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Evaluation of functional groups on amino acids in cyclic tetrapeptides in histone deacetylase inhibition

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Abstract The naturally occurring cyclic tetrapeptide, chlamydocin, originally isolated from fungus *Diheterospora chlamydosphoria*, consists of α-aminoisobutyric acid, L-phenylalanine, D-proline and an unusual amino acid (S)-2-amino-8-((S)-oxiran-2-yl)-8-oxooctanoic acid (Aoe) and inhibits the histone deacetylases (HDACs), a class of regulatory enzymes. The epoxyketone moiety of Aoe is the key functional group for inhibition. The cyclic tetrapeptide scaffold is supposed to play important role for effective binding to the surface of enzymes. In place of the epoxyketone group, hydroxamic acid and sulfhydryl group have been applied to design inhibitor ligands to zinc atom in catalytic site of HDACs. In the research for more potent HDAC inhibitors, we replaced the epoxyketone moiety of Aoe with different functional groups and synthesized a series of chlamydocin analogs as HDAC inhibitors. Among the functional groups, methoxymethylketone moiety showed as potent inhibition as the hydroxamic acid. On the contrary, we confirmed that borate, trifruoromethylketone, and 2-aminoanilide are almost inactive in HDAC inhibition.

Keywords Histone deacetylase inhibitor · Chlamydocin · Epoxyketone · Functional group design

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Introduction

Chemical modifications of histones by dynamic acetylation and deacetylation of ε-amino groups of lysine residues by histone acetyl transferases (HATs) and HDACs are involved in chromatin remodeling and play a fundamental role in epigenetics, the regulation of gene expression (Yoshida et al. 2003; Grozinger and Schreiber 2002; Kouzarides 1999; Hassig and Schreiber 1997). The level of modification of histone acetylation and its effects have received enormous interest; in parallel, growing evidences support their importance for basic cellular function such as DNA replication, transcription, differentiation, and apoptosis. The lysine residues are positively charged at physiological pH. Acetylation of lysine residues of histone neutralizes the positive charge and allows the DNA to unwind from the Histone core. This unwound DNA is transcriptionally active. On the other hand, deacetylation restores the ionic interaction and causes DNA to return to the transcriptionally repressed conformation. Deregulation of HATs and HDACs has been implicated in the formation and development of several human cancers by changing the expression pattern of various genes. It is therefore proposed that HDACs are a potential targets for the development of small molecular anticancer agents.

The HDAC enzymes have been divided into four groups based on function and DNA sequence similarity. The first two groups involve a zinc atom at the active center to employ metalloprotease-like mechanism for cleavage of acetyl group. As natural inhibitors of HDACs, two different types of metabolites have been reported. One group includes trichostatin A (Yoshida et al. 1990), which bears hydroxamic acid functional moiety at the end of the molecule. The second group, cyclic tetrapeptides, comprises trapoxins (Kijima et al. 1993), chlamydocin (Closse and

Fig. 1 Structures of chlamydocin and related inhibitors bearing different functional groups

Chlamydocin   1   2   3   4   5
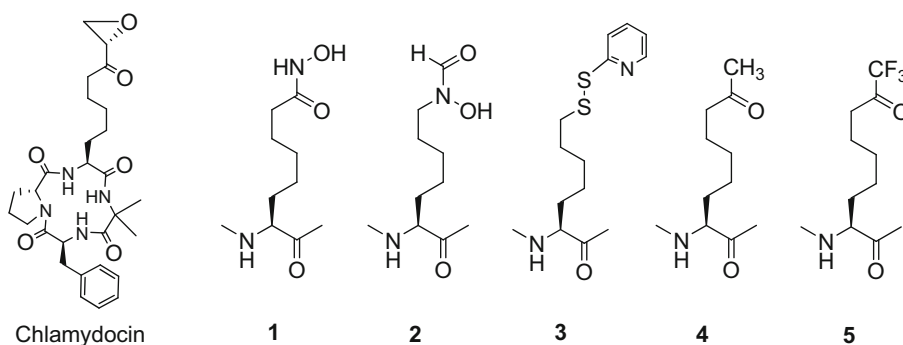
Hugenin 1974), Cyl-1 and Cyl-2 (Hirota et al. 1973a, b, c), FR-235222 (Xie et al. 2005), TAN-1746 (Yoshimura et al. 1995), and apicidins (Darkin-Rattray et al. 1996; Meinke and Liberator 2001; Han et al. 2000). These cyclic tetrapeptides have typically an α,β-epoxyketone, ketone, or hydroxyketone moiety, as a part of quite unusual amino acid in the cyclic peptides, which mimic an acetylated lysine residue and interact with the active-site zinc ion.

In the past decade, a considerable number of synthetic analogs have been reported as cyclic-tetrapeptide HDAC inhibitors. The design of these analogs have been done either by changing the amino-acids sequence in the cyclic tetrapeptide framework to achieve high affinity of the inhibitors with the enzyme surface and/or by the introduction of different type of functional groups as zinc ligands to achieve a strong binding affinity to the enzyme's active site. The functional groups introduced so far are mostly electron rich (having lone pair of electron or double bond) and included hydroxamic acid (**1**, Furumai et al. 2001), retro-hydroxamic acid (**2**, Nishino et al. 2004), hybrid disulfide (**3**, Nishino et al. 2003), methylketone (**4**, Bhuiyan et al. 2006), and trifluoromethylketone (**5**, Jose et al. 2004) as shown in Fig. 1. On the other hand, more functional groups have been introduced to the non-peptide HDAC inhibitors, such as 2-aminoanilide (Suzuki et al. 1999; Saito et al. 1999), thioacetate (Suzuki et al. 2004), borate (Suzuki et al. 2009), phosphate (Kapustin et al. 2003), and so on.

In order to enhance the binding capacity to HDACs as inhibitors, we attempted to derive novel artificial amino acids and introduce them into the chlamydocin scaffold. We designed and synthesized cyclic tetrapeptides (**6–11**) containing amino acids with functional groups shown in Fig. 2. o-Phenylenediamine was condensed to L-aminosuberoyl residue (**6**). The 2-amino-6-heptenoyl residue was converted to the corresponding boric acid (**7**). L-Amino-7-bromoheptanoyl residue was treated with sodium azide and then reacted with acetylene for triazolyl product (**8**). L-Lysine residue was condensed with acryloyl chloride and chloroacetic acid to afford **9** and **10**. The methoxymethylketone moiety in **11** was built from L-2-amino-8-nonenoyl residue.

Results and discussion

Chemistry

Our aim was to evaluate the possible inhibitory function of several chlamydocin analogs towards HDACs by introducing new and known functional groups in the chlamydocin framework based on the inhibition mechanism. Therefore, we proposed to synthesize chlamydocin analogs by replacing the epoxyketone moiety of chlamydocin by different functional groups. Synthesis of cyclic tetrapeptide was carried out according to the general Scheme 1 by the

Fig. 2 Structures of novel designed amino acid residues in the cyclic tetrapeptides (**6–11**) having different functional groups. **6** 2-aminoanilide, **7** boronic acid, **8** triazole, **9** acrylamide, **10** chloroacetamide, **11** methoxymethylketone (Mmk)

6   7   8   9   10   11
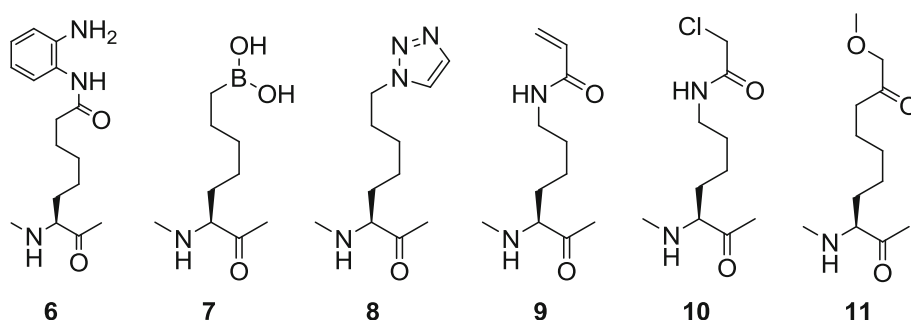
conventional solution phase method, starting from the Z-D-Pro-O^{t}Bu. After the removal of Z-protection by catalytic hydrogenation using H$_2$/Pd–C, free amine was extracted and coupled with Z-L-Phe-OH to give the protected dipeptide Z-L-Phe-D-Pro-O^{t}Bu. The Z group of the resulting dipeptide was deprotected by catalytic hydrogenation and coupled with Z-Aib-OH to yield the tripeptide Z-Aib-L-Phe-D-Pro-O^{t}Bu. The N-terminal of the tripeptide was deprotected and coupled with (S)-7-bromo-2-(*tert*-butoxycarbonylamino) heptanoic acid (Boc-L-7-bromo-Ahp-OH), (S)-2-(*tert*-butoxycarbonylamino)-6-heptenoic acid (Boc-L-Ae7-OH), Boc-L-Lys(Z)-OH, (S)-2-(*tert*-butoxycarbonylamino)-8-nonenoic acid (Boc-L-Ae9-OH) and (S)-8-(benzyloxy)-2-(*tert*-butoxycarbonylamino)-suberic acid (Boc-L-Asu(OBzl)-OH) to yield the relevant linear tetrapeptide. After deprotection of both the terminals by trifluoroacetic acid (TFA), the linear tetrapeptide TFA salt was then cyclized under high dilution conditions in dimethylformamide (DMF) using O-(7-azabenzotriazoyl-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) or benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) to give the cyclic tetrapeptide **12**, **13**, **15**, **17**, and **18**, respectively. The yield of cyclic tetrapeptides was 50–70% after purification by silica gel chromatography. Terminal moieties of the synthesized cyclic tetrapeptides were converted to several desired groups depicted in Schemes 2, 3, 4, 5, and 6.

Catalytic hydrogenation of **12** gave the corresponding acid which was coupled with *o*-phenylenediamine to obtain compound **6**. Regioselective addition of pinacolborane to the alkene group of compound **13** was successfully carried out, and the intermediate compound **14** was oxidized to boronic acid using sodium periodate to obtain compound **7**. Compound **16** was obtained by treating compound **15** with sodium azide. Treatment of **16** with acetylene yielded

compound **8**. The deprotection of Z group of compound 17 and subsequent treatment with acryloyl chloride and 2-chloroacetic acid yielded compounds **9** and **10**, respectively. The side chain double bond of **18** was oxidized to epoxide **19** using *m*-CPBA. Regioselective ring opening of the unsymmetrically substituted epoxide was carried with sodium methoxide which was employed for oxidation with Dess-Martin periodinane reagents to obtain compound **11**. All the final compounds were characterized by high resolution FAB-MS. Purity of the compounds was determined by HPLC analysis, and all the compounds showed purity above 97%.

Enzyme inhibition and evaluation of biological activity

The synthesized compounds were assayed for HDAC inhibitory activity using HDAC1, HDAC4, and HDAC6 enzymes prepared by 293T cells (Furumai et al. 2002). In addition, to know the inhibitory activity of these compounds in cell based condition, we carried out p21 promoter assay according to the literature (Bhuiyan et al. 2006). Detailed experimental procedure for the preparation and assay performed according to the reported methods. The results of HDAC inhibitory activity and the p21 promoter assay of chlamydocin (Furumai et al. 2001) and the synthesized compounds are shown in Table 1. For comparison, inhibitory activity of chlamydocin is also shown. Chlamydocin which showed good activity was taken as standard for this study.

In order to evaluate the inhibitory activity of triazole, we introduced this new group in chlamydocin scaffold and obtained compound **8**. But this compound was inactive which reveals that this group has no or very less binding affinities for zinc. More recently, on the basis of the catalytic mechanism, boronic acid containing HDAC inhibitors bearing an α-amino acid moiety have been reported (Suzuki et al. 2009). In order to compare the HDAC inhibitory activity, we introduced boronic acid in chlamydocin scaffold and synthesized compound **7** which showed potent activity against HDACs. As naturally occurring cyclic tetrapeptides having epoxyketone moieties are irreversible inhibitors, it has been proposed that epoxyketone moieties undergo nucleophilic attack in the active sites. Guided by this hypothesis, we synthesized compounds **9** containing acrylamide group and compound **10** having chloroacetamide group. Both groups are susceptible to nucleophilic attack, but these compounds displayed moderate activity. This might be due to the less zinc-binding affinities for these groups.

The cyclic tetrapeptide bearing methoxymethylketone (**11**) showed the highest HDAC inhibition except the hydroxamic acid (**1**), retrohydroxamate (**2**), and disulfide (**3**) in the series of functional groups examined in the
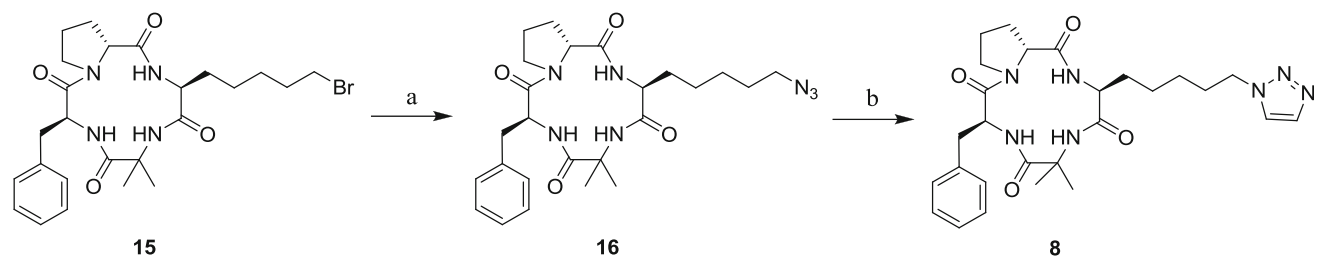

**Scheme 1** Synthesis of cyclic tetrapeptides

Scheme 2 Reagents and conditions: (**a**) MeOH, Pd–C, H$_2$, 12 h, 100%; (**b**) *o*-phenylenediamine, BOP, triethylamine, DMF, 12 h, 21%
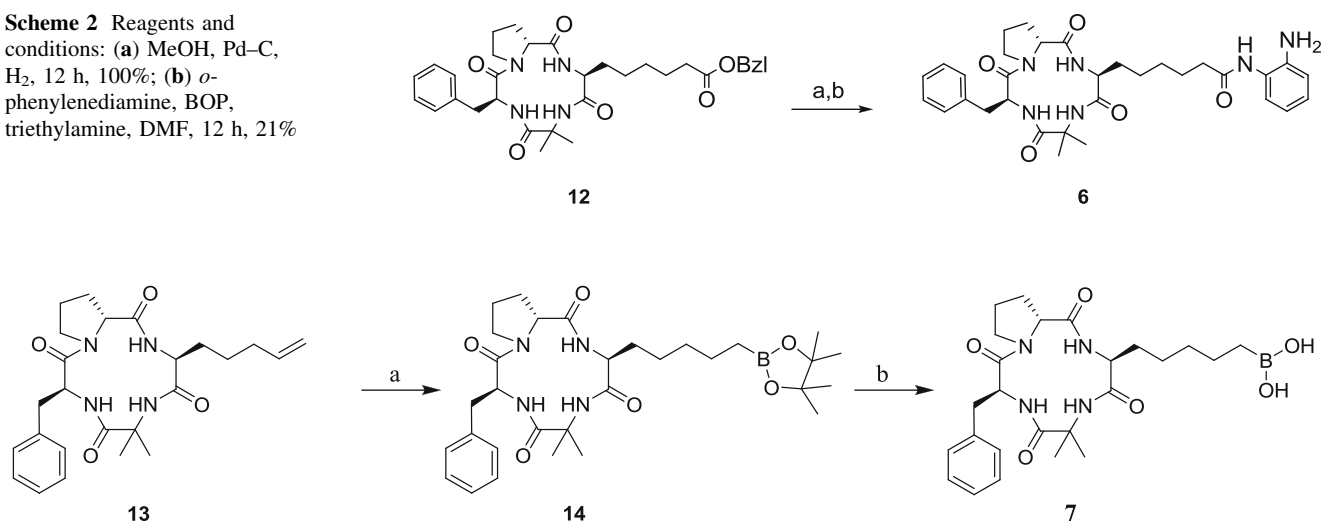

Scheme 3 Reagents and conditions: (**a**) bis(1,5-cyclooctadiene)diiridium(I)dichloride ([Ir(cod)Cl]$_2$), bis(diphenylphosphino)methane (DPPM), pinacolborane, DCM, 48 h, 90%; (**b**) NH$_4$OAc, NaIO$_4$, Acetone, H$_2$O, 20 h, 42%

Scheme 4 Reagents and conditions: (**a**) NaN$_3$, DMF, 0°C, 4 h, 100%; (**b**) CuI, triethylamine, acetylene, DMF, 120 h, 55%
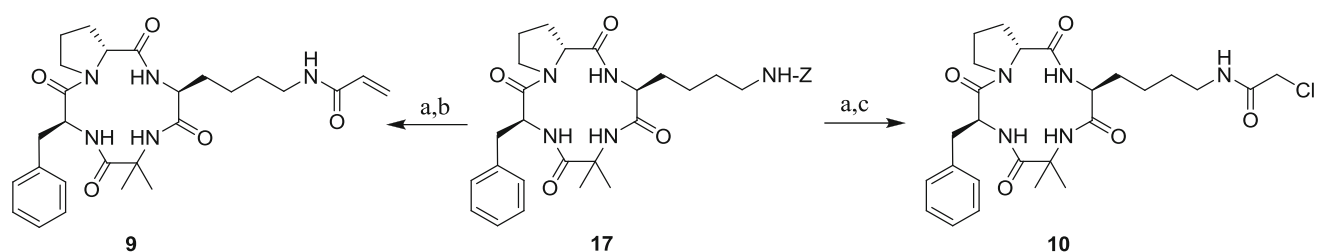

Scheme 5 Reagents and conditions: (**a**) AcOH, Pd–C, H$_2$, 12 h, 72%; (**b**) acryloyl chloride, triethylamine, DCM, 12 h, 66%; (**c**) 2-chloroacetic acid, DCC, DCM, 12 h, 67%
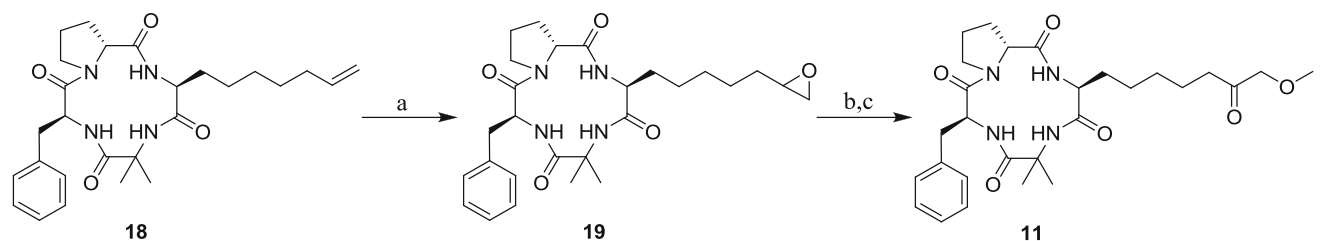
Scheme 6 Reagents and conditions: (**a**) *m*-chloroperoxybenzoic acid (*m*-CPBA), DCM, 15 h, 89%; (**b**) NaOMe/MeOH, 18 h, 68%; (**c**) Dess-Martin periodinane (DMP), DCM, 8 h, 85%

Table 1 HDAC inhibitory activity and p21 promoter assay data

No.	IC$_{50}$ (μM)			EC$_{1000}$ (μM)
	HDAC1	HDAC4	HDAC6	
Chlamydocin	0.00015	–	1.100	–
1	0.023	0.034	0.065	0.020
2	0.014	0.033	0.39	0.027
3	0.0040	0.0018	0.040	0.045
4	1.1	0.29	3.7	1.2
5	3.5	2.6	>100	30.1
6	24	33	>100	>25
7	2.1	0.15	21	3.6
8	65	52	>100	86
9	15	19	>100	>25
10	13	18	>100	>25
11	0.038	0.028	>100	0.043
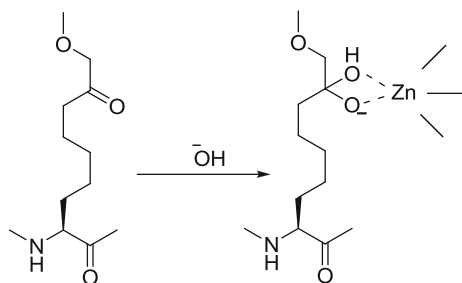
Fig. 3 Possible interaction of methoxymethylketone with Zn in HDAC

present study. The inhibition mechanism is estimated as shown in Fig. 3. The interaction of the ketone as acetate form is enhanced by methoxy group. The selectivity of cyclic tetrapeptide inhibitors for HDAC 1 and 4 over HDAC 6 could be explained by fact that HDAC 6 has a relatively wide rim (17.5 Å) compared to HDAC 1 (12.5 Å) (Butler et al. 2010). The tetrapeptide scaffold is well fit to the rim region of HDAC 1 and 4 which is less in the case of HDAC 6. Thus, for HDAC inhibition, we discovered novel functional group methoxymethylketone, which could be easily synthesized in three steps from 2-amino-8-nonenoic acid-containing cyclic tetrapeptide precursor.

Experimental

General

Unless otherwise noted, all solvents and reagents were of reagent grade and used without purification. Flash chromatography was performed using silica gel 60 (230–400 mesh) eluting with solvents as indicated. All compounds were routinely checked by thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC). TLC was performed on aluminum-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F254) with spots visualized by UV light or charring. Analytical HPLC was performed on a Hitachi instrument equipped with a chromolith performance RP-18e column (4.6 A 50 mm, Merck). The mobile phases used were A: H$_2$O with 0.1% TFA, B: CH$_3$CN with 0.1% TFA using a solvent gradient of A to B over 15 min with a flow rate of 2 mL/min, with detection at 220 nm. FAB-mass spectra and high-resolution mass spectra (HRMS) were measured on a JEOL JMS-SX 102A instrument. In positive-mode MS analysis using 3-nitrobenzyl alcohol (NBA) as a matrix, boronic acid is esterified with NBA, and the molecular weight detected corresponds to (M + 2NBA-2H$_2$O + H)$^+$. Amino acids were coupled using standard solution phase chemistry with 1-hydroxybenzotriazole (HOBt), dicyclohexyl-carbodimide (DCC), HATU or BOP.

Synthesis of linear tripeptide H-Aib-L-Phe-D-Pro-O^{t}Bu

To a cooled solution of H-D-Pro-O^{t}Bu (3.10 g, 18.1 mmol) and Z-L-Phe-OH (5.41 g, 18.1 mmol) in DMF (36.0 mL), HOBt.H$_2$O (2.77 g, 18.1 mmol) and DCC (4.48 g, 21.7 mmol) were added, and the mixture was stirred at room temperature for 12 h. After completion of the reaction, DMF was evaporated, and the residue was dissolved in ethyl acetate and washed with 10% citric acid, 4% sodium bicarbonate and brine, respectively, and then dried over anhydrous MgSO$_4$. After evaporation of the ethyl acetate, the residue was purified by silica gel chromatography using a mixture of chloroform and methanol (99:1)

to yield Z-L-Phe-D-Pro-O^{t}Bu (7.52 g, 92%, HPLC, rt 6.55 min) as a colorless oil. The protected dipeptide (7.52 g, 16.6 mmol) was dissolved in acetic acid (85 mL). Pd–C (0.85 g) was added, and the mixture was stirred under hydrogen atmosphere for 12 h. The reaction was monitored by TLC and HPLC. After completion of the reaction, Pd–C was filtered off, and the acetic acid was evaporated. The residue was dissolved in ethyl acetate, and the organic phase was washed with saturated sodium carbonate solution and dried over anhydrous Na$_2$CO$_3$. Evaporation of ethyl acetate gave H-L-Phe-D-Pro-O^{t}Bu (5.53 g, 83%, HPLC, rt 5.43 min) which was coupled with Z-Aib-OH (3.25 g, 13.7 mmol) following the same procedure described above to get Z-Aib-L-Phe-D-Pro-O^{t}Bu (7.01 g, 95%, HPLC, rt 8.03 min) as a white foam. The tripeptide Z-Aib-L-Phe-D-Pro-O^{t}Bu (1.06 g, 2.0 mmol) was subjected to hydrogenation in a similar manner as described for H-L-Phe-D-Pro-O^{t}Bu to obtain H-Aib-L-Phe-D-Pro-O^{t}Bu (0.880 g, 100%, HPLC, rt 4.56 min).

Synthesis of cyclic tetrapeptides

Cyclo(-L-Asu(OBzl)-Aib-L-Phe-D-Pro-) (**12**) The N-terminal free tripeptide H-Aib-L-Phe-D-Pro-O^{t}Bu (8.1 g, 20 mmol) was coupled with Boc-L-Asu(OBzl)-OH (7.56 g, 20 mmol) according to the method described above, and the fully protected crude linear tetrapeptide was purified by silica gel chromatography using a mixture of chloroform and methanol (99:1) to yield Boc-L-Asu(OBzl)-Aib-L-Phe-D-Pro-O^{t}Bu (9.7 g, 12.7 mmol, 64%). Boc-L-Asu(OBzl)-Aib-L-Phe-D-Pro-O^{t}Bu (9.6 g, 12.5 mmol) was dissolved in TFA (50 mL) at 0°C and kept for 3 h. After evaporation of TFA, the residue was solidified by precipitating with ether to yield H-L-Asu(OBzl)-Aib-L-Phe-D-Pro-OH as TFA salt (9.0 g, 12.5 mmol, 100%). To a volume of DMF (1,200 mL) solvent TFA salt (9.0 g, 12.5 mmol), HATU (7.13 g, 18.7 mmol) and *N,N*-diisopropylethylamine (DIEA) (7.61 mL, 43.7 mmol) were added in five aliquots with 30 min time interval while the solution was stirred vigorously. After the final addition, the reaction mixture was allowed to stir for an additional hour. Completion of the cyclization reaction was monitored by HPLC, and then DMF was evaporated under vacuo. The crude cyclic tetrapeptide was dissolved in ethyl acetate, and the solution was washed successively by 10% citric acid, 4% sodium bicarbonate, and brine. Finally, the ethyl acetate solution was dried over anhydrous MgSO$_4$ and filtered. After evaporation of ethyl acetate, the residue was purified by silica gel chromatography using a mixture of chloroform and methanol (99:1) to yield compound **12** (5.35 g, 9.0 mmol, 72%). HPLC, rt 7.98 min.

Cyclo(-L-Asu(2-aminoanilinyl)-Aib-L-Phe-D-Pro-) (**6**) Compound **12** (5.35 g, 9.0 mmol) was dissolved in methanol (45 mL) and Pd–C (0.45 g) was added. The solution was stirred under hydrogen overnight. After filtration of Pd–C, methanol was evaporated to yield *cyclo*(-L-Asu-Aib-L-Phe-D-Pro-) (4.5 g, 100%). HPLC, rt 4.68 min. To a cooled solution of *cyclo*(-L-Asu-Aib-L-Phe-D-Pro-) (0.20 g, 0.40 mmol) and o-phenylenediamine (0.21 g, 2.0 mmol) in DMF (2 mL), triethylamine (0.084 mL, 0.6 mmol) and BOP (0.265 g, 0.6 mmol) were added, and the mixture was stirred at room temperature for 12 h. After completion of the reaction, the compound was purified according to the procedure reported for **12** to yield compound **6** as solid crystal (0.05 g, 21%). HPLC, rt 6.03 min, HRMS (FAB) calcd for C$_{32}$H$_{43}$N$_6$O$_5$ [(M + H)$^+$] 591.3295, found 591.3278.

Cyclo(-L-Ae7-Aib-L-Phe-D-Pro-) (**13**) This compound was synthesized according to the procedure reported for compound **12** using Boc-L-Ae7-OH instead of Boc-L-Asu(OBzl)-OH. The product is white foam (0.768 g, 82%). HPLC, rt 7.91 min, LC–MS calcd for C$_{25}$H$_{34}$N$_4$NaO$_4$ [(M + Na)$^+$] 477.3, found 477.5.

Cyclo(-L-7-(pinacolboranyl)-Ahp-Aib-L-Phe-D-Pro-) (**14**) A solution of [Ir(cod)Cl]$_2$ (0.034 g, 0.05 mmol) and DPPM (0.038 g, 0.10 mmol) in anhydrous and degassed dichloromethane (DCM) (5.0 mL) was flashed with Ar for 5 min and stirred for 10 min at room temperature. After that, to the solution, *cyclo*(-L-Ae7-Aib-L-Phe-D-Pro-) (0.227 g, 0.50 mmol) and pinacolborane (113 μL, 0.75 mmol) were added, and the mixture was flashed with Ar for 5 min, then stirred at room temperature for 2 days. The reaction mixture was concentrated and purified according to the procedure reported for **12** to give compound **14** (0.260 g, 90%) as a white solid. HPLC, rt 8.94 min, LC–MS calcd for C$_{31}$H$_{48}$BN$_4$O$_6$ [(M + H)$^+$] 583.4, found 583.5.

Cyclo(-L-7-(boronyl)-Ahp-Aib-L-Phe-D-Pro-) (**7**) To a solution of *cyclo*(-L-7-(pinacolboranyl)-Ahp-Aib-L-Phe-D-Pro-) (0.200 g, 0.34 mmol) in acetone/H$_2$O (4 mL/2 mL), NaIO$_4$ (0.215 g, 1.0 mmol) and NH$_4$OAc (0.770 g, 1.0 mmol) were added, and the suspension was stirred at room temperature for 20 h. Then acetone was evaporated, and the reaction mixture was poured into water and extracted with ethyl acetate. Finally, the ethyl acetate solution was dried over anhydrous MgSO$_4$ and filtered. After evaporation of ethyl acetate, the residue was purified by silica gel chromatography using a mixture of chloroform and methanol (98:2) to yield compound **7** (0.070 g, 42%) as a white solid. HPLC, rt 6.10 min, LC–MS calcd for C$_{25}$H$_{37}$BN$_4$NaO$_6$ [(M + Na)$^+$] 523.3, found 523.5. HRMS (FAB) calcd for C$_{39}$H$_{48}$O$_{10}$N$_6$B [(M + 2NBA 2H$_2$O + H)$^+$ 771.3525, found 771.3587.

Cyclo(-L-*7-bromo-Ahp-Aib-*L-*Phe-*D-*Pro-*) (**15**) This compound was synthesized according to the procedure reported for compound **12** using Boc-L-7-bromo-Ahp-OH instead of Boc-L-Asu(OBzl)-OH. The product is white foam (0.42 g, 60%). HPLC, rt 6.19 min, LC–MS calcd for $C_{25}H_{36}BrN_4O_4$ [(M + H)$^+$] 535.2, found 535.3.

Cyclo(-L-*7-azide-Ahp-Aib-*L-*Phe-*D-*Pro-*) (**16**) To a cooled solution of *cyclo*(-L-7-bromo-Ahp-Aib-L-Phe-D-Pro-) (0.107 g, 0.20 mmol) in DMF (1 mL), NaN$_3$ (0.016 g, 0.25 mmol) was added, and the mixture was stirred gently for 4 h at room temperature. After completion of the reaction, DMF was evaporated, and the residue was dissolved in ethyl acetate and washed with brine and then dried over anhydrous MgSO$_4$. After evaporation of the ethyl acetate, the residue was precipitated with ether to yield compound **16** (0.10 g, 100%). HPLC, rt 6.09 min, HRMS (FAB) calcd for $C_{25}H_{36}N_7O_4$ [(M + H)$^+$] 498.2829, found 498.2822.

Cyclo(-L-*7-(1H-1,2,3-triazol-1-yl)-Ahp-Aib-*L-*Phe-*D-*Pro-*) (**8**) To a solution of *cyclo*(-L-7-azide-Ahp-Aib-L-Phe-D-Pro-) (0.10 g, 0.20 mmol) in DMF (2 mL), CuI (0.8 mg, 4 μmol) and triethylamine (0.22 mmol, 31 μL) were added. Acetylene gas was passed through the mixture for 120 h. DMF was evaporated, and the residue was dissolved in ethyl acetate and washed with brine and then dried over anhydrous MgSO$_4$. After evaporation of the ethyl acetate, the residue was purified by HPLC (30-55% CH$_3$CN, 50 mm column) to yield compound **8** (0.029 g, 55%). HPLC, rt 5.67 min, HRMS (FAB) calcd for $C_{27}H_{38}N_7O_4$ [(M + H)$^+$] 524.2985, found 524.3020.

Cyclo(-L-*Lys(Z)-Aib-*L-*Phe-*D-*Pro-*) (**17**) This compound was synthesized according to the procedure reported for compound **12** using Boc-L-Lys(Z)-OH instead of Boc-L-Asu(OBzl)-OH. The product is white foam (1.20 g, 71%).

Cyclo(-L-*Lys(2-propenoyl)-Aib-*L-*Phe-*D-*Pro-*) (**9**) Cyclo(-L-Lys(Z)-Aib-L-Phe-D-Pro-) (0.30 g, 0.5 mmol) was subjected to hydrogenation as described for H-L-Phe-D-Pro-O^{t}Bu to yield *cyclo*(-L-Lys-Aib-L-Phe-D-Pro-) (0.16 g, 72%). HPLC, rt 4.85 min. To a cooled solution of *cyclo*(-L-Lys-Aib-L-Phe-D-Pro-) (0.080 g, 0.17 mmol) in DCM (1 mL), triethylamine (35 μL, 0.25 mmol) and acryloyl chloride (20 μL, 0.25 mmol) were added and stirred for 12 h at room temperature. After completion of the reaction, DCM was evaporated, and the residue was purified as described for compound **12** to yield compound **9** (0.059 g, 66%). HPLC, rt 5.96 min, HRMS (FAB) calcd for $C_{27}H_{38}N_5O_5$ [(M + H)$^+$] 512.2873, found 512.2780.

Cyclo(-L-*Lys(2-chloroacetyl)-Aib-*L-*Phe-*D-*Pro* (**10**) To a cooled solution of 2-chloroacetic acid (0.048 g, 0.50 mmol)

in DCM (1 mL), DCC (0.052 g, 0.25 mmol) was added, and the mixture was stirred gently. After 2 h, *cyclo*(-L-Lys-Aib-L-Phe-D-Pro-) (0.080 g, 0.17 mmol) was added, and the mixture was stirred for 12 h at room temperature. After completion of the reaction, the compound was purified as described for compound **12** to yield compound **10** (0.063 g, 67%). HPLC, rt 6.07 min, HRMS (FAB) calcd for $C_{26}H_{37}ClN_5O_5$ [(M + H)$^+$] 534.2483, found 534.2532.

Cyclo(-L-*Ae9-Aib-*L-*Phe-*D-*Pro-*) (**18**) This compound was synthesized according to the procedure reported for compound **12** using Boc-L-Ae9-OH instead of Boc-L-Asu(OBzl)-OH. The product is white foam (3.36 g, 87%). HPLC, rt 9.34 min., HRMS (FAB) calcd for $C_{27}H_{39}O_4N_4$ [(M + H)$^+$] 483.2971, found 483.2971.

Cyclo(-L-*Ae9(epo)-Aib-*L-*Phe-*D-*Pro-*) (**19**) To a chilled solution of compound **18** (4.72 g, 9.77 mmol) in anhydrous DCM (200 mL) a solution of purified *m*-chloroperoxybenzoic acid (3.37 g, 19.54 mmol) in 100 mL of anhydrous DCM was added in aliquots over 30 min with stirring. The reaction mixture was allowed to stir for 18 h at room temperature. TLC and HPLC showed almost complete conversion of the olefin into epoxide. The solution was then washed with 4% sodium bicarbonate and brine followed by drying over anhydrous MgSO$_4$ and filtration. The compound was purified to yield *cyclo*(-L-Ae9(epo)-Aib-L-Phe-D-Pro-) (4.33 g, 89%). HPLC, rt 7.13 min. HRMS (FAB) calcd for $C_{27}H_{39}O_5N_4$ [(M + H)$^+$] 499.2920, found 499.2891.

Cyclo(-L-*Ae9(Mmk)-Aib-*L-*Phe-*D-*Pro-*) (**11**) The epoxide (499 mg, 1.0 mmol) was dissolved in 0.5 M sodium methoxide in methanol (2 mL). The reaction mixture was stirred at 28°C for 16 h and then quenched by adding acetic acid. Ethyl acetate was added to the reaction solution, and the compound was purified as described for compound **12** to yield the corresponding ether (361 mg, 68%). HPLC, rt 6.27 min, HRMS (FAB) calcd for $C_{28}H_{43}O_6N_4$ [(M + H)$^+$] 531.3183, found 531.3204. To a solution of vicinal alkoxy ether (318 mg, 0.60 mmol) in anhydrous DCM (12 mL) DMP (1.53 g, 3.6 mmol) was added. The reaction mixture was stirred at 25°C for 3 h. TLC and HPLC showed complete conversion of the starting alcohol. The reaction mixture was then diluted by adding diethyl ether (12 mL) followed by careful addition of a saturated solution of sodium bicarbonate containing 2.68 g of sodium thiosulfate pentahydrate (Na$_2$S$_2$O$_3$·5H$_2$O). After stirring for 10 min, the suspension became a clear solution. The organic layer was separated from the aqueous layer and purified as described for compound **12** to yield the corresponding ketone (270 mg, 85%). HPLC, rt 6.16 min. HRMS (FAB) calcd for $C_{28}H_{41}O_6N_4$ [(M + H)$^+$] 529.3026, found 529.3026.

Conclusion

In order to develop HDAC inhibitors with various functional groups, we replaced the epoxyketone moiety of chlamydocin scaffold with several known and new functional groups and evaluated these functional groups on the scaffold as HDAC inhibitory activity. The cell free and cell-based assay study showed that one of the functional groups potently inhibited HDACs in inhibition mechanism. Most of the functional groups have selectivity towards HDAC1 and HDAC4.

Conflict of interest The authors declare that they have no conflict of interest.

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