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An Overview of Naturally Occurring Histone Deacetylase Inhibitors

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Abstract

Histone deacetylases (HDACs) have recently emerged as key elements in epigenetic control of gene expression. Due to the implication of HDACs in a variety of diseases ranging from cancer to neurodegenerative disorder, HDAC inhibitors have received increased attention in recent years. Over the last few decades, a myriad of HDAC inhibitors containing a wide variety of structural features have been identified from natural sources. Here, we review the discovery, synthesis, biological properties, and modes of action of these naturally occurring HDAC inhibitors and consider their implications for future research.

Keywords

Epigenetics; histone deacetylase inhibitor; histone modification; isoform selectivity; natural product

1. Introduction

In the nucleus, DNA is highly ordered and tightly compacted in protein-DNA complexes known as chromatin [1]. The most fundamental subunit of chromatin, a nucleosome, consists of an octamer of four histone cores (H2A, H2B, H3, and H4) surrounded by 146 base pairs of DNA. The packing of DNA in this chromatin architecture plays an important role in gene expression. Epigenetics are the changes in gene expression that are not caused by variations in the DNA sequence, but rather by enzyme-mediated chemical modifications. These modifications cause fluctuations in the chromatin architecture [2]. Epigenetic factors have been implicated in a variety of normal physiological functions as well as diseases such as cancer, immunodeficiency, and various learning disabilities [3]. Epigenetic mechanisms are comprised of two major categories: DNA methylation and histone modification [2].

DNA methylation is mediated by DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b) [4]. DNMTs work through the transfer of a methyl group from *S*-adenosylmethionine (SAM) to the 5' position of a cytosine ring in CpG dinucleotides.

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Conflict of Interest

The author(s) confirm that this article content has no conflicts of interest.

Hypermethylated CpG regions in concert with histone modifications create heterochromatin states that are inaccessible to transcriptional factors (Fig. 1).

There are a variety of post-translational histone modifications, of which histone acetylation is one of the most studied. Histone acetylation is controlled by histone acetyl transferases (HATs) and histone deacetylases (HDACs) [5, 6]. HATs (MYST, GNAT, and p300/CBP) use acetyl-CoA as a cofactor to transfer acetyl groups to the ϵ -amino group of lysine residues on histone tails. This masks the positive charge of the lysine residue, reduces the interaction between histones and the negatively charged DNA backbone, and consequently increases gene transcription. HDACs remove the acetyl groups, restore the positive charge on the lysine residues, which increases interaction with the negatively charged DNA backbone, and represses gene expression (Fig. 2).

Another well characterized histone modification is the methylation of histone tails. Histone methylation can occur on lysine or arginine residues on N-terminal histone tails by SAM-dependent histone lysine methyl transferases (HKMTs) and arginine methyl transferases (PRMTs) [5]. HKMTs can mono-, di-, or trimethylate lysine residues and are very specific when it comes to their substrate and methylation degree. PRMTs are broken down into two types. Type I PRMTs are able to monomethylate or asymmetrically dimethylate an arginine residue, while type II PRMTs monomethylate or symmetrically dimethylate an arginine residue. Several lysine demethylases have been reported (such as LSD1 and JMJD2). These enzymes also have a high specificity for substrates and demethylation degree.

While histone acetylation and methylation are most widely documented, there are a variety of other reported modifications. Histone phosphorylation is regulated by kinases and phosphatases that predominantly add and remove phosphate groups from serine, threonine, and tyrosine residues on N-terminal histone tails [7]. Histone ubiquitination on lysine residues is controlled by the sequential action of three enzymes (E1, E2, E3) and removed by isopeptidase [8]. Other histone modifications include arginine citrullination, biotinylation, ribosylation, sumoylation, crotonylation, histone tail clipping, and proline isomerization [5, 9, 10].

Among these post-translational histone modifications (Fig. 3), the acetylation of histone tails has been examined extensively. HDACs specifically have garnered considerable attention from the scientific community due to their implication in early stages of tumorigenesis and cancer progression [11]. Therefore, this review will further investigate HDACs in more detail.

2. The History of HDACs

The beginning of HDAC research dates back to 1964, when Allfrey and co-workers reported a link between the acetylation state of histones and RNA synthesis [13]. Five years later, Fujimoto and co-workers postulated the existence of an unknown enzyme responsible for the deacetylation of histones in calf thymus extracts [14]. However, it was not until 1996 that the first HDAC would be discovered [15, 16]. An affinity matrix based on trapoxin B, a covalent HDAC inhibitor, was used to isolate a 46 kD protein responsible for the removal of the

acetyl groups from lysine residues. Sequencing of this protein revealed a close relationship to a yeast transcriptional regulator encoded by the RPD3 gene. The discovery of HDAC1 (also known as HD1) marked the first time a mammalian HDAC was cloned and characterized. Since then a total of 18 HDAC isoforms have been discovered [17, 18].

These isoforms are broken down into three different classes according to their sequence homology to yeast transcriptional regulators. Class I HDACs, consisting of HDACs 1, 2, 3, and 8, are related to the RPD3 gene. HDACs 4, 5, 6, 7, 9, and 10 are part of class II HDACs which are related to the HDA1 gene [17, 18]. Among them, HDACs 6 and 10 are subcategorized as class IIb because of their second catalytic domain [19, 20]. HDAC11 was originally classified as a class I HDAC, but was recently placed in its own class because of low sequence homology to other HDACs [18, 21]. These 11 HDACs all contain a Zn^{2+} in their active sites and are known as the zinc-dependent “classical” HDACs. The last class of HDACs, known as class III or sirtuins, is related to the yeast gene Sir2. These HDACs are fundamentally different in that they are NAD^+ -dependent proteins and are not inhibited by classical HDAC inhibitors [17]. These sirtuins are outside the scope of this review and will not be discussed.

Classical HDACs share a highly conserved active site [17, 18, 22]. Crystal structures of HDACs 1, 4, 7, and 8 reveal an active site that is shaped like an 11 Å-long channel made up of hydrophobic and aromatic residues [22–26]. At the bottom of this channel a catalytic triad consisting of a histidine residue and two aspartic acid residues keeps the Zn^{2+} in place. Acetylated lysines insert themselves into this channel and coordinate to the Zn^{2+} to catalyze the removal of the acetyl group by H_2O (Fig. 4). The high degree of sequence similarity suggests that other HDACs function by the same mechanism [17, 18, 22].

While the majority of HDACs have a single active site, HDACs 6 and 10 contain two catalytic domains, setting them apart from other class II HDACs [18–20]. However, unlike HDAC6, which has two functional active sites, HDAC10 only has a partial C-terminal domain that lacks key residues needed for enzymatic activity.

HDACs are ubiquitously expressed in all tissues. In order to perform their deacetylase function on histones, HDACs must be localized in the nucleus. Class I HDACs are almost exclusively found in the nucleus [18]. The remaining HDACs are found both in the nucleus and cytoplasm of the cell. Nuclear localization/export signals aid in the transport of these HDACs into and out of the nucleus.

The regulation of gene expression by HDACs has been examined closely for several decades. The implications of these enzymes in various diseases have only increased the interest in the regulation of their enzymatic function. This has led to the discovery and development of numerous HDAC inhibitors [27, 28]. Due to their broad chemical diversity and high target affinity and specificity, natural products have historically been a major source for many bioactive compounds [29]. This has been especially apparent for HDAC inhibitors. There have been a few reviews covering natural product HDAC inhibitors from their discovery to the synthesis of various analogues [27, 28]. Here, our main focus will be to provide a comprehensive resource by reviewing the discovery, synthesis, biological

properties, and mode of action of naturally occurring HDAC inhibitors of all classes, including recently discovered natural products not covered in previous reviews.

3. HDAC inhibitors

3.1. Linear inhibitors

3.1.1. Trichostatin A (TSA)—Due to advances in the field of epigenetics and further understanding of the role of HDACs in various disease states, numerous synthetic and natural product HDAC inhibitors have been studied in the last few decades. Trichostatin A (TSA, **1**, Scheme 1), isolated from *Streptomyces hygroscopicus* bacteria, was the first natural product HDAC inhibitor [30, 31]. It consists of an aromatic cap group, a conjugated diene linker region, and a hydroxamic acid tail.

Krebs/Fleming and co-workers reported the first racemic synthesis of TSA (**1**, Scheme 1) [32]. The synthesis proceeded through a Mukaiyama aldol reaction followed by a Wittig reaction. The resulting intermediate **5** was subjected to DDQ oxidation and subsequent treatment with hydroxylamine to complete the synthesis of ($\pm$)-**1** in 22% overall yield from known aldehyde **2** [32]. Since the original racemic synthesis, several enantioselective syntheses of TSA (**1**) have also been reported [33, 34].

Initial interest in TSA (**1**) came from its antifungal activity [30]. It was not until 1990 that TSA (**1**) was found to induce the accumulation of highly acetylated histones *in vitro*, suggesting the inhibition of HDAC activity [31]. It was also found that while natural TSA (**1**) is a very potent HDAC inhibitor, the synthetic *ent*-TSA does not show the same ability. In addition to HDAC inhibition, TSA (**1**) has been shown to induce cell differentiation in Friend leukemia cells and inhibit cell proliferation *in vivo* in rat mammary carcinoma models [35, 36].

TSA (**1**) continues to be particularly important in the development of HDAC inhibitors due to its crystal structure in the active site of the *A. aeolicus* HDAC homologue (Fig. 5) [22]. The crystal structure showed that TSA (**1**) non-covalently binds to the active site of HDACs. The terminal hydroxamic acid group is inserted into the active site and chelates the Zn²⁺ in a bidentate fashion. As a consequence, TSA (**1**) blocks the channel from its natural substrate.

The crystal structure also played a critical role in correcting the kinetic model of the HDAC inhibition of TSA (**1**). Previous kinetic data had predicted TSA (**1**) to be a non-competitive inhibitor of HDACs [31]. However, the clear depiction of the inhibitor in the active site of the enzyme prompted Davie and co-workers to reexamine the enzyme kinetics. The results showed that TSA (**1**) is in fact a competitive inhibitor [37].

TSA (**1**) remains one of the most potent HDAC inhibitors today and is often used as a comparison for other HDAC inhibitors. However, the downside of TSA (**1**) is its lack of HDAC isoform selectivity [38].

3.1.2. Psammaplin A—A family of psammaplins was isolated from the marine sponge *Psammaplysilla* sp. off the coast of Tonga [39]. From this family of monobromotyrosine

derived oxime containing cystamines, psammaplin A (**7**, Fig. 6) showed the most potent cytotoxicity against human colon, lung, and ovarian carcinomas [40]. Later, it was established that psammaplin A (**7**) and other members of the psammaplin family are very potent class I-selective HDAC inhibitors [41]. Additionally, psammaplin A (**7**) inhibits only H3 deacetylation without affecting tubulin acetylation levels. It is generally accepted that tubulin deacetylation is handled by class II HDACs and the lack of hyperacetylated tubulin supports the class I-selectivity of psammaplin A (**7**) (Fig. 6). Psammaplin A (**7**) was also shown to inhibit angiogenesis and bacterial growth. Recently, Sufrin and co-workers reported a concise synthesis of psammaplin A (**7**) (Scheme 2) [42].

It has been hypothesized that psammaplin A (**7**) is a prodrug that can undergo glutathione (GSH)-dependent disulfide bond reduction to release two thiols (Scheme 2) [41]. These thiols are able to coordinate to the Zn^{2+} in the active site of HDAC and deny access to the natural substrate.

3.1.3. Depudecin—Depudecin (**20**, Scheme 3) is a fungal natural product isolated from *Alternaria brassicicola* RF-328 found in soil samples in Okinawa, Japan [43]. Later, it was also found as a fungal metabolite of *Nimbya scirpicola* in paddy field weeds [44]. Depudecin (**20**) consists of six asymmetric centers and a bis-*trans*-epoxide moiety. The first synthesis of depudecin (**20**) was reported by Schreiber and co-workers (Scheme 3) [45].

2-Trimethylsilylthiazole was coupled to aldehyde **11** to afford *anti*-alcohol **12** through one-carbon homologation. In order to epimerize the configuration of the hydroxyl group of **12**, a sequence of oxidation and reduction was employed. After TBDMS protection, the thiazole ring was cleaved by standard procedures involving *N*-methylation, NaBH_4 reduction, and Cu (II)-assisted hydrolysis to form aldehyde **14**. Subsequent condensation with ketophosphate **15**, $\text{NaBH}_4\text{--CeCl}_3$ reduction, and TBDMS deprotection yielded diol **17**. Treatment with 2,2,2-trichloroethyl chloroformate was followed by treatment with anhydrous methanolic hydrochloric acid to yield *bis*-cyclic carbonate **18**. Treatment of **18** with excess dimethylsulfide in the presence of benzoyl peroxide followed by basic hydrolysis of the carbonates gave bis-methylthiomethyl-protected tetraol **19**. Conversion of **19** to a diacetoxo dichloride followed by base-mediated epoxide formation furnished the bis-*trans* epoxide moiety. Lastly, mercuric chloride-mediated MTM deprotection completed the synthesis of depudecin (**20**) in 19 steps from known D-threose derivative **11** (1.6% overall yield).

Depudecin (**20**) showed the ability to revert *ras/src* transformed NIH3T3 fibroblast cells to their normal flat phenotype (Fig. 7) [46]. While the connection is not entirely clear, it has also been reported that depudecin (**20**) is able to inhibit HDAC activity. In addition, depudecin (**20**) has exhibited antiangiogenic activity both *in vitro* and *in vivo* [47]. Because of its relatively low potency against HDAC1 ($\text{IC}_{50} = 4.7 \mu\text{M}$) [46], only limited work has been done on this particular natural product. However, it is believed that depudecin (**20**) binds covalently to nucleophilic residues in the active site of HDACs. The isoform selectivity of depudecin (**20**) is currently unknown.

3.1.4. Santacruzamate A—Recently, santacruzamate A (**22**, Scheme 4) was isolated from a marine cyanobacterium that morphologically resembles the genus *Symploca*.

Structurally, it contains an ethyl carbamate moiety, a linker region, as well as an aromatic cap group. Along with its isolation, Balunas and co-workers reported the first and only synthesis of santacruzamate A (**22**) (Scheme 4) [48].

Santacruzamate A (**22**) showed differential cytotoxicity towards HCT-116 colon cancer cells ($GI_{50} = 29.4 \mu M$) and Hut-78 cutaneous T-cell lymphoma cells ($GI_{50} = 1.4 \mu M$) while being inactive against normal human dermal fibroblast cells. It also possesses extremely high HDAC2-selectivity over HDACs 4 and 6 (Table 1) [48].

3.1.5. Suberoylanilide Hydroxamic acid (SAHA)—Continued interest and efforts in the field of HDAC research have also culminated in numerous synthetic HDAC inhibitors headlined by suberoylanilide hydroxamic acid (SAHA or vorinostat, **24**, Scheme 5). Even though SAHA (**24**) is not a natural product, it is worth mentioning here because of its excellent potency and wide application as a benchmark in HDAC inhibition research. SAHA (**24**) is a linear HDAC inhibitor that is structurally and functionally very similar to TSA (**1**, Scheme 1). It was discovered in a small molecule screen for inducers of differentiation of murine erythroleukemia (MEL) cells [49, 50]. Like TSA (**1**), it consists of an aromatic cap group and a hydroxamic acid tail. However, instead of a conjugated diene linker, SAHA (**24**) has a completely saturated linker region. SAHA (**24**) inhibits HDAC activity by chelating the Zn^{2+} with a hydroxamic acid moiety in a bidentate mode. This is supported by the crystal structure of SAHA (**24**) in the active site of an HDAC1 homologue from the bacterium *A. aeolicus* (Fig. 8) [22].

SAHA (**24**) has shown excellent *in vivo* efficacy against CWR22 prostate cancer in mouse models with limited toxicity to the animals [52]. This ability to inhibit tumor growth *in vivo* has eventually led to FDA approval of SAHA (**24**) for the treatment of cutaneous T-cell lymphoma, despite its low isoform selectivity [38]. Currently, it is one of two FDA approved HDAC inhibitors and is marketed by Merck under the name Zolinza® [49].

3.2. Cyclic Tetrapeptides

3.2.1. HC toxin and Cyl-1/2—HC toxin (**25**, Fig. 9) is the first cyclic tetrapeptide HDAC inhibitor isolated from natural sources [53]. It was isolated from the fungal plant pathogen *Helminthosporium carbonum*, which is known to cause corn leaf spot disease [54]. However, it was not until 1983 that the structure of HC toxin (**25**) was elucidated [55]. It consists of D-proline, D-alanine, L-alanine, and (2*S*)-amino-8-oxo-9,10-epoxydecanoic acid (Aoe). HC toxin (**25**) has been shown to inhibit HDAC activity in different organisms, including yeast, *Physarum* (mold), chicken, and maize [54]. However, the potency of HC toxin (**25**) is about an order of magnitude weaker than TSA (**1**, Scheme 1) [56]. This lack of potency along with the emergence of more promising cyclic tetrapeptide HDAC inhibitors has limited the investigation of HC toxin (**25**). Nevertheless, this first cyclic tetrapeptide HDAC inhibitor has served as a benchmark for future compounds of the same class.

Isolated from culture filtrates of the fungus *Cylindrocladium scoparium*, Cyl-1 (**26**) and Cyl-2 (**27**) are structurally related to HC toxin and possess the same Aoe residue (Fig. 9) [57–59]. Cyl-2 (**27**) is special due to its very potent HDAC1 inhibitory activity as well as its

lack of inhibition for HDACs 4 and 6 [60]. These results imply some level of class I-selectivity.

The Aoe residue of **25–27** is crucial to their HDAC inhibitory ability, because it mimics an acetylated lysine residue. While the absolute stereochemistry of the epoxides in **25–27** has not been determined, they play an important role in HDAC inhibition. The removal of these epoxides was shown to be detrimental to the HDAC inhibitory activity of HC-toxin (**25**) [61]. The α -keto epoxide moiety inserts itself into the active site of HDACs and covalently binds to it through epoxide opening by nucleophilic residues in the active site.

3.2.2 Chlamydocin—Chlamydocin (**32**, Scheme 6) was isolated from the fungus *Diheterospora chlamydosporia* and consists of L-phenylalanine, D-proline, 2-aminoisobutyric acid (Aib), and the Aoe group [62]. Chlamydocin (**32**) showed potent cytostatic activity against mastocytoma cells with an ED₅₀ value in the nanomolar range [62]. Chlamydocin (**32**) was also tested for its cell proliferation inhibition against various cancer cell lines. As shown in Table 2, all cell lines tested were very potently inhibited with IC₅₀ values in the nanomolar ranges [63]. It was also found that chlamydocin (**32**) was an extremely potent inhibitor of HDACs isolated from A2780 ovarian cancer cells with an IC₅₀ value comparable to that of TSA (**1**, Scheme 1). Chlamydocin (**32**) increased the levels of H3 and H4 acetylation in a dose-dependent manner. Additionally, it has been shown that treatment of A2780 cells with chlamydocin (**32**) induces p21^{cip1/waf1}-mediated apoptosis.

Schmidt and co-workers reported the first synthesis of chlamydocin (**32**, Scheme 6) [64]. The precursor of the Aoe residue **28** was coupled to tripeptide **29**. The resulting intermediate **30** was cyclized at the Aoe/proline residue juncture. Subsequent acetal cleavage, base-mediated epoxide formation, and oxidation completed the total synthesis of **32**.

Chlamydocin (**32**) is thought to covalently bind to HDACs through the epoxide moiety of the Aoe residue which inserts into the active site of HDAC. Chlamydocin (**32**) exhibits class I-selectivity as it inhibits HDAC1 more potently (IC₅₀ = 0.15 nM) than HDAC6 (IC₅₀ = 1,100 nM) [60].

3.2.3 Trapoxins—Trapoxins A and B (**33** and **34**, Fig. 10) are fungal natural products isolated from the fermentation broth of *Helicoma ambiens* RF-1023 [65]. They were determined to be cyclic tetrapeptides consisting of two L-phenylalanine residues, D-proline or D-pipecolinic acid, and an Aoe moiety (Fig. 10). Schreiber and co-workers reported the first total synthesis of trapoxin B (**34**) (Scheme 7) [16].

The precursor for the Aoe residue **35** was accessed from the mono-TIPS protected 2,3-(*O*)-isopropylidene-L-threitol in six steps. After coupling **35** to tripeptide **36**, BOP-mediated cyclization resulted in cyclic tetrapeptide **38**. Influenced by the total synthesis of chlamydocin (**32**, Scheme 6) reported by Schmidt and co-workers [64], the cyclization occurred between the N-terminal Aoe precursor and the C-terminal proline. Subsequent tosylation of the terminal hydroxyl group, acetal cleavage, base-mediated epoxide formation, and oxidation of the secondary hydroxyl group furnished the α -keto epoxide of the Aoe

residue to complete the synthesis of trapoxin B (**34**) in 15 steps from commercially available 2,3-(*O*)-isopropylidene-L-threitol (5.5% overall yield).

Trapoxins (**33** and **34**) were hypothesized to be strong antibiotic agents due to their structural similarity to previous antibiotic compounds such as WF-3161, HC-toxin (**25**), Cyl-1 (**26**), Cyl-2 (**27**), and chlamydocin (**32**) [65]. Surprisingly, trapoxins induced flattening of the *sis* oncogene-transformed NIH3T3 cells like TSA (**1**, Scheme 1) and depudecin (**20**, Scheme 3). The similarity in biological activity prompted testing of trapoxins (**33** and **34**) as HDAC inhibitors. As expected, trapoxins A and B (**33** and **34**) inhibited HDAC activity *in vitro*, resulting in hyperacetylation of histones [66]. It has been postulated that the α -keto epoxide moiety of trapoxins covalently binds to nucleophilic residues in the active site of HDACs, which is similar to the mechanism of depudecin and other epoxide containing cyclic tetrapeptide HDAC inhibitors. The removal of the α -keto epoxide by NaBH₄ has in fact proven to be detrimental to inhibitor activity. Trapoxins (**33** and **34**) are very potent class I and class IIa HDAC inhibitors with very low class IIb inhibitory activity [60].

Trapoxin B (**34**) has been especially important in HDAC inhibitor development due to its use as an affinity probe for the isolation of the first mammalian HDAC [15, 16]. In the study conducted by Schreiber and co-workers, the phenylalanine directly adjacent to the Aoe residue was replaced with a lysine to form a derivative named “K-trap” for the purposes of further functionalization and ultimate immobilization on an affinity matrix (Fig. 11). This position was chosen by examining the related cyclic tetrapeptide HDAC inhibitor, chlamydocin, where the lack of the phenylalanine residue did not result in a significant decrease in potency. Incubation of bovine thymus lysates with the K-trap affinity matrix led to the isolation of six major proteins between 45 and 50 kD. These proteins were not retained when incubated in the presence of trapoxin B (**34**) or TSA, indicating that the K-trap affinity matrix targeted the same protein as these known HDAC inhibitors. Further large scale purification of these bovine proteins led to the isolation of two bands of 46 and 50 kD. Sequencing of these proteins showed that the 46 kD-bovine protein was related to the yeast transcriptional regulator gene, RPD3 [15]. This protein was named histone deacetylase 1 (HD1, later changed to HDAC1) and was the first mammalian HDAC cloned and characterized.

3.2.4. Apicidins—Apicidins (**39–47**, Fig. 12) are a family of natural products that were isolated from cultures of the *Fusarium pallidoroseum* fungus in Costa Rica [67]. Apicidin (**39**) and apicidin A (**40**) were the first compounds of this family that were isolated and characterized. This discovery led to the targeted biological screening of fermentation extracts resulting in the isolation of apicidins B, C, D₁, D₂, and D₃ (**41–45**) [68]. Further analysis of the apicidin synthetase gene led to the isolation of apicidin E (**46**) [69] and overexpression of an apicidin-like gene cluster in *Fusarium fujikoro*i resulted in the discovery of apicidin F (**47**) [70]. All apicidins, except for apicidin A (**40**), contain *N*-methoxy-L-tryptophan. While apicidin B (**41**) contains D-proline, all other apicidins contain D-pipecolinic acid. Apicidins C and F (**42** and **47**) contain L-valine and L-phenylalanine, respectively, while the remaining apicidins consist of L-isoleucine at the corresponding

position. All apicidins (**39–47**) contain (2*S*)-amino-8-oxo-decanoic acid (Aoda) or its derivative.

Apicidins (**39–47**) drew initial interest due to their *in vitro* and *in vivo* antimalarial activity [67]. It was demonstrated that apicidin (**39**) and apicidin A (**40**) were potent HDAC inhibitors of the malarial Apicomplexan parasite as well as HDACs of the parasite *Eimeria tenella* [56, 67]. Subsequent testing of other apicidin congeners showed that apicidins B and C (**41** and **42**) were also very potent *E. tenella* HDAC inhibitors, while apicidins D₂ and D₃ (**44** and **45**) showed a significant reduction in HDAC inhibitory activity [68]. To date, no HDAC inhibitory activity has been reported for apicidins E and F (**46** and **47**).

The first total synthesis of apicidin A (**40**) was reported by Singh and co-workers (Scheme 8) [71]. The key step of their synthesis was the coupling of a primary radical, which was formed by treatment of iodide **51** with tributyltin hydride under photolysis conditions, and terminal alkene **52** to furnish the Aoda side chain **53**. Iodide **51** for this key step was easily prepared in four steps starting from *N*-Cbz protected glutamic acid **48**. Mono-methylation of the C-terminus followed by reduction of the glutamic acid side chain and conversion of alcohol **50** to the corresponding iodide provided the desired intermediate **51**. The Aoda side chain **53** was fully accessed in five steps from commercial glutamic acid **48** (23% overall yield). With **53** in hand, standard peptide synthesis procedures resulted in the acyclic precursor which was cyclized at the N-terminal Aoda and C-terminal pipecolic acid juncture to complete the synthesis of **40**.

Apicidin (**39**) has been shown to inhibit cell proliferation in various cancer cell lines through the induction of cyclin-dependent kinase (CDK) inhibitor p21^{cip1/waf1} [72]. Apicidin (**39**) is also able to revert the morphology of HeLa cells to normal cell morphology upon treatment. These observations have been attributed to the dose-dependent increase of H4 acetylation by apicidin (**39**).

The mechanism of HDAC inhibition by apicidins is very similar to that of other cyclic tetrapeptides. By inserting the acetylated lysine mimic into the active site of HDACs, the carbonyl functionality is able to chelate to the Zn²⁺ at the bottom of the active site and block the natural substrate from entering. This explains the lack of activity of apicidin D₂ and D₃ (**44** and **45**) as their lack of a ketone group reduces their binding affinity for Zn²⁺ [68]. In addition, while no experiments have demonstrated or ruled out the HDAC inhibition capabilities of apicidin E (**46**), the lack of a Zn²⁺ binding group most likely will prove detrimental to its ability to inhibit HDAC activity. Apicidin (**39**) has demonstrated impressive potency against HDACs 2, 3, and 8 (ED₅₀ = 120 nM, 43 nM, and 575 nM, respectively) while being inactive against other isoforms [38].

3.2.5. FR235222 and AS1387392—Fermentation of the fungus *Acremonium* sp. led to the isolation of the natural product FR235222 (**63**, Scheme 9) [73–75]. This cyclic tetrapeptide consists of (2*R*,4*S*)-L-methyl proline, L-phenylalanine, (2*S*)-isovaline, and (2*S*)-amino-8-oxo-(9*R*)-hydroxydecanoic acid (Ahoda) [75]. FR235222 (**63**) is a potent inhibitor of murine lymphocyte proliferation as well as HDAC activity in human T-cell leukemia Jurkat cells and mouse lymphoma EL-4 cells [73]. AS1387392 (LGP1, **64**,

Scheme 9), originally a synthetic analogue of FR235222 (**63**), was later naturally isolated from the same natural source as FR235222 (**63**) [76, 77]. The only structural difference is the lack of a methyl group on the proline residue. Both **63** and **64** showed similar IC₅₀ values for the inhibition of histone H4 deacetylation (60 and 50 nM, respectively) [76]. They also showed nanomolar IC₅₀ values in human HDAC inhibition as well as splenocyte proliferation [77].

Taddei/Gomez-Paloma and co-workers reported the first total synthesis of FR235222 (**63**), which was utilized for the synthesis of AS1387292 (**64**) as well (Scheme 9) [76, 78]. Synthesis of the TBDMS protected Ahoda residue **57** was achieved by one-carbon homologation of aldehyde **54** followed by Horner–Wadsworth–Emmons reaction, simultaneous debenzoylation/alkene reduction, and Boc protection of the primary amine. The second key to this synthesis was the preparation of the methylproline **61**. Dimethyl ester glutamic acid derivative **58** was methylated to yield a 4:1 mixture of diastereomers. Debzoylation of the desired diastereomer **59** and Boc-protection formed lactam **60**. Reduction of **60**, methyl ester cleavage, Boc-deprotection, and Fmoc-protection completed the synthesis of (2*R*,4*S*)-L-methyl proline (**61**) in seven steps from **58**. With these two building blocks in hand, standard peptide synthesis procedures were used to complete the synthesis of **63** and **64**.

While the modes of HDAC inhibition for FR235222 (**63**) and AS1387392 (**64**) are very similar to those of other cyclic tetrapeptides, it seems that the absolute stereochemistry of the hydroxyl group of the Ahoda residue plays a crucial role. Analogues with the opposite configuration showed a significant decrease in inhibitory activity demonstrating the importance of the configuration of the hydroxyl group [76]. It has been recently reported that treatment of human leukemia U937 cells with AS1387392 (**64**) induces tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis through caspase 8 activation [79].

3.2.6 Azumamides—Azumamides A–E (**65–69**, Fig. 13) were isolated from the marine sponge *Mycale izuensis* off the coast of Japan [80]. They consist of only D-amino acids, including one β-amino acid. The presence of a β-amino acid results in a 13-membered macrocycle instead of the 12-membered macrocycle found in other cyclic tetrapeptide HDAC inhibitors. The β-amino acid is either (*Z*)-(3*R*)-amino-(2*S*)-methyl-5-nonenedioic acid, 9-amide (Amnaa in **65**, **66**, and **68**) or (*Z*)-(3*R*)-amino-(2*S*)-methyl-5-nonenedioic acid (Amnda in **67** and **69**).

Azumamides (**65–69**) showed strong inhibitory activity against HDAC extracts from K562 human leukemia cells with IC₅₀ values ranging from 0.045–1.3 μM. Azumamide A (**65**) also showed dose-dependent inhibition of H3 and H4 deacetylation as well as cytostatic effects on WiDr colon cancer and K562 human leukemia cells [80].

Recently, Olsen and co-workers published total syntheses of **65–69** (Scheme 10) [81]. The key step of this synthesis used an Ellman-type Mannich reaction to set the stereochemistry of the β-amino acid precursor **72**. Ganesan and co-workers utilized a similar approach in the synthesis of azumamide A and E (**65** and **69**) [82]. Compound **72** was easily elaborated

through the standard organic transformations. The β -amino acid building block **75** was completed in seven steps from intermediate **70** (15% overall yield). Compound **75** was then used in standard solid phase peptide synthesis to complete the synthesis of azumamides A–E (**65–69**) [81].

Olsen and co-workers used these synthetic azumamides (**65–69**) to test their HDAC inhibitory activity against a full panel of recombinant HDAC enzymes (Table 3) [81]. These results showed that azumamides C and E (**67** and **69**) are the most potent HDAC inhibitors. Additionally, **67** and **69** also inhibited HDACs 10 and 11 efficiently while azumamides A, B, and D (**65**, **66**, and **68**) did not.

The relative potency of azumamides is most likely due to the nature of the β -amino acid. Azumamides C and E (**67** and **69**) contain carboxylic acid functionalities while the remaining azumamides contain amides. Amides are known to be weaker Zn^{2+} binding groups than carboxylic acids, therefore resulting in the observed reduced potency. Azumamides (**65–67**) insert the Amnaa or Amnda side chains into the active site of HDACs, where the carboxylic acid or amide is able to chelate the Zn^{2+} in a bidentate fashion. Therefore it is reasonable that the stronger Zn^{2+} binding group results in a higher potency [81]. This hypothesis was also further supported by changing the Zn^{2+} binding group to the more strongly chelating hydroxamic acid group. Ganesan and co-workers reported that this switch in Zn^{2+} binding group resulted in a significant increase in potency for HDAC inhibition [82].

3.2.7. Microsporins—Microsporins A and B (**78** and **79**, Scheme 11) were isolated from the marine fungus *Microsporium* cf. *gypseum* collected from the U.S. Virgin Islands [83]. They are cyclic tetrapeptides consisting of L-alanine, L-phenylalanine, D-pipecolic acid, and Aoda or (2*S*)-amino-8-hydroxydecanoic acid. Unfortunately, the stereochemistry of the secondary alcohol in microsporin B (**79**) has not yet been established. Along with its isolation, Silverman and co-workers also published the total synthesis of microsporin A (**78**) where they utilized solid phase peptide synthesis (Scheme 11) [83]. The site of cyclization was between the N-terminal Aoda and the C-terminal pipecolic acid.

Microsporins most likely insert the Aoda residue into the active site of HDACs like apicidin (**39**, Fig. 12). The ketone functionality is able to chelate to the Zn^{2+} while the cyclic cap group blocks the rim region of the active site. Microsporin B (**79**) showed lower potency in HTC-116 cytotoxicity, suggesting that the ketone is a stronger Zn^{2+} binding group than the hydroxyl group [83].

Both microsporin A and B (**78** and **79**) showed *in vitro* cytotoxicity against various cancer cell lines. In addition, microsporin A (**78**) showed slightly higher potency than SAHA (**24**, Scheme 5) in HDAC inhibition when tested against a mixture of HDACs extracted from HeLa cells as well as recombinant HDAC8 (Table 4) [83].

3.3. Cyclic Depsipeptides

3.3.1 FK228 and FR901375—FK228 (FR901228, romidepsin, **87**, Scheme 12) is the first cyclic depsipeptide HDAC inhibitor that has been discovered [84–86]. It was isolated

from the bacterial fermentation broth of *Chromobacterium violaceum* No. 968 and consists of L-valine, D-valine, (Z)-dehydrobutyrine, D-cysteine, and (3S)-hydroxy-7-mercapto-4-heptenoic acid [84, 85]. The last two groups are connected by an internal disulfide bond, forming a bicyclic system [85]. While FK228 (**87**) showed weak antimicrobial activity with minimum inhibitory concentrations of 18–180 μ M, it showed very potent *in vitro* antitumor activity (IC_{50} = 0.6–5.9 nM) with extremely low cytotoxicity in normal human and mouse fibroblast cell lines. In addition, FK228 (**87**) demonstrated the ability to revert the oncogenic morphological phenotype of *ras*-transformed NIH3T3 cells as well as the ability to inhibit *in vivo* tumor growth in mammary adenocarcinoma (MDF-7) and lung adenocarcinoma (A549) mouse models. The first synthesis of FK228 (**87**) was reported by Simon and co-workers (Scheme 12) [87].

A key step to this synthesis was the asymmetric synthesis of β -hydroxy mercaptoheptenoic acid **82**. This was accomplished through a titanium-mediated asymmetric aldol reaction with excellent stereoselectivity. Tetrapeptide **83** was accessed through standard peptide synthesis methods. Compound **83** then underwent DABCO-mediated elimination of the threonine residue for the conversion to (Z)-dehydrobutyrine. Subsequent coupling of hydroxy acid **82** to tetrapeptide **84** followed by macrocyclization through Mitsunobu conditions and iodine-mediated trityl deprotection and subsequent disulfide bond formation yielded the final product **87** in 14 steps from commercially available L-valine methyl ester (18% overall yield).

FK228 (**87**) has been found to be a prodrug that releases the active free dithiol (redFK228, **88**, Scheme 12) by reduction of the disulfide bond [88]. RedFK228 (**88**) is able to insert itself into the active site of HDAC and chelate to the Zn^{2+} in a monodentate fashion. This prodrug hypothesis has been supported by testing the HDAC inhibitory activity of both FK228 (**87**) and redFK228 (**88**). RedFK228 (**88**) shows a 20-fold increase in potency over FK228 (**87**). A similar level of increase in potency was also observed when the HDAC inhibitory activity of FK228 (**87**) was tested in the presence of dithiothreitol (DTT). Lastly, the methylation of thiols in redFK228 (**88**) significantly reduced its HDAC inhibitory ability, demonstrating the importance of the free thiol. FK228 (**87**) is a class I-selective HDAC inhibitor, demonstrated by its IC_{50} values for HDACs 1 (36 nM) and 2 (47 nM), compared to those of HDACs 4 (510 nM) and 6 (14,000 nM). The macrocycle of FK228 (**87**) most likely enables the inhibitor to leverage different interactions with the rim of the active site of HDACs to induce the class selectivity.

FK228 (**87**) has also shown potent antiangiogenic activity through the suppression of hypoxia inducible factor 1 α (HIF-1 α) [89]. Treatment of Lewis lung carcinoma cells with FK228 (**87**) in hypoxic conditions showed a dose-dependent decrease in HIF-1 α expression. Additionally, the same treatment resulted in the decrease of vascular endothelial growth factor (VEGF) mRNA as well as VEGF protein levels, demonstrating the antiangiogenic capabilities of FK228 (**87**). While the link between HDAC inhibition and antiangiogenesis is not clear, this biological property of FK228 (**87**) is of interest as HIF-1 α has also been implicated in certain cancers.

FR901375 (**94**, Scheme 13), isolated from the fermentation broth of *Pseudomonas chloroaphis* (No. 2522), is structurally related to FK228 (**87**) [90]. It is a 16-membered macrocyclic depsipeptide that contains an internal disulfide bond. It consists of two D-valines, D-cysteine, L-threonine, and (3*S*)-hydroxy-7-mercapto-4-heptenoic acid. The synthesis of FR901375 (**94**) was first reported by Janda/Wentworth Jr. and co-workers (Scheme 13).

Janda/Wentworth Jr. and co-workers utilized a chiral auxiliary to install the correct stereochemistry on β -hydroxy mercaptoheptenoic acid **82**. With compound **82** in hand, the easily accessible tetrapeptide **91** was coupled and cyclized under Mitsunobu conditions. Lastly, TBS deprotection and iodine-mediated trityl deprotection and subsequent disulfide bond formation completed the synthesis of FR901375 (**94**) in 13 steps from commercially available L-threonine (12.4% overall yield).

Structural similarities between FR901375 (**94**) and FK228 (**87**) suggest that FR901375 (**94**) is also a very potent HDAC inhibitor with possibly the same class I-selectivity. However, to date no HDAC inhibition studies have been reported for FR901375 (**94**). FK228 (**87**) on the other hand has garnered a tremendous amount of interest and, as mentioned before, is an FDA approved treatment for cutaneous T-cell lymphoma. It is marketed by Celgene under the name Istodax® [91].

3.3.2. Spiruchostatins—Spiruchostatins A–D (**95–98**, Fig. 14) were isolated from *Pseudomonas sp.* bacteria as gene expression-enhancing substances [92, 93]. Similar to FK228 (**87**, Scheme 12) and FR901375 (**94**, Scheme 13), **95–98** contain an internal disulfide bond that connects (3*S*)-hydroxy-7-mercapto-4-heptenoic acid and D-cysteine. They also contain D-alanine or D-valine as well as a statine or statine-like motif: (3*R*, 4*R*)-4-amino-3-hydroxy-5-methylhexanoic acid group for spiruchostatins A and D (**95** and **98**), (3*R*, 4*R*, 5*R*)-4-amino-3-hydroxy-5-methylheptanoic acid group for spiruchostatin B (**96**), and (3*R*, 4*R*)-4-amino-3-hydroxy-6-methylheptanoic acid group for spiruchostatin C (**97**). All spiruchostatins showed potent cancer cell growth inhibitory activity against various human cancer cell lines with GI₅₀ values in the nanomolar ranges (1.3–78 nM) [93, 94]. To date, five total syntheses for spiruchostatin A (**95**) [95–99], two for spiruchostatin B (**96**) [100, 101], and one for each spiruchostatin C and D (**97** and **98**) [93] have been reported. Of these, the synthesis of spiruchostatin A (**95**) by Ganesan and co-workers is particularly noteworthy (Scheme 14) [96].

In order to access β -hydroxy mercaptoheptenoic acid **103**, a chiral auxiliary was used with Vilarrasa's titanium-mediated aldol reaction. The synthesis of tripeptide **102** began with a Claisen condensation of Boc-protected valine **99** followed by diastereoselective reduction of the ketone **100** to the desired alcohol **101**. Coupling with D-cysteine and D-alanine resulted in the tripeptide **102** which was coupled to β -hydroxy mercaptoheptenoic acid **103**. Tce deprotection and Yamaguchi esterification resulted in the macrocyclic precursor **105**. Lastly, iodine-mediated disulfide bond formation and desilylation of the secondary hydroxyl group furnished spiruchostatin A (**95**) in 11 steps and 5.1% overall yield.

The mechanism of inhibition of spiruchostatins (**95–97**) is identical to that of FK228. Reduction of the disulfide bond releases two thiols, one of which is able to chelate to the Zn^{2+} in the HDAC active site [102]. Like FK228, spiruchostatins (**95–97**) are also class I-selective HDAC inhibitors. The IC_{50} values for HDAC1 were 320–636 fold lower than the IC_{50} values for HDAC6 [94].

3.3.3. Largazole—Largazole (**113**) is a marine natural product that was isolated from a cyanobacterium of the genus *Symploca* found in Key Largo, Florida [103]. It is a 16-membered depsipeptide macrocycle with a variety of unusual structural features, such as a 4-methylated thiazoline unit linearly fused to a thiazole, a 3-hydroxy-7-mercaptohept-4-enoic acid unit, and a thioester moiety. Largazole (**113**) showed strongly differential cytotoxicity towards transformed cancer cells. The growth of transformed mammary epithelial cells and fibroblastic osteosarcoma cells were inhibited at nanomolar concentrations ($\text{GI}_{50} = 7.7 \text{ nM}$ and 55 nM respectively), while non-transformed epithelial cells and fibroblast cells showed much higher inhibitory concentrations ($\text{GI}_{50} = 122 \text{ nM}$ and 480 nM respectively). Largazole (**113**) is also a very potent inhibitor of HCT116 (human colon cancer cells) proliferation ($\text{GI}_{50} = 44 \text{ nM}$) [104].

Due to its exciting differential cytotoxicity, largazole (**113**) has attracted strong interest from synthetic chemists, which has led to 11 total syntheses and various analogues syntheses [104–124]. The first total synthesis of largazole (**113**) was reported by Hong/Luesch and co-workers (Scheme 15) [104]. The first key step of the synthesis was the formation of the thiazole-thiazoline **108**. Condensation of (*R*)-2-methyl cysteine methyl ester hydrochloride (**107**) with nitrile **106** resulted in the thiazole-thiazoline methyl ester **108**. Coupling of **108** with known *syn*-aldol product **109** was followed by Yamaguchi esterification with Boc-protected valine. The key macrocyclization was comprised of hydrolysis of the methyl ester, Boc-deprotection, and HATU-mediated cyclization. Lastly, cross-metathesis with Grubbs' second-generation catalyst completed the total synthesis of **113** in eight steps from known nitrile **106** (19% overall yield) [104].

Like other cyclic depsipeptide HDAC inhibitors, largazole (**113**) contains a masked thiol group. However, largazole (**113**) is unique in that it contains a thioester rather than an internal disulfide bond. The hydrolysis of the thioester bond releases the largazole thiol (**114**) which is able to coordinate to the Zn^{2+} in the HDAC active site (Fig. 15) [104]. Largazole (**113**) is a class I-selective HDAC inhibitor (HDAC1 $\text{IC}_{50} = 25 \text{ nM}$, HDAC2 $\text{IC}_{50} = 21 \text{ nM}$, and HDAC3 $\text{IC}_{50} = 48 \text{ nM}$) that only weakly inhibits HDAC6 ($\text{IC}_{50} = 5,700 \text{ nM}$) [104, 106].

Recently, an HDAC8-largazole thiol crystal structure was reported, marking the first time a crystal structure of HDAC with a macrocyclic inhibitor was achieved (Fig. 16) [26]. From this crystal structure, it is apparent that the macrocycle of largazole thiol (**114**) is structurally very rigid as it does not undergo significant conformational changes upon binding with HDAC8. However, considerable conformational adjustments are required for HDAC8 in order to accommodate a large inhibitor such as largazole thiol (**114**). Specifically the Y100 residue rotates almost 180° upon binding with largazole thiol (**114**). This is not observed when smaller inhibitors are complexed with HDAC8. Additionally, several atoms in

largazole thiol (**114**) are involved in intermolecular interactions with the active site of HDAC8, as shown in Fig. 16. The information obtained from this crystal structure will undoubtedly be useful for future inhibitor designs.

Largazole (**113**) has demonstrated potent *in vivo* tumor growth inhibition in HCT116 xenograft mouse models (Fig. 17) [120]. HDAC inhibition in HCT116 cells by largazole (**113**) induced H3 hyperacetylation which consequently lead to G₂/M cell cycle arrest. Additionally, The induction of apoptosis *in vivo* was observed.

Largazole (**113**) possesses a variety of other biological activities as well. For example, it is able to induce a significant increase in alkaline phosphatase (ALP) expression levels as well as osteopontin (OPN) transcription levels in murine pluripotent mesenchymal precursor (C2C12) cells [125]. The ability to increase the expression of early phase markers of osteoblast differentiation in *in vitro* osteogenesis models was further supported in *in vivo* mouse and rabbit calvarial bone models (Fig. 18).

Additionally, largazole (**113**) has demonstrated the ability to sensitize EBV⁺ tumor cells to cytotoxicity in the presence of the antiherpes drug ganciclovir (GCV) with an optimal sensitizing concentration in the nanomolar ranges [126]. It has also been shown that largazole (**113**) inhibits the action of ubiquitin activating enzyme (E1) and consequently the ubiquitination of CDK inhibitor p27 [127]. Ubiquitination-dependent degradation of p27 is a phenomenon present in approximately 50% of all human cancers. Given largazole's anticancer activity, the inhibition of p27 ubiquitination is very interesting. The mode of inhibition of E1 is also extremely different as structure–activity relationship studies reveal that both the aliphatic tail and the macrocycle are needed to inhibit E1. Additionally, largazole (**113**) displays remarkable selectivity for human E1 over related enzymes such as SUMO E1. Largazole (**113**) also demonstrated induction of apoptosis in hepatic stellate cells (HSCs) in liver fibrosis as well as ameliorating liver fibrosis models *in vivo* [128].

3.3.4. Burkholdacs and Thailandepsins—Identification of a biosynthetic gene cluster in the bacteria *Burkholderia thailandensis* E264 has led to the isolation of seven structurally related compounds known as burkholdacs and thailandepsins (**115–121**, Fig. 19) [129–132]. These compounds are extremely similar to the spiruchostatin family. They contain D-amino acids, (3*S*)-hydroxy-7-mercapto-4-heptenoic acid, and a statine or statine-like moiety. All burkholdacs contain a methionine or methionine sulfoxide residue, while the thailandepsins contain methionine or norleucine [130–134]. Brady and co-workers discovered burkholdacs A and B (**115** and **116**) around the same time Cheng and co-workers discovered thailandepsins A–C (**115**, **116**, and **118**) [129–131]. Upon further investigation of the structures of burkholdacs A and B (**115** and **116**), it was concluded that burkholdac A (**115**) and thailandepsin C are the same compound, while burkholdac B (**116**) and thailandepsin A are identical [131, 133, 134]. Currently, only burkholdacs A and B (**115** and **116**) have been prepared synthetically [133, 134]. This was accomplished by employing the synthetic strategy used to prepare spiruchostatin A (**95**, Fig. 14) reported by Ganesan and co-workers.

Burkholdac B (**116**) is a very potent inhibitor of HDACs from HeLa extracts ($IC_{50} = 5$ nM) [134]. It also inhibits cell proliferation in MCF7 breast cancer cells with a GI_{50} of 60 pM. Burkholdac C (**117**) also showed similar anticancer activity when tested in the NCI-60 DTP Human Tumor Cell Line Screen. In 13 different cell lines, burkholdac C (**117**) showed total growth inhibition greater than FK228 (**87**, Scheme 12) [132]. In addition to burkholdac B (**116**), thailandepsin B (**118**) also showed broad-spectrum antiproliferative activities in the NCI-60 DTP Human Tumor Cell Line Screen with GI_{50} values in low nanomolar ranges for over 90% of the cell lines tested [130].

When **116** and **118** were tested for their HDAC inhibitory activity against nine different HDAC isoforms, a strong class I-selectivity was observed, especially for HDACs 1–3 (Table 5) [130]. They also demonstrated some HDACs 6 and 8 inhibitory activity, but only very weak inhibitory activity for all other isoforms. Indeed when other burkholdacs and thailandepsins were tested against HDACs 1, 4, and 6, a very similar class I-selectivity was observed [131]. Additionally, the reduction of the internal disulfide bond was examined. The resulting compounds demonstrated a very strong increase in potency in HDAC inhibition (Table 5). This further supports the prodrug hypothesis of these types of HDAC inhibitors. Upon reduction of the disulfide bond, the liberated thiol on the (3*S*)-hydroxy-7-mercapto-4-heptenoic acid group chelates to the Zn^{2+} and block the active site of HDAC.

4. Role of HDACs in other diseases

Since the discovery of TSA, HDAC inhibitors have been studied mostly in the context of potential anticancer therapeutics. However, HDACs have been implicated in a wide variety of other diseases including inflammatory, metabolic, autoimmune, and neurodegenerative diseases. Recently, the role of specific HDAC isoforms in these diseases has been elucidated [135].

For example, HDAC2 has been implicated in Alzheimer's disease, specifically in the cognitive decline of patients [136]. HDAC2 levels are elevated in the hippocampal area CA1 and the prefrontal cortex of CK-p25 mice. HDACs 1 and 3 are present in much lower levels in these areas of the brain responsible for memory formation and storage. Additionally, control mice do not show this increase in HDAC2 expression. Increased binding of HDAC2 to genes involved in learning, memory, and synaptic plasticity strongly correlates to the hypoacetylation of histone lysine residues at these genes. This shows a link between HDAC2 activity and the degradation of cognitive function. Most importantly, knockout studies of HDAC2 through short-hairpin-RNA showed improvement in synaptic plasticity and reversal of neurodegeneration associated memory impairments in murine models.

Similarly, HDAC4 has recently been implicated in Huntington's disease [137]. HDAC4 can bind to the mutant huntingtin (HTT) gene, which contains expanded polyglutamine (polyQ) stretches, and co-localize in cytoplasmic inclusions in the brain of Huntington's disease patients. These insoluble aggregates directly cause symptoms, such as chorea. HDAC4-knockdown studies showed a decrease in aggregate load, an increase in soluble HTT levels, and an improvement in neurological phenotypes in murine models, such as increased motor coordination and life span. In contrast, the knockdown of other HDAC isoforms did not

result in these phenotype improvements, highlighting the importance of HDAC4 in Huntington's disease.

HDAC7 has been implicated in inflammatory diseases [138]. This isoform synergizes with HIF-1 α to bind to the HIF-binding site on the Edn1 promoter gene in thioglycollate-elicited peritoneal macrophages (TEPM). This leads to an activation of Edn1 and the subsequent Toll-like receptor (TLR)-induced response. The inhibition of HDAC7 decreases this immune response in macrophages and leads to the inhibition of the inflammatory response.

In the presence of glucagon, HDACs 4, 5, and 7 work together with HDAC3 to activate gluconeogenesis [139]. Normally, these class IIa HDACs are excluded from the nucleus by serine phosphorylation through the action of AMP-activated protein kinases. However, in a fasting state, the release of glucagon leads to the dephosphorylation and translocation of HDACs 4, 5, and 7 to the nucleus. Subsequent recruitment of HDAC3 leads to the deacetylation of the FOXO transcription factors and ultimately gluconeogenesis. The knockout of HDACs 4, 5, and 7 leads to the decrease in blood glucose levels, showing the potential of these isoforms as targets for metabolic diseases such as type II diabetes.

Recently, HDAC6 has received a lot of attention due to its implications in a variety of different diseases including colitis, allograft rejection, charcot-marie-tooth disease (CMT), hypertension, and stroke [140]. The role of HDAC6 in cystic cholangiocytes has recently been elucidated [141]. HDAC6 is overexpressed in polycystic liver disease (PLD) cholangiocytes and a PLD rat model (PCK). The inhibition of HDAC6 in rat and human models led to a decreased proliferation of cholangiocytes as well as reduced liver and kidney cyst growth in PLD animal models.

The implications of specific HDAC isoforms in these diseases greatly increase the need for isoform-selective HDAC inhibitors. Such inhibitors can be used as probes for the elucidation of molecular pathways of these diseases. Additionally, isoform-selective HDAC inhibitors are able to limit off-target effects and consequently minimize unwanted side effects when used as therapeutics [142].

5. Isoform-selective HDAC inhibitors

There have been a number of synthetic HDAC inhibitors developed to date in efforts to develop an isoform-selective HDAC inhibitor. It has been generally accepted that efficient HDAC inhibitors should possess three structural features: (1) a Zn²⁺ chelating group (warhead), (2) a linker region, and (3) a hydrophobic "cap group" (Fig. 20) [143]. A large portion of these synthetically developed HDAC inhibitors are linear in nature and contain these three features. Libraries with variations in all three aspects have resulted in many different inhibitors that show preferences for one more isoforms of HDAC. However, a true isoform-selective HDAC inhibitor has not yet been developed.

One approach to isoform-selective inhibitor design is to utilize the scaffold of natural products. Natural products have been a great resource for potent HDAC inhibitors. Additionally, many of these naturally occurring HDAC inhibitors already exhibit class selectivity. Psammaplin A (7, Fig. 6) has been the focus of some of these efforts [144, 145].

The aromatic cap group was replaced with a variety of heterocycles in efforts to improve the isoform selectivity (Table 6). Although modest within HDACs 1, 2, and 3, some selectivity was accomplished among these three structurally very similar isoforms. Additionally, compared to the natural product, the class I-selectivity was increased significantly [145].

Although it is not a natural product, analogues of SAHA (**24**, Scheme 5) have also been tested for their isoform selectivity. Pflum and co-workers synthesized different C3 substitution analogues. The ethyl analogue showed a much increased selectivity for HDAC6 over HDACs 1 and 3 compared to SAHA (**24**) (Fig. 21) [146].

A tremendous amount of work has also been done with cyclic tetrapeptides and their corresponding analogues. Initially, Yoshida and co-workers developed a library of cyclic hydroxamic acid-containing peptides (CHAP) modeled after several cyclic tetrapeptide HDAC inhibitors such as HC-toxin (**25**), Cyl-1 (**26**), Cyl-2 (**27**), chlamydocin (**32**), and trapoxins (**33** and **34**) [60]. Unfortunately, in terms of isoform selectivity, little improvement over existing natural product inhibitors was observed. More recently, Ghadiri and co-workers prepared a large library of cyclic tetrapeptides consisting of a mixture of α - and β -amino acids to provide a detailed structure activity relationship for a $\alpha_3\beta$ ring type (Fig. 22) [147, 148]. Inspired by apicidin A (**40**, Fig. 12), a lead compound was designed based on preliminary structure–activity relationship studies. In order to design an efficient HDAC inhibitor, the following structure–activity relationship was found: 1) only D-amino acids can be present, 2) aa3 must be a β -amino acid, and 3) none of the amide nitrogens can be methylated. Based on these findings, a vast amount of cyclic tetrapeptides were prepared and tested against HDACs 1, 3, 6, and 8. The resulting structure–activity relationship is summarized in Fig. 22.

6. Conclusion

Here, we have reviewed a number of naturally occurring HDAC inhibitors and discussed their discovery, synthesis, biological properties, and mode of action. It is apparent that a tremendous amount of work has led to many developments and advances in the field of HDAC inhibitors. Natural products especially have had a large impact as they have been an excellent source for bioactive materials. The sheer number of HDAC inhibitors that have been reported is a testament to how valuable natural products have been. However, several challenges still remain. One of the most challenging tasks is the development of isoform-selective HDAC inhibitors. Isoform-selective HDAC inhibitors possess great potential as biochemical probes as well as promising therapeutics. While some advances in this area have been made, it is clear that a breakthrough is needed as the demand for isoform-selective HDAC inhibitors becomes more significant. Natural products will be a good place to start for these efforts due to their innate class selectivity. Utilizing the scaffolds of these natural products will prove to be invaluable in the efforts of developing an isoform-selective HDAC inhibitor.

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Fig. (1).
DNA methylation by DNMT (adapted from ref. [4]).

Fig. (2). Epigenetic mechanism of histone acetylation. Acetyl groups (Ac) are added and removed by HAT and HDAC, respectively, from lysine residues on histone tails, resulting in changes in transcription levels (adapted from ref. [6]).

Fig. (3).
Common post-translational modifications found in human histones (adapted from ref. [12]).

Fig. (4).
Proposed deacetylation mechanism of *Aquifex aeolicus* HDAC homologue (adapted from ref. [22]).

Fig. (5).
Crystal structure of *A. aeolicus* HDAC homologue with TSA (1) (adapted from ref. [22]).

Fig. (6).
Structure of psammaplin A (7) and its effect on acetylated H3 and tubulin levels (adapted from ref. [41]).

Fig. (7).
Effect of depudecin (**20**) on *v-ras* transformed NIH3T3 cell morphology. (A) *v-ras* transformed NIH3T3 cells. (B) *v-ras* transformed NIH3T3 cells treated with 4.7 μM of depudecin (**20**) (adapted from ref. [46]).

Fig. (8).
Crystal structure of *A. aeolicus* HDAC homologue with SAHA (**24**) (adapted from ref. [22]).

Fig. (9).

Structure of HC toxin (**25**), Cyl-1 (**26**), and Cyl-2 (**27**) [54, 57, 58].

Fig. (10).
Structure of trapoxins [65].

Fig. (11).
Synthesis of K-trap [15].

Apicidin (**39**, $n = 2$, $R^1 = \text{CH}_2\text{CH}_3$, $R^2 = \text{OCH}_3$)
 Apicidin A (**40**, $n = 2$, $R^1 = \text{CH}_2\text{CH}_3$, $R^2 = \text{H}$)
 Apicidin B (**41**, $n = 1$, $R^1 = \text{CH}_2\text{CH}_3$, $R^2 = \text{OCH}_3$)
 Apicidin C (**42**, $n = 2$, $R^1 = \text{CH}_3$, $R^2 = \text{OCH}_3$)

Apicidin D₁ (**43**, $R = \text{COCHOHCH}_3$)
 Apicidin D₂ (**44**, $R = \text{CH}((S)\text{-OH})\text{CH}_2\text{CH}_3$)
 Apicidin D₃ (**45**, $R = \text{CH}_2\text{CHOHCH}_3$)
 Apicidin E (**46**, $R = \text{CH}_2\text{CH}_2\text{CH}_3$)

Apicidin F (**47**)

Fig. (12).
 Structure of apicidins (**39–47**) [67–70].

Fig. (13).
Structure of azumamide A–E (**65–69**) [80].

Spiruchostatin A (**95**, R¹ = CH₃, R² = *i*-Pr)
Spiruchostatin B (**96**, R¹ = CH₃, R² = ((*S*)-1-methylpropyl)
Spiruchostatin C (**97**, R¹ = CH₃, R² = *i*-Bu)
Spiruchostatin D (**98**, R¹ = *i*-Pr, R² = *i*-Pr)

Fig. (14).
Structure of spiruchostatins [92, 93].

Fig. (15).
Mode of inhibition of largazole thiol (**114**).

Fig. (16). Crystal structure of HDAC8-largazole thiol (**114**) complex. (*atoms involved in intermolecular interactions with HDAC8) (adapted from ref. [26]).

Fig. (17).

(A) *In vivo* efficacy of largazole (**113**). (B) Xenograft tumor sections stained for caspase 3 expression in control mice. (C) Xenograft tumor sections stained for caspase 3 expression in mice injected with largazole (**113**). (adapted from ref. [120]).

Fig. (18).

Bone formation of largazole (**113**) in mouse calvarial bones. Treatment with (A) PBS, (B) largazole (10 μM), and (C) largazole (50 μM) (adapted from ref. [125]).

Fig. (19).

Structure of burkholdacs A–C (**115–117**) and thailandepsins A–F (**115**, **116**, **118–121**) [130–132, 134].

Fig. (20).
Structural features of HDAC inhibitors.

Fig. (21).
HDAC inhibition profile of SAHA (**24**) and SAHA analogues (adapted from ref. [146]).

Fig. (22).

Structure–activity relationship of $\alpha_3\beta$ ring type analogues (adapted from Structure–activity relationship of $\alpha_3\beta$ ring type analogues (adapted from ref. [147, 148])).

Scheme (1).

Total synthesis of (±)-TSA by Krebs/Fleming and co-workers [32].

Scheme (2).

Total synthesis of psammaplin A (**7**) by Sufrin and co-workers and disulfide reduction to form the active thiol [42].

Scheme (3).

Total synthesis of depudecin (**20**) by Schreiber and co-workers [45].

Scheme (4).

Total synthesis of santacruzamate A (**22**) by Balunas and co-workers [48].

Scheme (5).

Total synthesis of suberoylanilide hydroxamic acid (**24**) by Njar and co-workers [51].

Scheme (6).

Total synthesis of chlamydocin (**32**) by Schmidt and co-workers [64].

Scheme (7).

Total synthesis of trapoxin B (**34**) by Schreiber and co-workers [16].

Scheme (8).

Total synthesis of apicidin A (**40**) by Singh and co-workers [71].

Scheme (9).

Total synthesis of FR235222 (**63**) and AS1387392 (**64**) by Taddei/Gomez-Paloma and co-workers [76, 78].

Scheme (10).

Synthesis of β -amino acid building block for azumamide synthesis by Olsen and co-workers [81].

Scheme (11).

Total Synthesis of Microsporin A (78) by Silverman and co-workers [83].

Scheme (12).

Total synthesis of FK228 (**87**) by Simon and co-workers [87].

Scheme (13).

Total synthesis of FR901375 (**94**) by Janda/Wentworth Jr. and co-workers [90].

Scheme (14).

Total synthesis of spiruchostatin A (**95**) by Ganesan and co-workers [96].

Scheme (15).

Total synthesis of Largazole (**113**) by Hong/Luesch and co-workers [104].

Table 1.HDAC inhibitory activity of santacruzamate A (**22**) [48].

	IC ₅₀ (nM)		
	HDAC2	HDAC4	HDAC6
Santacruzamate A (22)	0.119	> 1000	434

Table 2.Inhibition of cancer cell proliferation by chlamydocin (**32**) [63].

Cell Line	IC ₅₀ (nM)
A2780 (ovarian)	0.36 ± 0.15
Malme-3M (melanoma)	45 ± 2.5
MCF-7 (breast)	5.3 ± 2.3
HT-29 (colon)	4.3 ± 0.8
HeLa (cervix)	14 ± 2
H1299 (lung)	4 ± 2
K562/C1000 (leukemia)	0.76 ± 0.06

Table 3.HDAC inhibitory activity of azumamides A–E (**65**–**69**) [81].

	IC ₅₀ (nM) ^a						
	HDAC1	HDAC2	HDAC3	HDAC8	HDAC6	HDAC10	HDAC11
Azumamide A (65)	>5000	>5000	3200	>5000	inactive ^b	inactive ^b	>5000
Azumamide B (66)	5000	3000	3000	inactive ^b	inactive ^b	inactive ^b	>5000
Azumamide C (67)	32 ± 1	40 ± 20	14 ± 1	>5000	2000	10 ± 4	35 ± 3
Azumamide D (68)	>5000	>5000	3700	inactive ^b	inactive ^b	inactive ^b	>5000
Azumamide E (69)	67 ± 7	50 ± 30	25 ± 1	4400	>5000	20 ± 12	60 ± 16

^aClass IIa HDAC inhibition data was omitted due to the inactivity of **65**–**69** against all isoforms.^b<50% inhibition at 50 μM.

Table 4.HDAC inhibitory activity of microsporin A (**78**) [83].

	HeLa HDACs IC ₅₀ (μM)	HDAC8 IC ₅₀ (μM)
Microsporin A (78)	0.14 ± 0.01	0.55 ± 0.01
SAHA (24)	0.30 ± 0.02	0.77 ± 0.03

Table 5.HDAC inhibitory activity of burkholdac B (**116**) and thailandepsin B (**118**) [130].

	IC ₅₀ (μM)							
	HDAC1	HDAC2	HDAC3	HDAC4	HDAC6	HDAC7	HDAC8	HDAC9
FK228 (87)	6.7	1.5	0.018	> 50	12	> 50	> 50	> 50
redFK228(88)	0.0053	0.0039	0.0053	0.47	0.33	3.2	0.026	12
Burkholdac B (116)	7.5	39	0.087	> 50	9.9	> 50	> 50	> 50
redBurkB	0.14	0.0035	0.0048	42	0.38	11	1.2	12
Thailandepsin B (118)	7.7	4.5	0.023	22	11	27	30	> 50
redThaiB	0.0065	0.0067	0.0094	18	0.61	24	1.0	30

Table 6.Isoform selectivity profiles of psammaplin A (**7**) and **122** [145].

<div><div> <p>Psammaplin A (7)</p></div><div> <p>HDAC1 selective analogue (122)</p></div></div>						
	IC ₅₀ (μM)					
	HDAC1	HDAC2	HDAC3	HDAC8	HDAC4	HDAC6
Psammaplin A (7)	0.001	0.006	0.009	1.3	6.3	0.36
122	0.021	0.14	0.068	4.2	> 10	> 10