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Chemistry and biology of mercaptoacetamides as novel histone deacetylase inhibitors

Bin Chen,[a] Pavel A. Petukhov,[a] Mira Jung,[b] Alfredo Velena,[b] Elena Eliseeva,[b] Anatoly Dritschilo[b] and Alan P. Kozikowski[a,*]

[a]*Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA*

[b]*Department of Radiation Medicine, Georgetown University, 3970 Reservoir Rd., NW, Washington, DC 20057, USA*

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Abstract—A series of mercaptoacetamides were designed and synthesized as novel histone deacetylase inhibitors with the aid of modeling. Their ability to inhibit HDAC activity and their effects on cancer cell growth were investigated. Some compounds exhibit better HDAC inhibitory activity than SAHA.

Histone deacetylases (HDACs) are a family of enzymes that regulate chromatin remodeling and gene transcription. They consequently control critical cellular processes, including cell growth, cell cycle regulation, DNA repair, differentiation, proliferation, and apoptosis.[1] The post-translational acetylation status of chromatin, which regulates chromatin structure, is determined by the competing activities of two classes of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs function to acetylate *N*-terminal lysine residues in nuclear histones, resulting in the neutralization of the positive charges on the histones and a more open, transcriptionally active chromatin structure, while HDACs function to deacetylate and suppress transcription.[2–5] Aberrant acetylation of histone tails emerging from HAT mutations or abnormal recruitment of HDACs has been linked to carcinogenesis.[6–9] HDAC inhibitors have been shown to reverse repression and to induce reexpression of differentiation inducing genes.[10] It is believed that HDAC inhibitors provide unique opportunities in the discovery of small molecule therapeutics for the treatment of cancer.

A variety of natural and synthetic compounds have been reported that show HDAC inhibitory activity and antitumor effects (Fig. 1).[11] Some of these inhibitors are currently in phase I/II clinical trials.[12] FK228 (or FR901228), a natural product of a rare family of bicyclic depsipeptides,[13] is currently the only member of the cyclic peptide class under clinical investigation.[14] It has been found that the reduced form of FK228, RedFK (FK228 + DTT), is more active against HDACs than FK228 itself.[15] The disulfide bond in FK228 is reduced in cells, releasing a free thiol that can bind to the zinc ion present at the bottom of a narrow binding pocket in the HDACs (Fig. 2c). Moreover, Nishino recently reported that cyclic tetrapeptides bearing a sulfhydryl group potently inhibit histone deacetylases at picomolar concentration levels.[16]

From this natural product lead, the crystal structure of HDAC enzyme[17] and the key elements of inhibitor–enzyme interaction, we designed (Fig. 2) and synthesized a range of mercaptoacetamides and investigated them for their ability to inhibit HDAC activity, and for their effects on cancer cell growth.

The design of mercaptoacetamides has been aided by the analysis of potential small molecules that may fit into the binding site of HDAC and their interactions with the HDAC protein. A homology 3D model of the HDAC1 protein was built using the HDLP protein (PDB:1C3S).[17,18] The sequence alignment of the HDAC1 and HDLP proteins were consistent with that published earlier.[17] The homology model of HDAC

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* Corresponding author. Tel.: +1 312 996 7577; fax: +1 312 996 7107; e-mail: kozikowa@uic.edu

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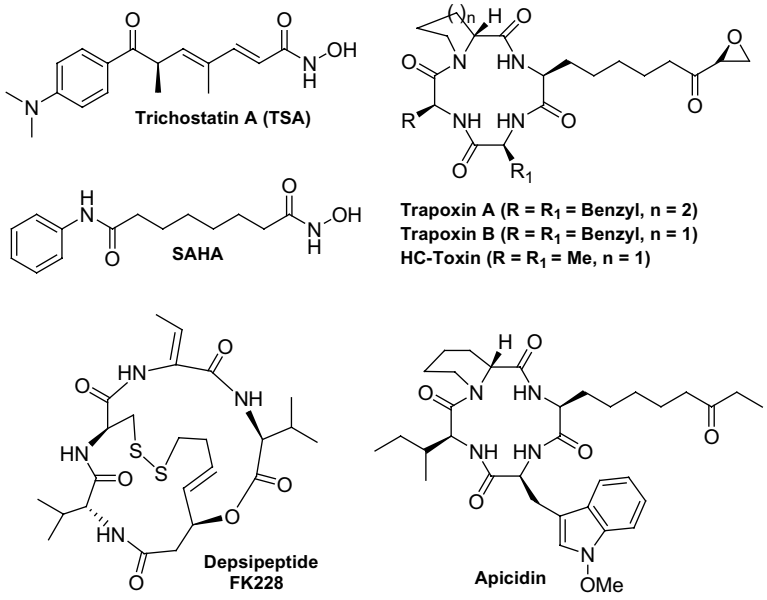


Figure 1. Structure of some known HDAC inhibitors.

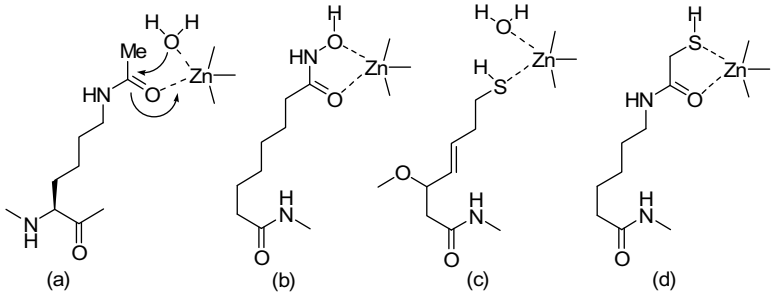


Figure 2. Possible mode of interaction of the zinc ion of HDAC with (a) acetylated lysine moiety of histone, (b) hydroxamate group of an HDAC inhibitor, (c) thiol group from FK228 + DTT, (d) our designed mercaptoacetamide-based inhibitors.

was subjected to relaxation (MD simulations) of the amino acid side chains followed by final optimization[19] using the CHARMm program (C29b1).[20,21]

All new ligands proposed during the rational drug design stage were docked to the binding site to ensure that they would fit into the binding site. The docking, scoring, and ranking were performed using the FlexX and CScore modules available in Sybyl6.91.[22] Most importantly, it was found that the HDAC binding site can accommodate head groups as large as thiol and mercaptoacetamide. The optimal linker according to the computer model of the HDAC binding site was between four and six CH_2 units. The binding mode of the mercaptoacetamide-based ligands was similar to that found for SAHA and TSA (Fig. 3). A detailed description of the computer-aided drug design protocols will be published elsewhere.

The mercaptoacetamides of general structure **4** containing spacers of varying length were synthesized starting from methyl mercaptoacetate **1**. This compound was protected by tritylation to give ester **2**, and reacted in turn with an alkyldiamine to provide the amine **3**. Intermediate **3** was coupled with a carboxylic acid and the trityl protecting group was removed to provide mercaptoacetamide **4** (Scheme 1).

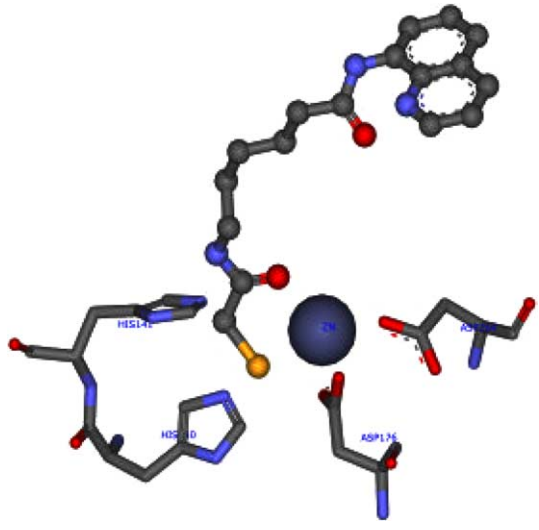
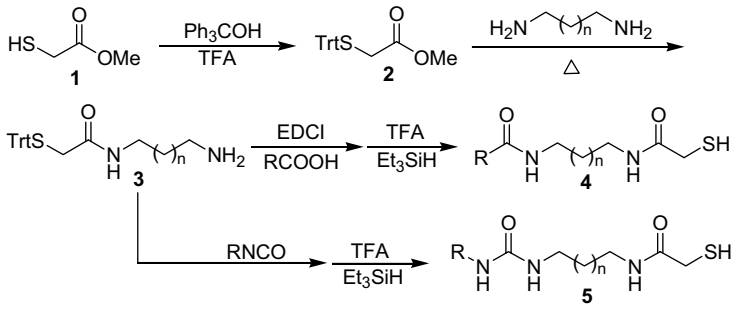


Figure 3. Compound **10b** docked into the binding site of HDAC1.



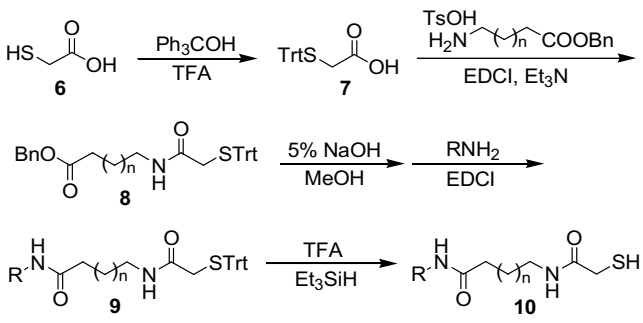
Scheme 1.

The preparation of mercaptoacetamides of structure **5** containing a urea spacer was brought about by reaction of amine **3** with an isocyanate, followed by the deprotection (Scheme 1).

Mercaptoacetamides of structure **10** were synthesized from mercaptoacetic acid **6** by trityl protection and condensation with an amino ester to afford **8**. Next, base hydrolysis of compound **8**, amide formation in the presence of EDCI, and deprotection of the trityl group gave mercaptoacetamide **10** (Scheme 2).

The in vitro HDAC inhibitory activity of these compounds was determined by using fluor-Lys as the substrate (BIOMOL). These data are displayed as 50% HDAC activity inhibition values in Table 1. Both TSA and SAHA were used as positive controls. From these data, it is apparent that the activity does show some dependence on chain length, with $n = 3$ or 4 being best,[23] and amide linkers being better than urea linkers. Substitution of the five methylene spacer with a *p*-xylylene unit as in **4k** gives comparable HDAC inhibitory activity. As observed previously, the optimum activity is achieved for R = *p*-dimethylaminophenyl in comparison to biphenyl, phenyl, or mercaptomethyl bearing ligands.

Compounds **10a**–**e** represent the reverse amide analogs of **4**. While **10a**, **e**, and **4c** have comparable activities, **10b** and **c** are particularly potent, and they show a clear dependence on the site of attachment of the thiol bearing appendage to the quinoline ring system. The aromatic cap of these HDAC inhibitors may thus be able to have a better interaction with the outside rim of the gorge region of the HDACs.



Scheme 2.

Table 1. HDAC inhibitory activity of mercaptoacetamides

Compound	50% HDAC activity inhibition (μM)
4a R = *p*-Me_2NPh, $n = 1$	0.80
4b R = *p*-Me_2NPh, $n = 2$	4.70
4c R = *p*-Me_2NPh, $n = 3$	0.20
4d R = *p*-Me_2NPh, $n = 4$	0.45
4e R = *p*-Biphenyl, $n = 1$	0.80
4f R = *p*-Biphenyl, $n = 2$	13.0
4g R = *p*-Biphenyl, $n = 3$	0.55
4h R = Ph, $n = 3$	1.1
4i R = Ph, $n = 4$	0.90
4j R = $HSCH_2$, $n = 4$	10.0
4k R = *p*-Me_2NPh, $(CH_2)_n$ = *p*-Ph	0.20
4l R = 8-Quinolinyl, $n = 3$	0.25
4m R = 3-Quinolinyl, $n = 3$	0.40
5a R = *p*-Me_2NPh, $n = 3$	1.7
5b R = *p*-Me_2NBn, $n = 4$	5.0
5c R = Ph, $n = 3$	0.75
5d R = Ph, $n = 4$	0.63
5e R = Bn, $n = 3$	1.0
10a R = *p*-Me_2NPh, $n = 3$	0.60
10b R = 8-Quinolinyl, $n = 3$	0.044
10c R = 3-Quinolinyl, $n = 3$	0.048
10d R = 6-Quinolinyl, $n = 3$	0.90
10e R = Ph, $n = 3$	0.30
TSA	0.0035
SAHA	0.080

To test the biological effects of these ligands, cytotoxicities were then determined following 24 h exposure of human cancer cell lines, including cervix carcinoma (HeLa), prostate cancer (PC-3), breast cancer (MCF-7) and squamous carcinoma (SQ-20B), to four compounds (Table 2). The IC_{50} values of these compounds range from 30 to 130 μM. as shown in Table 2. Compound **10b** shows potent cytotoxicity in these cancer cells.

Table 2. Antiproliferative activities of mercaptoacetamides

Compound	IC_{50} (μM) ± SD			
	HeLa	SQ-20B	MCF-7	PC-3
4c	108 ± 0.020	100 ± 0.093	125 ± 0.090	110 ± 0.081
4g	80 ± 0.018	130 ± 0.043	110 ± 0.044	100 ± 0.056
10b	30 ± 0.031	31 ± 0.024	42 ± 0.035	33 ± 0.021
10c	94 ± 0.016	100 ± 0.043	80 ± 0.020	50 ± 0.026

As is evident from the present work, some of these readily prepared mercaptoacetamides are potent HDAC inhibitors that show promising activity in inhibiting cellular proliferation. Issues relating to isoform selectivity as well as chemosensitization using these ligands are now under study.

Acknowledgments

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