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Tetrahedron Letters
Tetrahedron Letters 48 (2007) 4579–4583

# Cyclic disulfides as functional mimics of the histone deacetylase inhibitor FK-228

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Received 14 March 2007; revised 19 April 2007; accepted 25 April 2007
Available online 29 April 2007

**Abstract**—FK-228 is a potent histone deacetylase (HDAC) inhibitor with tremendous therapeutic potential against a wide array of human cancers. We describe the development of analogs that share FK-228's novel mechanism of activation and HDAC inhibition.


## 1. Introduction

Histone deacetylases (HDACs) convert neutral acetyl lysine side chains to their positively charged ammonium salts[1] and play a key role in the regulation of gene expression.[2–8] Histone deacetylation correlates most often with transcriptional repression; there are now numerous examples of human cancers believed to arise as a result of accidental transcriptional repression.[9] It is therefore no surprise that HDAC inhibitors are highly attractive anticancer agents. Among the most promising of these is the cyclic disulfide natural product FK-228 (**1**) which displays impressive anticancer and antiangiogenic activities by virtue of its capacity for HDAC inhibition.[10–13] FK-228 is currently in Phase II clinical trials for T cell lymphomas and other phase II projections involve non-Hodgkin's lymphoma, acute myelogenous leukemia, and pancreatic cancer.[14] Phase I clinical trials for thyroid and other advanced malignancies, combination therapy for lung cancer, and for leukemias are also underway. Alarmingly, the advancement of **1** through clinical trials has been hindered as a result of shortages of the natural product and its high degree of synthetic complexity.[13]

Importantly, HDAC inhibition by **1** is a consequence of disulfide reduction to **2**. The thiol-capped butene moiety likely binds an active site zinc (Fig. 1).[15] Dithiol **2** adheres to a tripartite structure characteristic of most effective HDAC inhibitors, as it contains an HDAC recognition or affinity element attached to an active site binding/inactivating group via a linker devoid of elaborate functionality.[16] The increased levels of disulfide reductants (i.e., glutathione, thioredoxin (Trx) and thioredoxin reductase (TrxR)) found in many cancer cells likely render such cells particularly susceptible to the actions of **1** and provide added enthusiasm for the pursuit of functional mimics of **1**.[17] Although advances have been made toward acyclic thiol and masked thiol-based HDAC inhibitors, the reductive mechanism displayed by **1** has not yet been mimicked by non-peptidic, more easily diversified scaffolds.[18] We report here, the synthesis and biological study of small, readily-diversifiable disulfide motifs that share FK-228's novel mechanism of activation and HDAC inhibition.

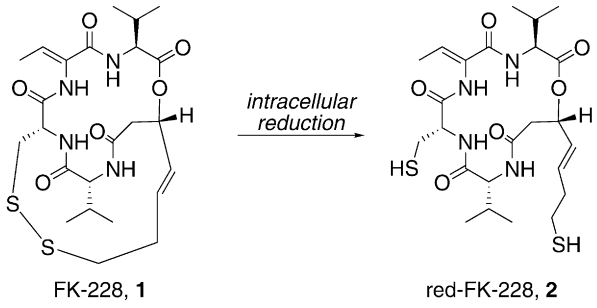


**Figure 1.** Mechanism of FK-228 activation en route to HDAC inhibition.

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doi:10.1016/j.tetlet.2007.04.123

## 2. Results and discussion

Our attention focused on cyclic disulfides **3**–**5**, bearing 4, 5, and 6 methylene unit-containing linkers between the proposed $Zn^{2+}$-binding thiol and the rest of the 'cap' linked molecular framework (Fig. 2). Based upon the elegant structure:activity studies of Yoshida and coworkers, we expected that different linker lengths would correlate to altered efficiencies of HDAC inhibition.[19] For the purposes of preliminary biological studies here, we have relegated efforts to the assessment of in vitro activities. We envisioned **3**–**5** to be important intermediates en route to an array of more elaborate cyclic disulfides. Methyl esters **3**–**5** would provide a handle with which to install lipophilic cap groups via simple amide-forming reactions with various primary and secondary amines. Our specific interest in lipophilic cap groups was motivated in large part by the vast experience of other groups.[8,16] In particular, crystal structures of histone deacetylase homolog protein (HDAC-like) bound to known HDAC inhibitors Trichostatin A (TSA) and SAHA highlight the importance of inhibitor interactions with a number of phenylalanine residues lining the enzyme channel leading to a deeply buried active site.[20] Moreover, amides derived from **3**–**5** would permit delineation of the impact of slightly different linker lengths between the presumed $Zn^{2+}$-binding thiol and the remainder of these simple agents. We envisioned a retrosynthesis wherein **3**–**5** would result from cyclization of di-*S*-trityl **6** using well-established thiol deprotection/oxidation conditions (Fig. 2). Compounds like **6** would be generated from simple peptide coupling of the suitably functionalized acids **7** and *S*-trityl cysteine methyl ester **8**.

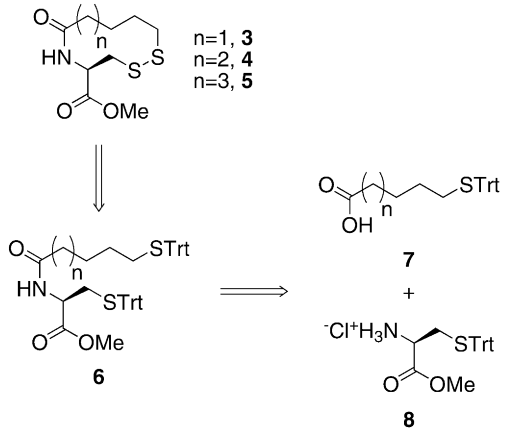
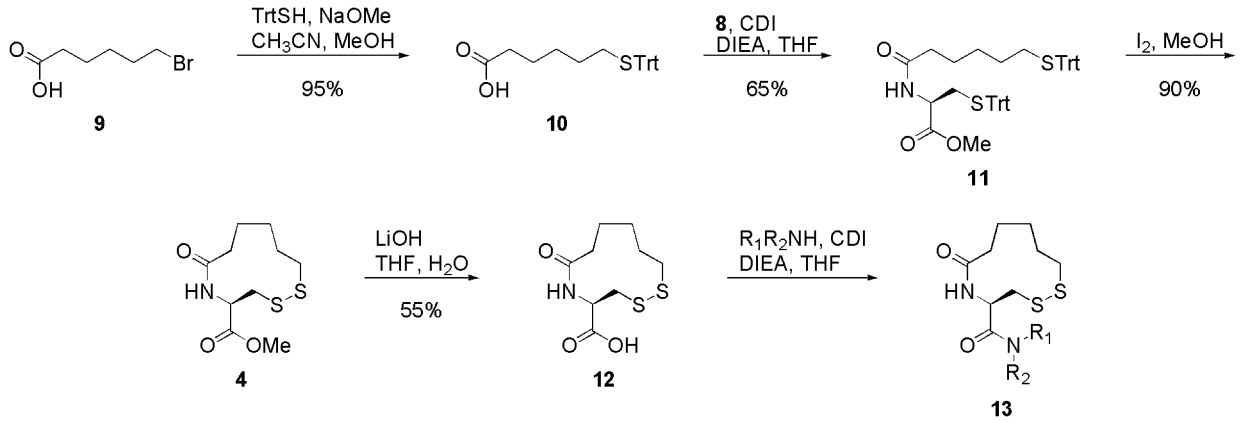



**Figure 2.** Envisioned disulfide core structures and relevant retrosynthesis.

A representative synthesis of disulfides **3**–**5** is shown in Scheme 1 using compound **4**. Commercially available bromoacids of type **9** were readily converted to their *S*-trityl carboxylic acids of type **10**; peptide coupling of **10** and *S*-trityl cysteine methyl ester **8** using 1,1′-carbonyldiimidizole (CDI) afforded di-*S*-trityl compounds such as **11**.[21] Cyclization of **11** to **4** was accomplished using $I_2$/MeOH, as previously reported in the total synthesis of FK-228 and related disulfides.[13,22] The purity of **3**–**5** was assessed to be >96% by $^1$H NMR. Saponification of **4** with LiOH provided acid **12** which, followed by peptide coupling to various amines, could produce an assortment of derivatives bearing different cap groups ancillary to the cyclic disulfide scaffold (**13**). Interestingly, it was found that, while core structures **3** and **4** could be readily hydrolyzed and further manipulated, the larger heterocycle **5** was not amenable to saponification. Attempts to produce amides from this substance were often complicated by significant decomposition. As such, only a limited number of amide-diversified analogs of **5** were successfully constructed.

We hypothesized that **3**–**5** would be cell permeable. Thus, **3**–**5** were tested for their ability to induce histone hyperacetylation in *Drosophila* S2 cells. *Drosophila*, with its small well-defined genome and amenability to gene ablation provides an excellent eukaryotic model.[23] *Drosophila* S2 cells were subjected to varying concentrations of methyl esters **3**–**5** for 48 h at ambient temperature, after which time nuclei were isolated and histones analyzed. Histone H4 lysine-acetylation status was assessed using Western blots utilizing anti-$(Ac)_4$-H4 antibody. Much to our delight, H4 hyperacetylation was observed in cells subjected to methyl esters **3**–**5**. Hyperacetylation was observed at disulfide concentrations as low as 20 μM (Fig. 3) and was particularly striking in cells sub-




**Scheme 1.** Synthesis of cyclic disulfides.

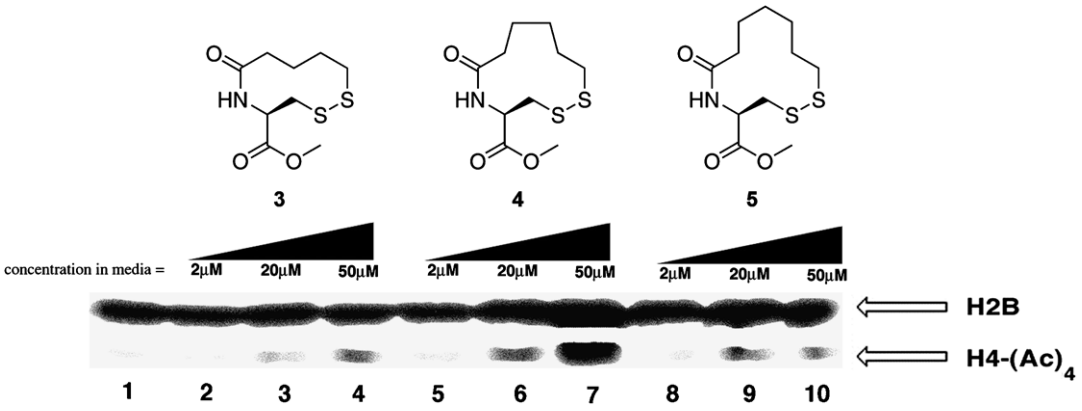



**Figure 3.** Cyclic disulfides induce histone H4 hyperacetylation in cells.

jected to disulfide **4** containing the 5 methylene-unit linker. The concomitant use of anti-H2B antibody revealed that alterations in $(Ac)_4$-H4 signal result from altered acetylation status and not variation in absolute protein loadings. Differences in H4 signal are clearly small molecule-dependent and not a result of uneven protein loading. These results confirmed that simple cyclic disulfides devoid of the relatively large and lipophilic cap groups characteristic of both natural and synthetic effective HDAC inhibitors can induce significant histone hyperacetylation in cells. Additionally, this suggested that diversified analogs like **13** may serve as good leads for new types of HDAC inhibitors inspired by FK-228.

The intracellular reduction of **1** to its corresponding dithiol **2** plays a crucial role in the agent's HDAC inhibition profile. We hypothesized that structurally simplified disulfides might capitalize on this activating event en route to HDAC inhibition. To test this hypothesis, methyl esters **3**–**5** and amide-diversified analogs **14**–**36** (Fig. 4) were assayed for HDAC inhibition using the Fluor-de-Lys fluorescence-based assay with HeLa cell extracts.[24] To emulate the reducing environment prominent within many tumor cells because of high glutathione, Trx and TrxR levels, Fluor-de-Lys assays were performed in the presence and absence of the reducing agent dithiothreitol (DTT).[17,25] Due to the limited availability of FK-228, we selected the highly potent, commercially-available and well-studied HDAC inhibitors Scriptaid and Trichostatin A (TSA) as positive controls. The results of this assay are depicted in Figure 4.

Most members of the library displayed a clear dependence upon DTT to effect HDAC inhibition. For instance, disulfide **22** bearing the 2,3-dimethoxybenzyl amine cap **E** is devoid of activity in the absence of DTT. However, in the presence of DTT, **22** (20 μM) exhibits substantial HDAC inhibition. There also ap-

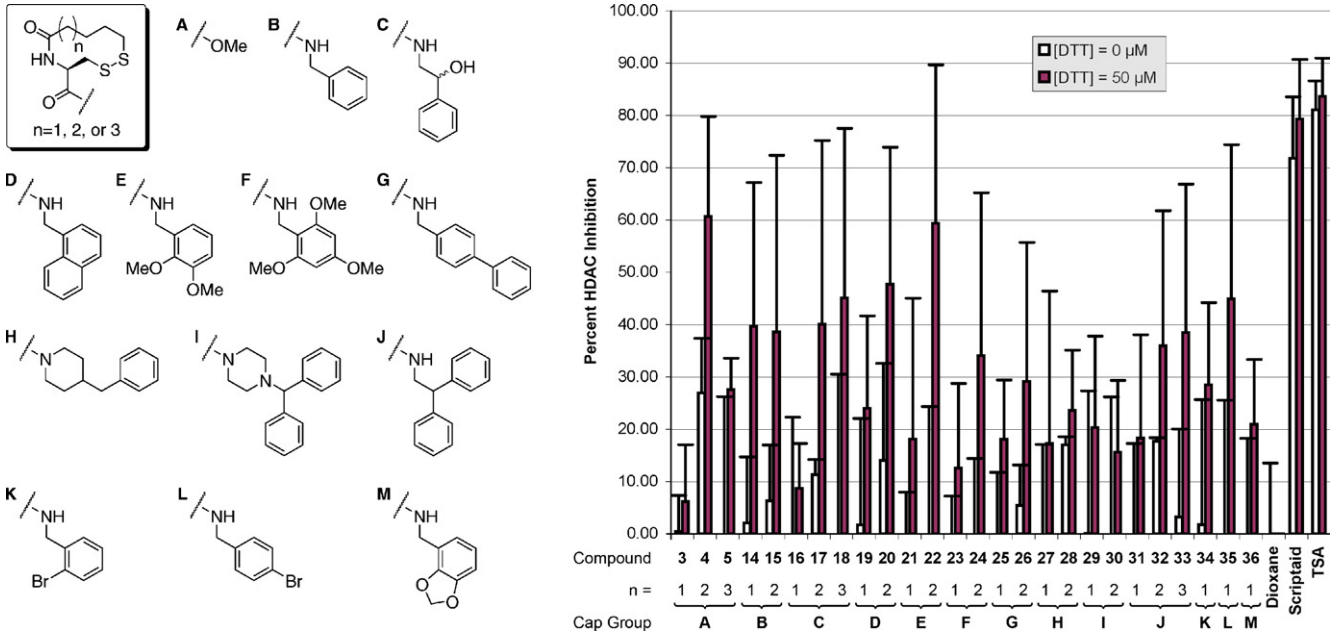



**Figure 4.** Dependence of redox-triggered alterations in HDAC activity on linker length and cap group.[26] The large standard deviations of these preliminary data have prompted us to assess HDAC inhibition using alternative methods. The results of these studies will be reported shortly.

peared to be a relationship between the number of methylene units and observed HDAC inhibition. This trend is exemplified through comparison of **23** and **24**. Although both compounds contain the same recognition/affinity group, 2,3-dimethoxybenzylamine, significantly greater HDAC inhibition is observed for **24** than **23**. A similar trend is observed for many other members of the tested cyclic disulfide panel. The cap group identity also appeared to play a role in the moderate HDAC inhibition activities displayed by members of the library. Even subtle changes in cap group structure led to significantly altered bioactivity. For example, **34** and **35** differ only in their bromide substitution pattern. Yet, these compounds differ significantly in their HDAC inhibition profiles. That such alterations in activity are observed with even subtle structural alteration from one compound to the next is supported by the well-established impact that both linker and cap identities have upon HDAC inhibitor effectiveness.[8,19] Overall, these data provide preliminary evidence that small cyclic disulfides may serve as more-easily constructed and readily-diversifiable mimics of **1**.

## 3. Conclusion

In conclusion, we have shown that simple functional mimics of **1** can be readily generated. Core methyl ester structures **3**–**5** induced H4 hyperacetylation in *Drosophila* S2 cells as determined by Western blot analyses of nuclear lysates, thus supporting early assertions of cell permeability by these synthetic agents. Methyl esters **3**–**5** can be diversified with a variety of cap groups to generate functional mimics of **1** that have moderate HDAC inhibition profiles. Consistent with the current body of structure:activity data relevant to HDAC inhibitors, we observe that even subtle structural changes have profound impact upon the degree of HDAC inhibition in in vitro assays. Synthetic efforts to produce new agents, as well as further studies with the structures in hand are ongoing and will be reported shortly.

## Acknowledgments

We thank the UW Advanced Opportunity Fellowship (AOF) program, the NIH sponsored Chemistry and Biology Interface (CBI) training grant for predoctoral support of J.A.R. and J.R.M. The generous contributions of WARF (through a Robert Draper Technology Innovation Award), the American Cancer Society, UW Graduate School and UW School of Pharmacy made this work possible and these agencies are accordingly thanked.

## Supplementary data

Experimental procedures and characterization for all synthetic compounds and experimental procedures used to obtain data in Figures 3 and 4. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.04.123.

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