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



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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

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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 IC₅₀ 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 IC₅₀ = 0.5 μ M against H460 cells, ranking **28** as one of the most potent HDAC inhibitors described so far.

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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 *ɛ*-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 alone¹⁰ or inserted in a macrocyclic fraction¹¹ 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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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 H₂ and Pd/C gave contemporary double bond reduction and deprotection to yield the free amino acid that was protected again at position 2 with Boc₂O to produce compound **4**. Coupling with aniline in the presence of DMTMM¹³ 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**



Macrocyclic ether based SAHA derivatives 7-Lactam-car Ref. 12 This work

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



Scheme 2. (a) Ref. 11. (b) $(MeO)_2OPCH_2COOMe$, LiCl, DIPEA, CHCl₃, rt, 12 h, 79%. (c) Boc₂O, H₂ (6 bar), Pd(OH)₂/C (10% mol), MeOH, rt, 12 h, 62%. (d) (i) PhNH₂, DMTMM, NMM, THF, rt, 12 h, 96%; (ii) NaOH aq 1 M, MeOH, rt, 12 h; (iii) BnONH₂, 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%.

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 NH₂OH 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), Ilb (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 μ M solution of the fluorogenic tetrapeptide RHKK(Ac) substrate (from p53 residues 379–382) or in the presence of a 50 μ M 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 3. (a) (i) Lactam carboxylic acids DMTMM, NMM, THF, rt, 12 h, 76–82%; (ii) H₂ (1 bar) Pd/C, MeOH, rt, 12 h, 52%. (b) (i) Lactam carboxylic acids DMTMM, NMM, THF, rt, 12 h 72–89%; (ii) NH₂OH, NaOH, MeOH/H₂O, rt, 12 h, 52–77%.



Scheme 4. (a) ArNH₂, PyBOB, DIPEA, DMF/DCM, rt 12 h. (b) (i) Methyl acrylate Grubbs Cat. 2nd generation, DCM, rt, 12 h; (ii) H₂, (1 bar) Pd/C MeOH, rt. (c) (i) NaOH 1 M in MeOH, rt then BnONH₂ EDC, Et₃N, DCM rt; (ii) TFA, DCM, 0 °C to rt; (iii) lactam carboxylic acids, PyBOP, DIPEA, DMF/DMF rt; (iv) H₂ (1 bar) Pd(OH)₂/C EtOH/EtOAc rt.

membered lactams were included in the structure resulting in a general ten times increase of the inhibition potency on HDACs. The δ and β -lactams **9** and **27** showed a promising activity in the range 5-100 nM on Class I enzymes, while on Class IIb and IV they resulted less potent (although more active than SAHA, see Table 1). The γ lactams **10** and **11** also inhibited all HDAC isoforms much better than SAHA although with more modest IC₅₀ values with respect to 9 and 27. The configuration of the stereocentre present in the lactam was irrelevant for the activity as the diastereomers 75/ 2'R(10) and 7S/2'S(11) showed a comparable inhibition on all the investigated isoforms. The relative position of the C=O and NH parts of the lactam has some influence on the activity as demonstrated by the reduced inhibition recorded with compound 12 and 13 (compared with 9 and 10). In this inverted lactam series, the introduction of a bulky substituent close to the lactam connection point gave a product (14) with the inhibition potency aligned with the first more active variants. However, due to the more elaborated structure, also complicated by the presence of two



Scheme 5. Reference linear compound. Products **31–35** were prepared following the same synthetic procedure already described for SAHA (see Ref. 13).

stereocenters, this substituted variant was not further investigated. Then, different selected amines were introduced at the amide position of the Cap group in order to assess a possible additive effect on the inhibition activity that was always compared with the 7-unsubstituted SAHA like analogous compounds **31–35** that were prepared following a reported general procedure (Scheme 5).¹⁵

The molecule that included a m-CF₃ aniline and a γ -lactam showed high activity with one digit nanomolar inhibition on all Class I HDACs tested, proving to be one of the most potent molecule described so far. In fact, moving from SAHA to the linear m-trifluoromethylanilide **32**, the introduction of the lactam improves the activity of **28** on Class I HDACs and HDAC6 to very interesting IC₅₀ values, with maximum of 2 nM. An analogous trend was observed with the phenyl-ethylamide derivative, where the introduction of the lactam resulted in an increase of activity (**16** and **17** vs the linear analogue **35**). Finally, introduction in the amide position of an aliphatic amine instead of an aromatic one, gave less active compounds if compared with the previous tested arylamides (**31** and **15**).

These products were also studied for their effects on tumor cell lines and again the introduction of an amido-lactam in position 7 of the SAHA structure resulted in more active molecules compared with the unsubstituted analogues. The *meta*- and *para*-substituted anilides with the γ -lactam in position 7 were highly cytotoxic under sub-micromolar concentrations with again a peak of activity for compound **28** (also **29** and **30**) that showed a IC₅₀ = 0.5 μ M against the H460 cell lines.

In conclusion, SAHA variants containing (S)-7-amino carboxylactams exhibited nanomolar IC_{50} values against different HDAC

Table 1

Different isoform inhibitory activities and in vitro cytotoxicity of lactam based 7-aminosuberoylamide hydroxamic acids

Entry	Product	HDAC (IC ₅₀ nM)							$(IC_{50} \ \mu M)$
		HDAC1	HDAC2	HDAC3	HDAC6	HDAC8	HDAC10	HDAC11	H460
1	SAHA	258	921	350	29	243	456	362	3.4
2	9	25	89	20	5	97	51	47	2.5
3	10	43	142	67	8	161	93	75	4.0
4	11	59	143	89	6	137	125	37	5.4
5	12	131	689	116	19	251	244	255	5.1
6	13	72	238	74	4	118	147	150	4.0
7	14	33	110	14	4	123	88	70	2.4
8	27	34	89	62	3	93	113	135	2.2
9	31	1790	4390	3330	75	1410	4890	2630	21
10	15	1450	5040	5660	31	2130	6830	1440	2.4
11	32	63	165	185	6	113	374	75.1	2.5
12	28	2	8	2	2	32.9	10.6	55.5	0.5
13	33	197	239	216	22	523	503	1600	0.8
14	29	7	27	11	3	52.0	30.3	15.5	0.5
15	34	200	589	452	12	336	649	78.6	4.1
16	30	32	93	66	4	156	163	127	0.6
17	35	1140	5160	3610	25	376	1350	1130	18.2
18	16	102	956	539	8	131	227	262	8.2
19	17	77	180	144	6	31.6	210	47.8	4.9

isoforms, pointing out that the presence of a cyclic amide on the solvent exposed capping group area has a particular influence on the affinity with the enzyme. Combined with some suberoylamide fragments, the lactam introduction yielded extremely active compounds on Class I and Class IIb isoforms, corroborated with a very promising antitumor activity on selected cancer cell lines. Compound **28** (ST8078AA1) can be considered an interesting lead compound as well, suitable for simple modifications in order to develop new potent antitumor molecules as HDAC inhibition.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013. 11.072.

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