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Discovery and Mechanism of Natural Products as Modulators of Histone Acetylation

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Abstract

Small molecules that modulate histone acetylation by targeting key enzymes mediating this posttranslational modification – histone acetyltransferases and histone deacetylases – are validated chemotherapeutic agents for the treatment of cancer. This area of research has seen a rapid increase in interest in the past decade, with the structurally diverse natural products-derived compounds at its forefront. These secondary metabolites from various biological sources target this epigenetic modification through distinct mechanisms of enzyme regulation by utilizing a diverse array of pharmacophores. We review the discovery of these compounds and discuss their modes of inhibition together with their downstream biological effects.

Keywords

Histone acetylation; histone deacetylases; histone acetyltransferases; natural products; secondary metabolites

INTRODUCTION

Natural products are secondary metabolites derived from marine and terrestrial micro- and macro- organisms that are not deemed essential for survival but are utilized mainly for specialized functions such as chemical defense, communication, predation, and reproduction. Many of these small molecules have distinct protein targets, capable of modulating protein activity and localization. In addition, natural product-based compounds present high structural diversity and have been a validated source of new pharmaceutical agents, either used as the natural product itself or in modified form [1–3]. Natural products drug discovery is initiated through a phenotypic or target-based screen using a bioactivity-guided or structure-directed fractionation to obtain in high purity the bioactive constituents for structure elucidation and mechanism of action studies. In particular, many available treatments for cancer are based on natural products, with ~70% of drugs introduced from 1981–2010, showing diverse mechanisms of action [3]. Some of the more recently approved natural products drugs for cancer have non-DNA epigenetic targets such as histone deacetylases (HDACs).

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CONFLICT OF INTEREST

H.L. is co-founder of Oceanyx Pharmaceuticals, Inc., which is negotiating licenses for HDAC inhibitor related patents and patent application.

The genetic origins of cancer from mutations leading to gain of function of oncogenes and loss of function of tumor suppressors are widely understood [4, 5]. In addition, epigenetic changes – modifications to the structural organization of DNA resulting in alterations in gene expression, but which are independent of the DNA sequence – have been recognized to lead to genomic instability and contribute to tumor progression [6, 7]. Epigenetic changes, which include DNA methylation, covalent histone modifications, and nucleosome remodeling affect the basic unit of the chromatin structure, the nucleosome, and hence dictate access to genetic information and gene expression [8]. Covalent modifications on DNA and histones are dynamically regulated by proteins that introduce and remove these modifications, making them potential targets for chemotherapeutic intervention for the treatment of cancer. Histone acetylation and DNA methylation are epigenetic events that are validated targets for the treatment of cancer. The drugs vorinostat (Zolinza[®]) and romidepsin (Istodax[®]) Fig. (1) are histone deacetylase inhibitors and approved for the treatment of cutaneous T-cell lymphoma. Azacitidine (Vidaza[®]), marketed for myelodysplastic syndromes, is a DNA methyltransferase inhibitor.

In this review, we focus on natural products that modulate histone acetylation either through the canonical histone deacetylases (HDACs) or histone acetyltransferases (HATs) derived from both phenotypic and target-based screening. We will discuss details of their discovery, isolation, mechanism, biological activity, and screening rationale.

I. HISTONE POSTTRANSLATIONAL MODIFICATIONS

The nucleosome is composed of ~146 bp of DNA wrapped around a histone octameric core [9]. This nucleoprotein structure is highly stable due to numerous interactions between the DNA and histone protein. These include (1) salt bridges and hydrogen bonding interactions between basic amino acids with the negatively charged phosphate groups of DNA, (2) hydrogen bonding interactions between peptide and DNA backbone, and (3) nonpolar interactions between amino acid side chains and deoxyribose sugar of DNA [9, 10]. The octameric histone core is composed of two copies of four different types of histones – H2A, H2B, H3, and H4 – and is characterized by amino terminal domains that appear as tails protruding from the central histone core and are rich in basic amino acids [9, 10]. These histone tails can undergo posttranslational modifications which include acetylation, methylation, and ubiquitination of lysine residues, methylation of arginine residues, and phosphorylation of serine residues [11]. These covalent changes affect gene expression by altering the overall structure of the chromatin with modified interaction between the histone core and DNA as well as the recruitment of transcription factors. The dynamic interplay between the enzymes that introduce and remove these posttranslational modifications as well as crosstalk among various covalent modifications on the same or different histone tail determines the global levels of these covalent changes [12]. Histone posttranslational modifications are regarded to occur in specific combinations thought to comprise a histone code that determines the downstream cellular effects [13].

Histone acetylation of lysine residues involves the introduction of an acetyl group to the terminal amino group of the lysine side chain. This reaction is catalyzed by HATs using acetyl-CoA as the cofactor. This posttranslational modification converts the positively charged amine side chain of lysine to a neutral amide and thus neutralizes the charged interaction between the lysine residues of histones and negatively charged phosphate groups of DNA. The result is a less compact chromatin structure and increased accessibility by transcription factors and RNA polymerase, ultimately promoting gene expression Fig. (2) [11]. The deacetylation of modified lysine residues, carried out by HDACs, has been regarded to cause a negative effect on gene expression. Known acetylation sites on histones include lysine residues of histone H3 (K9, K14, K18, K23, K56), histone H4 (K5, K8, K12,

K16), H2A (K5), and histone H2B (K5). Global profiling of the cellular acetylome suggested that HDACs and HATs also contribute to the posttranslational modification of a plethora of non-histone proteins involved in various cellular pathways [14, 15].

II. ENZYMATIC MEDIATORS OF HISTONE ACETYLATION

A. Histone Deacetylases

The HDAC family is comprised of four classes of enzymes (Classes I–IV) that catalyze the deacetylation reaction of histones and non-histone protein substrates. Class I, II, and IV HDACs are metalloenzymes that catalyze the reaction in a Zn^{2+} -dependent manner, while Class III HDACs are NAD^+ -dependent enzymes and hence are considered non-canonical HDACs [16]. Class I HDACs, which include HDAC1, 2, 3, and 8, are ubiquitously expressed, primarily found in the nucleus and act as transcriptional corepressors [16–18]. Class II HDACs, on the other hand, are able to shuttle in and out of the nucleus and have tissue-specific functions [17]. Class IIa HDACs have an N-terminal extension with regulatory functions, and include HDAC4, 5, 7, and 9, while Class IIb HDACs, including HDAC6 and 10, are characterized by two catalytic domains [18, 19]. The only Class IV HDAC is HDAC11, which was previously classified as Class I and is predominantly nuclear. However, it coprecipitates with the mainly cytoplasmic HDAC6.

The protein folding of canonical HDACs is conserved and consists of a single α domain comprising a central parallel β -sheet flanked by several α -helices [18, 20, 21]. Structural analysis of HDACs was based mostly on cocrystallization with small molecule inhibitors [20–24]. Sequence alignment of the various HDAC isoforms suggested a highly homologous active site, characterized by a catalytic Zn^{2+} metal ion and His-Asp dyads at the bottom of an 11 Å tubular channel that accommodates the natural acetylated lysine substrate Fig. (3) [18, 20, 21]. Divergent amino acid residues among HDAC isoforms are located mainly at the rim of the tubular channel Fig. (3) [18, 20–22, 25]. Predominant HDAC isoforms, based on sequence annotations, were utilized for alignment using SATCHMO and Clustal Omega [26, 27].

The catalytic Zn^{2+} ion is held in place by Asp178, Asp267, and His180 (HDAC8 numbering) [18, 20, 21]. The acetylated lysine residue, on the other hand, is coordinated to the Zn^{2+} ion and the hydroxy group of Tyr306 [18, 20]. This arrangement then favors the $\text{S}_{\text{N}}2$ attack of a water molecule to the carbonyl of the acetyl group to give a tetrahedral intermediate that collapses to liberate the unmodified lysine and acetate Fig. (4) [28].

B. Histone Acetyltransferases

HATs are classified according to their cellular localization and are divided into three families – MYST, GNAT and p300/CBP – all of which possess a cofactor binding site for acetyl-CoA and bromodomains [29]. The MYST family of HATs, which include MOF, MOZ, and ACTR, uses a direct attack mechanism for acetyl transfer where, following acetyl-CoA binding, the lysine substrate is deprotonated by an active site Glu residue and leads to formation of the ternary complex Fig. (5) [28, 30]. The GNAT family which includes GCN5 forms a ternary complex during the acetylation of the lysine substrate, although the intermediate has not been experimentally observed [31]. The crystal and solution structure of the catalytic domain of GCN5 has been previously reported and supported a single-step reaction mechanism [32]. The backbone NH of Leu126 forms hydrogen bonding interactions with the carbonyl oxygen of the cofactor acetyl-CoA and stabilizes the partial positive charge on the carbonyl moiety of acetyl-CoA, hence favoring the nucleophilic attack of the amino group of the lysine substrate [32]. Based on the enzymatic data and crystal structure of the p300/CBP family, the acetylation reaction is

distinct from other HATs, which proceeds according to the Theorell-Chance mechanism where the ternary complex has an extremely short half-life [33].

III. DEVELOPMENT OF MODULATORS OF HISTONE ACETYLATION

While acetylation of histones H3K9 and H3K14 are marks of transcriptionally active genes [34], comprehensive analysis of cancer cell lines and primary tumors showed a global loss of acetylation and trimethylation at H4K16 and H4K20, respectively [35]. Furthermore, the pattern of histone posttranslational modification has been demonstrated to predict prostate cancer recurrence [36]. Dysregulation of these covalent modifications can be linked to aberrant expression and/or enzymatic activity of histone-modifying enzymes in tumor cells. More than 75% of human cancer cell lines have higher expression of Class I HDACs compared to normal cells, and 5–40% of various solid tumors showed overexpression of these HDAC isoforms depending on tissue source [37]. Different members of Class I HDAC isoforms are emerging to play distinct roles in tumor progression, with HDAC1 and HDAC2 being linked to tumor aggressiveness and increase in cell proliferation, with HDAC2 also related to sensitization of cancer cells towards chemotherapy [38, 39].

A. Screening Approaches

The majority of natural HDAC inhibitors was discovered through phenotypic screens for cell viability and differentiation (Table 1). Cell viability screens with transformed and non-transformed cell lines provide a quantitative assessment of antiproliferative effects. Chemical agents with potent growth-inhibitory activity against transformed cells with minimal cytotoxicity on non-transformed cells are ideal. Cell transformation assays, on the other hand, require transfection of normal cells with oncogenic proteins such as *Ras*, *v-sis*, or human papillomavirus [40]. Transformed cell lines undergo changes in morphology and growth characteristics that are monitored by microscopy following cellular staining. Reversion of the altered phenotype to normal would be observed for compounds with potential antitumor activity. Chemical agents that induce differentiation or cytotoxicity do not necessarily target HDACs and hence would require subsequent determination of their mechanism of action and direct cellular target. The similarity in structure and/or phenotype induced by a natural product can provide insights on potential targets.

Inhibition of HDACs can be determined through assays using whole cells, purified nuclear extracts, or purified enzymes which are incubated with the inhibitor, and the progress of the deacetylation reaction is monitored by fluorescence, colorimetric or scintillation measurement generated by either the product or substrate of the enzymatic reaction [41]. Whole cell lysates can also be profiled for histone hyperacetylation by probing with specific antibodies followed by fluorography. Trichostatin A and trapoxin were among the first natural products to be verified to inhibit HDACs by demonstrating the accumulation of acetylated histones and increased [³H] acetate turnover [42, 43]. The cocrystal structure of trichostatin A with the histone deacetylase-like protein (HDLP) [44] and affinity chromatography purification of HDACs with trapoxin [45] further corroborated the direct target of these seemingly unrelated compounds as HDACs. The structurally disparate cyclic depsipeptide FK228 and trichostatin A both caused the same effect on SV40 transcriptional activation, suggesting that they may affect the same pathway, and were demonstrated to both directly target HDACs [46]. Another common strategy is target-based screening using purified HDAC enzymes, which offers the advantage of immediately knowing the direct molecular target of a small molecule. However, false negatives can arise in the event of insufficient bioactivation of HDAC inhibitors that exist as prodrugs.

HDAC inhibitors were also identified in pathway-based screens for target genes (i.e., *p21*) or for modulators of protein signaling pathways such as Wnt that could be indirectly

affected by HDACs, *via* downstream cellular effects [47, 48]. The p21 protein is a negative regulator of the cell cycle that is commonly downregulated in cancers and is inducible following treatment with HDAC inhibitors. Therefore, it has been commonly utilized as a biochemical marker for HDAC inhibition [49].

Modulators of HAT activity have been mostly derived from *in vitro* enzymatic screening using HeLa-derived histones as substrates, recombinant p300 or p300/CBP, and [³H] acetyl-CoA [50, 51]. Scintillation counting of the reaction mixture and SDS-PAGE profiling of the acetylated histones with concomitant detection by fluorography were performed to determine the extent of radiolabelling and consequently the progress of the acetylation reaction [50]. These target-based screens immediately provided the direct protein target of the compounds.

B. Structural Diversity and Biological Source of Modulators of Histone Acetylation

HDAC inhibitors from Nature have been derived from diverse source organisms of both terrestrial and marine origins. Purification of these inhibitors from the corresponding producer was accomplished mainly through bioactivity-guided approaches, employing either phenotypic- or target gene-based assays. Known natural HDAC inhibitors can be classified into (1) linear compounds, (2) cyclic tetrapeptides, and (3) cyclic depsipeptides with a prodrug moiety (Table 1). HDAC inhibitors are characterized by a Zn²⁺ binding motif, a linker moiety that mimics the natural substrate of HDAC, and a cap group that can interact with regions >5 Å from the enzyme's active site and can be exploited for isoform-selective HDAC inhibition Fig. (2). Hydroxamates, mercapto (thiol), epoxide, keto, carboxylic acid, amide and hydroxy functionalities are common “warhead” moieties utilized by natural product HDAC inhibitors to chelate the catalytic Zn²⁺ ion and/or active site amino acid residues. The linker moiety is a 4- or 5- carbon linear chain that occupies the tubular channel and is critical for optimal interaction between the “warhead” moiety and the active site residues of the enzyme. Cinnamoyl, aromatic, or heteroaromatic ring systems have been described as alternative linker residues in synthetic HDAC inhibitors [52]. Linkerless HDAC inhibitors were also reported and access the catalytic center through a unique pocket in HDAC8 [53]. The cap moiety among natural product HDAC inhibitors exhibits the greatest structural diversity – ranging from a phenyl group, multiple peptide residues and peptide-polyketide hybrid units. A large and highly functionalized cap residue enables interaction between nonconserved amino acid residues of HDACs and the inhibitor, thus promoting pronounced Class I HDAC isoform selectivity, as in the case of the cyclic depsipeptides FK228 and largazole [54]. In contrast, trichostatin A and SAHA, which utilize a small and unfunctionalized phenyl ring as cap residue, cannot access divergent amino acid residues distant from the HDAC active site, which lowers the isoform selectivity for these compounds. The diet-derived butyrate is a weak modulator of deacetylase activity compared to other natural HDAC inhibitors discussed in this review, requiring high μM to low mM levels for activity [55]. Butyrate was initially suggested to be a noncompetitive HDAC inhibitor; however, more recent studies suggested its capability to bind to the HDAC active site *via* its carboxylic acid “warhead” and a C₃H₇ spacer [55, 56]. Modulation of HDAC inhibitory activity of these compounds can be achieved through modification of essential moieties to obtain optimal interaction between the enzyme and inhibitor. A review of the synthetic efforts for these natural HDAC inhibitors has recently been published [57].

Fewer natural products-derived and synthetic HAT inhibitors have been documented. With the variable mechanisms and structures of these enzymes, a general pharmacophore for HAT inhibitors or activators has not yet been established. The chemistry, molecular interactions with HDACs and downstream cellular effects of natural product HDAC inhibitors will be presented in the following sections, together with a glimpse at HAT modulators. Aside from the cyclic depsipeptide romidepsin (FK228), which gained approval for clinical use in 2009,

other natural product HDAC inhibitors and HAT modulators described here are currently at various stages of clinical and preclinical development, mainly for cancer therapy.

1. Linear HDAC Inhibitors—Trichostatins Fig. (6) are bacteria-derived compounds from *Streptomyces* spp. characterized by a para-*N,N*-dimethylbenzaldehyde linked to a conjugated C7 alkyl chain and a terminal functionality as a hydroxamic acid or hydroxamic acid-derived, amide or carboxylic acid. To date, only trichostatin A purified from mycelia of *Streptomyces hygroscopicus* [58] with a terminal hydroxamic acid moiety, has been demonstrated to have potent HDAC inhibitory activity from this group of compounds. Like trichostatin A, both trichostatin C and trichostatic acid induced the differentiation of Friend leukemia cells, while trichostatin D prevented the transformed cell phenotype induced by human papillomavirus [59–61].

The ferric chelate of trichostatin A, trichostatin B Fig. (6), was isolated as an artifact from Fe-supplementation in the culture broth [58]. Related natural products are two glycosyl hydroxamate derivatives of trichostatin A, the isomeric trichostatins C and D Fig. (6), which are -D- and -D-glucopyranoside derivatives, respectively [60, 62]. These compounds can be hydrolyzed to give trichostatic acid Fig. (6) as a semisynthetic product, but which was also purified from *Streptomyces sioyaensis* [61]. The hydroxamic acid moiety of trichostatin A is substituted by an amide functionality in trichostatin RK and JBIR-17 Fig. (6) [47, 63].

Depudecin is a polyketide-derived HDAC inhibitor bearing a highly oxygenated C11 linear chain with multiple double bond, two hydroxy substituents, and two epoxide groups Fig. (6) [64]. It was initially isolated from the soil-derived fungus strain RF-328 identified as *Alternaria brassicicola* and later from the weed pathogen *Nimbya scirpicola* as a phytotoxin [64, 65].

The cytotoxic extracts of marine sponges belonging to the Order Verongida and their associations yielded the psammaphin family of compounds which to date has at least 25 related structures derived from bromotyrosine and modified cysteine [66–70]. The most active in this series is psammaphin A Fig. (6), a dimeric structure possessing a disulfide bond and an oxime functionality [70]. It can undergo glutathione-dependent reduction to liberate the monomeric unit, each possessing a sulfhydryl group [71].

2. Cyclic Tetrapeptide HDAC Inhibitors—The cyclic tetrapeptide HDAC inhibitors are characterized by a 12- or 13- membered ring structure that is made up of at least three hydrophobic and/or aromatic α -amino acids and a fourth residue being a nonproteinogenic modified β - or γ -amino acid Figs. (7, 8). The nonproteinogenic residue is a C₉/C₁₀ alkyl chain that acts as a lysine mimic and possesses a terminal functional group as the Zn²⁺ binding motif. The absolute configurations of cyclic tetrapeptides were determined from acid hydrolysis of these compounds to liberate the corresponding α -amino acid components, followed by enantioselective HPLC separation employing a chiral column or by Marfey's type analyses using a chiral derivatizing reagent such as 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA), 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (FD LA), or 1-fluoro-2,4-dinitrophenyl-5-L-valinamide (FDVA) [72, 73]. Other stereocenters were assigned by X-ray crystallography and/or NMR measurements following modification of secondary alcohols with a chiral anisotropic reagent to form diastereomeric β -methoxy- β -trifluoro-methylphenyl acetate (MTPA) esters [74].

Trapoxins A and B Fig. (7) from the culture broth of *Helicoma ambiens* RF-1023[75] are related to the previously isolated antibiotics HC-toxin [76], chlamydocin [77], WF-3161 [78], and Cyl-2 [79]. These cyclic tetrapeptides are characterized by a Pro or pipercolinic acid (Pip) moiety, at least one unnatural α -amino acid having D-configuration, and the

nonproteinogenic amino acid as 2*S*-amino-8-oxo-9*S*,10-epoxydecanoic acid (Aoe) [75]. Trapoxin A is composed of D-Pip, 2 × L-Phe, and (2*S*,9*S*)-Aoe moieties, while trapoxin B bears a D-Pro instead of D-Pip Fig. (7). Similar to depudecin, the terminal epoxy functional group is essential for HDAC inhibition, and reduction of this moiety by NaBH₄ treatment rendered the analog inactive [43, 45].

Microsporins A and B Fig. (7), closely related to the trapoxins, were derived from culture extracts of the marine-derived fungus *Microsporium* cf. *gypseum* using a cytotoxicity-directed fractionation [80]. Instead of the Aoe moiety, microsporins A and B bear a 2*S*-amino-8-oxodecanoic acid (Aoda) and 2*S*-amino-8-hydroxydecanoic acid, respectively, as the nonproteinogenic moiety [80]. Other units include D-Pip, L-Phe and L-Ala [80].

Apicidin and apicidin A Fig. (8) were the first members of this group of compounds isolated from cultures of the endophytic fungus *Fusarium pallidoroseum* derived from the branches of *Acacia* sp. and *Langularia racemosa* [81]. They consist of the residues D-Pip, L-Ile, 2*S*-amino-8-oxo-decanoic acid (Aoda), and *N*-methoxy-L-Trp in apicidin or L-Trp in apicidin A Fig. (8) [81]. Targeted isolation of other apicidin congeners Fig. (8) yielded apicidins B, C, and D₁–D₃. Apicidin B bears D-Pro instead of D-Pip, while apicidin C contains L-Val instead of L-Ile [82]. Apicidins D₁–D₃ Fig. (8) have modifications in the Aoda residue, with apicidin D₁ being 9-hydroxyapicidin, D₂ the dihydroapicidin, and D₃ the 9-hydroxy-8-deoxyapicidin [83].

Screening for new inhibitors of T-cell activation from the fungus *Acremonium* sp. yielded FR235222 Fig. (8) as a potent inhibitor of lymphokines IL-2 and IL-4 production [84]. This compound also inhibited lymphocyte blastogenesis without cytotoxicity towards primary rat hepatocytes [85]. FR235222 bears the two unusual α -amino acid units (2*R*,4*S*)-MePro and (2*S*)-isovaline, L-Phe and 2*S*-amino-8-oxo-9*R*-hydroxydecanoic acid (Ahoda) [86]. An analog of FR235222, AS1387392/LGP1 Fig. (8), bearing a Pro unit instead of (2*R*,4*S*)-MePro, was recently isolated from the same *Acremonium* sp. collection that yielded FR235222, although it had been previously reported as a synthetic compound [87, 88]. Demethylation of the Pro unit was well-tolerated and did not have any detrimental effects on the biological activities of FR235222 while at the same time simplifying the synthesis of this compound [87]. Both compounds were determined to have the same absolute configuration and comparable HDAC inhibitory activity [87, 88].

Azumamides A–E Fig. (7), derived from the marine sponge *Mycale izuensis* [89], are unique from other cyclic tetrapeptides by having three unnatural α -amino acids with D-configuration and by the absence of a Pro or Pip residue that is common among trapoxins, microsporins, apicidins and FR235222. They also have a β -amino acid derived non-proteinogenic residue, giving it a 13-membered ring system instead of the usual 12-membered ring for cyclic tetrapeptides [89]. Azumamides are characterized by two nonpolar amino acids either as D-Val or D-Ala, an aromatic amino acid either as D-Tyr or D-Phe, and a nonproteinogenic α -amino acid either as 3*R*-amino-2*S*-methyl-5-nonenedioic acid, amide (Amnaa) or 3*R*-amino-2*S*-methyl-5-nonenoic-1,9-diacid (Amnda) [89].

3. Cyclic Depsipeptide HDAC Inhibitors with a Prodrug Moiety—Cyclic depsipeptide HDAC inhibitors are of mixed peptide–polyketide origin and possess a prodrug functionality that acts as a protecting group for the bioactive moiety Fig. (9). Prodrugs are biologically inactive and require activation through a variety of mechanisms such as hydrolysis, reduction, or rearrangement to render the relevant functional groups for inhibitory activity [90]. The protecting groups usually improve cellular stability of the inhibitor and prevent cellular sequestration or untimely release prior to reaching the site of action. Upon activation of the known HDAC inhibitor prodrugs, a common 3*S*-hydroxy-7-

mercapto-4-heptenoic acid moiety is liberated that acts as the lysine mimic and the terminal sulfhydryl group as the Zn^{2+} binding motif Fig. (9).

FK228, also known as depsipeptide, FR901228 or romidepsin, now marketed under the trade name Istodax[®], was isolated from the fermentation broth of *Chromobacterium violaceum* No. 968 which showed weak antimicrobial activity but reversed the *Ras*-transformed phenotype to normal [91]. FK228 is characterized by two Val units with opposite configurations, D-Cys, (*Z*)-dehydrobutyrine, and 3*S*-hydroxy-7-mercapto-4-heptenoic acid Fig. (9) [92]. The compound is a bicyclic depsipeptide with an internal disulfide bond that is reduced in the presence of glutathione to liberate a sulfhydryl (thiol) moiety that can act as the Zn^{2+} binding group [93]. Although the reduction of the disulfide bond of FK228 liberates two thiol groups, only one of these can be appropriately positioned to access the active site of HDACs as determined by the spacer length between the macrocycle and thiol.

Spiruchostatins Fig. (9) isolated from *Pseudomonas* sp. are structurally similar to FK228, possessing a bicyclic depsipeptide structure with an internal disulfide bond formed between D-Cys and 3*S*-hydroxy-7-mercapto-4-heptenoic acid moieties [94]. Spiruchostatins A and B are characterized by a D-Ala and a statine (4*S*-amino-3*S*-hydroxy-6-methyl-heptanoic acid) unit instead of the two Val units and (*Z*)-dehydrobutyrine groups present in FK228 [94]. Another related compound is FR901375 Fig. (9), purified from *Pseudomonas chloroaphilis* No. 2522, with a tetrapeptide scaffold made up of 2 × D-Val, D-Cys, and L-Thr and together with 3*S*-hydroxy-7-mercapto-4-heptenoic acid moiety [95].

Largazole Fig. (9) is a natural HDAC inhibitor recently described by our group from a marine cyanobacterium *Symploca* sp. using a cancer cytotoxicity-guided purification of the crude extract [96]. Largazole is a cyclic depsipeptide characterized by several unique structural features such as a 4*R*-methylthiazoline fused to a thiazole ring and 3*S*-hydroxy-7-mercapto-4-heptenoic acid like in FK228 and spiruchostatins. However, the sulfur atom has a different oxidation status and is protected as a thioester moiety that only requires hydrolysis to activate the prodrug [96, 97].

4. Modulators of Histone Acetyltransferase Activity—Inhibitors and activators of HATs are mostly derived from plants that were previously shown to have anticancer activity and were identified through HAT-targeted assays [50]. The polyphenolic compounds curcumin and epigallocatechin gallate (EGCG) from turmeric and green tea, respectively, are both inhibitors of p300/CBP [98, 99]. These compounds have various documented pharmacological effects and are widely regarded as nutraceuticals. A bioactivity-guided fractionation of the cashew nut shell liquid using the HAT-targeted assay yielded a mixture of unsaturated anacardic acids as the bioactive components, inhibiting p300 and p300/CBP-associated HAT activity [50]. Hydrogenation of this mixture retained HAT-inhibitory activity and yielded one compound, anacardic acid (2-hydroxy-6-penta-decylbenzoic acid) Fig. (10) [50]. Based on results from molecular docking on the p300/CBP active site, anacardic acid mimics the HAT cofactor acetyl-CoA, supporting its non-specific HAT-inhibitory activity [100]. This compound, however, has poor cellular permeability [50].

The polyisoprenylated benzophenone garcinol Fig. (10) was isolated from the fruit rind of *Garcinia indica* possessing low micromolar IC_{50} values against both p300 and p300/CBP HATs [101, 102]. This compound was previously determined to be an antioxidant and, like other members of this class of compounds, has a polysubstituted bicyclic nonane ring that is highly oxygenated and prenylated Fig. (10). Garcinol is a cell-permeable HAT inhibitor and acts as a noncompetitive and competitive inhibitor upon variation of acetyl-CoA and histone concentration, respectively [101, 103]. Screening of other natural polyisoprenylated

benzophenones for HAT-modulatory activity showed that structurally related compounds, *viz.* guttiferones A and E Fig. (10) and clusianone, can also inhibit the p300 HAT activity [102]. Structure–activity relationship studies and molecular docking of garcinol on the p300 HAT domain suggested that the phenol moiety facilitates binding to the acetyl-CoA (cofactor) pocket, while the prenylation pattern affects the specificity and binding to allosteric sites of HATs [103]. On the other hand, the polyisoprenylated benzophenone nemorosone Fig. (10) stimulated p300/CBP activity and enhanced cellular histone acetylation levels [102]. Although nemorosone bears the same substituents as the other polyisoprenylated benzophenone HAT inhibitors, the substitution pattern is different, suggesting that regulation of HAT activity by polyisoprenylated benzophenone is highly tunable.

IV. BINDING MODES OF HDAC INHIBITORS

Probing for the essential features of HDAC inhibitors as well as their binding modes was accomplished primarily through X-ray cocrystallization [21, 23, 44] and molecular docking studies using HDAC homology models or derived crystal structures of HDACs or histone deacetylase-like protein (HDLP) [104, 105]. The majority of characterized natural product HDAC inhibitors compete with Lys for the substrate binding site, chelate the catalytic Zn^{2+} ion and interact with active site amino acid residues. This binding is noncovalent; except for the epoxide-bearing compounds, which covalently modify these enzymes. Natural product HDAC inhibitors also feature multiple stereocenters with defined absolute configurations, and the spatial orientation of functional groups of HDAC inhibitors also promotes optimal interaction with the enzyme. Elucidation of the binding modes and key molecular interactions between natural HDAC inhibitors and enzymes has greatly aided in the design of potent inhibitors and, at the same time, isoform selective agents. A complete assessment of the isoform selectivity of small molecule HDAC inhibitors is limited due to variable assay systems utilized to assess direct enzyme inhibition as well as partial profiling for inhibitory activities against the eleven canonical HDACs.

A. Noncovalent Inhibitors

1. Linear Compounds—Much of the understanding of the structural aspects of HDACs and their inhibitors was derived from successful cocrystallization of the hydroxamate-based HDAC inhibitors trichostatin A Fig. (6) and SAHA Fig. (1) with the histone deacetylase-like protein (HDLP) from *Aquifex aeolicus* [44]. Although SAHA is a synthetic compound originally designed to understand the growth arrest and terminal differentiation effects of dimethyl sulfoxide, the similarity in structure between the natural product trichostatin A and SAHA suggested a similar mechanism of action between these two compounds [106]. It was previously demonstrated that trichostatin A caused the accumulation of acetylated histones in a stereospecific manner, with the natural (*R*)-trichostatin A being biologically active while the synthetic (*S*)-trichostatin A was inactive [42]. The terminal hydroxamic acid moiety of trichostatin A and SAHA is a strong chelator of the active site Zn^{2+} , binding in a bidentate fashion Fig. (11) [21, 44]. The aliphatic chain in these compounds occupies the tubular channel of HDACs and mimics the natural acetylated lysine substrate of HDACs [21, 44]. The conjugated double bonds of trichostatin A form favorable π -stacking interactions with Phe141 and Phe198 residues of the enzyme, while the terminal dimethylaminophenyl moiety interacts with the entrance of the binding pocket on the surface groove of HDLP [44]. Whereas SAHA and trichostatin A are potent HDAC inhibitors, these compounds do not display particular selectivity towards different HDAC isoforms since enzyme–inhibitor interactions are restricted to residues that are common among different HDAC isoforms.

2. Cyclic Tetrapeptides—The cyclic tetrapeptides apicidins, microsporins, azumamides, and FR235222 Figs. (7 and 8) chelate the active site Zn^{2+} ion through the terminal carbonyl, hydroxy and/or amino functional group of the nonproteinogenic α - or β -amino acid. These functionalities have weaker binding affinities compared to thiol and hydroxamic acid groups. The presence of the rigid ring system in these HDAC inhibitors together with at least one aromatic amino acid in the backbone structure are critical for enzyme–inhibitor interaction and π - π stacking interactions as suggested by molecular docking and structure–activity relationship studies on this class of compounds.

NMR experiments, energy minimization, molecular dynamics, and X-ray crystallography of the macrocyclic ring of apicidin Fig. (8) suggest that it is fairly rigid and, depending on the solvent used for solution NMR, would exhibit two β -turns or a γ -turn [107]. Semisynthesis of apicidin analogs to probe the role of the Trp residue and the Aoda moiety yielded 17 indole modified analogs, 10 Trp replacement moieties, and 32 Aoda modified analogs. From these, modification of the Trp residue to a non-indole moiety was detrimental to activity, while the introduction of a bulky aryl or alkyl substituent to the indole group was able to increase the potency of this class of compounds [108–111]. The Aoda group is also critical for activity, with the exact location of the keto group being the primary determinant for biological activity [110]. The length of the aliphatic chain and a non-sterically hindered terminal functionality modulate the bioactivity. Improvement was observed with modification of the keto group of the Aoda side chain to hydroxamic acid or epoxy groups that are characteristic for other HDAC inhibitors [110].

Solution structure analysis of FR235222 Fig. (8) by ROESY indicated a predominantly *trans*-MePro configuration and hydrogen bonding interactions between the Phe and Ahoda moiety [112]. Modification of the Phe unit by *N*-methylation was unfavorable, suggesting that the conversion of Phe to *N*-Me-Phe could lead to a change in conformation of the macrocycle of FR235222 and therefore unfavorable interactions between HDAC and the inhibitor. Modification of the Ahoda side chain of FR235222 and AS1387392 Fig. (8) by inversion of the configuration at C9 and modification of the terminal functionality to a guanidino or amide group were also detrimental to biological activity [87, 112, 113]. The binding interactions of FR235222 with HDLP interpreted using ICM3.2 software indicated interactions between the terminal β -hydroxy-keto group of the Ahoda side chain with the active site metal ion [112]. FR235222 also participates in hydrogen bonding interactions with His131 and Asp168, amino acid residues that are part of the catalytic His-Asp dyads involved in the deacetylation reaction [112]. Hence, the inversion of the C9-configuration of the Ahoda side chain would be unfavorable for hydrogen bonding interactions with these catalytic amino acid residues [112, 114]. Replacement of the isovaline group with Trp and MeAla improved the HDAC inhibitory activity, presumably due to increased hydrophobic interactions of these moieties with nonpolar amino acids in the HDAC binding pocket. Based on docking studies, the Trp analog of FR235222 had its indole side chain occupying the binding pocket surrounded by Gln192, Tyr186, Ala197, Phe200, and Lys267, thus providing additional π - π stabilizing interactions between the HDAC–inhibitor complex [114].

Molecular docking studies of azumamide E on the co-crystal structure of HDLP using Autodock 3.0.5 showed that the carboxylic acid moiety of azumamide E chelates the Zn^{2+} ion and also creates hydrogen bonding interactions with His131, His132, and Tyr297 [115]. The Amnda side chain of azumamide E Fig. (7) interacts with the 11 Å tubular channel of HDLP and establishes a π - π interaction. The macrocycle forms van der Waals and hydrogen bonding interaction with His170, Tyr196, Ala197, Phe198, and Phe200 of HDLP [115]. Synthesis of other azumamide E analogs such as the enantiomer (–) azumamide E and (2*R*, 3*S*)-azumamide E with selective inversion of the Amnda stereogenic centers lowered the

HDAC inhibitory activity compared to the natural product [115]. Docking studies with these two analogs showed sub-optimal interactions with the enzyme compared to the natural product [115].

3. Cyclic Depsipeptides—Cyclic depsipeptide HDAC inhibitors show HDAC isoform selectivity because of their large and functionalized ring system that can interact with divergent regions of HDACs farther than 5 Å from the enzyme's active site. While the sulfhydryl group present in largazole thiol and in the reduced forms of FK228, spiruchostatins, and FR901375 Fig. (9) binds to the active site Zn^{2+} in a monodentate fashion, additional binding interactions between the enzyme and this class of HDAC inhibitors through the rigid cyclic structure add to the potent inhibitory activity of this class of compounds. The most active natural product HDAC inhibitor to date is largazole thiol liberated from largazole [54, 116].

Based on NOESY experiments and Monte-Carlo conformational searching, the macrocyclic ring structure of largazole is rigid and flat [117]. Crystallization of the macrocycle showed that the fused thiazole-thiazoline moieties have a planar arrangement that moves the nitrogen groups of the thiazole and thiazoline rings toward the center of the macrocycle [118]. The NH group of L-Val forms hydrogen bonding with the thiazoline ring and thus would presumably be necessary to maintain the conformation of the macrocycle in optimal contacts with the HDAC enzyme [118]. Several total syntheses [97, 117–121] and preparation of synthetic analogs of largazole [117, 122–127] were published in the last few years, with the aim of improving the HDAC inhibitory activity and HDAC isoform selectivity through modification of the various components of the macrocyclic ring structure. Aside from the thiol group of largazole, other critical features for activity include the length of the aliphatic chain of the 3*S*-hydroxy-7-mercapto-4-heptenoic acid moiety, the configuration of the carbon–carbon double bond and the acylated ester methine carbon of the heptenoic acid, and the cyclic depsipeptide core Fig. (9) [97, 122]. Well-tolerated modifications include the removal of the methyl substituent on the thiazoline ring as well as replacement of the amino acid component of largazole [121–123, 127]. However, *N*-methylation of both the L-Val and Gly-derived units of largazole was detrimental to activity [127]. The amino acid component of largazole, L-Val, was modified to an aromatic, basic, acidic, and nonpolar amino acid, leading to different extents in potency decrease [126, 127].

Based on the recently published X-ray cocrystal structure with HDAC8 and molecular docking using HDAC1 homology models, the thiol moiety of largazole was demonstrated to chelate the catalytic Zn^{2+} ion with a tetrahedral geometry and also form hydrogen bonding interaction with Tyr306 Fig. (11) [23, 126, 127]. The depsipeptide ring system occupies the entrance to the enzyme active site and while, the conformation of the ring system of largazole is preserved upon binding to the enzyme, the L1 and L2 loop of HDAC8 undergoes significant conformational changes to accommodate the inhibitor [23]. Based on sequence alignment, this region is highly divergent among different HDAC isoforms Fig. (3).

B. Covalent HDAC inhibitors

The preceding examples of natural product HDAC inhibitors are all competitive but noncovalent inhibitors. Only the epoxide-bearing natural compounds trapoxins and depudecin are covalent HDAC inhibitors [43, 128]. The electrophilic epoxide moiety of trapoxins and depudecin Fig. (6, 7) is susceptible to nucleophilic attack by an active-site amino acid residue of HDAC. Incubation of [^3H] acetylated histones and monitoring for [^3H] acetic acid release in the presence of HDACs and trapoxin A showed a non-linear reaction rate suggestive of a covalent HDAC inhibitor or a slowly dissociating HDAC–

inhibitor complex [45, 129]. Total synthesis of trapoxin B together with [³H] trapoxin and a trapoxin-based affinity reagent K-trap enabled the purification and molecular characterization of HDACs. Depudecin was also demonstrated to compete with the K-trap coumarin dye conjugate [128].

V. BIOLOGICAL EFFECTS OF HISTONE ACETYLATION MODULATORS

The antitumor activity of HDAC inhibitors is primarily due to promotion of histone hyperacetylation that induce transcriptional activation, but is also due to acetylation of non-histone proteins such as transcription factors (p53, NF- κ B, STATs), cytoskeletal proteins (α -tubulin) and chaperone proteins (Hsp90). While the downstream cellular effects of HDAC inhibitors on histone acetylation directly affects gene transcription, changes in the cellular acetylome of non-histone proteins can induce secondary transcriptional effects, perturb protein levels by modifying protein stability and affect protein-protein interactions and subcellular localization of proteins. Proteins relevant to cancer that are known to be posttranslationally modified by acetylation are shown in (Table 2). Interestingly, treatment of MV4-11, Jurkat and A549 cells with SAHA only affected approximately 10% of the cellular acetylome, despite SAHA being a pan-selective HDAC inhibitor [14]. This mass spectrometry-based analysis of the cellular acetylome indicates that small molecule HDAC inhibitors can affect hyperacetylation of both histones and non-histone protein targets of HDACs to varying extents. The effects of natural product HDAC inhibitors on acetylation of non-histone proteins is, however, not well-documented. The cellular consequences of HDAC inhibition on both histone and non-histone proteins and consequently on gene expression and protein levels and localization lead to cell cycle arrest, decreased angiogenesis, immune modulation, and programmed cell death selectively in cancer cells.

A. Trichostatin A

Trichostatin A showed potent *in vivo* activity in a carcinogen-induced rat mammary model and caused the induction of tumor differentiation to a benign phenotype [153]. Despite potent bioactivity *in vitro*, trichostatin A did not proceed to clinical development. Trichostatin A is rapidly metabolized, with mean plasma half-lives of 6.3 min and 9.6 min for low-dose (0.5 mg/kg) and high-dose (80 mg/kg), respectively, following a single intraperitoneal injection [154]. This compound undergoes *N*-demethylation to yield *N*-monomethyl trichostatin A, oxidative deamination to yield trichostatic acid, and reduction of the hydroxamic acid to yield *N*-monomethyltrichostatin A amide [154]. Aside from *N*-monomethyl trichostatin A, none of the other biotransformation products of trichostatin A inhibits HDACs. The plasma half-life and further biotransformation pathway of *N*-monomethyl trichostatin A are unclear, while *N*-monomethyltrichostatin A amide exists as the major plasma metabolite after 1h [154].

B. Apicidin

Apicidin showed cytotoxicity towards cancer cells and induced cell death by caspase-3 mediated apoptosis. Conversion of procaspase 3 to the effector protease was observed with concomitant cleavage of poly(ADP-ribose) polymerase, a downstream effect of the activation of caspase 9 [155, 156]. Cytochrome C was released in the cytosol and correlated with the onset of apoptosis and activation of caspase 3 in response to apicidin treatment [155]. *In vivo* testing of apicidin using human endometrial cancer cells implanted in BALB/C mice showed a significant reduction in tumor growth compared to control group [157]. Staining of tumor sections showed decreased expression of VEGF and PCNA, decreased blood vessel formation, and large areas of necrosis [157]. Staining of tumor sections for histone acetylation and p21 expression showed upregulation compared to the control group [157]. Apicidin had a serum half-life of 1.0 ± 0.1 h following intravenous injection of 1 mg/

kg apicidin to rats [158]. Gene expression profiling of MDA-MB-435 cells treated with apicidin using DNA microarray analysis of 19,000 genes indicated effects on genes involved primarily in cell signaling [159]. Cell signaling modulators *SMAD3*, *SMAD4*, and negative cell cycle regulator *p21* were upregulated, while positive regulators of the cell cycle such as the cyclin-dependent kinases *CDK1*, *CDK2*, *CDK4*, and cyclins A2 (*CCNA2*), B1 (*CCNB1*), B2 (*CCNB2*) were downregulated with apicidin treatment [159].

C. FR235222

In vitro studies showed that FR235222 induced apoptosis in leukemia (U937 and Jurkat) and prostate (LnCap) cell lines [160, 161]. This compound induced G1 cell cycle arrest and apoptosis in a concentration- and time-dependent manner, which has been related to modulation of annexin A1, a Ca^{2+} -dependent protein that binds to phospholipids and has been implicated in signal transduction, growth, and differentiation [160, 161]. FR235222 also induced cleavage of poly(ADP-ribose) polymerase and p21 expression, with concomitant decrease of cyclin E. An analog of FR235222, LGP1, was recently shown to promote apoptosis through a TRAIL-mediated pathway that activates caspase 8 [162].

D. FK228

The disulfide prodrug moiety of FK228 improves its cell permeability and stability. It has a half-life of >12 h and 4.7 h in growth medium and serum, respectively, compared to the bioactive species redFK228 which has a half-life of 0.54 h and <0.3 h in growth medium and serum, respectively [93]. FK228 showed potent inhibitory activity both *in vitro* and *in vivo* against a wide panel of solid and hematopoietic malignancies while maintaining selectivity over normal cells [163–166]. Of note, renal derived cancer cell lines ACHN and A-498 were particularly resistant to FK228 treatment [166]. The difference in gene expression profiles of FK228-resistant and sensitive cell lines enabled the prediction of markers towards FK228 sensitivity [166]. Genes that are predictors of sensitivity had at least two-fold differences in expression in sensitive and resistant cell lines. FK228-resistant cell lines with natively low expression of the dual specificity protein phosphatase *MKPI* gene showed upregulation upon FK228 treatment [166]. Induction of the pro-apoptotic caspase 9 gene was observed for FK228-sensitive cell lines [166]. Comprehensive gene expression profiling of human esophageal cancer cell lines showed expression level changes for genes involved in cell cycle, transcription (transcription factors), cytoskeleton, signal transduction, and metabolism [167]. Proteomic profiling of H322 lung cancer cells also affirmed changes in expression levels of proteins involved in signal transduction, cytoskeleton, transcription, protein synthesis and degradation [168]. Using this approach, a novel target identified was thioredoxin reductase, which is involved in apoptosis [168]. While FK228 is approved for the treatment of cutaneous T-cell lymphoma, it is undergoing several Phase I and Phase II clinical trials as a single agent or in combination therapy for various solid and blood malignancies [169].

E. Spiruchostatins

Like FK228, spiruchostatins require disulfide reduction for activity. Cytotoxicity screening using a panel of 39 cancer cell lines showed mean IC_{50} values of 15 nM and 5.6 nM for spiruchostatins A and B, respectively [170]. The most susceptible cancer cell line was the lung-derived NCI-H522 cell line, while the colon-derived HCT-15 cells were least susceptible to spiruchostatin treatment [170]. A dose-dependent increase in acetylation of histones H3 and H4 was observed for MCF7 and BT474 breast cancer cell lines [171]. Dose-dependent G2/M cell cycle arrest was observed for MCF7-treated cells with concomitant increase in *p21* promoter activation [171].

F. Largazole

Cytotoxicity testing of largazole showed potent inhibitory activity against cancer cell lines and, at the same time, superior selectivity for transformed over non-transformed cells [96]. Largazole is rapidly hydrolyzed in serum and cell lysates to the active largazole thiol by a protein-assisted mechanism, with the active species having good stability [127]. NCI60 screening of largazole showed that certain colon cancer cell lines were particularly susceptible to treatment, and an HCT116 xenograft mouse model was adopted for *in vivo* studies [127]. Largazole did not show acute toxicity and was able to retard tumor growth in test animals compared to control group, caused an upregulation of p15 and induction of caspase 3, and also downregulated levels of HER2, cyclin D1, IRS-1, and pAKT in the tumor [127]. A transcriptomic analysis with largazole-treated HCT116 cells showed upregulation of genes involved in chromatin assembly, negative regulation of cell cycle, and transcription, with downregulation of genes involved in positive regulation of transcription, nucleic acid metabolism, intracellular protein cascade, RNA biosynthesis, metabolism and epidermal growth factor receptor activity [127].

VI. CHALLENGES AND FUTURE AVENUES FOR NATURAL PRODUCTS-DERIVED MODULATORS OF HISTONE ACETYLATION

Secondary metabolites from terrestrial and marine micro- and macro- organisms have yielded several families of potent and structurally diverse HDAC inhibitors. The trichostatin analog SAHA and the natural product FK228 have already reached the patient bedside, and several other natural product HDAC inhibitors are currently at different stages of clinical and preclinical assessment. The clinical development of HDAC inhibitors has been precluded primarily by their metabolic instability, thus leading to poor *in vivo* activity despite potent *in vitro* antiproliferative and HDAC inhibitory activity [172]. Fortunately, Nature has provided the cyclic depsipeptide prodrug HDAC inhibitors, which at least partially circumvent this limitation through chemical functionalities that act as protecting groups, and the highly functionalized ring systems of this class are more resistant to biotransformation. Newer generations of synthetic HDAC inhibitors are designed based on natural products and address the problem of *in vivo* potency through optimization of pharmacokinetic-pharmacodynamic properties [172].

A smaller group of HAT modulators has so far been described from natural sources and the majority is in the early stages of preclinical development. These compounds have nonetheless demonstrated that other non-HDAC epigenetic enzymes can be successfully targeted by secondary metabolites and pose an attractive area of drug development. Natural product HAT modulators have been utilized to elucidate the chemistry and structure of HATs, in much the same way as trapoxins and trichostatin A were also utilized as molecular tools to purify and understand the mechanism and structures of HDACs. As such, development of natural products-derived compounds as molecular probes to elucidate the function and rationale for both HAT and HDAC selectivity is an avenue for exploration, as this has not been fully determined. In addition, combination therapy of these inhibitors with other agents that have non-overlapping mechanisms of action is also actively explored.

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Fig. 1.
FDA-approved HDAC inhibitors for cutaneous T-cell lymphoma.

Fig. 2.

The dynamics of histone acetylation by the key enzymes HATs and HDACs. Posttranslational modifications on nucleosomes affect the chromatin structure, which can exist as compact (upper) or open (lower) conformations. DNA is tightly bound to the histone octameric protein in the compact chromatin. Acetylation (Ac) on histone tails promotes an open chromatin structure and thus gene expression by neutralizing the charged interactions between histones and DNA. This epigenetic process can be regulated through small molecule HDAC inhibitors. The generic HDAC inhibitor structure is shown, consisting of a Zn²⁺ chelating moiety, a Lys chain mimic and a cap group. Modification of these critical features can modulate the potency and isoform selectivity of HDAC inhibitors.

Sequence alignment of HDACs. **(A)** Alignment of the catalytic regions of HDAC1-11 showed high sequence homology. The residues involved in catalysis (shaded in gray) and Zn^{2+} ion chelation (marked with *) are conserved among different isoforms. The residue number (R_n) of the first amino acid of selected peptide sequences is also indicated. **(B)** The amino acid residues that make up the tubular channel of HDACs are conserved among members of each class, with lower homology between Class II-IV, in comparison to the catalytic center. **(C)** Representative regions of HDACs which are $>5 \text{ \AA}$ from the enzyme active site are shown. These are highly divergent among the different isoforms. For **(B)** and **(C)**, amino acid residues in HDAC8 within $3.2 \text{ \AA}$ and shifted with the binding of the HDAC inhibitor largazole are indicated in bold (L31, D101, Y111, F208, Y306) [23]. Conformational changes in these divergent regions (Leu31-Lys36, Leu98-Tyr111) were

observed with largazole binding [23]. Alignments were performed separately for each class using SATCHMO [27]. Default minimum affinity values were used for Class I, and 0.5 for Class IIa and Class IIb alignments. Each “profile” alignment was sequentially aligned using Clustal Omega at default parameters. Some manual editing (removal of gaps) of the alignments was performed using BioEdit 7.1.3 [26]. NCBI Protein Database accession IDs: HDAC1 (GI13128860), HDAC2 (GI293336691), HDAC3 (GI13128862), HDAC8 (GI8923769), HDAC4 (GI153085395), HDAC5 (GI62750349), HDAC7 (GI169234807), HDAC9 (GI30795204), HDAC6 (GI13128864), HDAC10 (GI20070354), HDAC11 (GI217272877).

Fig. 4.
Mechanism of histone deacetylation catalyzed by canonical HDACs.

Fig. 5. Mechanism of histone acetylation catalyzed by HATs. The kinetics of Lys acetylation varies among the three major families of HATs.

Fig. 6.
Linear HDAC inhibitors and members of the trichostatin class of compounds.

Fig. 7.
Trapoxin, microsporin, and azumamide families of cyclic tetrapeptides.

Fig. 8.
Apicidin, apicidins A–C, D₁–D₃ and FR235222.

Fig. 9.
Metabolic activation of cyclic depsisepptides bearing a prodrug moiety.

Fig. 10.
Modulators of histone acetyltransferase activity.

Fig. 11.

Binding of SAHA and largazole thiol to HDAC. The HDAC active site is accessed by SAHA and largazole thiol through the tubular channel. **(A)** SAHA utilizes its hydroxamate moiety to bind to the catalytic Zn^{2+} ion (shown as sphere) of HDACs. This functional group also interacts with the amino acid residues His143, His144 and Asp267, involved in enzyme catalysis. **(B)** The catalytic Zn^{2+} ion of HDACs (shown as sphere) is chelated by the “warhead” mercapto (thiol) group of largazole thiol. The highly functionalized macrocycle of largazole interacts with divergent regions of HDACs, giving pronounced Class I isoform selectivity. SAHA and largazole thiol were docked into a homology model of HDAC1 using AutoDock Vina [104, 105].

Table 1**Structural Diversity and Biological Source of Modulators of Histone Acetylation**

Compound	Class of Compound	Biological Origin	Source Organism	Target/Phenotypic Assay
HDAC inhibitors				
Trichostatin A	Linear Hydroxamic Acid	Microbial	<i>Streptomyces</i> sp.	Transformation
Depudecin	Linear Polyketide with Epoxy Functionality	Microbial	<i>Alternaria brassicicola</i> <i>Nimbya scirpicola</i>	Transformation
Psammaplin A	Linear Bromotyrosine	Marine Sponge	<i>Psammaplysilla</i> sp. <i>Aplysinella rhax</i>	Cytotoxicity, Wnt Signaling
Trapoxins A and B	Cyclic Tetrapeptide	Microbial	<i>Helicoma ambiens</i>	Transformation
Apicidin	Cyclic Tetrapeptide	Microbial	<i>Fusarium pallidroseum</i>	Antiplasmodial
FR235222	Cyclic Tetrapeptide	Microbial	<i>Acremonium</i> sp.	Lymphokine Production
Azumamides A–E	Cyclic Tetrapeptide	Microbial	<i>Mycale izuensis</i>	Antitumor
Microsporins A and B	Cyclic Tetrapeptide	Microbial	<i>Microsporium</i> cf. <i>gypseum</i>	Cytotoxicity
FK228	Cyclic Depsipeptide	Microbial	<i>Chromobacterium violaceum</i>	Transformation
Spiruchostatins A and B	Cyclic Depsipeptide	Microbial	<i>Pseudomonas chloroaphilis</i>	TGF- like Phenotype
FR901375	Cyclic Depsipeptide	Microbial	<i>Chromobacterium violaceum</i>	Transformation
Largazole	Cyclic Depsipeptide	Marine Cyanobacterium	<i>Symploca</i> sp.	Cytotoxicity
HAT modulators				
Curcumin	Polyphenol	Terrestrial Plants	<i>Curcuma longa</i>	Target-based HAT Assay
EGCG	Polyphenol	Terrestrial Plants	<i>Camelia sinensis</i>	Target-based HAT Assay
Anacardic Acid	Modified Fatty Acid	Terrestrial Plants	<i>Anacardium occidentale</i>	Target-based HAT Assay
Garcinol	Polyprenylated Benzophenone	Terrestrial Plants	<i>Garcinia indica</i>	Target-based HAT Assay
Isogarcinol	Polyprenylated Benzophenone	Terrestrial Plants	<i>Garcinia</i> sp.	Target-based HAT Assay
Guttiferones A and E	Polyprenylated Benzophenone	Terrestrial Plants	<i>Garcinia</i> sp. <i>Rheedia</i> sp. <i>Symphonia</i> sp.	Target-based HAT Assay
Nemorosone	Polyprenylated Benzophenone	Terrestrial Plants	<i>Clusia</i> sp.	Target-based HAT Assay

Table 2

Selected Cancer-Relevant Non-Histone Proteins Posttranslationally Modified by Acetylation

Protein	Known Acetylation Sites	Proposed Cellular Effects	References
p53	Lys305/320/370/372 Lys373/381/382/386	Modulates interaction with Mdm2 and consequently affects p53 stability. Complete deacetylation of p53 abrogated <i>p21</i> expression.	[130–135]
NF- B	p65: Lys221/310 p65: Lys122/123 p50: Lys431/440/441	Positive/negative effect on expression of NF- B target genes, depending on acetylation site.	[136–140]
-tubulin	Lys40	Affects dynamics of -tubulin polymerization and the aggresome-mediated protein degradation pathway.	[141–147]
STAT3	Lys685	Contributes to STAT3 dimerization, which consequently initiates downstream signaling pathways.	[148, 149]
HSP90	Lys69/100/292/327/478 Lys554/558	Prevents association of HSP90 with client proteins and co-chaperones.	[150–152]