Cyclic tetrapeptides with thioacetate tails or intramolecular disulfide bridge as potent inhibitors of histone deacetylases

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ABSTRACT

Two thioacetate tails were introduced to the chlamydocin- and CHAP31-related cyclic tetrapeptides. An intramolecular disulfide bridge could be formed in the CHAP31-related cyclic peptides. Both the thioacetate-tailed and disulfide-bridged peptides were potent histone deacetylase inhibitors in the presence of sulfhydryl compound. Potent p21 promoter inducing activity was also observed in vivo.

Histone deacetylases (HDACs) play a prominent role in the regulation of gene transcription by histone deacetylation. Unusual deacetylation of histone is sometimes linked to carcinogenesis. Therefore, novel HDAC inhibitor may lead to a novel cancer drug such as vorinostat. Current challenges include the discovery of the selective inhibitor to target cancer.

The naturally occurring FK-228 (Fig. 1) displays impressive anticancer and anti-angiogenesis activities through the HDAC inhibition, and is now approved as a cancer drug (Istodax®). FK228 is a bicyclic depsipeptide with intramolecular disulfide bridge, which is reductively activated after uptake into the cells generating the sulfhydryl groups. Inspired by the stability of FK228 due to its prodrug nature, several HDAC inhibitors with the disulfide bond have been proposed with more simplified structure for the ease of synthetic study. We have reported sulfur containing cyclic tetrapeptides with disulfide bonds (SCOPs, Fig. 1) based on CHAP31 (cyclo(-L-Asu(NHOH)-d-Tyr(Me)-L-Ile-D-Pro-)); Asu, 2-aminosuberic acid) skeleton (Fig. 1). Here we report the synthesis of a bicyclic tetrapeptidyl inhibitor with an intramolecular disulfide bridge. These cyclic peptides with disulfide bonds are activated in a similar manner to FK228 and inhibit HDACs by the coordination of the sulfhydryl tails to Zn2+ ions.

First, a cyclic tetrapeptide 3 (Fig. 2) with the thioacetate side-chains in the neighboring amino acids was designed, based on the ‘LDLD’ amino acid configuration of CHAP31. The linear tetrapeptide Boc-l-Ab7-d-Aob7-l-Phe-d-Pro-OtBu (1, Ab7, 2-amino-7-bromohexanoic acid; Aob7, 2-amino-4-(2-bromoethoxy)butyric

Figure 1. Natural and synthetic HDAC inhibitors with cyclic peptide structure.
(acid) was synthesized from d-Pro-OBu in the solution phase, that is, the stepwise deprotections of the N-termini and DCC (N,N-dicyclohexylcarbodiimide)-HOBt (1-hydroxybenzotriazol) couplings. After TFA deprotection of 1, the obtained free peptide (H-L-Ab7-D-Aob7-L-Phe-D-Pro-OH) TFA salt was treated with HATU (1H-7-azobenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) in DMF-diisopropylethylamine under high dilution conditions (4.0 mmol L⁻¹ peptide concentration). The desired cyclic peptide 2 was obtained in fair yield (72%) after silica-gel column chromatography. Such a high cyclization yield suggested that the peptide conformation of 2 with the ‘LDLD’ amino acid configuration was not strained.

The bromoalkyl sidechains of 2 were transformed to thioacetates (3) by KSAc in DMF (71%). After TFA deprotection of 1, the obtained free peptide (H-L-Ab7-D-Aob7-L-Phe-d-Pro-OH) TFA salt was treated with HATU (1H-7-azobenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) in DMF-diisopropylethylamine under high dilution conditions (4.0 mmol L⁻¹ peptide concentration). The desired cyclic peptide 2 was obtained in fair yield (72%) after silica-gel column chromatography. Such a high cyclization yield suggested that the peptide conformation of 2 with the ‘LDLD’ amino acid configuration was not strained.

The bromoalkyl sidechains of 2 were transformed to thioacetates (3) by KSAc in DMF (71%). The reaction of 3 with methylamine in methanol generated the sulfhydryl groups, which were successively I₂-oxidized to yield the desired bicyclic peptide 4. This intramolecular disulfide formation took place with moderate yield (36%) without extreme dilution (50 mmol L⁻¹ peptide concentration).

Next, cyclic tetrapeptides with the thioacetate sidechains in the opposite amino acids (Fig. 3) were synthesized, based on the ‘LAibLD’ amino acid configuration of chlamydocin (cyclo-L-Aoe-L-Aib-L-Phe-L-Pro-); Aoe, 2-amino-8-oxo-9,10-epoxydecanoic acid; Aib, 2-amino-2-methylpropionic acid). Linear tetrapeptides Boc-L-Ab7-Aib-L-XXX-d-Pro-OBu; XXX = Ab7 for 5, Aob7 for 6) were synthesized in a similar manner to 1. TFA deprotection and HATU cyclization under high dilution conditions (4.0 mmol L⁻¹ peptide concentration) afforded the cyclic peptides 7 and 8 in fair yields (74% and 60%, respectively) after silica-gel column chromatography. Compounds 7 and 8 were transformed to 9 and 10 bearing the thioacetal sidechains, respectively. Unfortunately, the methylamine treatment and successive I₂-oxidation did not afford the bicyclic peptide with the intramolecular disulfide bridges. All the new compounds were characterized by 1H, 13C NMR and FAB-MS including the high resolution MS. The purity of the compounds was checked by HPLC and was >98%.

Figure 3 shows the HPLC analyses of the reaction mixture of I₂-oxidation. The CHAP31-related 3 (–X = –SH) generated 4 with some oligomeric byproducts. In 3, the sidechains of L-amino acid and that of d-amino acid in the neighboring positions may come close with each other. Contrary, the oxidation of 10 (–X = –SH) formed only oligomers. In 9 and 10, the sulfur tails at the opposite positions may spread from the cyclic peptides, therefore, the sulfur groups are separated with each other.

1H NMR spectra of 3 and 4 (Fig. 4) indicates that the cyclic peptide framework little changed upon the intramolecular disulfide bridging. The amide-NH protons of 3 (δ 7.11, Aob7; 6.58, Phe; 6.30, Ab7) slightly shifted in 4 (δ 7.15, 6.71, 6.40). The CαH of 3 and 4 were also similar with each other. (Note: slight shift of the CαH of Aob7 and Ab7 are caused by the change of –SAc to –SS–.)

The J values of NH/CαH, which reflect the dihedral angles of the...
amino acid residues,11 were all 10 Hz in 3 and 4. Thus, no conformational change was detected in the reaction of 3 to 4 in 1H NMR. In the CD measurements, 3 and 4 also showed a similar spectra (0.1 mmol L−1 in methanol, data not shown). Positive Cotton effect at 253 nm, negative one at 243 nm, positive one at 228 nm and negative one at 212 nm. This fact also supported that the intramolecular disulfide bridging in 4 caused little conformational distortion.

Table 1 shows the HDAC inhibitory activities of the bicyclic peptides with the intramolecular disulfide bridge (4) and the cyclic peptides with the thioacetate groups (3, 9, 10). The in vitro activities of peptides were tested using the enzymes (HDAC1, HDAC4 and HDAC6) and a fluorophore-modified substrate. For HDAC1 and HDAC4, the cyclic peptides 3, 9 and 10 were moderately effective inhibitors with μmol L−1-range IC50 (50% inhibitory concentration). Probably, the thioacetate branches of these peptides weakly coordinated to Zn2+ ions in the enzyme active sites. No tail existed in the bicyclic peptide 4 that can coordinate to Zn2+ ion, therefore, 4 did not inhibit HDAC1, HDAC4 and HDAC6 in the absence of dithiothreitol (DTT).

The cyclic peptide 4 showed excellent inhibitory activities after treatment with DTT, IC50 of 0.010 μmol L−1 for HDAC1 and 0.0059 μmol L−1 for HDAC4, while DTT showed no effect in HDAC1 inhibition. Reduction of 4 by DTT generated the sulphydryl groups, which inserted into the enzyme active site pocket. The inhibition of 4 in the presence of DTT is still less than CHAP31 (IC50 3.32 nM)12 or chlamydocin (IC50 0.15 nM).13 However, Compound 4 is probably stable in the blood (relatively oxidative conditions) due to its disulfide bridge. Such a potent inhibitor with nmol L−1 level activity with improved stability is greatly beneficial to the practical application as a cancer prodrug. Interestingly, the addition of DTT also caused 10 times increased the inhibitory activities of 3, 9 and 10, suggesting that the thioacetate groups may be in some extent hydrolyzed under the assay conditions to generate sulphydryl groups. The less effective nature of 3, 9 and 10 compared to 4 in the presence of DTT suggests the incomplete generation of sulphydryl groups. It may be noted that these cyclic peptide inhibitors (3, 4, 9 and 10) were less effective for HDAC6, even in the presence of DTT. Because HDAC4 is more frequently expressed in tumor tissues, this target sensitivity is valuable.14

The cyclic peptides were examined for in vivo p21 promoter assay, in which the effect of the peptide inhibitors on a cell expression was measured.9 The cyclic peptide 3 and the bicyclic peptide 4 showed similar EC1000 (effective concentration of 10 times increased induction) values. These produgs were transformed to the sulfhydryl derivative by the glutathione-reduction or protease-hydrolysis in the cell, therefore, they showed similar activity under the cellular assay conditions. In this assay, the cyclic peptides with the chlamydocin framework, 3 and 10, were more efficient inhibitors than the CHAP31-based peptides, 3 and 4. The relationship between HDAC inhibitory activity and p21 promotion activity should be studied in future.

In summary, the bicyclic tetrapeptide 4 based on the CHAP31 framework with an intramolecular disulfide bridge was for the first time successfully synthesized. In the chlamydocin-related cyclic peptides, the intramolecular disulfide bridges were not formed. Biochemical assays showed that 4 is a potent inhibitor of HDAC in the presence of DTT in vitro. Interestingly, the precursor cyclic peptides with the thioacetate ligands (3, 9, 10) moderately inhibited the action of HDAC. Compounds 3, 4, 9 and 10 were also active in vivo p21 promoter inducing activity.

References and notes