Review

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HDAC8: a multifaceted target for therapeutic interventions

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Histone deacetylase 8 (HDAC8) is a class I histone deacetylase implicated as a therapeutic target in various diseases, including cancer, X-linked intellectual disability, and parasitic infections. It is a structurally well-characterized enzyme that also deacetylates nonhistone proteins. In cancer, HDAC8 is a major 'epigenetic player' that is linked to deregulated expression or interaction with transcription factors critical to tumorigenesis. In the parasite *Schistosoma mansoni* and in viral infections, HDAC8 is a novel target to subdue infection. The current challenge remains in the development of potent selective inhibitors that would specifically target HDAC8 with fewer adverse effects compared with pan-HDAC inhibitors. Here, we review HDAC8 as a drug target and discuss inhibitors with respect to their structural features and therapeutic interventions.

HDAC8: a unique class 1 deacetylase

HDACs are 'eraser' enzymes that cleave acetate from acetylated ε-amino groups of lysines in histones and other proteins. Deacetylation of histones causes global chromatin condensation and transcription repression [1]. HDACs participate in critical signaling networks and their deregulation has been linked to many diseases [2], including cancer, neurodegenerative disorders, metabolic dysregulation, and autoimmune and inflammatory diseases; hence, HDACs continue to be interesting drug targets [3].

While most disease-oriented research has focused on HDAC 1–3 and 6 [4], lately there has been an increasing interest in the isotype HDAC8 as a drug target [5]. This is a zinc-dependent class I HDAC containing 377 amino acids (aa, 42 kDa) [6–8] that is ubiquitously expressed and that can localize to either the nucleus (primary site) or the cytoplasm [6–9]. HDAC8 diverged from other class I enzymes early in evolution, indicating a discrete functional specialization [10]. It is X-linked in humans, and its activity is independent of additional co-factors [6–8]. The C-terminal (aa 50–111) protein-binding domain of other HDACs is not present in HDAC8 [11]. The L1 loop in the proximity of the active site is highly flexible and can undergo conformational changes to accommodate different substrates [11]. The presence of serine 39 phosphorylation near the active site is special to HDAC8 and negatively regulates its activity [11,12]. Some unique nonhistone proteins [13–15], such as cohesin, estrogen receptor α (ERRα), or cortical actin-binding protein (cortactin), have been linked with HDAC8. Thus, it controls diverse processes, such as sister chromatid separation, energy homeostasis, microtubule integrity, and muscle contraction [9,13,14]. Consequently, it is not surprising that HDAC8 has a multifaceted role in human pathophysiology. Mutations leading to changes in its structure and activity are reported in Cornelia de Lange syndrome (CdLS) [16]. In cancer, HDAC8 is either deregulated, overexpressed, or is reported to interact with transcription factors [17–20]. Moreover, HDAC8 is correlated with childhood neuroblastoma and T cell lymphoma [21–23]. In parasitic and viral infections, HDAC8 inhibition leads to reduced infection [24,25]. Here, we review the evidence of the crucial importance of HDAC8 in many diseases, present available inhibitors with a focus on the structural basis of inhibition, and highlight the potential for therapeutic intervention by HDAC8 inhibition.

HDAC8 substrates

Although both core histones (preferentially H3 and H4) and histone-tail derived peptides are known to be deacetylated *in vitro* [6,7,12], histones remain controversial *in vivo* as bona fide HDAC8 substrates. While deacetylation occurs specifically at acetylated lysines (Kac) 14, 16, and 20 on histone H4-derived peptides [6,8,26] (Figure 1), many

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Glossary

AT-rich interactive domain-containing protein 1A (ARID1A): encoded by the *ARID1A* gene. ARID1A is a transcription factor, a part of the switch/sucrose nonfermentable (SNF/SWI) chromatin-remodeling complex, and is thought to recruit the complex to DNA to activate transcription of repressed genes. The protein contains two domains: a DNA-binding domain that binds AT-rich sequences of the DNA, and the C terminus, which can activate transcription in a glucocorticoid-dependent fashion. The gene is mutated in many cancers and the protein functions as a tumor suppressor.

Cortical actin-binding protein (cortactin): acts as a regulator of actin dynamics and promotes actin polymerization. F-actin interacts with cortactin on a special repeat region. Acetylation of nine lysine residues in this repeat region inhibits F-actin interaction, while deacetylation promotes F-actin binding and positively regulates actin dynamics by promoting polymerization.

c-AMP response element-binding protein (CREB): a transcription factor that binds specifically to c-AMP response elements in DNA and regulates genes such as those encoding somatostatin and c-fos. It is activated by phosphorylation by protein kinase A and is involved in the maintenance of circadian rhythm and in Huntington's disease. This protein can be deactivated by phosphatases, such as PP1, which is a serine threonine phosphatase.

Differentiated embryo-chondrocyte expressed gene 1 (DEC1): a basic helix-loop-helix transcription factor that acts as a transcriptional repressor by recruiting HDACs as a co-repressor. It can transactivate several genes, such as that encoding survivin. It is a target of the p53 family of proteins and modulates their activity. Functionally, DEC1 is important in circadian rhythm, senescence, cellular differentiation, and apoptosis. Upregulation of DEC-1 has been associated with gastric cancer.

Estrogen receptor α (ERRα): encoded by the gene *ESR1*. It is a nuclear receptor activated by estrogen and overexpressed in estrogen-associated cancers, such as breast cancer.

Inv(16) fusion protein: a product of the most frequent chromosomal translocation in AML-1. It fuses core binding factor β with smooth muscle myosin heavy chain, resulting in an oncogenic chimeric protein. This translocation disrupts AML-1 function. The C-terminal assembly complex domain of the smooth muscle myosin heavy chain region can associate with co-repressors, such as HDAC8, and repress transcription.

Nuclear receptor co-activator 3 (NCOA3): encoded by *NCOA3* gene and belongs to the SRC-1 family of transcriptional coactivators. The protein has an intrinsic acetyltransferase activity and can acetylate histones to enhance transcription. NCOA3 protein increases nuclear receptor transcription. Overexpression and gene amplification of NCOA3 has been associated with estrogen receptor-positive breast and ovarian cancers.

Retinoic acid induced 1 (RAI1): encoded by the *RAI1* gene. The protein is a transcription factor. It may be a part of a complex and acts as a transactivator. RAI1 is associated with neuronal disorders, such as Smith–Magenis syndrome.

Suppressor of cytokine signaling 1/3 (SOCS1/3): cytokine-induced negative regulators of cytokines that act by inhibiting the JAK/STAT pathway. They are also known to inhibit inflammatory responses in macrophages and microglia. Overexpression of these proteins is associated with breast cancer.

TAp73: an isoform of P73, a member of the p53 family. It is transcribed from the upstream P1 promoter and the protein acts as a regulator of p53 target genes. It is a tumor suppressor with additional functions in innate immunity and septic shock. It is often overexpressed in cancer and is associated with enhancement of the pentose phosphate pathway, as well as with proliferation in tumors.

Thyroid hormone receptor-associated protein 3 (THRAP3): encoded by the *THRAP3* gene. It is a transcription factor associated with RNA splicing and degradation. It has a crucial role in diabetes gene programming and can also regulate DNA damage.

Zinc finger Ran-binding domain-containing protein 2 (ZRANB2): encoded by the *ZRANB2* gene. It is part of a supraspliceosome and acts as a splicing factor of many genes, several of which are involved in cancer development. It is upregulated in grade III ovarian serous papillary carcinoma.

recent cellular experiments have failed to demonstrate histones as *in vivo* substrates of HDAC8 [15,27]. Initially, overexpression studies with HDAC8 showing lower levels of acetylated H3 and H4 in cells indicated histones as classical substrates [7,12]; however, inactivity in HDAC8 immunoprecipitated with an acetylated H4 peptide has been also reported [7]. Current data on the absence of global histone H3 hyperacetylation in HDAC8-specific inhibitor treated cells additionally suggest that HDAC8 has other major cellular substrates [7,23] and prefers specific gene regions in histones (H3) for deacetylation, as shown by ChIP experiments [28].

The controversies between *in vitro* and *in vivo* status of histones may arise from several caveats. While overexpression may change the normal deacetylase activity, multiple washing in immunoprecipitation might wash away the substrate (if loosely associated) or might have other HDACs as contaminants (due to >30% similarity in aa sequences of class I HDACs [29,30]). Additionally, global acetylation modifications in histones might mask the specific deacetylation pattern of H3 by HDAC8. Moreover, given that other HDACs also act on histones, a counter regulation may conceal the effect. Hence, cell-based inhibition approaches combined with mass spectrometry are emerging as alternative methods to identify '*in cellulo*' substrates of HDAC8 [15]. Thus, histones cannot be called general bona fide substrates of HDAC8, but there is evidence that some histone hyperacetylation is observed upon HDAC8 inhibitor treatment [27].

A quest for further substrates identified multiple partners, including structural maintenance of chromosomes 3 (SMC3), p53, ERRα, and inv(16) fusion protein [13,14,31,32] (Figure 1; see Glossary). The latest additions are retinoic acid induced 1 (RAI1), zinc finger, RAN-binding domain containing 2 (ZRANB2), nuclear receptor co-activator 3 (NCOA3), thyroid hormone receptor-associated protein 3 (THRAP3), AT-rich interactive domain-containing protein 1A (ARID1A), and cortactin [9,15]. These proteins have diverse physiological functions, from regulation of the cell cycle, carcinogenesis, to muscle contraction, indicating an intricate role of HDAC8 in cellular homeostasis. While direct deacetylation occurs in some of these proteins, such as SMC3, HDAC8 may act as a scaffold for other candidates and recruit these proteins in complexes. A special example could be the inv(16) fusion protein, where HDAC8 might act both as a deacetylase and an interacting scaffold [32]. As a scaffolding protein, association between HDAC8 and cAMP responsive element binding protein (CREB) provides an alternative example [33]. Both protein phosphatase 1 (PP1) and CREB co-precipitate with HDAC8; overexpression of HDAC8 decreases CREB phosphorylation as well as its activity, suggesting HDAC8 as a scaffolding platform for increased interaction of PP1 phosphatase and CREB [33]. It is difficult to differentiate the deacetylation versus scaffolding functions of HDAC8 *in vivo*, because the enzyme–substrate interactions might be short lived. While knockout or knockdown of HDAC8 could provide a mechanistic clue, such experiments would not present direct proof of enzyme association or activity (global deletion of HDAC8 in mice after birth is not lethal [34]). Catalytic dead mutants in cellular contexts might add more insight to distinguish between deacetylation and scaffolding roles of HDAC8.

Interestingly, in some of the proteins mentioned above, HDAC8 exhibits selectivity for certain deacetylation sites. Distinct motifs, such as arginine-Kac129 (RKac) in ERRα, RSKacFE in inv(16) fusion protein, and RHKK in p53 are shown to be preferred by HDAC8 [13,35,36] (Figure 1). A similar KRHR motif in H4 is also deacetylated by HDAC8 [37]. In general, an N-terminal arginine at position -1 to the Kac and an aromatic ring (e.g., phenylalanine) at the C-terminal position +1 constitute the most effective deacetylation sites [35,38]. Serine at -1 usually diminishes this

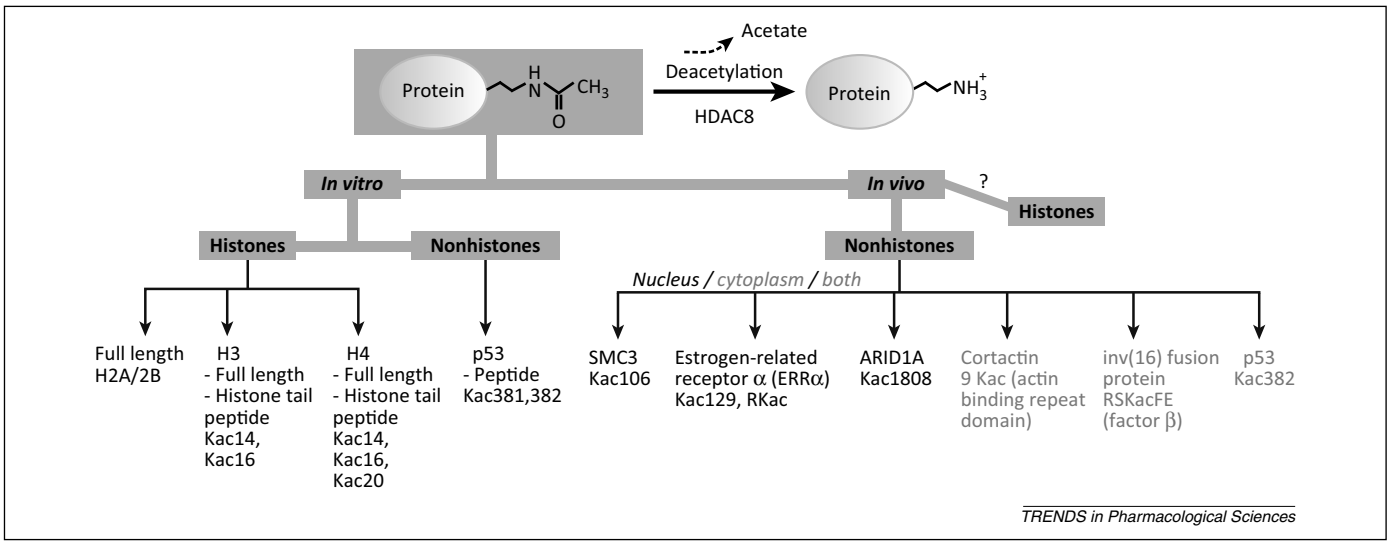

Figure 1. Protein deacetylation and substrates of histone deactylase 8 (HDAC8). *In vitro*, HDAC8 can deacetylate both histone [6–8,12] and nonhistone substrates, such as a tetrapeptide derived from p53 [39]. *In vivo*, nonhistone proteins seem to be major deacetylation substrates [9,13–15,31,32], while histones remain controversial as bona fide substrates of HDAC8. Representative nuclear and cytoplasmic nonhistone substrates are shown here. RKac and RSKacFE are specific motifs associated with HDAC8. Abbreviations: Kac, acetylated lysine; RKac, arginine at −1 position to Kac; RSKacFE, arginine-serine-Kac-phenyl-glutamic acid.

preference [35]. Furthermore, the presence of a conserved Asp101 in the rim of the active site is also known to critically contribute to substrate positioning (in a *cis* conformation) [39].

In *in vitro* screening, trifluoracetylated substrates have often been used to boost deacetylation rates [40,41], but acetylated tetrapeptides with a C-terminal fluorophore also work [39]. It has been shown that, in many cases, IC_{50} values are very similar but may differ with different substrates by up to 20-fold in potency for certain inhibitors [42].

HDAC8 and diseases

Cancer

HDAC8 is expressed in a variety of adult cancer tissues, such as colon, breast, lung, and pancreas, as well as in childhood cancer tissues, such as neuroblastoma [43,44]. The level is similar in cancer and corresponding healthy tissues, with a tendency towards higher expression in cancer tissues [31,43]. Knockdown of HDAC8 by RNA interference (RNAi) inhibits proliferation of human lung, colon, and cervical cancer cell lines (Figure 2), and the upregulation of HDAC8 promotes proliferation and inhibits apoptosis in hepatocellular carcinoma [31,45].

Microarray analyses of adult T cell leukemia/lymphoma (ATL) revealed that HDAC8 expression in these tumor cells is regulated by the transcription factor SRY (sex determining region Y)-box 4 (SOX4) [46–48], which directly activates the HDAC8 promoter [49] (Figure 2). HDAC8 knockdown suppresses growth of ATL cell lines [49] and the treatment of T cell-derived lymphoma and leukemic cells (e.g., Jurkat, HuT78, and Molt-4) with the HDAC8 specific inhibitor PCI-34051 induces calcium-mediated and caspase-dependent apoptosis [23] (Figure 2). However, solid cancer cell lines, such as A549 (lung), RKO (colon), U87 (glioma), and MCF-7 (breast), do not respond to such treatment with apoptosis [23]. Nevertheless, HDAC8 is among the HDAC family members that are upregulated in invasive breast tumor cells and the enforced overexpression of HDAC1, 6, or 8 supports invasion and matrix metallopeptidase 9 (MMP-9) expression of MCF-7 breast cancer cells [18].

In normal development, HDAC8 controls patterning of the skull in cranial neural crest cells of mice. Consequently, deletion of HDAC8 in mice leads to perinatal (P1) lethality due to skull instability [34]. In neural crest-derived neuroblastoma, HDAC8 expression correlates with advanced tumor stage and poor outcome, and selective inhibition induces differentiation, shown by the outgrowth of neurite-like structures [44]. HDAC8 inhibitors showed advantages over pan-HDAC inhibition with regard to toxicity and efficacy in a preclinical neuroblastoma model [21,50,51]. Targeting of other HDAC isotypes in neuroblastoma results in completely different phenotypes (apoptosis or blocked autophagy) [52–54].

Mechanistically, HDAC8 has been shown to affect telomerase activity by protecting the human ever-shorter telomeres 1B (hEST1B) protein against ubiquitin-mediated degradation, a function dependent on the phosphorylation status of HDAC8 [55]. HDAC8 also associates with the inv(16) fusion protein and other co-repressors to repress acute myeloid leukemia-1 (AML-1)-regulated genes (such as *p21*), which might result in abnormal hematopoietic cell proliferation, as seen in leukemia [32,56]. Furthermore, HDAC8 cooperates with the transcription factor differentiated embryo-chondrocyte expressed gene 1 (DEC1) to enhance TAp73 expression, thus differentially controlling this tumor suppressor [20]. HDAC8 also downregulates suppressor of cytokine signaling 1/3 (SOCS1/3) expression, altering Janus kinase 2/signal transducer and activator of transcription (JAK2/STAT) signaling; inhibition of HDAC8 led to SOCS1/3-dependent reduction in cell growth and clonogenic activity of hematopoietic cells derived from patients with myeloproliferative neoplasms [57,58]. Additionally, HDAC8 mediated regulation of Bcl-2-modifying factor (BMF) via cooperation with STAT3 has also been

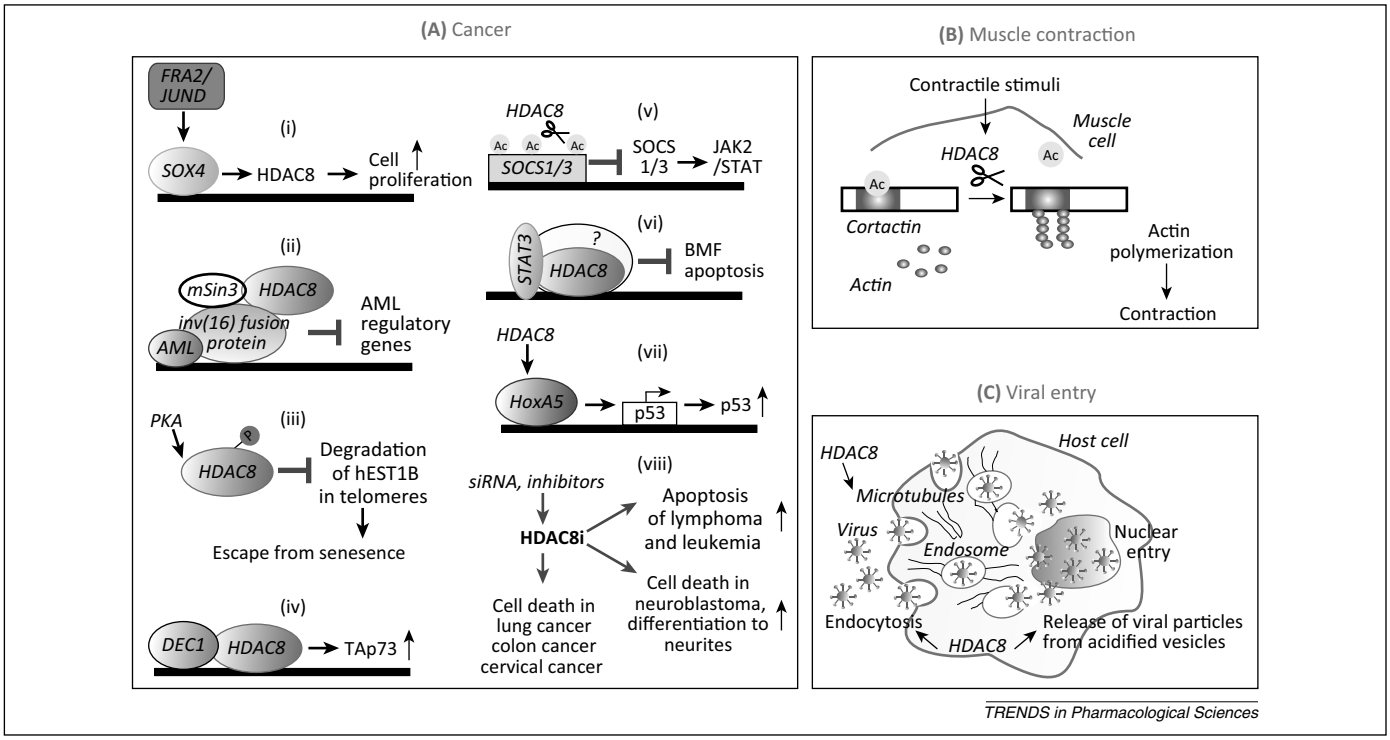

Figure 2. Overview of selected biological roles of histone deactylase 8 (HDAC8). Human HDAC8 is associated with: **(A)** cancer [19,20,23,31,32,44,49,55,57–59]; **(B)** muscle contraction [9]; and **(C)** entry of viruses [25]. (A) Representative molecular interactions of HDAC8 with crucial proteins in cancer. (i) Induction of HDAC8 expression by *SOX4*, a direct target gene of FRA2/JUND [49]. (ii) HDAC8 associates with co-repressors, such as mSin3A and, AML-1, represses AML regulatory genes, and affects hematopoietic cell proliferation [32]. (iii) Phosphorylated HDAC8 protects hEST1B from ubiquitin-mediated degradation affecting total telomerase activity. As a result, telomeres escape shortening and, consequently, cells escape senescence [55]. (iv) HDAC8 associates with DEC1 to upregulate Tap73 expression and control the balance of this tumor suppressor [20]. (v) HDAC8 represses SOCS1/3 expression, which in turn enhances JAK2/STAT expression and cell growth [58]. (vi) HDAC8 interacts with STAT3 to repress BMF transcription [59]. (vii) HDAC8 regulates *HOXA5* gene transcription to control p53 expression [19]. (viii) Inhibition of HDAC8 by siRNA or inhibitors leads to cell death, apoptosis, and differentiation of cells. (B) Upon contractile stimulation, HDAC8 deacetylates cortactin to promote actin polymerization and smooth muscle contraction [9]. (C) HDAC8 is associated with enhanced endocytosis of late-penetrating viruses by controlling acidification of vesicles and microtubule integrity in host cells, concurrent with increased infection [25]. Abbreviations: HDAC8i, HDAC8 knocked down or inhibited by inhibitors; PKA, protein kinase A. For additional definitions, please see the main text.

reported [59] (Figure 2). BMF has an important function in the execution of apoptosis triggered by the metabolite methylselenopyruvate, which is an inhibitor of HDAC8 [60]. Another important mechanistic pathway demonstrates a link between HDAC8 and the tumor suppressor p53 [19,31] (Figure 2). Depletion of HDAC8 decreased homeobox A5 (HoxA5)-dependent expression of wild type (WT) and mutant p53 [19]. HDAC8 is required for the expression of p53, regardless of whether it is WT or mutated. However, depletion or inhibition of HDAC8 only affects proliferation of those cells harboring a p53 mutation. This suggests that HDAC8 inhibitors are reasonable as an adjuvant for tumors carrying a mutant p53. Interestingly, although co-localization studies with ChIP [20] or overexpression and/or siRNA knockdowns showed association of these proteins with HDAC8, it remains unresolved whether they are direct deacetylation targets. It is tempting to speculate that HDAC8 provides a scaffolding platform for signaling complexes that ferry inside the nucleus to act on specific loci of DNA, affecting expression of particular genes critical to cancer.

Schistosomiasis

Besides cancer, HDAC8 has been lately discovered as an important target to fight the 'neglected' parasitic disease schistosomiasis [24] (Figure 3). This is a parasitic infection caused by *Schistosoma* flatworms that affects over 200 million people [61]. Severe symptoms stem from the reaction of the body to chronic exposure to rejected eggs [62]. Control depends on the administration of a single drug, praziquantel, which poses a threat of resistance [63,64]. Moreover, praziquantel is ineffective towards immature worms, thus enabling the persistence of the infection [64], which adds to the need of new approaches in drug treatment.

The sequencing and annotation of the genomes of parasites has allowed new approaches to drug development, including the targeting of epigenetic processes. This is especially true for HDACs, which, as already mentioned, have been exploited for drug development in other pathologies, notably cancer [65,66]. Death of schistosome larvae and adult worms in culture via the induction of apoptosis after treatment with trichostatin A (TSA), a pan-inhibitor of HDACs, indicated HDAC targeting as a possible strategy in schistosomes for drug development [67].

Schistosomes have three class I and three class II HDACs [68], among which HDAC8 (smHDAC8 in *Schistosoma mansoni*) is the least conserved compared with its human ortholog, exhibiting amino acid substitutions and insertions in the catalytic pocket [68,69]. smHDAC8 is the most abundant class I transcript, being expressed at high levels during almost all stages of the life cycle [69]. Transcript knockdown of smHDAC8 using RNAi in invasive

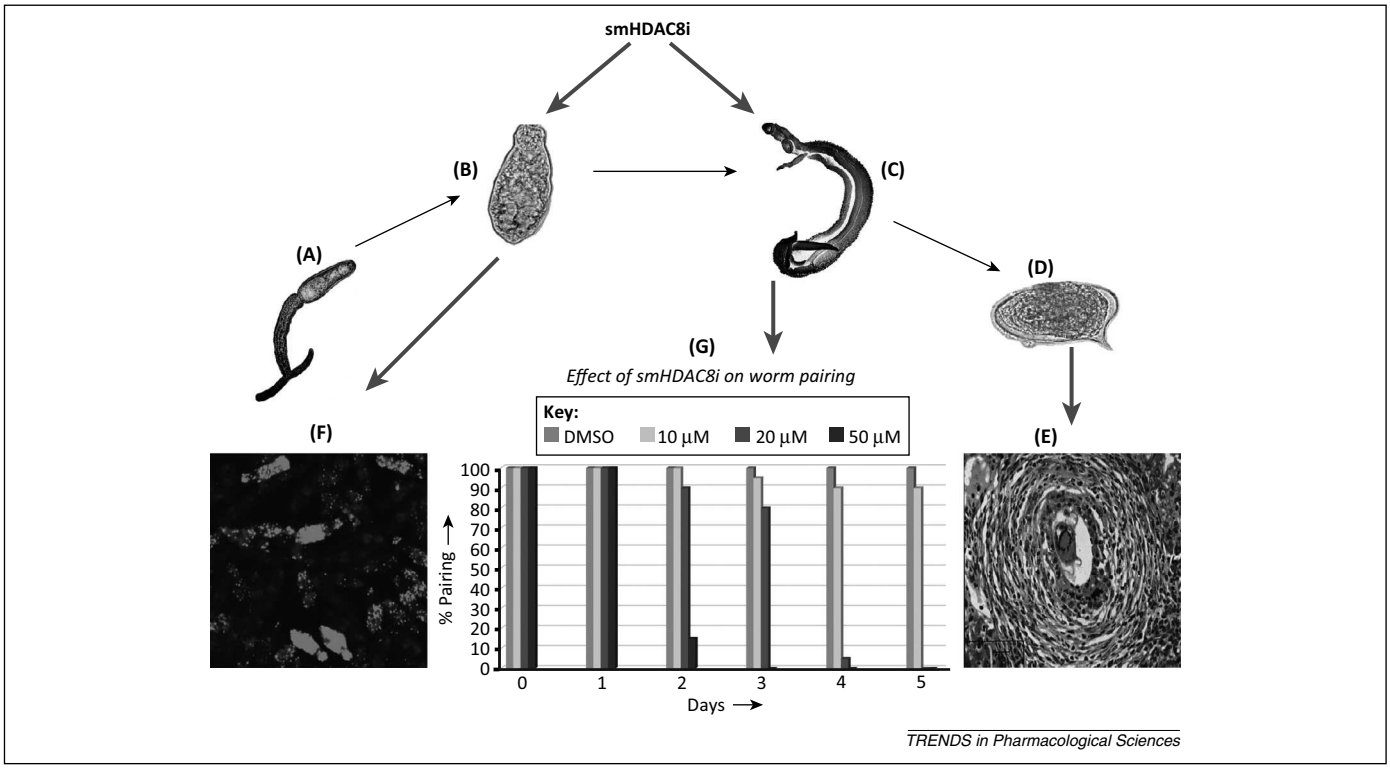

Figure 3. Schistosome targets of *Schistosoma mansoni* histone deactylase 8 (smHDAC8) inhibitors. Human hosts are infected by free-living cercariae **(A)**, which lose their tails to become schistosomula **(B)**. These larvae migrate within the host bloodstream, mature in the hepatoportal system, and form pairs of male and female worms **(C)**, which then localize to the mesenteric veins (in the case of *S. mansoni*), where female worms lay hundreds of eggs **(D)** daily. A proportion of these eggs circulates and becomes trapped in the capillary system, particularly in the liver, where they provoke an inflammatory granulomatous reaction **(E)**, responsible for disease pathology. The administration of smHDAC8 inhibitors (smHDAC8i) in culture affects both schistosomula, causing apoptosis and death [**(F)** pink TUNEL staining], and adult worms, causing marked changes in the reproductive organs, separation of worm pairs **(G)** and arrest of egg laying [24]. Life-cycle stages in the fresh-water snail intermediate host are not shown.

schistosome larvae (schistosomula) significantly reduced the recovery of adult worms and eggs from infected mice, and chemical inhibition induced unpairing of adult worms, demonstrating the validity of the target [24] (Figure 3).

Other diseases

Viral infections. A prominent role of HDAC8 has been deciphered recently in Influenza A and Uukuniemi virus entry [25]. These late-penetrating viruses use endocytosis to enter host cells, and this is followed by aci-activated penetration from late endosomes [25,70]. HDAC8 (and HDAC3) is associated *in vitro* with enhanced endocytosis, acidification, and penetration of these viruses (Figure 2), while HDAC1 produces the opposite results. Downregulation of HDAC8/HDAC3 by siRNA showed a reduction in viral infectivity [25]. This involved the pathway from early endosomes to lysosomes, resulting in ineffective endocytosis and insufficient acid conversion of the viruses [25]. Depletion of HDAC8 evokes rearrangement of the microtubule network, causing centrosome splitting, loss of centrosome-associated microtubules, and disorientation of centripetal movements of late endosomes and/or lysosomes. Thus, HDAC8 is critical to centrosome architecture and cohesion that controls endosome mobility. However, it remains obscure whether deacetylation of the centrosome itself, of microtubules, or of regulators of centrosome cohesion influences viral entry.

CdLS. HDAC8 is one of the causative genes in CdLS, (a rare genetic disease causing congenital malformations) and has been identified as the SMC3 deacetylase [14,71,72]. SMC3 is a cohesin subunit that binds sister chromatids together [73]. HDAC8 deacetylates chromatin-released acetylated SMC3 in early mitosis, facilitating cohesin recycling for subsequent cell cycles [14]. Lack of HDAC8 activity induces an accumulation of acetylated cohesins with reduced affinity towards chromatids and subsequently leads to defective transcription with clinical symptoms as seen in CdLS [14]. Loss-of-function HDAC8 mutations, mainly in conserved residues [74], are predominantly missense, occur mostly *de novo*, are associated with severely skewed X inactivation, and show strong loss of activity [16]. One hundred percent inactive mutants demonstrate major structural changes within the catalytic active site or in the surrounding residues [16]. It has been proposed that the loss of HDAC8 activity correlates with the proximity of the mutated residues to the active site [74]. The activity of most mutants is restored by a specific activator of HDAC8, *N*-(phenylcarbamothioyl)benzamide, except where the mutation is closest to the active site, tempting the authors to speculate that the activator might stabilize the enzyme conformation for substrate binding, which is not possible when the mutation is close to the active site [74].

Structural aspects and inhibitors of HDAC8

Selective HDAC8 inhibitors (HDAC8i) with characterization in advanced preclinical models are still scarce. Examples are several benzhydroxamates (Figure 4A, Table 1), such as the 'linkerless' inhibitor **1**, compound **2**, the 12-membered macrocycle-based phenyl hydroxamate **3**, and triazole-containing compounds, such as NCC149 (**5**) and **6** [75–78]. Triazoles such as **5** or **6** induced hyperacetylated cohesin in HeLa cells and suppressed growth of T cell lymphoma cells. *Ortho*-aryl *N*-hydroxycinnamides, such as **4**, are one of the most potent nanomolar and selective HDAC8i *in vitro* (Table 1) and showed

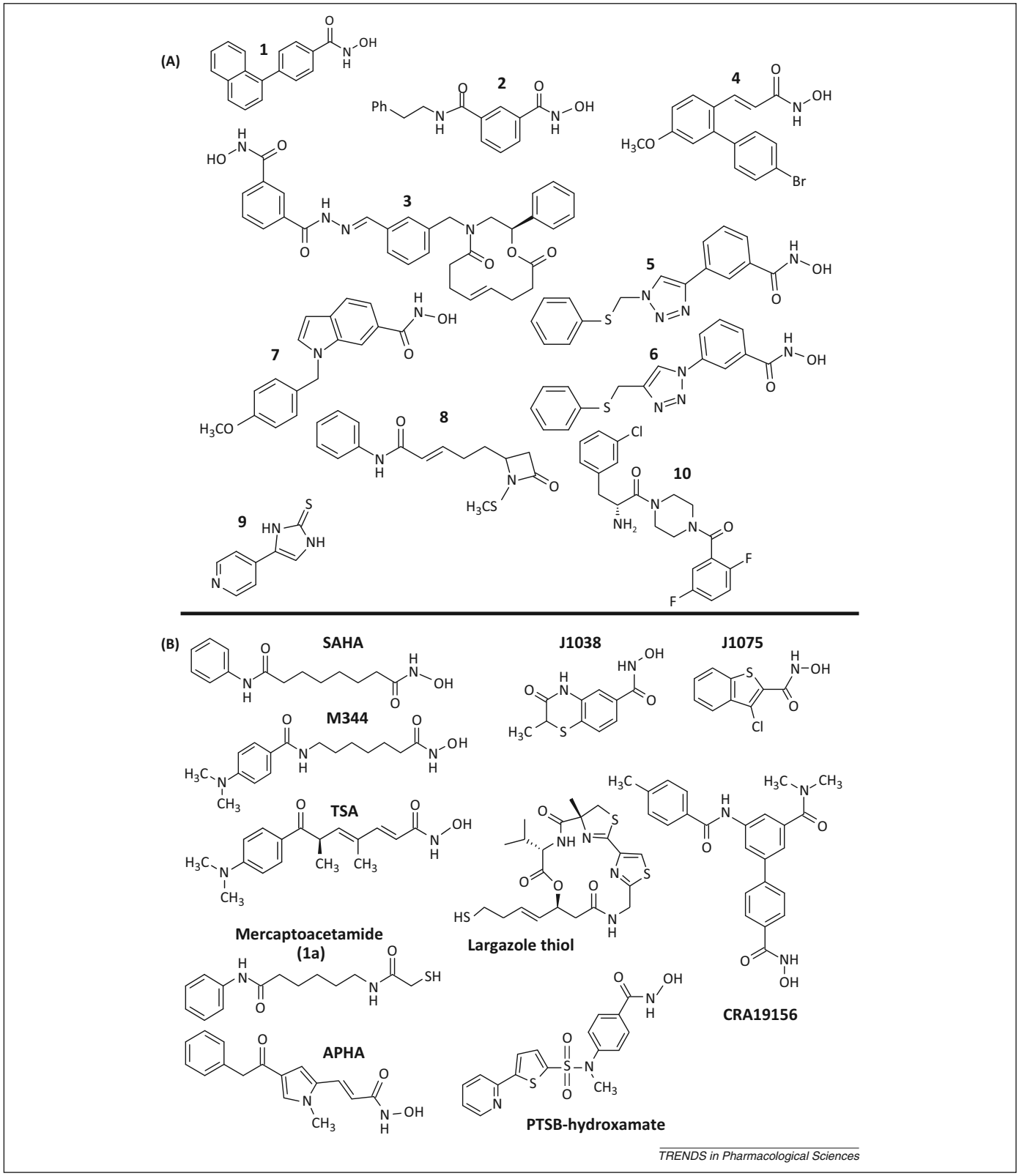

Figure 4. Inhibitors of histone deactylase 8 (HDAC8). **(A)** Specific HDAC8 inhibitors [23,75–82]. **(B)** Inhibitors co-crystalized with HDAC8 [11,24,39,45,74,82,87,96–98].

Table 1. IC_{50} values of HDAC8i against class I and class II HDACs[a]

Compound	Class I HDACs					Class II HDACs		Refs
	HDAC1	HDAC2	HDAC3	HDAC8	smHDAC8	HDAC4	HDAC6	
1	>100 μM	–	–	0.3 μM	–	–	55 μM	[75]
2	12 μM	9 μM	23 μM	0.12 μM	–	>33 μM	0.036 μM	[76]
3	–	3.6 μM	15 μM	0.023 μM	–	––	–	[77]
4	4.5 μM	>20 μM	4.8 μM	5.7 nM	–	>20 μM	>20 μM	[79]
5	38 μM	>100 μM	68 μM	0.070 μM	–	44 μM	2.4 μM	[78]
6	>100 μM	>100 μM	>100 μM	0.053 μM	–	>100 μM	2.2 μM	[78]
7	4 μM	>50 μM	>50 μM	0.01 μM	–	–	2.9 μM	[23]
8	>1000 μM	>1000 μM	>1000 μM	4.53 μM	–	>1000 μM	>1000 μM	[80]
9	ni[a]	–	ni[a]	0.5 μM	–	–	–	[81]
10	>30 μM	>30 μM	–	200 nM	–	–	>30 μM	[82]
J1038	23.6 μM	–	12.3 μM	0.97 μM	1.48 μM	–	3.5 μM	[24]
J1075	19.4 μM	–	1.9 μM	3.1 μM	4.32 μM	–	37.4 μM	[24]
(1a)	–	–	–	200 μM	50 μM	–	–	[87]
SAHA	117 nM	–	123.8 nM	0.4 μM	1.6 μM	–	72.9 nM	[24,87]
M344	43.1 **nM**	–	17.62 **nM**	2.9 μM	2.4 μM	–	81.6 **nM**	[24,87]

[a]Abbreviations: ni, no inhibition; –, values not reported.

antiproliferative effects towards several human lung cancer cell lines [79]. The indole PCI34051 (**7**) is also a nanomolar and highly selective inhibitor (> 200-fold compared with other HDACs). It exhibited limited cellular activity in T cell leukemia cells and no activity in other tumor cells [23,79] (Figure 4A). Other chemical classes include the azetidinone **8**, the cyclic thiourea SB-379278A (**9**), and the amino acid derivative **10** [80–82]. Compounds **1**, **8**, and **9** show moderate HDAC8 selectivity (not tested for all HDAC isoforms) and inhibitory activity within the micromolar range (Table 1). The benzhydroxamate **2** is a nanomolar HDAC8i but inhibits HDAC6 more potently. Compounds **3** and **10** were reported as nanomolar HDAC8i. HDAC8 exhibits weaker binding affinities for known pan-HDAC inhibitors, such as suberoylanilide hydroxamic acid (SAHA) and TSA (Figure 4B).

Clinically, isoform-specific or selective HDAC8i are likely to be relevant in treatment of T cell leukemia and in neuroblastoma, where HDAC8 is the only correlated HDAC. It remains to be seen whether inhibition of one HDAC isoform can minimize adverse effects (such as diarrhea, and hematological and cardiac toxicities) as seen in patients treated with pan-HDAC inhibitors, such as SAHA. However, the functional redundancy of the other class I isoforms or shared targets might mask the selective profile of such inhibitors. For example, while PCI-34051 seems to have already excellent potency and selectivity, further selective HDAC8 inhibitors with different scaffolds are necessary for advanced preclinical evaluation. This is necessary to distinguish between HDAC8-related-specific versus compound-specific efficacy and safety. Further potent and selective inhibitors would also clarify whether the inhibition profiles obtained with pure HDAC isotypes are mirrored *in vivo* using distinct hyperacetylation substrates or mass spectrometric evaluation. It has been shown that IC_{50} values of HDAC inhibitors differ if one uses either recombinant subtypes or HDACs in a more native setup (e.g., complexes) [83].

HDAC8 has high flexibility and, hence, is able to accommodate a range of diverse inhibitors, exemplified by the 29 X-ray human HDAC8 structures in the Protein Data Bank (PDB, http://www.wwpdb.org/) (Figure 4B). In addition to acetyl-lysine-containing peptidic substrates, a variety of pan-HDACi have been co-crystallized with HDAC8, including SAHA, TSA, M344, CRA19156, aroyl pyrrole hydroxy amide (APHA), PTSB-hydroxamate, and largazole (Figure 4B, Table 2). The structures show the same architecture as other HDAC members (for a detailed review, see [84,85]) (Figure 5A,B). The zinc ion is coordinated by D178, D267, and H180. The remaining two sites are occupied by zinc-binding groups of the inhibitors or the inhibitor and one water molecule. The active site tunnel comprises lipophilic amino acids. Notably, two phenylalanines (F152 and F208) contribute strongly to formation of the tunnel. The rim of the tunnel is formed by several loop regions (named L1–L4) of variable sizes and conformations that differ among HDAC isoforms. Analysis of the HDAC8 structures revealed several conformations of the L1 (from S30 to K36) and L2 (P91 to T105) loops (Figure 5A). Both are not always resolved in the X-ray structures, indicating an increased flexibility. L1 has been shown to adopt two different conformations (colored blue and magenta in Figure 5A), which dramatically change the size of the binding pocket. Given the flexibility of L1 and L2, the crystallized inhibitors TSA, APHA, and PTSB-hydroxamate occupy different areas at the rim of the active site.

Recently, the structure of smHDAC8 was solved, in apo and inhibited forms (Table 2). Using HDAC-biased chemical scaffolds, initial hits were quickly discovered for smHDAC8 [68,86]. The structure of smHDAC8 is similar to that of hHDAC8, but active-site differences were recognized in the primary structure [e.g., human methionine hM274 is a histidine (smH292) in *S. mansoni*].

First hits were the pan-HDAC inhibitors SAHA and M344 (Figure 4B) [24]. A structural change was observed, at least in the apo and SAHA-bound smHDAC8 structures, in the side chain of a conserved active-site phenylalanine

Table 2. Inhibitors or substrates co-crystallized with HDAC8

PDB ID	HDAC8	Inhibitor	Substrate	Form	Res.	Year	Refs
1T64	Human	TSA		WT	1.90	2004	[11]
1T67	Human	M344		WT	2.31	2004	[11]
1T69	Human	SAHA		WT	2.91	2004	[11]
1VKG	Human	CRA19156		WT	2.20	2004	[11]
1W22	Human	PTSB-hydroxamate		WT	2.50	2004	[45]
2V5W	Human	–	Acetylpeptide	Y306F	2.00	2007	[39]
2V5X	Human	Hydroxamate		S39D	2.25	2007	[39]
3EW8	Human	M344		D101L	1.80	2008	[96]
3EWF	Human	–	Acetylpeptide	H143A	2.50	2008	[96]
3EZP	Human	M344		D101N	2.65	2008	[96]
3EZT	Human	M344		D101E	2.85	2008	[96]
3FO6	Human	M344		D101A	2.55	2008	[96]
3F07	Human	APHA		WT	3.30	2008	[96]
3F0R	Human	TSA		WT	2.54	2008	[96]
3MZ3	Human	M344		WT/Co^{2+}	3.20	2010	[97]
3MZ4	Human	M344		D101L/Mn^{2+}	1.85	2010	[97]
3MZ6	human	M344		D101L/Fe^{2+}	2.00	2010	[97]
3MZ7	human	M344		D101L/Co^{2+}	1.90	2010	[97]
3SFF	human	Amino acid derivative		WT	2.00	2011	[82]
3SFH	human	Amino acid derivative		WT	2.70	2011	[82]
3RQD	human	Largazole thiol		WT	2.14	2011	[98]
4BZ5	*S. mansoni*	–		WT apo[a]	1.78	2013	[24]
4BZ6	*S. mansoni*	SAHA		WT	2.00	2013	[24]
4BZ7	*S. mansoni*	M344		WT	1.65	2013	[24]
4BZ8	*S. mansoni*	J1038		WT	2.21	2013	[24]
4BZ9	*S. mansoni*	J1075		WT	2.00	2013	[24]
4CQF	*S. mansoni*	Mercaptoacetamide		WT	2.30	2014	[87]
4QA0	human	SAHA		C153F	2.24	2014	[74]
4QA1	human	M344		A188T	1.92	2014	[74]
4QA2	human	SAHA		I243N	2.38	2014	[74]
4QA3	Human	TSA		T311M	2.88	2014	[74]
4QA4	human	M344		H344R	1.98	2014	[74]
4QA5	human	–	Acetylpeptide	A188T/Y306F	1.76	2014	[74]
4QA6	human	–	Acetylpeptide	I243N/Y306F	2.05	2014	[74]
4QA7	human	–	Acetylpeptide	H334R/Y306F	2.31	2014	[74]

[a]Apo-structure without inhibitor, listed for comparison.

(hF152, smF151). The smF151 side chain is inserted in a hydrophobic pocket, away from the active site of the enzyme (flipped-out conformation) (Figure 5C). By contrast, in hHDAC8 (and in all other hHDACs whose structure has been solved), this residue is locked in a conformation (flipped-in), where it participates in the formation of the active-site pocket [24]. This structural difference is due to sequence and structural changes observed in loops surrounding the HDAC8 active site. Binding of M344 to smHDAC8 induced a flipping-in of smF151, showing that the side chain of this phenylalanine can freely change the conformation even if the flipped-out conformation appears preferred [24].

Virtual screening using these structural data enabled the identification of new linker-less, hydroxamate-based inhibitors of smHDAC8. Among them, J1038 and J1075 (Figure 4B) led to structural data in complex with smHDAC8 [24]. The two inhibitors used different specific features of the smHDAC8 active site (Figure 5D,E). J1038 not only interacts specifically through a salt bridge with the side chain of smH292, but also induces a flipping-in of smF151 (Figure 5D). By contrast, J1075 does not interact with smH292, but forces smF151 into its flipped-out conformation (Figure 5E). Also, a tyrosine residue, smY341, attains an intermediate conformation between those observed in the apo and inhibitor-bound forms of smHDAC8, thus preventing this residue interacting with the hydroxamate group of the inhibitor. Despite the bulkiness of these inhibitors, the IC_{50} values were similar for both smHDAC8 and hHDAC8 (Table 1), whereas pan-HDAC inhibitors, such as SAHA and M344, are more potent on other hHDACs (200–500 times on hHDAC1, 100–150 times on hHDAC3, and 50–200 times on hHDAC6; Table 1) [24].

In a second study, an inhibitor bearing a mercaptoacetamide (1a) warhead (Figure 4B) showed specificity for smHDAC8 over hHDAC8 (Table 1) [87]. The structure in complex with smHDAC8 revealed a specific binding mode of its warhead to the catalytic zinc and a flipping-in of the smF151 side chain, whereas smH292 was not involved in inhibitor binding (Figure 5F) [87]. Both linkerless hydroxamates and mercaptoacetamide induced apoptosis in cultured worms.

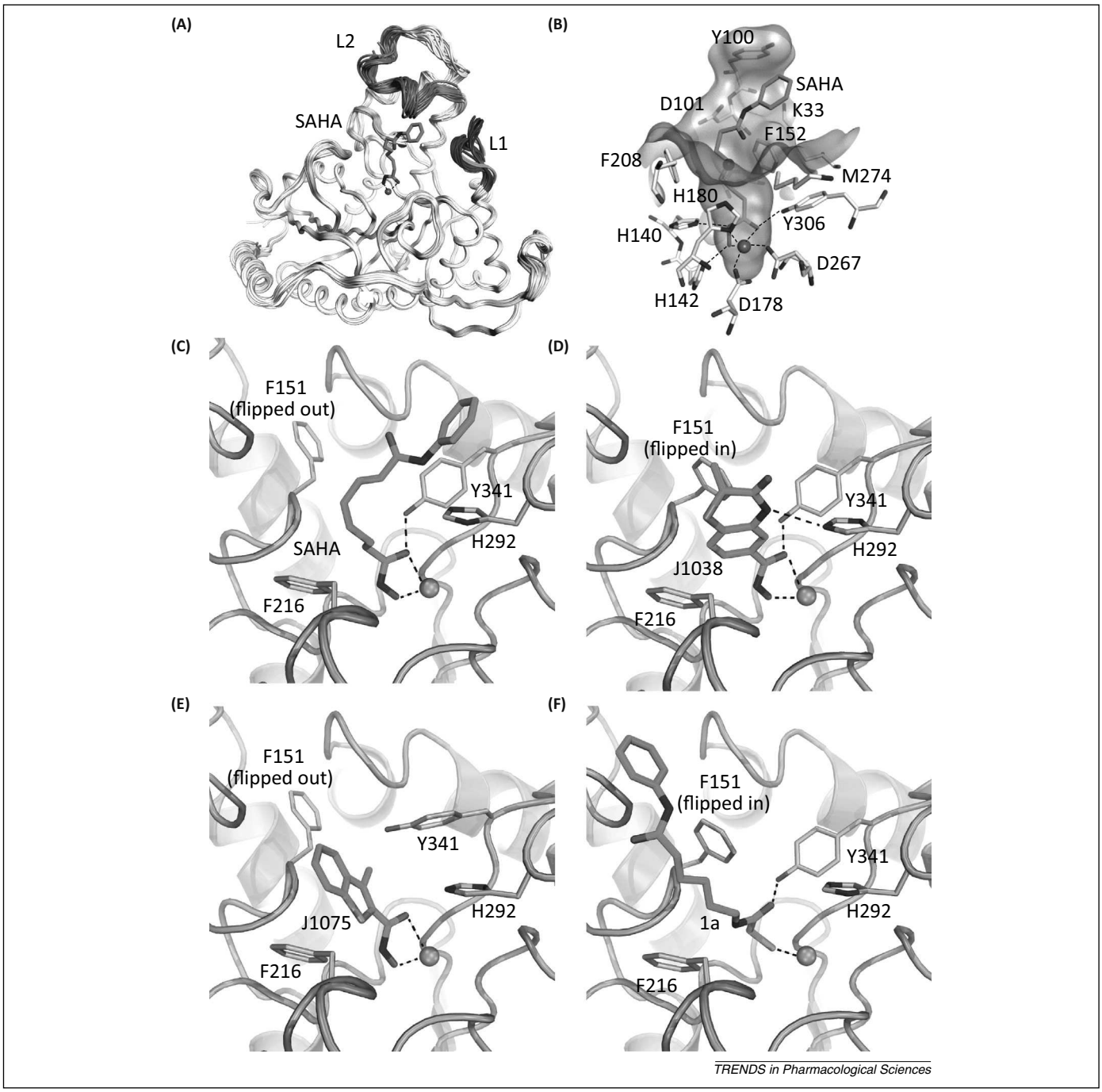

Figure 5. Structural specificities for inhibition of human and *Schistosoma mansoni* histone deactylase 8 (HDAC8). **(A)** Superimposition of all human HDAC8 structures from the Protein Data Bank (PDB). Only the protein backbone is shown as a white ribbon. The two main clusters of the flexible loop L1 are colored dark blue and magenta, respectively. In cases where the loop L1 is not fully resolved in the X-ray structures, it is colored cyan. Flexible loop L2 is colored orange. **(B)** Interaction of the inhibitor SAHA (green) at the HDAC8-binding site (PDB ID 4QA2). The molecular surface of the binding pocket is displayed. Hydrogen bonds and metal interactions are shown as broken lines. **(C–F)** Close-up view of the active site of *S. mansoni* HDAC8 (smHDAC8) with bound inhibitors: suberoylanilide hydroxamic acid (SAHA) (C), J1038 (D), J1075 (E), and 1a (mercaptoacetamide) (F) [24,87]. The secondary structure elements of smHDAC8 are shown as light-brown ribbons. Important smHDAC8 active site side chains and inhibitors are shown as sticks and are labeled. Carbons are shown in gray (smHDAC8) and green (inhibitors), nitrogens in blue, and oxygens in red. Hydrogen and coordination bonds are shown as broken lines.

Concluding remarks

HDAC8 has emerged as an attractive target for isotype-selective drug development in a variety of diseases, such as cancer, parasitic and viral infections, as well as neurodegenerative diseases. Despite promising initial target validation and pharmacological intervention studies, many questions remain. New nonhistone substrates are still being discovered [88], and the function of HDAC8 in normal physiology has to be defined further. A recent example is a role of HDAC8 in bone differentiation [84], which may also open up a new therapeutic area. Data on different subcellular locations (nuclear versus cytoplasmic) of HDAC8 [43,89] implicate a context-dependent action of its inhibitors and may help to define specific disease subsets, such as in cancer, for treatment.

It has been shown that HDAC8 can be targeted selectively and available HDAC8–inhibitor structures will aid future development immensely. However, the demonstrated flexibility of HDAC8 may yet make such approaches difficult, especially in the design of species-selective inhibitors. For selectivity on the human isotype versus smHDAC8, differences in the active site (see above) pave a clear path towards selective inhibitors; however, for use in humans, the lack of structural data especially on HDAC6 makes rational optimization of selectivity towards that subtype difficult. HDAC inhibitors have already shown promise for treatment of apicomplexan diseases, such as malaria (caused by *Plasmodium* spp.) or trypanosomiasis [90–92]. Structure-based studies on smHDAC8 inhibitors may be considered a model approach for species-selective inhibitors in such diseases [24,87] and may also extend to, for example, fungi [93]. For antiviral therapy, preclinical tests of specific HDAC8 inhibitors will provide valuable insight into its druggability in this context. For infectious diseases, the role of HDAC8 in the immune response by repressing interleukin β gene expression [94] or in the toxin-induced resistance of macrophages [95], has to be considered.

Thus, HDAC8 remains not only a multifaceted and challenging, but also a promising target for pharmacological intervention.

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