.89 (m, 3 H), 2.73 (dd, *J* = 4.5, 16.2 Hz, 1 H), 2.37 - 2.17 (m, 4 H), 2.11 - 1.87 (m, 5 H), 0.98 (d, *J* = 6.3 Hz, 6 H); MS (ESI): found: $[M + H]^+$, 543.5.

Microfluidic chip-based KDAC inhibition assay

Substrate A, a (FAM)-labeled peptide purchasable from PerkinElmer (Broad Substrate A, Product number CLS960006), was synthetized in house (see Supporting Information section S7) and used for KDAC1 assays, while the others were purchased from PerkinElmer: two (FITC)-labeled peptides (p53 Acetylated Peptide and Histone 4 Acetylated Peptide, Product Number 760512 and 760513, respectively) were used as substrates to test compounds against KDAC 3 and 6, respectively, and a (FAM)-labeled peptide (Broad Substrate B, Product Number CLS960007,) was employed as substrate for KDAC8 assays. All KDACs were purchased from BPS Bioscience.

Compounds were tested in duplicate in a 10-point dose curve with 3–fold serial dilution starting from 30uM; in the case of SAHA a 15-point dose curve with 3-fold serial dilution starting from 30μM was performed when testing on KDACs 3 and 6. Purified KDACs were incubated with 1uM of p53 Acetylated Peptide, Histone 4 Acetylated Peptide, Broad Substrates A or B and SLAs for 60 min at room temperature, in KDAC assay buffer that contained 25 mM Tris-HCl (pH 8.0), 137mM NaCl, 2.7 mM KCl, 1mM $MgCl_2$, and 0.01% BSA. Reactions (in duplicates) were terminated by the

addition of a stop buffer containing 100mM HEPES, 0.015% Brij-35, 10 mM EDTA, 0.1% CR-3 and 1.5μM of the known pan-KDAC inhibitor Panobinostat[67, 68] (LBH589, purchased from ApexBio Technology). Fluorescence intensity of electrophoretically separated substrate and product was detected using the Labchip EZ Reader and the data were analyzed by non-linear regression using GraphPad Prism 6.01 software[69] to afford IC_{50} values from dose-response experiments. The percentage of inhibition at 30uM is reported when its value is less than 50% at that concentration. As standard compounds (positive controls), eight well known KDAC inhibitors: Entinostat (MS-275),[56] TSA,[17] Tubastatin A,[34] SAHA,[4] purchased from Selleck Chemicals, PCI-34051[32] (purchased from Cayman Chemical Company) , together with T247[33] (synthetized in house, see Supporting Information section S8) as well as Largazole[70] (thioester) and one of its analog (herein designated SD-L-256,[57] generously supplied by Prof. Robert Williams of the Department of Chemistry at Colorado State University), were used (see **Table 1**, **Figures 6-7** and Supporting Information **Figure S3** for dose-response curves).

Molecular Modeling. All calculations were performed on two MacPros (dual 2.67GHz six core Intel Xeon X5650) cluster (24 CPU total) running GNU/Linux Mint 17.1 64-bit operating system.

KDAC8 co-crystal structure (PDB code 3RQD)[43] was retrieved from the Protein Data Bank (PDB).[71] Solvent molecules, buffer and non-interaction ions were removed, and hydrogens added considering a neutral pH (7.4). A minimization process was then performed by means of GROMACS 5.0.2:[72] 1) ligand topology was computed using the ACPYPE/Antechamber tool;[73-75] 2) periodic boundary conditions were applied in the *x, y* and *z* directions using a cubic box; 3) 5000 steepest descend minimization steps were performed using the AMBER99SB-ILDN force field[76] with TIP4P as the water model. Na^+ and Cl^- were employed as counter ions. Marvin software was used for drawing and characterizing the chemical structures of compounds **14a** and **25a**, Marvin 14.11.3.0, 2014, ChemAxon (http://www.chemaxon.com). The protonation states were assigned considering a

physiological pH. To generate random conformations of compounds **14a** and **25a,** to be used as input structures for docking calculations, the OpenBabel suite (The Open Babel package, version 2.3.2 http://openbabel.org)[77] was employed to: 1) generate a best conformer (after 250 geometry optimization steps) out of 250 conformers using the obconformer tool; 2) further optimize the obtained geometry by applying the obminimize tool using first the steepest descent algorithm followed by the conjugate gradients algorithm with a default number of steps and force-field (2500 and MMFF94, respectively). Molecular docking of compounds **14a** and **25a** has been performed using PLANTS[60] (v1.2, PLP scoring function) to generate 10 conformation for each docked ligand. Search speed, number of ants, evaporation factor and iteration scaling factor were set as SPEED1, 20, 0.15 and 2, respectively. Due to the stochastic nature of PLANTS, each experiment has been repeated 10 times, to release a total of 100 conformations to be analyzed.

Supporting Information

Detailed experimental procedures, purity and spectral data of compounds, additional results and dose-response curves.

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Abbreviation.

BD, Best Docked poses; COMBINEr, enhanced comparative binding energy analysis; CTP, cyclic tetrapeptide; DIPA, diisopropylethylamine; DPPA, diphenylphosphoryl azide; ECRD, Experimental Conformation Re-Docking; FITC, fluoresceine isothiocyanate; HA_RMSD*h*, Hungarian symmetry-corrected heavy-atom RMSD; HAART, highly active antiretroviral therapy; KAT, Lysine acetyltransferase; KDAC, lysine deacetylase; KDACI, lysine deacetylase inhibitor; LTR, long terminal repeat; O^{t}Bu, ortho-tert-butyl; LiOH, lithium hydroxide; MeOH, methanol; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EtOAc, ethylacetate; DMSO-*d6*, deuterated dimethyl sulfoxide; HCl, hydrochloric acid; RCRD, Random Conformation Re-Docking; SAHA, suberoylanilide hydroxamic acid; SLA, simplified largazole analog; TSA, trichostatin A.

References

1. Lysine deacetylases are generally referred to as histone deacetylases (HDACs), a historical imperative as epigenetic modification of histones was described in 1964 by Allfrey et al.[2], thus, the terms, histone acetylases (HATs), deacetylases(HDACs), methylases (HMTs), etc. It has been shown by proteomics, however, that over 1700 proteins in cells besides histones undergo dynamic acetylation[3], thus, the current preference for the term lysine acetylase (KAT), deacetylase (KDAC), or methylase (KMT).

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