Bioorganic & Medicinal Chemistry Letters 24 (2014) 61–64

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Lactam based 7-amino suberoylamide hydroxamic acids as potent HDAC inhibitors

Maurizio Taddei [a,*], Elena Cini [a], Luca Giannotti [a], Giuseppe Giannini [b,*], Gianfranco Battistuzzi [b], Davide Vignola [b], Loredana Vesci [b], Walter Cabri [b,†]

[a] Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, Via A. Moro 2, 53100 Siena, Italy
[b] R&D Sigma-Tau Industrie Farmaceutiche Riunite S.p.A., Via Pontina Km 30,400, I-00040 Pomezia, Roma, Italy

ARTICLE INFO

Article history:
Received 22 October 2013
Revised 25 November 2013
Accepted 29 November 2013
Available online 4 December 2013

Keywords:
HDAC
Anticancer
Pan-inhibitor
Hydroxamic acid
Heterocycles

ABSTRACT

A series of SAHA-like molecules were prepared introducing different lactam-carboxyamides in position 7 of the suberoylanilide skeleton. The activity against different HDAC isoforms was tested and the data compared with the corresponding linear products, without substituent in position 7. In general, this modification provided an effective reinforcement of in vitro activity. While the lactam size or the CO/NH group orientation did not strongly influence the inhibition, the contemporary modification of the suberoylamide fragment gave vary active variants in the lactam series, with compound **28** (ST8078AA1) that showed IC_{50} values between 2 and 10 nM against all Class I HDAC isoforms, demonstrating it to be a large spectrum pan-inhibitor. This strong affinity with HDAC was also confirmed by the value of IC_{50} = 0.5 μM against H460 cells, ranking **28** as one of the most potent HDAC inhibitors described so far.

Epigenetic regulation of gene expression is a mutable dynamic process that can contribute to human diseases, driven by epigenetic protein families that include the histone deacetylases (HDACs).[1] HDACs are a group of enzymes found in several organisms as bacteria, fungi, plants, and animals. They belong to the huge class of so-called 'lysine-deacetylase', a class of enzymes that work removing acetyl groups from ε-amino-lysine residues on many different substrates (histone, nonhistone nuclear and cytoplasmic proteins),[2] the acetylation/deacetylation ratio working as a regulatory signaling network in cells. Over the last years, great efforts have been spent on the HDAC inhibitors in the oncology field:[3] so far two drugs (Vorinostat and Romidepsin) have emerged for new promising anticancer treatments approved by the US Food and Drug Administration (FDA). Vorinostat was the first-in-class FDA approved HDAC-inhibitor to treat cutaneous T-cell lymphoma, in patients who have received at least one prior systemic therapy.[4] It works as a pan inhibitor, active against the eleven HDAC human isoforms. Recently many efforts have been dedicated to find power selective HDAC inhibitors, although researchers are divided with regard to the assessment of particular HDAC isoforms bound to specific cancers.[3b] Despite such intense efforts, there is still a large medical need for pan inhibitors since it has been demonstrated that the various cancer diseases do not involve the same HDAC isoforms.[5] The availability of a new generation of HDAC inhibitors, more powerful than those available today, and nontoxic such as Trichostatin A, could represent an opportunity for a clinical use as single agent, unlike what happens now where the HDAC inhibitors are mainly used in combination with other cytotoxic agents. Looking to SAHA (suberoylanilide hydroxamic acid, the active principle of Vorinostat) with medicinal chemists eyes, the molecule is characterized by a very simple chemical structure, represented by a pharmacophoric model that consists of three parts, a zinc-binding group—ZBG (hydroxamic acid), a linker (poly-methylene) and a cap-group (phenyl ring). The introduction of substituents on the methylene chain has been extensively studied leading to more powerful and/or more selective new derivatives.[6] Over the years several authors, such as Breslow and coworkers, have reported a series of 7-substituted ethers, amines and amide derivatives of SAHA with excellent inhibitory activities.[7,8] Other researchers, through a molecular dynamic simulations of HDAC/inhibitor complexes have demonstrated that interaction between the protein surface and inhibitor play an important role.[9] Efforts in the same general direction were made, also in our laboratory, to probe specifically the influence on enzyme binding, studying different 7-alkoxy derivatives alone[10] or inserted in a macrocyclic fraction[11] with the goal to highlight the role of different substituents in this position.

* Corresponding authors. Tel.: +39 0577234275; fax: +39 0577234333 (M.T.); tel.: +39 0691393640; fax: +39 0691393638 (G.G.).
E-mail addresses: maurizio.taddei@unisi.it (M. Taddei), giuseppe.giannini@sigma-tau.it (G. Giannini).
† Present address: Indena, SpA, viale Ortles 12, I-20139 Milano, Italy.

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http://dx.doi.org/10.1016/j.bmcl.2013.11.072

We report here the results relative to the introduction of new amido lactams in position 7 of the SAHA skeleton. The insertion of these 'peptide resembling fragments' gave a strong increase of inhibition of different HDAC isoforms together with a general high value of cytotoxicity against selected tumor cell lines (see Scheme 1).

The molecules described here were prepared following two different synthetic approaches in order to optimize the introduction of the hydroxamic acid function in the presence of polar lactam/amide structures. The aldehyde **2**, derived from (*S*)-glutamic acid **1**,[12] was transformed into alkene **3** via Horner–Wadsworth–Emmons reaction (Scheme 2). Treatment with H_2 and Pd/C gave contemporary double bond reduction and deprotection to yield the free amino acid that was protected again at position 2 with Boc_2O to produce compound **4**. Coupling with aniline in the presence of DMTMM[13] followed by carboxymethyl hydrolysis and coupling with *O*-benzyl hydroxylamine gave compound **5** that was finally submitted to Boc removal to yield amido amino hydroxamate **6**. Alternatively intermediate **4** was coupled with cyclopentylamine or phenethyl amine (using always DMTMM as the coupling agent) followed by Boc removal to give amido amino esters **7** and **8** (Scheme 2).

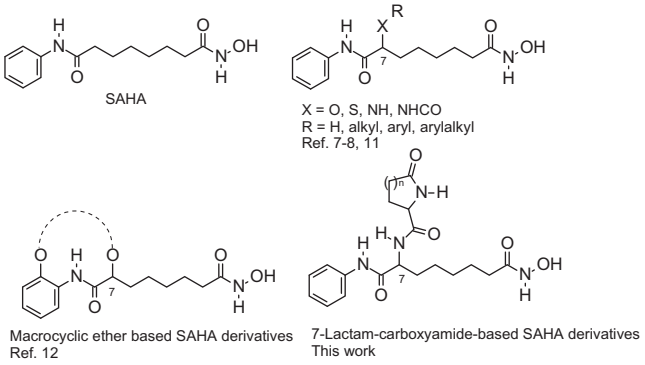
On scaffold **6**, the introduction of different lactam carboxylic acids was carried out using DMTMM as the coupling agent, followed by hydrogenolysis of the benzyl protection to form the anilide lactam hydroxamic acids **9**–**14**. The coupling of **7** and **8** with (2*S*)-6-oxo-2-piperidinecarboxylic or (2*S*)-5-oxo-2-pyrrolidine-carboxylic acids was instead followed by direct transformation of the ω-methylcarboxylate into hydroxamic acids with aqueous NH_2OH to yield compounds **15**–**17** (Scheme 3).

As an alternative, commercially available (*S*)-2-*tert*-butoxycarbonylamino-hept-6-enoic acid **18**, was coupled with the proper aromatic amines to obtain compounds **19**–**22**. (Scheme 4), Then chain elongation was achieved by olefin metathesis with methyl acrylate (Grubbs 2 Rh complex) followed by reduction of the double bond (Scheme 4). The obtained methyl esters (**23**–**26**) were hydrolyzed with NaOH in MeOH and the resulting carboxylic acids were coupled with *O*-benzyl hydroxylamine using PyBOP, DIPEA. Boc removal with TFA, gave the 7-amino suberoyl derivatives that were reacted with the proper lactam–carboxylic acids. The final benzyl removal by hydrogenolysis generated hydroxamic acids **27**–**30**.

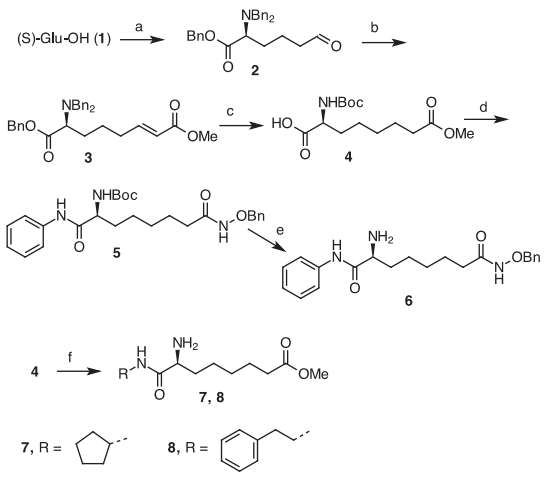
The amino lactam substituted suberoylamide hydroxamic acids **9**–**17** and **27**–**30** were evaluated for their activity against Class I (1–3, 8), IIb (6, 10) and IV (11) HDAC isoforms. Class IIa HDACs were avoided due to their lower sensitivity in this assay. HDAC profiling was performed in the 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.[14]

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.

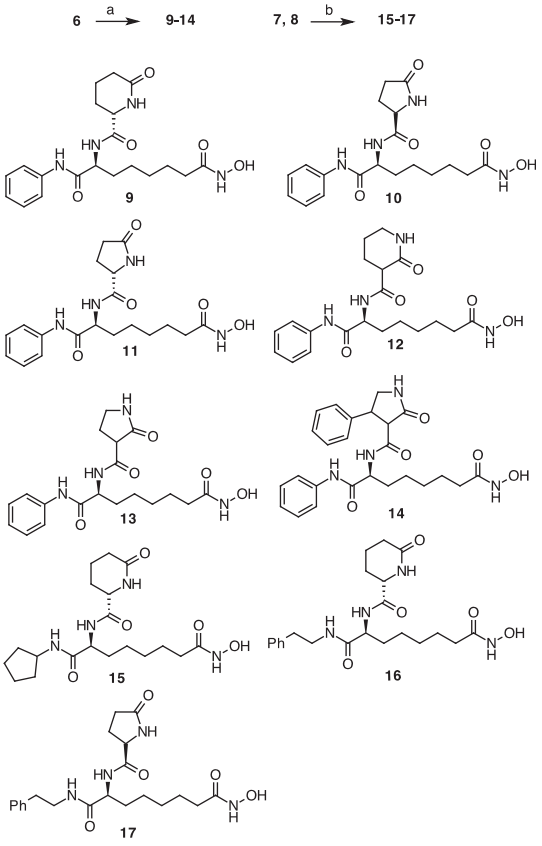
A first exploration was done keeping intact the original SAHA structure and introducing different lactam amides on the stereogenic center in position 7 (*S* configuration). Six, five and four



Scheme 1. SAHA like HDAC inhibitor carrying substituents at position 7.



Scheme 2. (a) Ref. 11. (b) $(MeO)_2OPCH_2COOMe$, LiCl, DIPEA, $CHCl_3$, rt, 12 h, 79%. (c) Boc_2O, H_2 (6 bar), $Pd(OH)_2/C$ (10% mol), MeOH, rt, 12 h, 62%. (d) (i) $PhNH_2$, DMTMM, NMM, THF, rt, 12 h, 96%; (ii) NaOH aq 1 M, MeOH, rt, 12 h; (iii) $BnONH_2$, DMTMM, NMM, THF, rt, 12 h, 75%. (e) TFA, DCM, rt, 1 h, 79%. (f) (i) Cyclohexyl amine or phenethylamine, DMTMM, NMM, THF, rt, 12 h, 86% and 90%, respectively; (ii) TFA, DCM, rt, 1 h, 80%.



Scheme 3. (a) (i) Lactam carboxylic acids DMTMM, NMM, THF, rt, 12 h, 76–82%; (ii) H_2 (1 bar) Pd/C, MeOH, rt, 12 h, 52%. (b) (i) Lactam carboxylic acids DMTMM, NMM, THF, rt, 12 h 72–89%; (ii) NH_2OH, NaOH, $MeOH/H_2O$, rt, 12 h, 52–77%.