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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5343–5346

Novel histone deacetylase inhibitors: cyclic tetrapeptide with trifluoromethyl and pentafluoroethyl ketones

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Received 21 June 2004; revised 2 August 2004; accepted 7 August 2004
Available online 3 September 2004

Abstract—Cyclic tetrapeptides containing trifluoromethyl and pentafluoroethyl ketone as zinc binding functional group were synthesized as potent HDAC inhibitors. Evaluation by human HDAC inhibition assay and p21 promoter assay showed that these inhibitors are promising anticancer agents.

Post-translational modification of *N*-terminal tails of histone proteins by reversible acetylation with histone acetyl transferase (HAT) and histone deacetylase (HDAC) enzymes is involved in chromatin remodeling and plays a crucial role in gene expression.[1] The inappropriate recruitment of HDACs has been clearly linked to carcinogenesis and the inhibition of HDACs results in transcriptional reactivation, cell-cycle arrest and terminal differentiation of transformed cells. HDAC inhibitors have potential for the prevention and treatment of cancer and emerged as an attractive target as anticancer drugs.[2]

A number of structurally diverse natural and synthetic compounds have been so far reported exhibiting HDAC inhibitory activity.[2] These compounds consist of a hydrophobic scaffold with a spacer, that is attached to a functional group which can interact with zinc ion present in the active site pocket. Compounds containing hydroxamic acid as a functional group is reported as the most potent inhibitor for HDACs, which include the natural product trichostatin A.[3] In our previous work, we synthesized cyclic hydroxamic acid-containing peptides (CHAPs) and found that they have very high HDAC inhibitory activity.[4] Other HDAC inhibitors have thiol,[5] epoxide,[6] carboxylic acid,[7] and amide[8] as zinc binding functionalities. Very recently, we reported retrohydroxamates as potent HDAC inhibitors.[9] The catalytic mechanism for the deacetylation of acetylated lysine and various reported ligands of cyclic tetrapeptide inhibitors of HDAC are shown in Figure 1. Many of inhibitors have potent antitumor effect in vivo in tumor bearing animals. However, the potential for drug development could be limited due to many reasons such as low potency, lack of selectivity, cytotoxicity, low solubility in aqueous vehicle and low stability in cell culture. Poor pharmacokinetic properties associated with the hydroxamic acid group in matrix metalloproteinases inhibitors[10] prompted us to explore the possibility of other zinc binding functional groups for cyclic tetrapeptides.

Trifluoromethyl ketones are potent inhibitors of aspartyl[11] and serine[12] proteases, by formation of stabilized hemiketals and hemithioketals at the active site. A recent report on HDAC inhibitors showed that trifluoromethyl ketone attached to aromatic amides has micromolar inhibitory activity for HDAC.[13] We designed and synthesized cyclic tetrapeptides containing trifluoromethyl ketone as HDAC inhibitors. We speculate that the cyclic tetrapeptide trifluoromethyl ketone and related compounds can give better inhibitory activity. The functional groups selected for this study is

Keywords: Histone deacetylase; Inhibitor; Cyclic tetrapeptide; Trifluoromethyl ketone; Pentafluoroethyl ketone.
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doi:10.1016/j.bmcl.2004.08.016

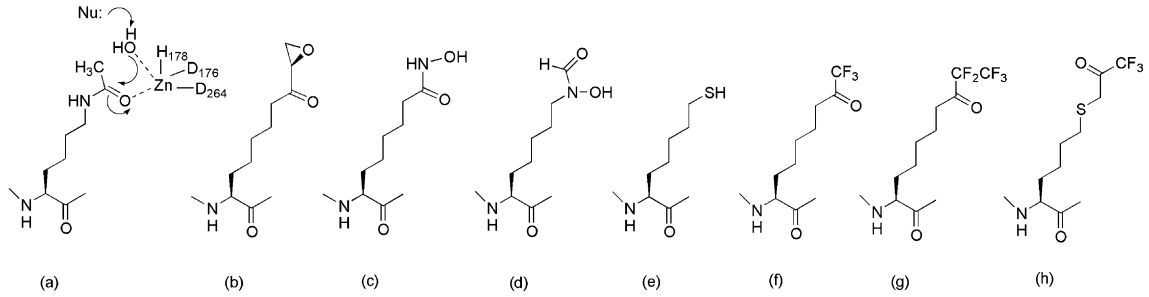


Figure 1. Binding of substrate to zinc ion in HDAC1, reported ligands of cyclic tetrapeptide inhibitors and proposed functional groups. (a) Binding with substrate, (b) ketoepoxide, (c) hydroxamic acid, (d) retrohydroxamic acid, (e) sulfhydryl, (f) trifluoromethyl ketone, (g) pentafluoroethyl ketone, (h) trifluoromethyl ketone with a sulfur atom in the spacer.

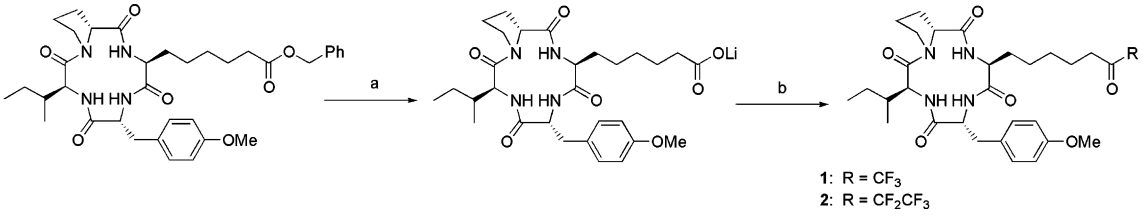
shown in Figure 1. The hydrophobic scaffold for the proposed inhibitor is the cyclic tetrapeptide portion of CHAP31, which was found to have excellent antitumor activity.[4]

The cyclic tetrapeptide *cyclo*(-L-Asu(OBzl)-D-Tyr(Me)-L-Ile-D-Pro-) was synthesized by the reported procedure, where Asu is aminosuberic acid.[4a] The benzyl side chain protection of the cyclic tetrapeptide was removed by hydrolysis with lithium hydroxide and the resulted lithium salt was then treated with trifluoroacetic anhydride under modified Dakin–West conditions[14] to give the desired trifluoromethyl ketone **1** in 40% yield. Using pentafluoroacetic anhydride, cyclic tetrapeptide containing pentafluoroethyl ketone **2** was also synthesized (Scheme 1).

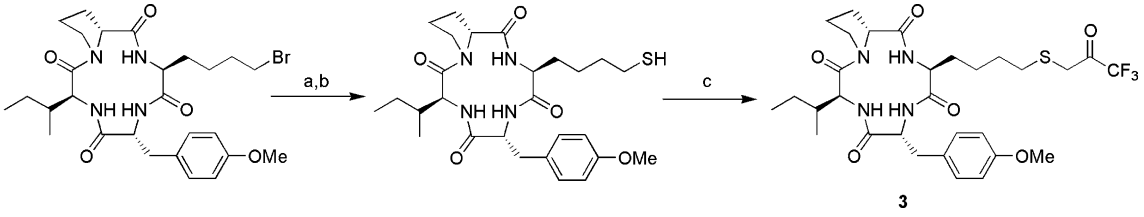
Next we synthesized a cyclic tetrapeptide containing 1,1,1-trifluoro(3-thio)acetone as the functional group. For this, we used a cyclic tetrapeptide containing an L-amino-6-bromohexanoic acid (L-Ab6).[15] The cyclic tetrapeptide, *cyclo*(-L-Ab6-D-Tyr(Me)-L-Ile-D-Pro-) was synthesized by the reported procedure.[5] This cyclic peptide is treated with potassium thioacetate to yield thioacetate derivative, and the product was then treated with ammonia to obtain the thiol derivative. The thiol derivative is reacted with 3-bromo-1,1,1-trifluoroacetone to yield the proposed compound **3** (Scheme 2).[16]

HDAC inhibitory activity of these electrophilic ketones depends on the degree of hydration of the ketone, which can chelate with the zinc ion present in the deep pocket of HDAC binding site. We verified the degree of hydration using HPLC and ^{19}F NMR studies. HPLC of compound **1** showed a single peak at 8.0 min indicating that the compound is completely hydrated under the HPLC conditions.[17] Compound **2** showed two peaks, one at 8.8 min and the other at 10.5 min in the ratio of 3:7 indicating that the tendency to form hydrate in the case of **2** is less (Fig. 2). Compound **3** shows the similar behavior as **1** with a single peak at 8.2 min.

^{19}F NMR of **1** shows only one singlet at δ −82.5 in $CDCl_3$. In methanol **1** shows a doublet at δ 82.0 indicating hemiacetal formation. In a mixture (7:3) of methanol and water, a new peak was observed at δ −86.5 due to the hydrate formation. The intensity ratio of 1:2 for the peaks at δ −82 and −86.5 indicate the strong tendency of trifluoromethyl ketone to form hydrate. On the other hand, under similar conditions, a lower rate



Scheme 1. Reagents and conditions: (a) LiOH, H_2O, THF; (b) for **1**, pyridine, trifluoroacetic anhydride for **2**, pyridine, pentafluoropropionic anhydride.



Scheme 2. Reagents and conditions: (a) AcSK, DMF; (b) NH_3/MeOH; (c) $BrCH_2COCF_3$, DMF.

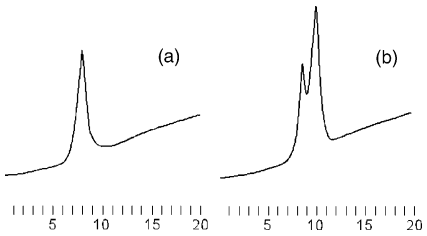


Figure 2. (a) HPLC chart for **1** and (b) **2**. The conditions for HPLC analysis is given in Ref. 17.

of hydration was observed from the ^{19}F NMR of compound **3**. Similar differences in the degree of hydration of trifluoromethyl ketones and pentafluoromethyl ketones were observed by Peet and co-workers during their study on human neutrophil elastase inhibitors.[12]

The compounds synthesized were tested for the HDAC inhibitory activity using HDAC1, HDAC4, and HDAC6 prepared from 293T cells.[18] The results of HDAC inhibitory activity and p21 promoter assay of the compounds are shown in Table 1. For comparison the activity of trichostatin A is also shown. All three compounds show HDAC inhibitory activity in nanomolar concentrations. The activity of compound **3** is higher than the other two compounds probably due to the presence of the C–S–C bond, that may provide a different mode of ligation with Zn ions. More studies are needed to find the binding nature of **3** within the enzyme pocket. When comparing the activity between compounds **1** and **2**, trifluoromethyl ketone is more active than pentafluoroethyl ketone. This may be ascribed to the presence of the bulky pentafluoroethyl group that causes a decrease in activity. Since Frey and co-workers described on the instability of trifluoromethyl ketone inhibitors in their work, we carried out in vivo stability tests of inhibitor **1**. The inhibitor was stable under the p21 promoter assay conditions though not much better than trichostatin A (Fig. 3).

Although the mechanism of inhibition on these inhibitors is not completely clear, it seems that the hydration of the ketone is very important for the inhibitory activity. Based on this observation, we propose the mode of interaction of the functional groups of these inhibitors with the zinc ion present in the active site of HDAC as shown in Figure 4.

Table 1. HDAC inhibition and p21 promoter assay data for the compounds

Compound	IC_{50} (μM)				EC_{50} (μM)
	HDAC1	HDAC4	HDAC6	HDAC8	
1	0.73	0.61	1.44	0.37	3.09
2	0.85	NT[a]	5.70	0.78	31.10
3	0.047	0.19	0.18	0.23	6.64
TSA[b]	0.022	0.020	0.028	0.040	0.77

[a] Not tested.
[b] Trichostatin A.

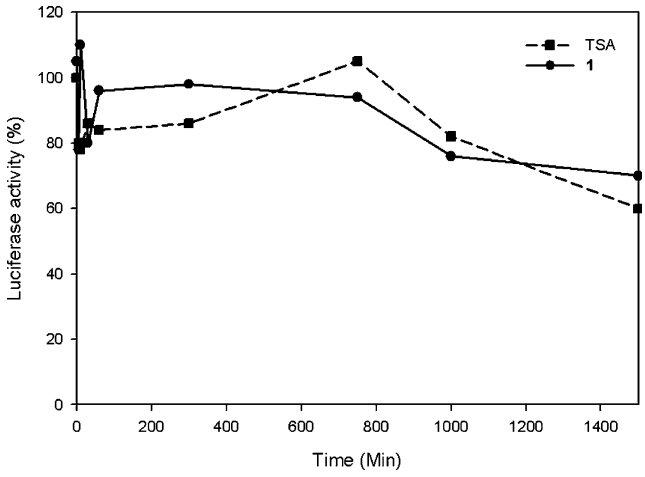


Figure 3. In vivo stability test of **1** and trichostatin A.

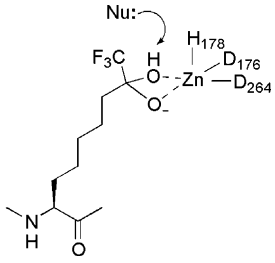


Figure 4. Proposed mode of interaction of the inhibitor functional groups with Zn in the active site of HDAC1.

In summary, in order to find novel non-hydroxamate HDAC inhibitors, we designed and synthesized CHAP31 based electrophilic ketones. These inhibitors have displayed potent HDAC inhibition activity in vivo and in vitro. These results further confirm the hypothesis that structural modification of the terminal residues of HDAC inhibitors can lead to potent HDAC inhibitors that may have potential as anticancer agents. Further studies on compound **1** and **3** are under way.

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17. HPLC was performed on a Hitachi instrument equipped with a chromolith performance RP-18e column (4.6×100 mm, Merck). The mobile phases used were A: H_2O with 10% CH_3CN and 0.1% TFA, B: CH_3CN with 0.1% TFA using a solvent gradient of A to B over 15 min with detection at 220 nm with a flow rate of 2 mL/min.
18. (a) For enzyme preparation and assay, see: Furumai, R.; Matsuyama, A.; Kobashi, M.; Lee, K.-H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. *Cancer Res.* **2002**, *62*, 4916–4921; (b) Matsuyama, A.; Shimazu, T.; Sumida, Y.; Saito, A.; Seigneurin-Berny, D.; Yoshimatsu, Y.; Osada, H.; Komatsu, Y.; Nishino, N.; Khochbin, S.; Horinouchi, S.; Yoshida, M. *EMBO J.* **2002**, *21*, 6820–6831.