PharmacologyOnLine December 30, 2014

Archives • 2014 • vol.3 • 184-189

STEROIDAL HYDROXAMIC ACID DERIVATIVES: A STRUCTURALLY NOVEL CLASS OF HDAC INHIBITORS

Rosario Randino[1]; Elena Cini[2]; Maurizio Taddei;[2] Manuela Rodriquez[1*]

[1] Dipartimento di Farmacia, Università degli Studi di Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, Italy
[2] Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, Via Aldo Moro, 2, 53100 Siena, Italy

[*]mrodriquez@unisa.it

Abstract

Some original chimeric structures formed by a hydroxamic acid moiety separated from a steroid nucleus (derived from cholanoic or abietic acids) by a seven atoms linker have been prepared and tested against the 11 HDAC isoforms. The products derived from abietic acid show promising results with a particular activity against Class IIb tubuline deaceyilating HDAC6.

Key words: HDAC inhibitors, Cancer, Steroids, Hydroxamic acid

Introduction

Important cellular events can be regulated by mechanisms inheritably affecting specific phenotypes without altering the underlying genotypes such as chemical modifications of histones and DNA, which are proposed to convey a part of the so-called epigenetic information. [1-4] The interest of biomedical research toward epigenetics is increasing in recent years. The changes in histone modifications and DNA methylation, commonly found in human cancers, have encouraged the study and development of epigenetic drugs. [5,6] So now methylation and acetylation are the two primarily mechanism under investigation. Acetylation is one of the best-studied histone modifications, which takes place at the histones ε-amino group of lysine residues. Histone acetylation and deacetylation are regulated by the activity of two sets of enzymes, histone deacetylases (HDACs) and histone acetyltransferases (HATs) that, in addition to histone proteins, have many non-histone proteins as a substrate including proteins of transcription complexes. [7-9] By targeting these key histone-modifying enzymes, it has been possible to affect important downstream cellular events. Accordingly, HDAC inhibitors are very promising chemotherapeutic agents capable of regulating gene expression, as well as proteins in pathways that regulate cell proliferation, cell migration, cell death and angiogenesis. [10-12] Eighteen HDACs, subdivided into four classes, have been identified in humans. Class I (HDACs 1, 2, 3, and 8), class IIa (HDACs 4, 5, 7, and 9), class IIb (HDACs 6 and 10), and class IV (HDAC11) operate as zinc dependent enzymes, have distinct gene expression patterns, and have different cellular location and function.

A common pharmacophoric model, generally accepted for the HDAC inhibitors, shows key features involved into active site interactions: a capping group (CAP), which plays the role of a surface recognition domain; a zinc-binding moiety (ZBM), responsible for the crucial Zn ion-complexation event in the catalytic pocket (commonly a hydroxamic acid); the linker domain, a straight-chain connecting the two parts, which has the appropriate length in order to be correctly inserted in the long and narrow channel of the enzyme active site, and to project the zinc chelating element at an optimal distance for the metal-chelation process (Scheme 1). The amino acid sequence, that surround the catalytic site of the different HDACs has greater sequence diversity compared with the other domains. The CAP is generally a hydrophobic structure that interacts with these amino acids, and thus, might be manipulated to develop new selective HDAC inhibitors (HDACis).

Several HDAC inhibitors (HDACis) reached clinical trials, and the first approved by FDA was suberoylanilide hydroxamic acid (SAHA) (Scheme 1), Zolinza® from Merck & Co., Inc., followed by depsipeptide (FK228) Istodax®, both for use in cutaneous T-cell lymphoma (CTCL). [13] SAHA is a pan-inhibitor, since it is equipotent toward all the HDAC isozymes, with a relatively small CAP group linked through a polymethylene chain to the strong Zn binder hydrohamic acid. Other natural products (as FR235222 in Scheme 1) [14] are much highly potent inhibitors due to the strong interaction of the macropeptide with the external rime of the enzyme.

Even if some data suggest that selectivity toward a specific HDAC might be useful to find more selective inhibitors with limited side effects, hydroxamic acid remains the strongest zinc chelating moiety in spite of its metabolic and pharmacokinetic issues. The accommodation of the ZBG into the HDAC catalytic site is a crucial step of the inhibition process and is finely controlled by its zinc coordination ability and by key interactions with the surrounding protein residues.

Following our interest in this field, [15] we focused on the idea to replace the cap group in order to discover new hydroxamate small-molecule HDAC inhibitors and to investigate on the additional interactions at the rime of the tube-like pocket level in order to identify key elements for HDAC inhibition. Steroids caught our attention, as they appear hydrophobic, bulky and drug-like enough to be considered as CAP groups. We report here the first example of steroidal hydroxamic acids and their activity against the whole panel of HDAC isoforms, including the anticancer activity of the most active one.

Methods

Compounds **1-4** were prepared following standard processes and completely characterized through ^{1}H, ^{13}C NMR, and high resolution mass spectrometry. HDAC profiling was performed in the presence of a 50 μM solution of the fluorogenic tetrapeptide RHKK(Ac) substrate (from p53 residues 379–382) or in the presence of a 50 μM solution of its diacetylated analogue RHK(Ac)K(Ac) for HDAC8. Upon its deacetylation, the fluorophore was released

giving rise to fluorescence emission, which was detected by a fluorimeter, and the IC_{50} values of the compounds were calculated from the resulting sigmoidal dose-response inhibition slopes. [16] The cytotoxic effects of the most promising compound (1-100 μM) in DMSO solution were evaluated on H460 cell lines by MTT assay. The assay is based on the observation that viable cells have the ability to metabolize a water-soluble tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma), into an insoluble formazan salt. Reduction of cell viability by more than 30% is considered a cytotoxic effect, otherwise the Ic50 value is considered not available (NA). The concentration of each compound which inhibit cell viability of 50% (Ic50) was determined using Prism 5.0 (GraphPad Software Inc.). IC50 values were presented as means ± SEM of three independent experiments carried out by triplicate.

Chemistry

As the hydroxamic acid mojety needs to be separed from the bulky steroidal CAP group by a spacer of at least 6-7 atoms, two sets of structures were prepared. Compounds **1** and **2** were obtained by standard amide coupling between b-alanine methyl ester and 3-hydroxy-cholanoic acid and 3,11-dihydroxy-cholanoic acids respectively followed by transformation of the methyl ester into hydroxamic acid. [16] Analogously, starting from abietic acid or dihydroabietic acid and 6-aminocaproic acid methyl ester, amide bond formation and introduction of the hydroxamic acid moiety gave steroidal hydroxamic acids **3** and **4** (Scheme 2).

Biochemistry

The activity of compounds **1-4** was tested against the 11 Human HDAC isoforms and their IC50 was evaluated. The results are listed in Table 1. [17]

Result and Discussion

The two sets of structures show different behaviour. The cholanoic acid derivatives carry a not completely apolar connecting unit due to the presence of the amide bond. This structural feature may have an influence during the accommodation in the tube-like pocket located just before the enzyme catalytic site. Compound **1**, having also more apolar CAP group, is almost inactive, while the introduction of deoxycholic acid as CAP bearing an additional OH increased the inhibitory activity of the molecule although in a micromolar range.

Better results were obtained connecting the smaller resin acids with a classic SAHA-like linker. The abietic acid derivative **3** showed a general promising inhibition on almost all the enzymes tested with a particular activity against the ClassIIb isoform HDAC6. This behavior was confirmed by results obtained with the more “flat” dehydroabietic acid derivative **4** that showed a two digit increment of activity against HDAC6 reaching a good level of selectivity. These promising properties of the resin derivatives **3** and **4** were confirmed by testing citotoxic activity against human non-small cell lung cancer cells (table 2).

Also in this assay dehydroabietic acid derivative **4** showed a better antiproliferative effect on human non-small cell lung cancer cell model.

In conclusion we have demonstrated that it is possible to use steroidal structures as CAP groups for the preparation of HDAC inhibitors. The presence of the steroid seems to increase the selectivity against the tubuline associated HDAC isoforms. Although very preliminary, these results introduce a new class of molecules in the arsenal of HDAC targeting compounds. The extension of this approach to other biologically relevant steroids is currently underway in our laboratories and will be reported in due course.

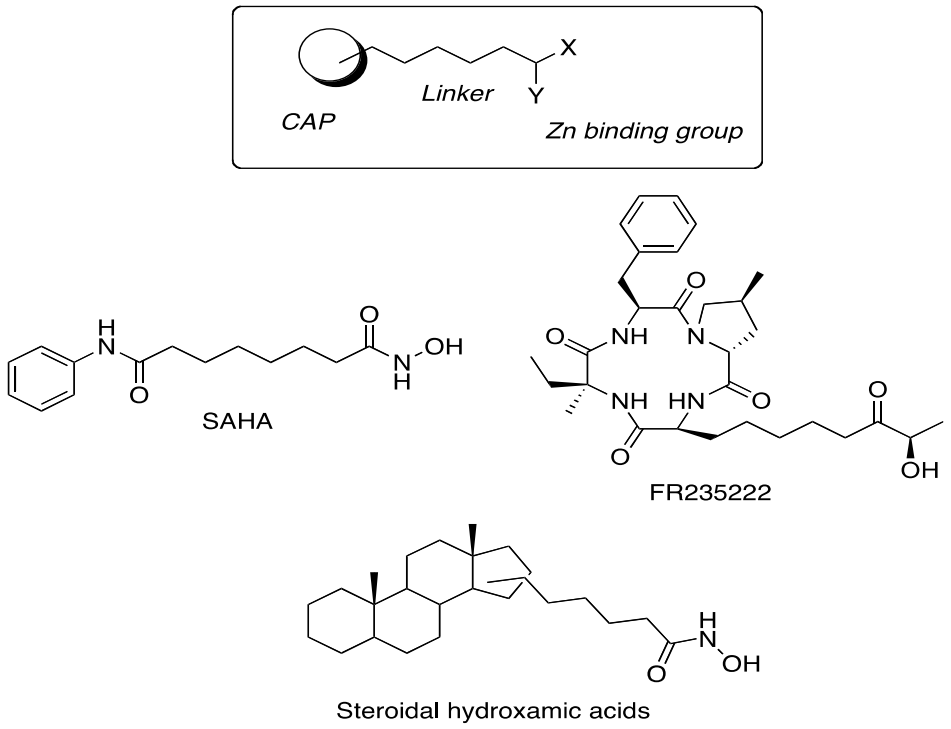
Acknowledgments

Authors thank Sigma-Tau S.p.A. Pomezia (Roma) for partial financial support and for the biological assays.

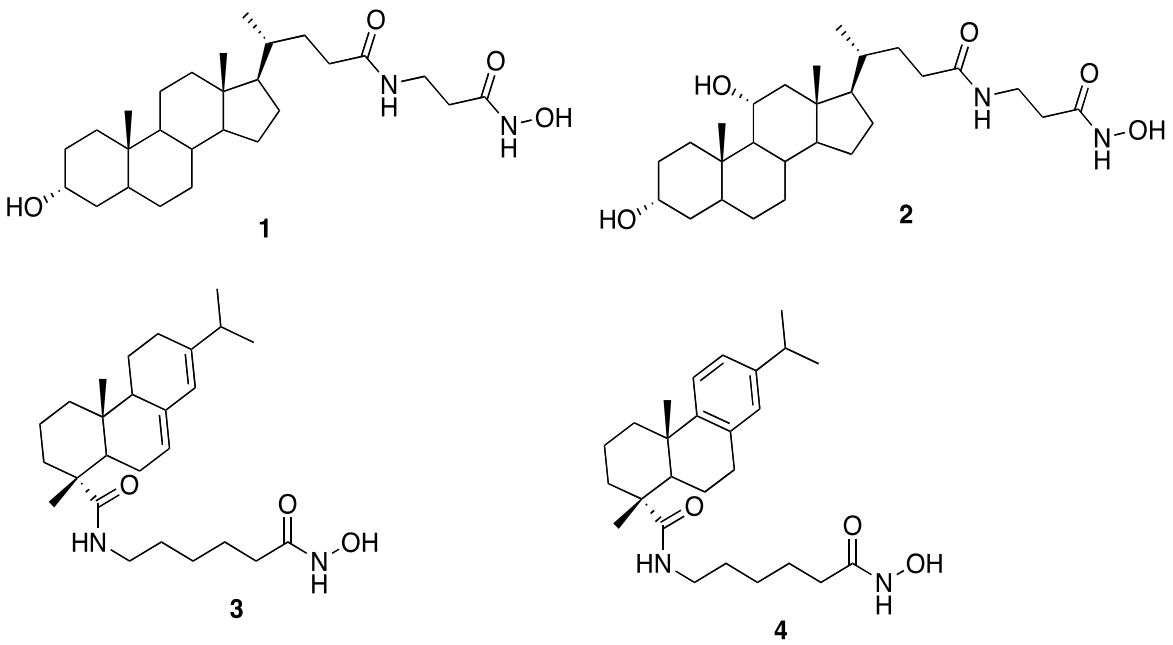
References

1. Biel, M.; Wascholowski, V.; Giannis, A., Epigenetics–an epicenter of gene regulation: histones and histone-modifying enzymes. *Angew. Chem., Int. Ed. Engl.* 2005*, 44*, 3186-3216.
2. Rodriquez, M.; Aquino, M.; Bruno, I.; De Martino, G.; Taddei, M.; Gomez-Paloma, L., Chemistry and biology of chromatin remodeling agents: state of art and future perspectives of HDAC inhibitors. *Curr. Med. Chem.* 2006*, 13*, 1119-1139.
3. Petrella A, Fontanella B, Carratù A, Bizzarro V, Rodriquez M, Parente L., Histone Deacetylase Inhibitors in the Treatment of Hematological Malignancies. Mini-Reviews In Medicinal Chemistry, 2011, *11*, 519-527.
4. Giannini, G.; Cabri, W.; Fattorusso, C.; Rodriquez, M., Histone deacetylase inhibitors in the treatment of cancer: overview and perspectives. *Future Medicinal Chemistry*, 2012, *4*, 1439-1460.
5. Kristeleit R, Stimson L, Workman P, Aherne W. Histone modification enzymes: novel targets for cancer drugs. *Expert. Opin. Emerg. Drugs*., 2004, 9, 135-54.
6. Marks PA, Richon VM, Miller TA, Kelly WK. Histone deacetylase inhibitors: new targeted anticancer drugs. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. Cancer: *Principles & Practice of Oncology. 7th ed. Philadelphia, Pa: Lippincott Williams & Wilkins* 2005, 439–45.

7. Peserico A, Simone C. Physical and Functional HAT/HDAC Interplay Regulates Protein Acetylation Balance. *J. Biomed. Biotechnol.,* 2011, 371832.
8. Glozak MA, Sengupta N, Zhang X, Seto E. Acetylation and deacetylation of non-histone proteins. *Gene*, 2005, 363, 15-23.
9. Kim SC, Sprung R, Chen Y, et al. Substrates and functional diversity of lysine acetylation revealed by a proteomics survey. *Mol. Cell.*, 2006, 23, 607-18.
10. Kim, M. S.; Kwon, H. J.; Lee, Y. M.; Baek, J. H.; Jang, J. E.; Lee, S. W.; Moon, E. J.; Kim, H. S.; Lee, S. K.; Chung, H. Y.; Kim, C. W.; Kim, K. W., Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. *Nat. Med.* 2001*, 7*, 437-443.
11. Marks, P.; Rifkind, R. A.; Richon, V. M.; Breslow, R.; Miller, T.; Kelly, W. K. *Nat. Rev. Cancer* 2001*, 1*, 194-202.
12. Ruefli, A. A.; Ausserlechner, M. J.; Bernhard, D.; Sutton, V. R.; Tainton, K. M.; Kofler, R.; Smyth, M. J.; Johnstone, R. W., The histone deacetylase inhibitor and chemotherapeutic agent suberoylanilide hydroxamic acid (SAHA) induces a cell-death pathway characterized by cleavage of Bid and production of reactive oxygen species. *Proc. Natl. Acad. Sci. U.S.A.* 2001*, 98*, 10833-10838.
13. a)Deal watch: Celgene acquires Gloucester Pharmaceuticals, gaining approved HDAC inhibitor. Nat. Rev. Drug Discovery 2010, 9, 94; b) Grant, S.; Easely, C.; Kirkpatrick, P. Vorinostat. Nat. Drug Discovery Rev. 2007, 6, 21–22.
14. Rodriquez, M.; Terracciano, S.; Cini, E.; Settembrini, G.; Bruno, I.; Bifulco, G.; Taddei, M.; Gomez-Paloma, L. Total synthesis, NMR solution structure, and binding model of the potent histone deacetylase inhibitor FR235222. *Angew. Chem., Int. Ed. Engl.* 2006*, 45*, 423-427.
15. a) Botta CB, Cabri W, Cini E, De Cesare L, Fattorusso C, Giannini G, Persico M, Petrella A, Rondinelli F, Rodriquez M, Russo A, Taddei M., Oxime amides as a novel zinc binding group in histone deacetylase inhibitors: synthesis, biological activity, and computational evaluation. J. Med. Chem. *54*, 2165-2182. b) Gomez-Paloma, L.; Bruno I.; Cini, E.; Khochbin, S.; Rodriquez, M.; Taddei, M.; Terracciano, S.; Sadoul, K.; Design and Synthesis of cyclopeptide analogues of the potent Histone deacetylase inhibitor FR235222. ChemMedChem, 2007, 2, 1511-1519; c) Cini E.; Bifulco G.; Menchi G.; Rodriquez M.; Taddei M., Synthesis of Enantiopure 7-Substituted Azepane-2-Carboxylic Acids as Templates for Conformationally Constrained Peptidomimetics. Eur J Org. Chem, 2012, *11*, 2133-2141; d) Naldini A.; Filippi I.; Cini .; Rodriquez M.; Carraro F.; Taddei M., Downregulation of Hypoxia-related Responses by Novel Antitumor Histone Deacetylase Inhibitors in MDAMB231 Breast Cancer Cells. Anti-Cancer Agents in Medicinal Chemistry, 2012, *12* (*4*), 407-413; e) Petrella, A.; D'Acunto, C. W.; Rodriquez, M.; Festa, M.; Tosco, A.; Bruno, I.; Terracciano, S.; Taddei, M.; Gomez-Paloma, L. Effects of FR235222, a novel HDAC inhibitor, in proliferation and apotosis of human leukemia cell lines: role of annexin A1. Eur. J. Cancer. 2008, 44, 740–749; f) Di Micco, S.; Terracciano, S.; Bruno, I.; Rodriquez, M.; Riccio, R.; Taddei, M.; Bifulco, G. Molecular modeling studies toward the structural optimization of new cyclopeptide-based HDAC inhibitors modeled on the natural product FR235222. Bioorg. Med. Chem. 2008, 16, 8635–8642; g) D'Acunto, C. W.; Carratù, A.; Rodriquez, M.; Taddei, M.; Parente, L.; Petrella, A., LGP1, a histone deacetylase inhibitor analogue of FR235222, sensitizes promyelocytic leukemia U937 cells to TRAIL-mediated apoptosis. Anticancer Research, 2010, *30*, 887-894; h) D'Acunto, C. W.; Fontanella, B.;, Rodriquez, M.; Taddei, M.; Parente, L.; Petrella, A., Histone deacetylase inhibitor FR235222 sensitizes human prostate adenocarcinoma cells to apoptosis through up-regulation of annexin A1. Cancer Letters, 2010, *295*, 85-91.
16. Massaro A, Mordini A, Reginato G, Russo F, Taddei M Microwave-assisted transformation of esters into hydroxamic acids. Synthesis 2007, 3201-3204.
17. HDAC screening was performed by Reaction Biology Corp. (Malvern, PA).



Scheme 1. Active HDAC inhibitors and general structure of steroidal hydroxamic acids.



Scheme 2 Steroidal hydroxamic acids.

Table 1. Activity of steroidal hydroxamic acid derivatives against the 11 Human HDAC isoforms (IC50, μM).[a]

	IC_{50} (μM)										
	HDAC1	***HDAC2***	***HDAC3***	***HDAC4***	***HDAC5***	***HDAC6***	***HDAC7***	***HDAC8***	***HDAC9***	***HDAC10***	***HDAC11***
1						17		7			
2	87		27	59	94	3.6	13	35			
3	14	34	6	45	26	0.4	43	2	30	24	21
4	5	23	5	8	8	0.08	16	1	7	7	6
SAHA	0.3	0.9	0.4	0.5	0.4	0.03	0.3	0.2	0.3	0.5	0.4

IC50 values > 100 mM are not reported

Table 2. Citotoxic activity of resin derivatives **3** and **4**.

Compound	H460 cells IC50 (µM)
3	14.6±0.7
4	10.7±0.4