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# Histone deacetylase 10: A polyamine deacetylase from the crystal structure to the first inhibitors

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**Abstract**

Polyamine deacetylase activity was discovered more than 40 years ago, but the responsible histone deacetylase 10 (HDAC10) was described only recently. HDAC10 is a class IIb HDAC, as is its closest relative, the α-tubulin deacetylase HDAC6. HDAC10 has attracted attention over the last 2 years due to its role in diseases, especially cancer. This review summarises chemical and structural biology approaches to the study of HDAC10. Light will be shed on recent advances in understanding the complex structural biology of HDAC10 and the discovery of the first highly selective HDAC10 inhibitors.

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**Keywords**

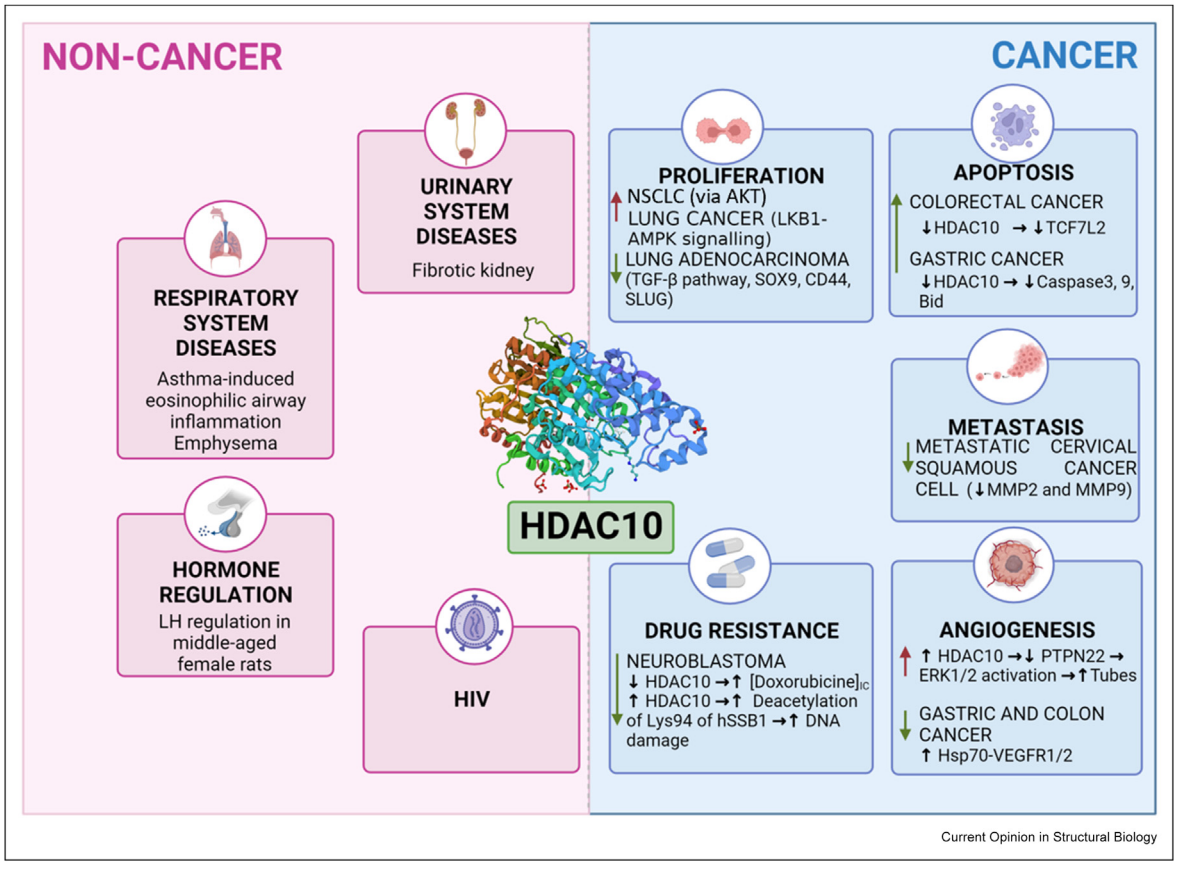
HDAC10, Polyamine deacetylase, Crystallography, HDAC10 substrates, HDAC10 inhibitors.

## Introduction

Polyamine deacetylase (PDAC) activity was discovered more than 40 years ago [1,2], but until recently, the precise enzymes responsible for eukaryotic PDAC activity were unknown. In 2002, four groups independently described histone deacetylase 10 (HDAC10) [3–6], now classified as a class IIb HDAC, together with its closest relative, the α-tubulin deacetylase HDAC6 [7]. Indeed, HDAC6 is the primary off-target isoform, and HDAC6-selective inhibitors often possess potent HDAC10 inhibition activity [8]. HDACs are well-known epigenetic players that catalyse the deacetylation of histones as well as an increasing number of non-histone targets [9]. It took more than 15 years to discover that, in contrast to the other HDAC family members, HDAC10 specifically hydrolyses $N^8$-acetylspermidine but not $N^1$-acetylspermidine, without significantly influencing histone acetylation levels [10]. Thus, the long-sought PDAC [11] was identified as described in the seminal paper by Hai et al. [10]. Class IIb enzymes are distinct from the other classes of isozymes as they poorly deacetylate histones and are localised in the cytoplasm and not in the nucleus [12]. HDAC10-knockout mice are living and develop normally without showing signs of disease [13].

HDAC10 has been shown to play a crucial role in several key aspects of cancer biology, including cell proliferation, apoptosis, metastasis, angiogenesis and drug resistance [14]. Its effects vary depending on the type of cancer as it can increase proliferation in non-small-cell lung cancer [15] and lung cancer via the protein kinase B and liver kinase B1-5' adenosine monophosphate-activated protein kinase pathways [16] but decreases it in lung adenocarcinoma via the transforming growth factor-β pathway [17]. In colorectal and gastric cancer, low levels of HDAC10 increase apoptosis by depleting transcription factor 7 like 2 or by increasing levels of Caspase-3 and Caspase-9 [18,19]. HDAC10 also plays a role in angiogenesis by promoting the binding of Hsp70 to vascular endothelial growth factor 1/2 receptor (VEGFR1/2), leading to the proteasomal-dependent degradation of VEGFRs in colon and gastric cancer [20], and by decreasing the PTPN22 expression [21]. HDAC10 is involved in metastasis by reducing the expression of matrix metalloprotease 2 and 9 (MMP2 and 9) in metastatic cervical squamous cancer cells [22] and in drug resistance by regulating the accumulation of doxorubicin and acetylation of hSSB1 in neuroblastoma [23,24]. In addition to its double-sided role in cancer [14,25–27], HDAC10 is implicated in various non-cancerous diseases, including respiratory [14,28] and urinary system diseases [14,29], hormone regulation [14,30], neurodegenerative [14,31,32] and HIV infection [14,33]. Figure 1

**Figure 1**




Involvement of HDAC10 (protein structure: PDB ID 5TD7) in cancer [14–24] and non-cancer diseases [25–28,32]. HDAC, histone deacetylase.

summarises the diseases and relative pathways in which HDAC10 is (very likely) involved.

## General structural properties of HDAC10 and its unique substrate selectivity

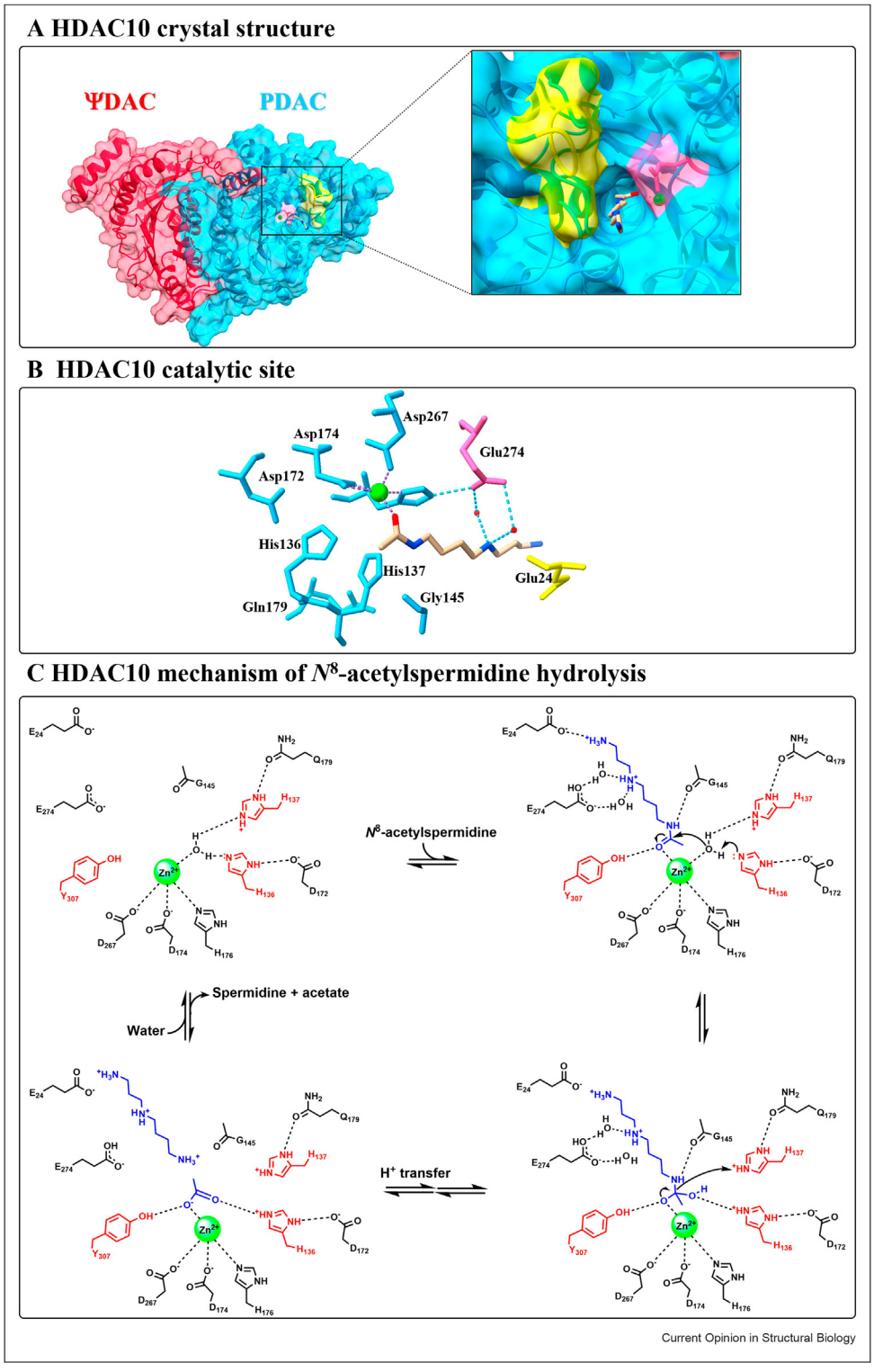
Its structure and catalytic function probably make HDAC10 a fascinating member of the human 11 metal-dependent HDACs [34,35]. In 2017, an X-ray crystal structure of HDAC10 from *Danio rerio* (zebrafish) was described in a seminal paper [10], HDAC10 possesses two α/β arginase-deacetylase folds similar to those of HDAC6 [36]. It is notable that in HDAC6, both domains are catalytically active, while in HDAC10, one is a smaller and inactive domain and this named as a pseudodeacetylase domain (see Figure 2a and b) [36,37].

The catalytically active PDAC domain possesses two very particular and unique features in terms of a negatively charged gatekeeper residue Glu274 in *dr*HDAC10 (Glu272 in *h*HDAC10), and a $3_{10}$helix containing the consensus sequence $P^{23}$(E, A)$CE^{26}$ (the PEACE motif) which plays a crucial role in the substrate selectivity by constricting the active site. Indeed, HDAC10 has a remarkable selectivity for long, slender polyamines, such as $N^8$-acetylspermidine, acetylputrescine, or acetylcadaverine, while the classical $N^{\varepsilon}$-acetyl-L-lysine residue HDAC substrate is practically not deacetylated [34,37,38].

The gatekeeper Glu274 at the entrance of the active site enables the binding of the protonated cationic polyamine via two water-mediated hydrogen bonds (see Figure 2a and b) [34], while other conserved glutamate and aspartate residues across the active site additionally contribute to the recognition of the positively charged substrate [36].

The preference of HDAC10 for $N^8$-acetylspermidine over $N^1$-acetylspermidine can be rationalised via the position and orientation of the secondary amino group — a distance of 4 methylene units between the secondary amine, and the amide function is preferred for substrate recognition [36,39]. Trifluoracetylated lysines are described as good HDAC substrates in literature [40,41], but considering the unique PDAC activity of

**Figure 2**




(**a**) The structure of HDAC10 consists of two domains: a polyamine deacetylase domain (PDAC, sky blue) and a catalytically inactive pseudoacetylase domain (ΨDAC, red). $N^8$-Acetylspermidine is bound to the PDAC active site, which contains a zinc ion, shown as a green sphere. The substrate binding site is zoomed in the square. The «PEACE» motif $3_{10}$ helix [$P^{23}$(E, A)$CE^{26}$] that sterically constricts the active site is in yellow and the negatively charged gatekeeper E274 is in pink sticks. (**b**) Catalytic site of HDAC10 with Glu274 in pink and Glu24 in yellow. The two water-mediated hydrogen bonds between Glu274 in pink and $N^8$-acetylspermidine in dark yellow are shown with the water molecules depicted as red balls (PDB ID 7KUQ) [14]. (**c**) Mechanism for HDAC10 catalysed hydrolysis of $N^8$-acetylspermidine (in blue) based on the structural studies of Herbst-Gervasoni and Christianson. Crucial residues (H136, H137 and Y307) are in red (Figure adapted from the study by Herbst-Gervasoni and Christianson [34]). HDAC, histone deacetylase.

HDAC10, they are not suitable substrates for use in inhibitor screens [39]. Appropriate assay systems are needed to develop isozyme-selective HDAC inhibitors (HDACi). Herp et al. have recently described a robust and selective HDAC10 inhibition assay suitable for high-throughput screening approaches that uses a novel polyamine-based substrate, *i.e.* Ac-spermidine-aminocoumarin [39]. Their synthetic substrate only reacts with a stop solution containing naphthalene-2,3-dialdehyde and Mesna (see Figure 3) once it is deacetylated, giving a benzisoindole that quenches the fluorescent group on the substrate. Thus, the more intense the fluorescence signal, the higher the inhibition potency of a potential HDAC10 inhibitor (HDAC10i) (Figure 3a and b).

As an alternative to the substrate-based assays, inhibitor-based assay systems using either the time-resolved fluorescence energy transfer or the bioluminescence energy transfer technology [42,43] can be applied to screening. However, the competitive displacement of a fluorescent HDAC10-probe with a putative inhibitor has potential drawbacks compared to assays based on turnover [44].

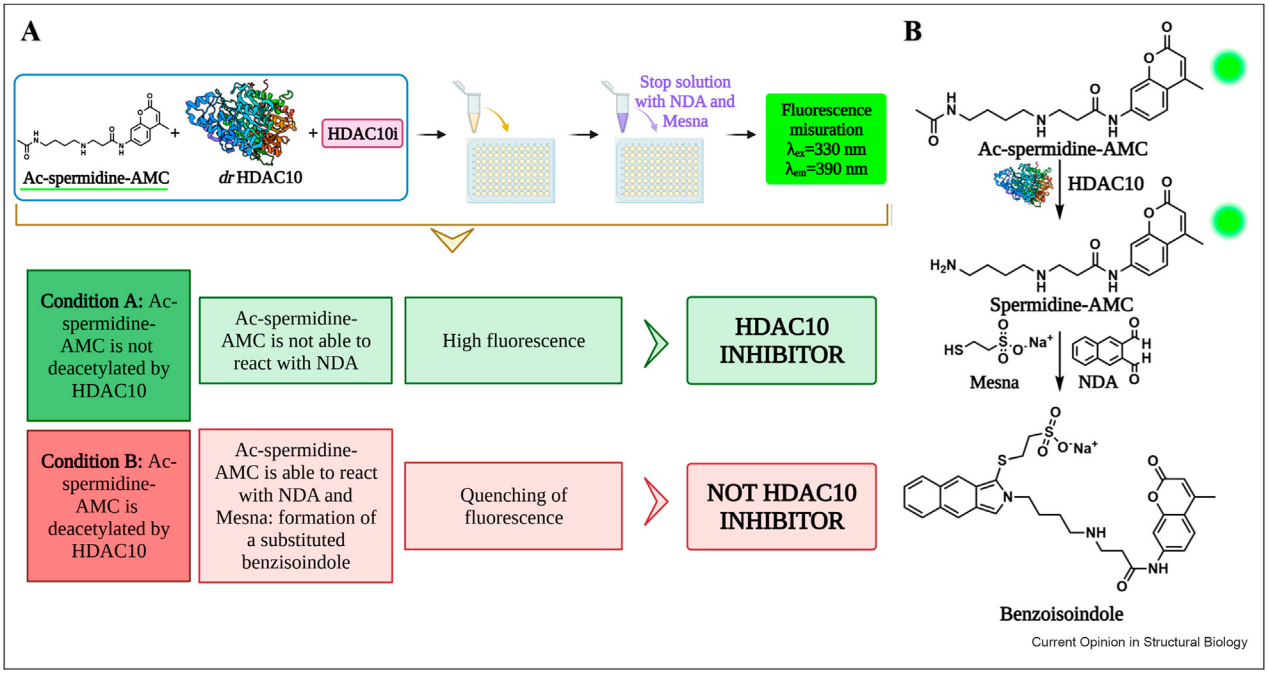
## Mechanism of amide bond hydrolysis

A mechanism for the hydrolysis of $N^8$-acetylspermidine by Herbst-Gervasoni and Christianson has been recently proposed in part based on the results arising from trapping of intermediates in mutated HDAC10 proteins with substitution of catalytic residues in the active site [34]. Their structural and biochemical studies reveal that the tandem histidine residues H136 and H137 play a crucial role together with tyrosine Y307 [34]. This mechanism is similar to the deacetylation catalysed by class I HDACs as well as by HDAC6 [36,45], where also a tyrosine is employed to polarise the amide carbonyl of the substrate and to stabilise the oxyanionic tetrahedral intermediate. One of the tandem histidines acts as a base promoting the reaction of the zinc-coordinated water molecule with the carbonyl group of the substrate, while the second histidine acts as an acid facilitating exit of the leaving amino group from the tetrahedral intermediate (Figure 2c) [34].

## HDAC10 inhibitors and isozyme selectivity

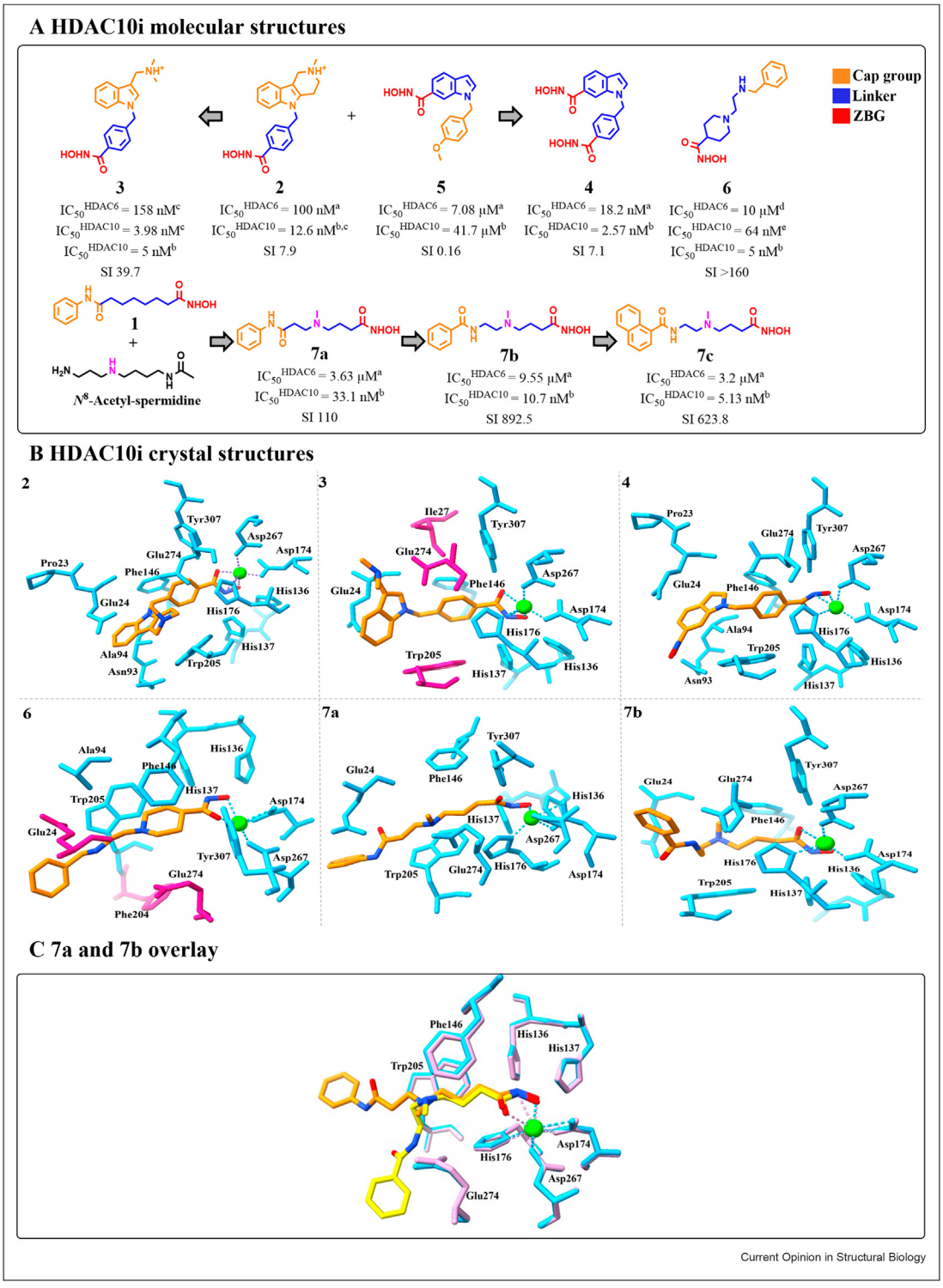
Some HDACi (vorinostat (SAHA, **1**), romidepsin (FK228), belinostat (PXD101), tucidinostat (chidamide, CS055) and panobinostat (LBH589)) have been

**Figure 3**




Workflow of a robust and selective HDAC10 inhibition assay (**a**) A microplate-based assay system in a homogeneous environment: Ac-spermidine-AMC is incubated with *dr*HDAC10 (PDB ID 7U69), and a stop solution containing naphthalene-2,3-dialdehyde (NDA) is added. The fluorescence is then measured ($\lambda_{ex}$ = 330 nm, $\lambda_{em}$ = 390 nm). If Ac-spermidine-AMC does not react with NDA, the fluorescence remains high at 390 nm. However, if Ac-spermidine-AMC is deacetylated by HDAC10, NDA reacts with Spermidine-AMC in the presence of a nucleophile (such as Mesna) to form a substituted benzisoindole, which intramolecularly quenches the fluorescence at 390 nm. (**b**) Reactions involved in the assay in A [39]. AMC, aminocoumarin; HDAC, histone deacetylase.

**Figure 4**




(**a**) selected HDAC10i: structures, $IC_{50}$ values (obtained by [a]HDAC Glo enzymatic assay, [b]FRET assay, [c]BRET assay, [d]ZMAL, [e]Ac-spermidine-AMC assay) and Selectivity Index (SI) HDAC10/HDAC6. For each inhibitor, the ZBG is in red, the linker in blue, and the cap group in orange. (**b**) Crystal structures views of HDAC10 inhibitors in orange (N in blue, O in red). Crucial binding residues are in magenta, and the zinc ion is a green ball. PDB ID: 6WBQ (**2**, tubastatin A), 6WDV (**3**, DKFZ-480), 6VNQ (**4**, in the original manuscript 3a), 7U59 (**6**, in the original manuscript 48a), 7SGJ (**7a**, DKFZ-711), 7SGK (**7b**, DKFZ-728). Reference $IC_{50}$ values for **2**, **5** (PCI-34051), **3** [50], **4** [51], **6** [39], **7a**, **7b** and **7c** (DKFZ-748) [35]. (**c**) Overlay of **7a** (yellow) and **7b** (orange). PDB ID: 7SGJ in blue (**7a**), 7SGK in lilac (**7b**) [35]. BRET, bioluminescence energy transfer; FRET, fluorescence energy transfer; HDAC, histone deacetylase.

approved to treat various types of cancer and others are in different states of preclinical and clinical development for treatment of cancer and other diseases [46]. Two clinically used HDACi (romidepsin and tucidinostat) possess a limited isozyme specificity; thus, design is heading towards isozyme-specific HDACi aiming to develop less toxic drugs and to understand the complex biology and disease aetiology [46]. The structure of most HDACi follows a pharmacophore model consisting of a zinc-binding group (ZBG), a linker and a cap group [46–48]. In recent years, more selective HDACi have been developed, including via structure-based design approaches based on X-ray crystal structures [49].

HDAC10 possesses unique structural features, which have been recently exploited in the design and development of selective HDAC10i (Figure 4) [34,35,37,39,50,51]. The selectivity of the inhibitors of HDAC10 over the other HDAC isozymes has been improved by enhancing specific interactions with the HDAC10 unique gatekeeper residue Glu272/Glu274 and the PEACE motif [34,50,51]. As described, HDAC10i usually chelate the catalytic zinc ion in a bidentate manner and make characteristic hydrogen bonds with histidine and tyrosine residues.

The first HDAC10i to be investigated were among the potent and rather selective HDAC6 inhibitors. This is perhaps not surprising as HDAC6 and 10 belong both to the class IIb HDACs, sharing a general sequence identity of >45%, while one of the substrate binding sites is even higher, reaching about 68% [19,39]. Notably, tubastatin A (**2**), originally developed as an HDAC6 selective inhibitor, has been discovered to be an even more potent HDAC10 inhibitor by about 8-fold ($IC_{50}^{HDAC10}$ = 12.6 nM, $IC_{50}^{HDAC6}$ = 100 nM) [34,50].

Crystallographic studies of **2** and the more potent and selective analogue DKFZ-480 (**3**) ($IC_{50}^{HDAC10}$ = 5 nM, $IC_{50}^{HDAC6}$ = 158 nM [37]) reveal that not only slender polyamine substrates but also rather bulky capping groups are capable of entering the active site of HDAC10, reflecting flexibility in the PEACE motif as inhibitors [34,37]. Indeed, the HDAC10 $3_{10}$helix helps to hold the inhibitor at the active site in place while the positively charged free amine makes electrostatic interactions with the gatekeeper Glu274, and the indole is positioned to hydrophobic interactions with Ile27 and Trp205 [37].

Considering that dual HDAC8/10 inhibitors might be beneficial in neuroblastoma treatment, Morgen et al. prepared the bis-hydroxamate inhibitor **4** (3a in the original manuscript) [51], which merges elements of the HDAC6/10 inhibitor **2** and the HDAC8 inhibitor PCI-34051 **(5)**. The two hydroxamates were inserted at the shared N-benzylindole core of **2/5** [51]. Indeed, bis-hydroxamate **4** ($IC_{50}^{HDAC10}$ = 2.57 nM [51], $IC_{50}^{HDAC6}$ = 18.2 nM [51]) possesses higher selectivity towards HDAC10 with respect to HDAC8 than the parent compounds. However, due to poor cell permeability, **4** was not very active in cells; thus, further optimisation is necessary, for example, one of its hydroxamates could be substituted by another ZBG group [52]. Crystallographic studies of **4** with HDAC10 reveal the flexibility of the PEACE region [51], consistent with the previous studies [34,37].

Herp et al. used their high-throughput HDAC10 screen to develop HDAC10i [39]. They optimised the CAP group of piperidine-4-hydroxamates described in earlier work by Zeyen et al. [53]; the oligoamine substrate was mimicked *via* linking of an amino group with an ethylene spacer to a piperidine, with capping with a lipophilic benzyl group [39]. One of their most potent compounds, **6** (48a in the original manuscript) [39] ($IC_{50}^{HDAC10}$ = 64 nM [39], $IC_{50}^{HDAC6}$ = 10 μM [39]), was crystallized in complex with HDAC10.

Compared to the other HDAC10i mentioned above, in the case of **6**, the PEACE motif undergoes only a slight conformational shift, possibly facilitating the interaction of the secondary ammonium cation with Glu274. The nitrogen of the piperidine interacts with Glu274, while the cap group exhibits π–π stacking interactions with Phe204 [39].

Recently, an innovative drug-design approach on the well-known pan-HDACi **1** has been applied. Inspired by the slender long-chain polyamine substrates of HDAC10 [54], Steimbach et al. 'walked' a methyl-amino group across the alkyl chain of **1**, aiming to improve isozyme selectivity. Indeed, γ-aminohydroxamate DKFZ-711 (**7a**) was an isozyme-selective HDAC10 hit, exhibiting a 3.5-fold increase in HDAC10 potency with respect to **1** while reducing the activity towards other tested HDACs [35]. Optimisation of the cap group of the aza-SAHA derivative **7a** ($IC_{50}^{HDAC10}$ = 33.1 nM [35], $IC_{50}^{HDAC6}$ = 3.63 μM [35]) led to the reverse amide DKFZ-728 (**7b**), which was more selective towards HDAC10 ($IC_{50}^{HDAC10}$ = 10.7 nM [35], $IC_{50}^{HDAC6}$ = 9.55 μM [35]). Additional SAR led to 1-naphthylamide DKFZ-748 (**7c**) ($IC_{50}^{HDAC10}$ = 5.13 nM [35], $IC_{50}^{HDAC6}$ = 3.2 μM [35]) which manifests a gain in potency towards HDAC10 without loss of selectivity [35].

Interestingly, **7a** and **7b** possess slightly different binding modes related to the inverted amide function (Figure 4) [35]. The tertiary ammonium cation of these HDAC10i was expected to interact directly with the gatekeeper Glu274 as for polyamine substrates (see also Figure 2b), but instead, crystallography reveals it interacts via cation–π interactions with Trp205, probably serving as an anchor for the orientation of **7a** or **7b** in the active site. Class I HDACs possess at position 205 a phenylalanine instead of a tryptophan; thus, the

nonpolar surfaces might not be compatible with the polar amine in the linker of the DKFZ compounds **7a-c** [36]. Instead, the **7a** or **7b** anilide cap group interacts with Glu274 in both cases; however, the presence of a benzamide at the same place seems to be preferred as **7b** (and the related **7c**) has a higher HDAC10 affinity than **7a** (Figure 4). Recently, it has been shown that HDAC10 inhibition by **7b** and by the related **7c** phenocopies the effect of HDAC10 knockout, *i.e.*, they suppress colon cancer cell growth mediated by spermidine [55].

The crystal structures of HDAC10i obtained to date reveal that HDAC10 is tolerant of the size and substitution patterns of the CAP group, reflecting the flexibility of the PEACE motif, enabling the modulation of physiochemical properties. Note, however, that to retain HDAC10 isozyme-selectivity, an amine-containing linker that is able to interact with the gatekeeper glutamate is required.

## Concluding remarks

Like other HDACs, HDAC10 is involved in numerous biological pathways, exhibiting complex functions some with clinical relevance in particular to cancer [14,54]. By contrast with most other HDACs, HDAC10 possesses a preference for polyamine substrates such as $N^8$-acetylspermidine [10,36]. Over the last 5 years, knowledge of HDAC10 has rapidly improved from a structural perspective. Numerous crystal structures are now available, covering not only the natural substrates but also the first potent and selective HDAC10i. Isoenzyme selectivity of inhibitors is likely important to reduce side effects and to study the biological functions of HDACs. Indeed, HDAC6 is the primary off-target isoform of HDAC10i; inhibitors thought to be HDAC6-selective often also possess potent HDAC10 inhibitory activity. Indeed, assays for HDAC10 have only recently become available [8,39]. Compared to other HDAC isoforms, relatively little is known about aberrant HDAC10 regulation and its interplay with other (epi)enzymes in diseases. The new small molecule tools described in the present review will help understand the complex biology. Recently, evidence has been presented that HDAC10i are endowed with low cytotoxicity, so very likely, undescribed clinically relevant features of HDAC10 are waiting to be discovered [35]. Selective HDAC10 degraders and HDAC10 mutation experiments might help to shed light on unanswered questions relating to HDAC10 function [56]. A multidisciplinary approach involving chemical biology, structure-based drug design and physiology is necessary to boost understanding of the polyamine metabolome and its related HDAC10 biology and (patho)physiological functions, with one goal to define the clinical relevance of HDAC10 as a disease treatment target.

## Author contributions

Chiara Lambona, Clemens Zwergel: investigation, visualisation and writing – original draft. Antonello Mai, Sergio Valente: writing - review and editing. Rossella Fioravanti: conceptualisation, validation, supervision, writing – review and editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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