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Research paper

Design and biological evaluation of tetrahydro-β-carboline derivatives as highly potent histone deacetylase 6 (HDAC6) inhibitors

Michel Leonhardt [a], Andreas Sellmer [a], Oliver H. Krämer [c], Stefan Dove [d, 1], Sigurd Elz [a], Birgit Kraus [b, 1], Mandy Beyer [c, 1], Siavosh Mahboobi [a, *, 1]

[a] Institute of Pharmacy, Department of Pharmaceutical/Medicinal Chemistry I, University of Regensburg, 93040, Regensburg, Germany
[b] Institute of Pharmacy, Department of Pharmaceutical Biology, University of Regensburg, 93040, Regensburg, Germany
[c] Institute of Toxicology, University Medical Center Mainz, 55131, Mainz, Germany
[d] Institute of Pharmacy, Department of Pharmaceutical/Medicinal Chemistry II, University of Regensburg, 93040, Regensburg, Germany

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ABSTRACT

Various diseases are related to epigenetic modifications. Histone deacetylases (HDACs) and histone acetyl transferases (HATs) determine the pattern of histone acetylation, and thus are involved in the regulation of gene expression. First generation histone deacetylase inhibitors (HDACi) are unselective, hinder all different kinds of zinc dependent HDACs and additionally cause several side effects. Subsequently, selective HDACi are gaining more and more interest. Especially, selective histone deacetylase 6 inhibitors (HDAC6i) are supposed to be less toxic. Here we present a successful optimization study of tubastatin A, the synthesis and biological evaluation of new inhibitors based on hydroxamic acids linked to various tetrahydro-β-carboline derivatives. The potency of our selective HDAC6 inhibitors, exhibiting IC_{50} values in a range of 1–10 nM towards HDAC6, was evaluated with the help of a recombinant human HDAC6 enzyme assay. Selectivity was proofed in cellular assays by the hyperacetylation of surrogate parameter α-tubulin in the absence of acetylated histone H3 analyzed by Western Blot. We show that all synthesized compounds, with varies modifications of the rigid cap group, were selective and potent HDAC6 inhibitors.

1. Introduction

Histone alterations play a key role for the epigenetic regulation of cellular mechanisms [1]. The enzymes modifying histones are divided into two families based on their molecular function: Histone acetyltransferases (HATs) transfer acetyl groups to the *N*-terminal tails of lysine residues, whereas HDACs cleave acetyl groups from lysine residues in proteins (histones and non-histone proteins) [2]. Moreover, unlike other deacetylases, HDAC6 exerts the deacetylase activity on nonhistone proteins, including α-tubulin, heat shock protein (Hsp)90, cortactin, and peroxiredoxin [3,4]. Besides, HDACs are important for posttranslational modifications through the influence on chromatin remodeling, cell signaling, gene expression, RNA modification and several other cellular processes [1]. Hence, the balance between acetylation and deacetylation is crucial for the cellular function. Moreover, imbalance is related to several maladies including cancer, Alzheimer's disease, autoimmune- and inflammatory diseases [5–10]. Additionally, overexpression of HDACs leads to dysfunction of the cell cycle and problems in differentiation [11,12]. This displays the impact of HDACs on the general cell status.

Therefore, the modulation of posttranslational processes gains more and more interest in regard to the development of new therapies. HDACi target the imbalance between acetylation and deacetylation and are suggested as a new therapeutic approach for the treatment of the mentioned diseases [13–15]. Recently, the Food and Drug Administration (USA) has approved different HDACi for the treatment of various cancer diseases. Vorinostat (SAHA, **1**), the first registered HDACi was approved 2006 for the treatment of cutaneous T-cell lymphoma (CTCL) [16], followed by romidepsin (FK-228), belinostat (PXD-101) (peripheral T-cell lymphoma; PTCL), and panobinostat (LBH-589) (multiple myeloma) [17–19].

HDACi also seem to be valuable for the treatment of other ailments than cancer. The utility of HDACi represents a promising

* Corresponding author.
E-mail address: Siavosh.mahboobi@chemie.uni-regensburg.de (S. Mahboobi).
[1] These senior authors contributed equally.

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approach especially regarding the therapy of inflammatory diseases: Tubastatin A (**2b**) showed anti-inflammatory and antirheumatic effects in a mouse model. These effects are attributed to isoform selective inhibition of HDAC6, which is suggested to cause IL-6 modulation, hence influencing inflammatory cytokines [20,21].

In general, HDACs are divided into four classes (I - IV) and two sub-families: The nicotinamide adenine dinucleotide (NAD) dependent class III needs NAD^+ as cofactor. In contrast, class I (HDAC1, 2, 3 and 8), II and IV (HDAC11) are zinc dependent. Furthermore, class II is separated into IIa (HDAC4, 5, 7, 9) and IIb (HDAC6 and 10) [22,23]. HDAC6 consists of 1215 amino acids and shuttles between the nucleus and the cytoplasm. Whereas it is mainly located in the cytoplasm which is ensured by an amino-terminal nuclear export signal (NES) and a cytoplasmic retention signal providing its stable anchoring in the cytoplasm [24,25]. This isoform is unique among the HDAC family because it has two catalytic active domains, a zinc finger motif and mainly modifies relevant non-histone proteins - for instance α-tubulin, HSP90 and cortactin [1,20]. Surprisingly, it was shown that HDAC6 targets nuclear proteins but has no *in vivo* activity against histones [25].

The zinc binding group (ZBG) is part of the pharmacophore model for HDACi (Fig. 1), it chelates the central zinc ion of the enzyme and thereby causes inhibition. Mostly, a hydroxamic acid is the preferred ZBG due to its strong Zn^{2+}-ion chelation, but also sulfur containing or other ZBG groups can be used [4]. The ZBG is connected to a surface recognition region (head or cap group) by a linker. This linker is a crucial structural element directing the ZBG through a hydrophobic channel [26]. The head group interacts with the amino acid residues at the active binding site, it is therefore a key factor for achieving further isoform selectivity [13]. An overview of several selective HDAC6 inhibitors (**2c**-**2i**) is compiled in Fig. 2 (for Review see Refs. [4,27]).

Non-selective histone deacetylase inhibitors effect all Zn^{2+}-dependent classes of HDACs (pan-HDACi) and have a critical toxicity profile. Moreover, they cause a number of side effects in contrast to isoform selective inhibitors [26]. Remarkably, HDAC6 specific inhibitors in particular cause less side effects [29]. This is supported by a study in which knock out mice did not show major health issues when lacking HDAC6 [30]. Nevertheless, there is no HDAC6 selective inhibitor approved. The first published selective HDAC6i was tubacin [31] (**2a**). Due to its high lipophilicity and heavy molecular weight, it was not applicable as a drug. Nowadays, tubacin (**2a**) is used as a research tool helping to learn more about structure-activity relationships for next generation compounds [14,15].

Butler, Kalin et al. [32] showed that tubastatin A (**2b**) inhibits HDAC6 with an IC_{50} of 15 nM and has neuroprotective effects. Potency and selectivity are achieved by a rigid tetrahydro-γ-carboline capping group attached to a hydroxamic acid by a hydrophobic benzyl linker [32]. Bulky head groups fit into the larger active binding sight of HDAC6, compared to the other HDACs. Furthermore, its specificity is supported by interaction of the bulky head group with amino acid residues at the active binding site [14,32]. The second generation of tetrahydro-β- and tetrahydro-γ-carbolines showed that derivatization of the cap group leads to compounds with a good inhibitory activity and high selectivity [33]. Based on these results, we synthesized a series of tetracyclic tetrahydro-β-carboline derivatives gaining more information about potency and selectivity against HDAC6.

2. Results and discussion

2.1. Chemistry

Two different types of bulky head groups were synthesized in order to improve potency and provide iso-enzyme selectivity (Fig. 3).

The synthesis of the tetrahydro-β-carboline structure providing head 1 is illustrated in Scheme 1. Tryptophan (**4**) was esterified with methanol according to the literature [34–39]. In the following, **5** was converted by Pictet-Spengler [40] reaction with formaldehyde yielding **6** [41–43]. **6** was reacted with either *N*-succinimidyl-*N*-methyl-carbamate or various isocyanates affording intermediates **7a**–**d**, which were directly reacted further. Cyclization was

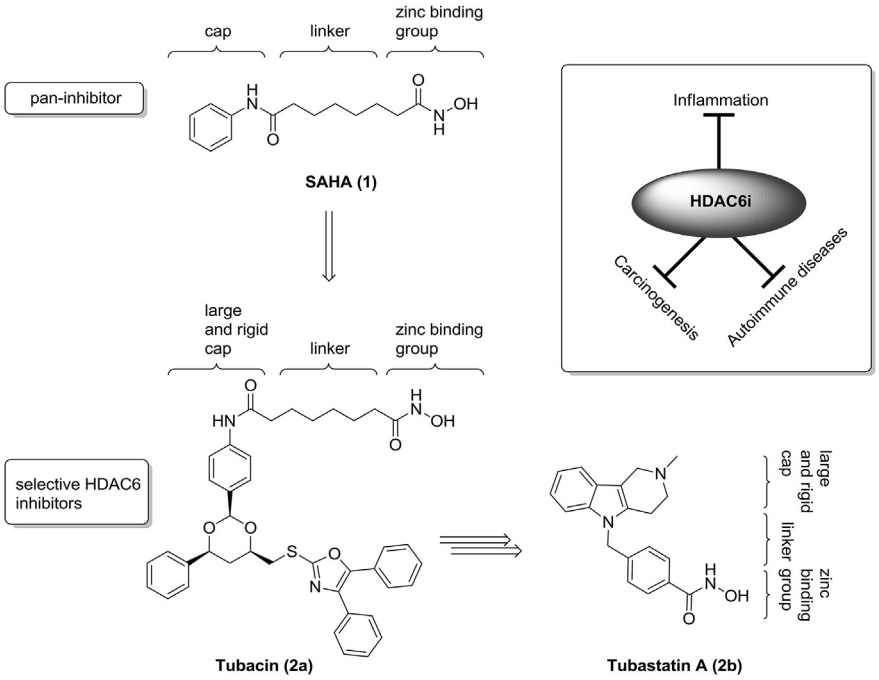

Fig. 1. Schematic illustration of the pharmacophore of HDACi.