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Histone deacetylase inhibitors: synthesis of cyclic tetrapeptides and their triazole analogs

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ABSTRACT

Synthesis of nine macrocyclic peptide HDAC inhibitors and three triazole derivatives is described. HDAC inhibitory activity of these compounds against HeLa cell lysate is evaluated. The biological data demonstrate that incorporation of a triazole unit improves the HDAC inhibitory activity.

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During the cell cycle, post-translational modifications to the ε-amino-terminal tails of histone proteins are made by a number of different enzymes, including histone acetyl transferases (HATs) and histone deacetylases (HDACs).[1] Histone tails contain ~40 lysine residues, which are acetylated by HATs. Acetylation induces a conformational change within chromatin, allowing the transcriptional machinery access to DNA thus promoting gene expression.[1,2] HDACs repress the gene expression by deacetylating the lysine tails, allowing the positively charged lysines to bind tightly to the negatively charged DNA and denying the transcriptional machinery access to genes, thereby repressing gene expression. Thus, these post-translational modifications play a key role in directing gene expression, and can create a phenotype that is unrelated to changes in DNA.[3] Inappropriate up-regulation of HDACs' silences specific tumor suppressor genes, which are responsible for cell proliferation, differentiation, and apoptosis.[4,5] Molecules that interfere with HDAC activity have shown a great promise as anticancer agents as they inhibit this silencing process and allow tumor suppressor genes to be transcribed and control the cell's growth.[6–8] With a number of HDAC inhibitors in clinical trials and suberoylanilide hydroxamic acid (SAHA, Zolinza®) recently approved by the FDA for the treatment of cutaneous T cell lymphoma (CTCL), HDAC inhibition proves to be a worthy strategy for cancer therapy.[9]

HDAC inhibitors consist of three components: (1) the active site metal-binding unit, (2) surface recognition domain, and (3) a linker that connects the two domains.[10] They operate by binding the surface recognition domain located at the rim of the HDAC pocket and placing the metal-binding unit within the pocket (Fig. 1).[11] HDAC inhibitors can be divided into five structural categories: short chain fatty acids, hydroxamic acids, electrophilic ketones, benzamides, and cyclic peptides.[12] These five structural categories are known to inhibit the three classes of metal-dependent HDACs.[13] There are 11 metal-dependent HDACs currently known, and it is unclear which isoforms are responsible for silencing tumor suppressor genes. Given the uncertainty of the role played by the individual HDACs, chemotherapy has focused on inhibitors that target endogenous HDACs, which are multiple isoforms.

Romidepsin (Istodax®), a cyclic peptide, has recently been approved as an HDAC inhibitor against CTCL, indicating that cyclic peptides are pharmacophores of interest in this field.[14] In this Letter, we describe the synthesis of twelve HDAC inhibitors. Nine of these tetrapeptides were synthesized and modifications were focused on the surface recognition domain and incorporating diverse metal-binding units: acetyl lysines, trifluoroacetyl lysines, and guanidine moieties. The poor solubility of these macrocycles, coupled with the fact that rigid structures bind more effectively to protein targets than their structurally similar flexible counterparts, led us to incorporate triazole units within the surface recognition domain in three molecules.[15] We anticipated that the presence of a triazole will rigidify the macrocyclic structure, restricting bond rotations. This may lead to a more potent inhibitor if the metal-binding unit is placed in the correct orientation within the HDAC pocket. In the triazole molecules, only 2 of the 3 metal-binding units were incorporated into their design: acetyl lysines and trifluoroacetyl lysines as, during the course of this work, we found that the guanidines were poor inhibitors.

The design of compounds **2–9** was based on our lead compound **1** (Fig. 1).[16] Compound **2** was generated by modifying **1**, replacing the acetyl lysine metal-binding unit with a guanidine unit. Reversing the stereochemistry from L to D of the homoarginine in **2** produced **3**, while incorporation of arginine and a six-membered ring at positions 3 and 4, respectively, gave **4**. Integrating a six-membered ring or a triazole at position 4 into our lead structure gave compounds **5** and **6**, respectively. Finally, substitution of the acetyl moiety with a trifluoroacetyl moiety as the metal-binding unit and subsequent inclusion of a six-membered ring or triazole unit at position 4 produced compounds **7**, **8**, and **9**, respectively.

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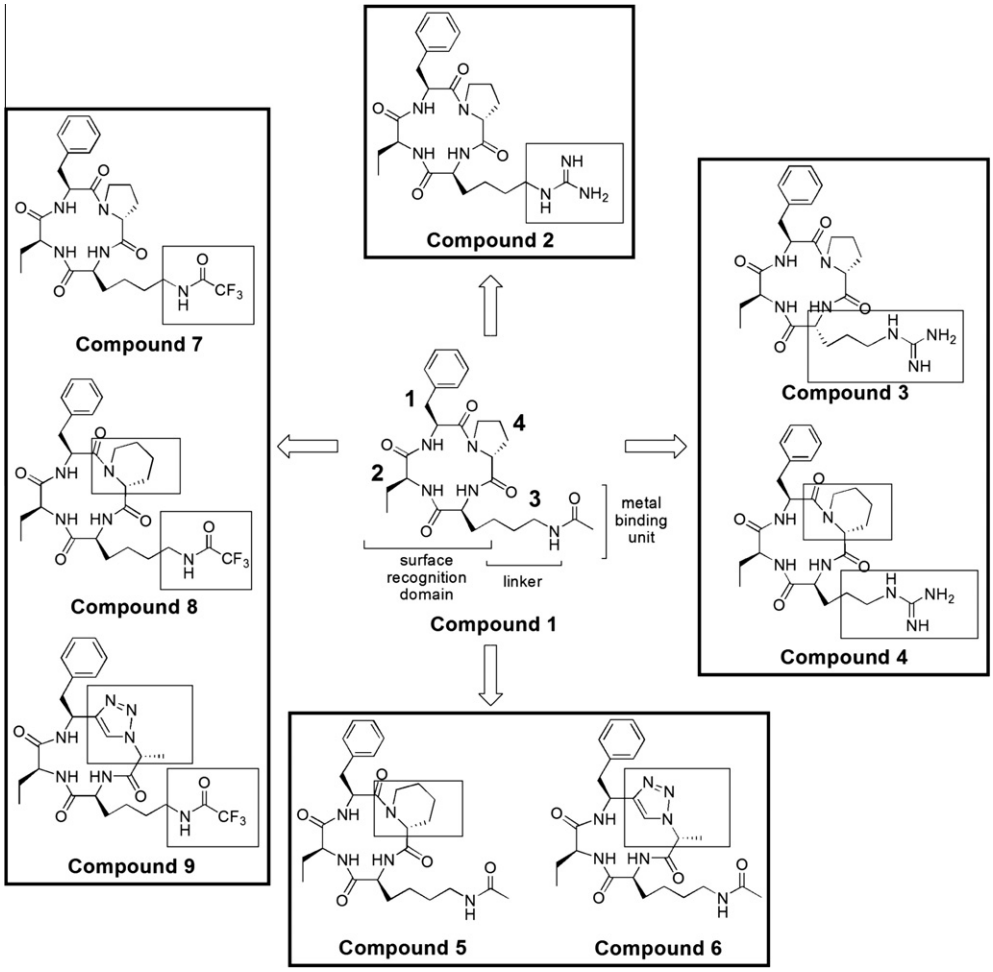


Figure 1. Peptide HDAC inhibitors based on lead compound **1**.

A second series of compounds were synthesized, where these were based on apicidin, a cyclic tetrapeptide HDAC inhibitor with low nanomolar protozoan histone deacetylase inhibition.[17] Four compounds were made (Fig. 2), where all the four employed a free tryptophan as opposed to the methoxy-protected indole present in Apicidin. Both compounds **10** and **11** contain an acetyl lysine as the metal-binding unit, and **10** includes a proline unit at position 4 whereas **11** contains a triazole unit. Compounds **12** and **13** replaced the six-membered ring at position 4 of Apicidin with a D-proline and incorporated a trifluoroacetyl moiety as the metal-binding unit. Derivative **12** maintained the L-stereochemistry of the tryptophan, while **13** reversed it to a D-tryptophan.

Syntheses of our peptide HDAC inhibitors were completed via a convergent solution-phase route (Scheme 1), which allowed for an easy substitution of amino acids at each position.[16] TBTU and DIPEA were used to form the dipeptide fragments 1–2 and 3–4, where acid-protected residues 1(a–d) and *N*-Boc-protected residue 2(a–c) furnished dipeptide MeO-1-2-Boc, and acid-protected residues 3(a–d) and *N*-Boc-protected residues 4(a,b) were coupled to give dipeptides MeO-3-4-Boc (84–98% yield) (Scheme 1). Dipeptide acids of MeO-1-2-Boc were deprotected with lithium hydroxide (52–80% yield), whereupon they were coupled to free amine dipeptides MeO-3-4 to give linear tetrapeptides (41–90% yield depending on the substrate). The linear tetrapeptide was acid and amine deprotected using standard conditions, whereupon it was cyclized by dissolving it in a 1:1 ratio of CH_2Cl_2 and CH_3CN (0.007–0.1 M). Addition of DIPEA (8–10 equiv) and three coupling agents (HATU, DEPBT, and TBTU 2 equiv total) to the reaction

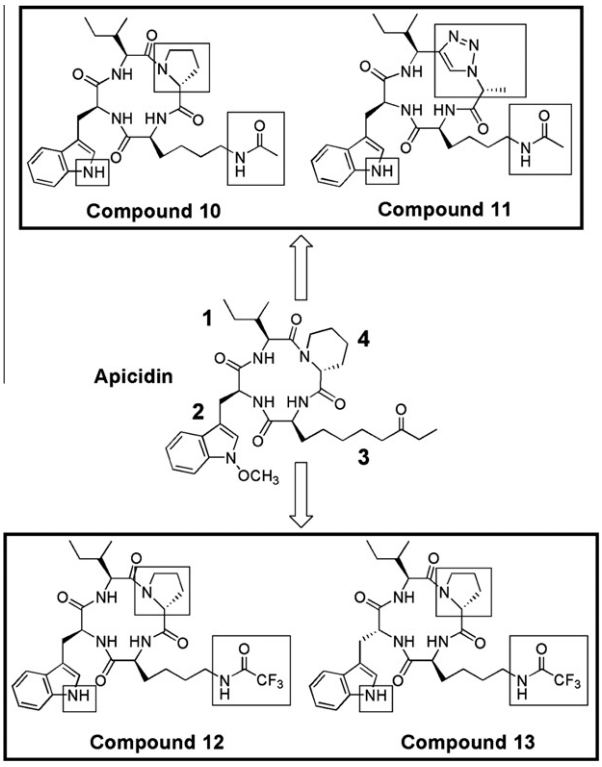
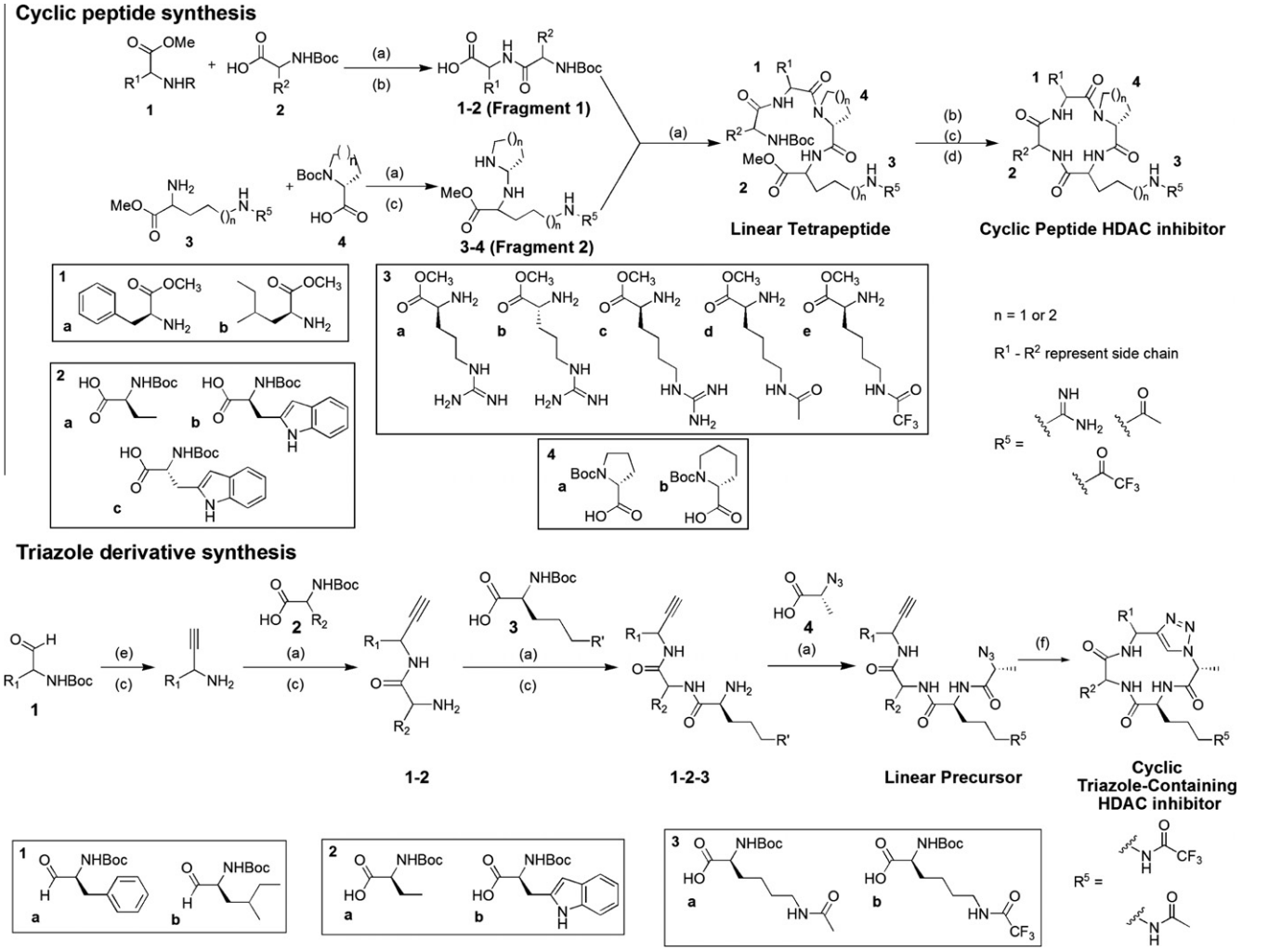


Figure 2. Peptide HDAC inhibitors based on apicidin.



Scheme 1. Cyclic peptide and triazole analog synthesis. Reagents and conditions: (a) TBTU, HATU, and/or DEPBT (1.2 equiv total), DIPEA (3–6 equiv), CH_2Cl_2 (0.1 M), yields 41–98%; (b) LiOH (4–8 equiv), CH_3OH (0.1 M), yields 52–80%; (c) TFA (20–25%), anisole (2 equiv), CH_2Cl_2 (0.1 M); (d) TBTU, HATU, and DEPBT (2.0 equiv total), DIPEA (8–10 equiv), CH_2Cl_2/CH_3CN (1:1, 0.1–0.007 M), yields 3–45%; (e) K_2CO_3 (3 equiv) *p*-TsN_3 (3 equiv), dimethyl(2-oxypropyl)phosphonate (3 equiv), CH_3CN/CH_3OH (1:1, 0.25 M), yields 42–73%; (f) $CuSO_4$ (1.5 mM), sodium ascorbate (45 mM), CH_3OH/H_2O (1:1, 0.005 M), yields 3–9%.

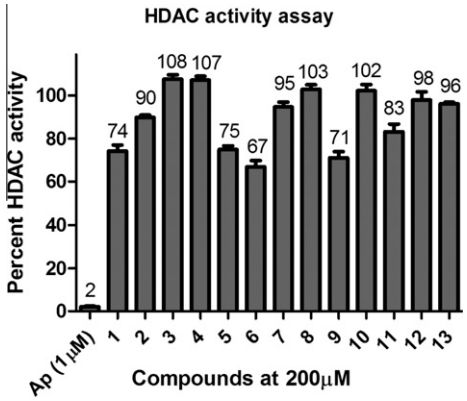


Figure 3. HDAC inhibition assays, where each column represents percent of HDAC activity from the average of three independent trials at 200 μM of apicidin and compounds **1**–**13**, respectively. HDAC inhibition activity was relative to the DMSO control.

produced a clear solution. The reactions were complete in approximately 2–4 h. Work-up with methylene chloride and ammonium chloride, concentration in vacuo, purification via flash chromatography, followed by HPLC furnished final products, which were confirmed via LC/MS and ^{1}H NMR (yields ranged from 3% to 45% depending on substrate). For compounds **2**–**4** deprotection of amines on residues 3(a–b) was accomplished via hydrogenolysis following cyclization. Compounds were dissolved in EtOH (0.1 M) and treated with H_2 gas in the presence of pure Pd/C. The reaction mixtures were stirred for 3–5 h under H_2, followed by filtration with Celite, to yield final derivatives.

Synthesis of triazole containing HDAC inhibitors followed a linear approach (Scheme 1) whereby each residue was sequentially added onto the molecule in a solution-phase. An amino aldehyde residue 1(a–b) was converted to its corresponding alkyne with the Bestmann–Ohira reagent (42–73% yield).[18] The alkyne was amine deprotected with TFA and subsequently coupled to residue 2(a–b) to give 1-2-Boc (92–98% yield). Treatment of 1-2-Boc with TFA afforded free amine 1–2. This intermediate was coupled to residue 3(d–e) to furnish 1-2-3-Boc (74–92% yield), which was amine deprotected to furnish 1-2-3. The linear precursor was obtained upon coupling 1-2-3 with residue 4 (66–93% yield).[19] To generate our final triazole containing macrocycles, we employed a Cu(I)-catalyzed azide-alkyne cycloaddition as reported by Sharpless and coworkers.[20] Sodium ascorbate and copper sulfate were dissolved in water and added to reaction flask with 10% of the solvent mixture

1:1 ratio of CH_3OH and H_2O (0.005 M). The linear precursor was dissolved in the remaining solvent mixture and added dropwise to the reaction flask overnight. Upon completion of the reaction, CH_3OH was removed under reduced pressure and the product was extracted with CH_2Cl_2, concentrated in vacuo, and purified via flash chromatography, followed by HPLC to furnish final products. Final compounds were confirmed via LCMS and ^1H NMR (yield ranged from 3% to 9% yield).

Upon completion of the synthesis, we tested our molecules in HDAC inhibition assays. The twelve compounds were assayed at 200 μM concentration against endogenous HDACs from HeLa cell lysates using a fluorogenic substrate, as previously described.[16] Apicidin (Fig. 2) was used as a positive control (2% ± 0.3% deacetylase activity at 1 μM) and DMSO was used as a negative control (set to 100% deacetylase activity) (Fig. 3). Compounds **6** and **9** were the most potent, and inhibited the deacetylase activity 67% ± 3% and 71 ± 3% at 200 μM, respectively. Both the potent compounds contained a 1,2,3-triazole unit at position 4 of the macrocycle.

Although our HDAC Inhibition data show that our compounds are not tremendously potent we can draw several conclusions as our compounds showed a distinctive structure–activity relationship (SAR). First, we conclude that despite precedence[21] guanidine is not a good metal-binding unit, while both acetyl and trifluoro acetyl metal-binding units have similar effects on inhibiting HDAC activity. Second, changes from 4 to 5 atoms on the linker do not affect HDAC inhibition and third, it appears that there is no difference between compounds when a five- or six-membered ring is placed at position 4. Finally, fourth, there is generally improved HDAC inhibition for molecules containing the triazoles (comparing compounds **1** and **5** to **6**, **7** and **8** to **9**, and **10** to **11**).

In conclusion, our data suggest that triazole-containing macrocycles are more effective HDAC inhibitors than their structurally related peptide analogs. These data support our hypothesis that the presence of a triazole, which rigidifies the macrocyclic structure and restricts bond rotation, leads to a more potent inhibitor presumably because it places the metal-binding unit in an appropriate orientation within the HDAC pocket. This fact as well as the other trends noted above will be utilized to design new HDAC inhibitors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.06.050.

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