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Review article

Synthesis and applications of benzohydroxamic acid-based histone deacetylase inhibitors

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ABSTRACT

This paper provides an overview of the synthesis and biological activity of the most representative benzohydroxamic acid-based histone deacetylase inhibitors published to date. Benzohydroxamic acids comprise an important class of HDAC inhibitors, and recently several of these structures have been evaluated in clinical trials for the treatment of a variety of cancers. In this overview, benzohydroxamic acids were divided in four different classes based on their reported selectivity towards zinc-dependent HDACs: a first and major class consists of HDAC6 selective inhibitors, a second class deals with pan-HDAC inhibitors, a third class comprises HDAC8 selective inhibitors and a fourth, minor class includes dual HDAC6/8 selective inhibitors. Through this approach, structure-activity relationships were identified for each class, which could help future researchers in the design and development of novel benzohydroxamic acid-based HDAC inhibitors.

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1. Introduction

The hydroxamic acid functional group can be considered as a privileged scaffold in several fields of chemistry due to its excellent metal-chelating properties. Metal chelation can occur through a monoanionic hydroxamato form or a dianionic hydroximato form in an *O,O*'-bidentate fashion. As a consequence, hydroxamic acids are ideal ligands for binding the active site of nickel- or zinc-containing metalloproteins (e.g. histone deacetylases, matrix metalloproteases, ureases and carbonic anhydrases), and they form a class of siderophores (iron-sequestering molecules secreted by microorganisms) as well. Hydroxamic acids are also used in heavy metal extraction procedures, nuclear fuel reprocessing and as chiral ligands in asymmetric synthesis [1].

This review will exclusively focus on the synthesis and biological activity of benzohydroxamic acids as histone deacetylase inhibitors. The development of benzohydroxamic acids indeed involves an important and active field within HDAC inhibitor design, and many

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research teams from industry and academia are currently participating in this quest.

Histone deacetylases (HDACs) have been discovered as a class of enzymes which regulate the removal of acetyl groups from lysine residues of histones, consequently playing an important regulatory role in epigenetics [2]. In following studies, other proteins have also been identified as HDAC substrates, and therefore these enzymes are more correctly referred to as lysine deacetylases or KDACs [3]. In total, four classes of HDACs can be identified (HDAC I-IV). HDAC classes I, II and IV employ Zn^{2+} as an essential cofactor while HDAC class III, also known as the Sirtuin class, needs NAD^{+} to exert activity. Since the focus of this review is directed towards hydroxamic acids targeting zinc-containing HDACs, the Sirtuin class will not be discussed here. In total, eleven zinc-containing isoforms have been discovered, which were subdivided via their homology to yeast HDACs (Class I: HDAC1, 2, 3 and 8, Class IIa: HDAC4, 5, 7 and 9, Class IIb: HDAC6 and 10, Class IV: HDAC11) [4]. Due to the involvement of these isoforms in modern-day diseases such as cancer, neurodegenerative diseases and inflammatory disorders, a lot of effort is currently being devoted to the development of safe and efficient histone deacetylase inhibitors (HDACi's) [5,6]. In that regard, several HDACi's have reached the patient, with vorinostat, the first clinically approved anti-cancer HDACi for the treatment of cutaneous T cell lymphoma, as a leading example [7,8]. HDAC inhibitors typically consist of (i) a zinc-binding group complexing the zinc atom in the catalytic pocket of the enzyme, (ii) a linker unit filling the tubular space between the catalytic pocket and the outer surface of the enzyme, and (iii) a cap-group for interaction with the outer protein surface. This review is oriented towards the medicinal chemistry of benzohydroxamic acids as privileged structures in HDAC research and will encompass the synthesis and biological activities of the most representative HDACi's bearing a hydroxamic acid zinc-binding group directly connected to a phenyl ring. The biological part will mainly focus on the observed *in vitro* HDAC1-11 inhibitory activities of the presented structures, whereas further biological applications in the field of oncology, immunology,…, will be touched only briefly. This approach will provide insights into the selectivity that can be observed when designing functionalized benzohydroxamic acids and will give an overview of available synthetic routes to obtain this kind of structures.

2. Benzohydroxamic acid-based HDAC inhibitors

This overview is based on a classification of benzohydroxamic acids in terms of their reported selectivities. As a result, four groups of inhibitors were identified: a major group of HDAC6 selective inhibitors, a group of non-selective pan-inhibitors, and two smaller groups, one consisting of HDAC8 selective inhibitors and one containing dual HDAC6/8 selective inhibitors. When reading the appropriate literature, one will notice that the term 'selectivity' is interpreted differently by various authors, and therefore the following questions arose when writing this review. Can one claim an inhibitor to be selective for a specific zinc-containing HDAC isoform if not all IC_{50}-values for each of the eleven HDAC isoforms have been determined? When is an inhibitor selective over another HDAC isoform, in other words, can a certain threshold value be employed? Is determination of the selectivity based on the purified HDAC isoforms an accurate representation of the selectivity, or should the IC_{50}-values be determined based on the selectivity against the in cell existing HDAC complexes? [9] Can conclusions be made by comparing IC_{50}-values resulting from different assays, or should dissociation constants (K_i) be used? These important questions should be taken into account when reading the chapters below. In order to avoid any ambiguity concerning the interpretation of the term 'selectivity', an inhibitor will be denoted here as selective towards another isoform if it holds at least a tenfold lower inhibition value (K_i or IC_{50}) over the other isoform.

2.1. Selective benzohydroxamic acid-based HDAC6 inhibitors

The selective inhibition of HDAC6 is a 'hot topic' in medicinal chemistry, exemplified by the impressive group of benzohydroxamic acids presented in Fig. 1. When overviewing compounds **1**–**20**, it is noticeable that the majority accommodate a rather voluminous cap-group, *para*-substituted with respect to the hydroxamic acid functionality and in close proximity to the phenyl linker. This voluminous cap-group is never directly attached to the phenyl linker, implying that at least one atom (carbon or nitrogen) resides between the cap-group and the phenyl unit. This distance is most likely necessary to avoid a steric clash between the large cap-group and the protein, suggesting that this additional atom is part of the linker unit filling the tubular space to the catalytic pocket. Another feature which emerges when inspecting this group of molecules is that several members share the following common structure: a heterocyclic scaffold linked through a methylene bridge to the benzohydroxamic acid unit (structures **9**–**19**). For each inhibitor depicted in Fig. 1, the biological activity and synthetic pathway will be discussed below. When presenting an overview on benzohydroxamic acids as HDAC inhibitors, the simplest representative, *i.e.* benzohydroxamic acid **1** itself, must be included in the discussion as well (Fig. 1). The search for selective HDAC6 inhibitors able to penetrate the blood-brain barrier encouraged Wagner and co-workers to design the smallest possible pharmacophore still demonstrating effective HDAC6 selectivity and activity [10]. Therefore, the concept of ligand efficiency was used, defined as the HDAC6 activity over the number of non-hydrogen atoms, which is a known valuable tool in drug design to compare differently sized molecules with similar activity values. Compounds possessing a high ligand efficiency have a higher probability to demonstrate improved pharmacokinetic properties as central nervous system drugs. In that regard, benzohydroxamic acid **1** (a commercially available hydroxamic acid) has been evaluated as HDAC inhibitor and showed good potency and selectivity for HDAC6 (Table 1) and holds a high ligand efficiency, due to the small size of the molecule. The selectivity of this compound was further confirmed in a Western Blot assay in HeLa cells, measuring the acetylation status of α-tubulin (a substrate of HDAC6) and histone H3 (a substrate of class I HDACs).

In the same study several other substituted benzohydroxamic acids were synthesized as well, with compound **2** demonstrating the most pronounced HDAC6 activity (IC_{50} = 0.004 μM, Table 1, Scheme 1) [10]. All benzohydroxamic acids reported in this article bear a carbamoyl group directly linked to the benzohydroxamic acid scaffold, similar as in structure **2**, and were prepared via the following procedure (no yields reported). Amide **22** was synthesized from acid **21** using peptide coupling chemistry with HATU (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate) as acid-activating reagent. Subsequently, without purification, ester **22** was treated with an excess of hydroxylamine over 12 h to yield *N*-hydroxyphtalamide **2** as a white solid.

Lee et al. have developed HPOB **3** (*N*-hydroxy-4-{2-[(2-hydroxyethyl)(phenyl)amino]-2-oxoethyl}benzamide), a highly selective HDAC6 inhibitor containing a free alcohol and a *N*-phenylamide in the cap-region (Scheme 2) [11]. The selective inhibition of HDAC6 is illustrated in Table 1. HPOB demonstrated low nanomolar potency for HDAC6 and micromolar potency for all other zinc-containing HDACs. In a cellular environment, HPOB effectively inhibited HDAC6 by acetylating α-tubulin and peroxiredoxin, two known substrates of HDAC6, and little or no acetylation of histone