HDAC Inhibitors-New Generation of Target Specific Treatment

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Abstract: Histone Deacetylases (HDACs) enzymes are critical in regulating gene expression and transcription. They also play a fundamental role in regulating cellular activities such as cell proliferation, survival and differentiation. Inhibition of HDACs has generated many fascinating results including a new strategy in human cancer therapy. HDAC Inhibitors (HDACIs) like SAHA, TSA are emerging as new promising drugs for various anti-inflammatory and CNS-disorders. This review, along with chemical classification, emphasizes on the therapeutic potential of various HDACIs against different diseases.

Keywords: Histone Deacetylase, HDAC inhibitor, chemistry, therapeutic applications.

1. INTRODUCTION

Epigenetics is nothing but the regulation of gene transcription and it is defined as the reversible heritable changes in gene activity that occur without a change in the sequence of nuclear DNA [1]. Regulation of gene transcription occurs by various mechanisms which include [2], (1) DNA methylation, (2) Post-translational histone modifications (primarily acetylation but also includes methylation, phosphorylation, poly-ADP-ribosylation, ubiquitinylation, sumoylation, carbonylation and glycosylation), (3) RNA-associated silencing.

The sudden changes in DNA methylation and histone modifications commonly found in human tumors, have inspired various laboratories and pharmaceutical companies to develop and study epigenetic drugs. The discovery of a dynamic group of nuclear proteins in the chromatin that regulates transcription of many genes came about with the discovery of the histone deacetylases (HDACs) in 1996 when HDAC 1 was identified using the HDAC inhibitor trapoxin [3]. One of the most promising groups of epigenetic drugs is the HDACIs, which have different biochemical and biologic properties but have a single common activity: induction of acetylation in histones [4]. This rapidly evolving field offers exciting new opportunities for investigating the molecular and cellular mechanisms underlying various poorly understood biological phenomena as well as providing new approaches to the diagnosis and treatment of complex clinical disorders such as cancer, asthma, chronic obstructive pulmonary disease (COPD), various kinds of inflammation, etc. [5,6].

2. ACYLATION AND DEACYLATION OF HISTONES IN THE CELL

The fundamental subunit of chromatin is called nucleosome, which is composed of an octamer of four core histones, i.e. H3 and H4 tetramer and two H2A and H2B dimers, surrounded by 146 base pair (bp) of DNA. Among the four histone tails, H3 and H4 are targeted for various post-translational modifications [6]. Transcription in eukaryotic cells is influenced by the manner in which DNA is packaged [7]. The binding of histones to DNA is controlled or regulated by various enzymes present in the cell.

Enzyme Histone acetyltransferases (HAT) add an acetyl group to the histone proteins, releasing the restricted access to the DNA imposed by the histones. Due to this access to the DNA, transcription begins. When the gene no longer needs to be transcribed, enzyme HDAC removes the acetyl group. The removal of the acetyl group enables histones to bind to the DNA, causing restricted access to the DNA again. Thus, histone can exhibit in one of the two antagonist forms, acetylated or deacetylated. Deacetylation leads to the removal of an acetyl group from the ε-amino groups of the lysine side-chain of the histone molecule [8].

The mechanism of these two enzymes i.e. HAT and HDAC in regulating the DNA structure is depicted in Fig. (**1**) [9].

HDACs also regulate the acetylation of numerous non-histone proteins such as transcription factors p53, STAT1 and NF-κB as well as α-tubulin, Hsp90 and Ku70 [10].

3. HDAC SUPERFAMILY OF PROTEINS

Histone deacetylase superfamily is classified into four classes with overall 18 members.

Class I containing HDACs 1–3 and 8, shows similarity to yeast transcriptional regulator Rpd3p deacetylases enzyme, whereas class II (HDACs 4–7, 9 and 10) are similar to yeast Hda1 deacetylases enzyme [11] and class IV (HDAC 11 only) are structurally related to both, class I and II HDACs. Class III HDACs shows sequence similarity to the yeast Sir2, and includes NAD+-dependent deacetylases. The class III HDACs are not sensitive to the inhibition by HDAC inhibitors and their pivotal roll in deacetylation of cell cycle proteins like p53 [12] is still not very clear.

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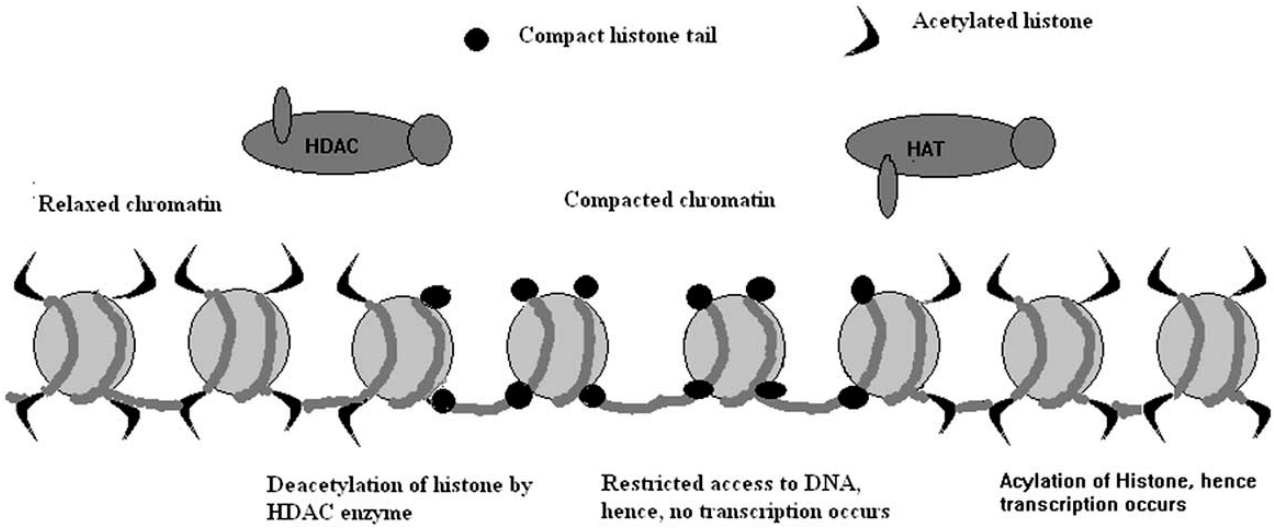


Fig. (1). Schematic representation of HAT and HDAC enzymes regulating transcription.

Class I, II, and IV are referred as ''classical" HDACs as they are Zn^{2+} dependent enzymes which are readily subjected to chelation by Zn^{2+} chelating compounds such as hydroxamic acids. Class III members are named as sirtuins. Therefore, the term ''HDAC inhibitor" is commonly used for compounds that target the ''classical" HDACs i.e., I, II, and IV class [13]. HDAC class I family is found primarily in the nucleus and ubiquitously expressed throughout all human tissue [14]. Class 1 enzymes contain 350–500 amino acids in length. HDAC 1 and HDAC 2 only display activity within a complex of proteins. Three protein complexes have been characterized that contain both HDAC 1 and HDAC 2: Sin3, NuRD and Co-REST [15]. HDAC 3 is found within the nuclear receptor co-repressor (N-coR) and Silencing Mediator for Retinoid and Thyroid receptors (SMRT) repressor complex [16]. HDAC 8 consists largely of the catalytic domain [17]. Its function is not yet known. Class II enzymes can be found in the nucleus and cytoplasm. Their presence is observed in skeletal muscle and the tissues of brain and heart. Class II HDAC family members are further subdivided into IIA and IIB. HDAC 4, 5, 7, 9 belong to class IIA. They regulate nuclear-cytoplasmic shuttling and specific DNA-binding. HDAC 6 and 10 belong to class IIB. HDAC 6 functions as α-tubulin deacetylase [18] and HSP90 deacetylase [19] thus regulates cell motility, adhesion and chaperone function. HDAC 10 is structurally related to HDAC 6 but its exact function is still under investigation. The ySir2 is the prototype of the Class III family of HDAC enzymes [20]. The studies have revealed that class III HDACs governs diverse biological functions as their activities are not limited to histone regulation [21].

4. MECHANISM OF ACTION OF HDACs

The HDAC enzymes mainly remove the acetyl group from the histones comprising the nucleosome. The active site consists of a gently curved tubular pocket with a wider bottom [22]. Removal of an acetyl group occurs *via* a charge-relay system consisting of two adjacent histidine residues, two aspartic residues (located approx. 30 amino acids from the histidine and separated by approx. 6 amino acids), and one tyrosine residue (located approx. 123 amino acids downstream from the aspartic residues) [22, 23]. An essential component of the charge-relay system is the presence of a Zn^{2+} ion. This atom is bound to the zinc binding site at the bottom of the pocket. HDACIs function by displacing the zinc ion and thus rendering the charge-relay system dysfunctional as shown in the Fig. (**2**) [24].

5. HDAC INHIBITORS (HDACIs)

HDAC inhibitors are a structurally diverse group of agents, comprising both natural and synthetic compounds. They bind to the catalytic pocket of the HDAC enzyme, with the long aliphatic chain inserting into the pocket while the polar group chelates the catalytically indispensable zinc ion. Drugs which inhibit classical HDAC enzymes are generally considered as HDAC inhibitors. There are three characteristics shared by all HDAC inhibitor drugs, which are as follows [25]:

1) Large hydrophobic region, which binds to the hydrophobic part of the enzyme near the active site.

2) An aliphatic chain, usually consisting of 5 to 6 carbons attached to the hydrophobic region.

3) An active functional group, which is attached to the other end of the aliphatic chain, interacts with the zinc ion and the residues at the active site to disrupt the enzymatic activity of HDAC.

Required structural features of HDAC inhibitors are depicted in the Fig. (**3**) [25].

Based on these characteristics, various chemical classes of drugs are developed and many of them are still in the various phases of clinical trial.

6. CLASSIFICATION OF HDACIs

HDAC inhibitors are classified as follows:

1. Hydroxamic acids (e.g., trichostatin A (TSA) [26, 27], suberoylanilide hydroxamic acid (SAHA) [28], and oxamflatin [29])

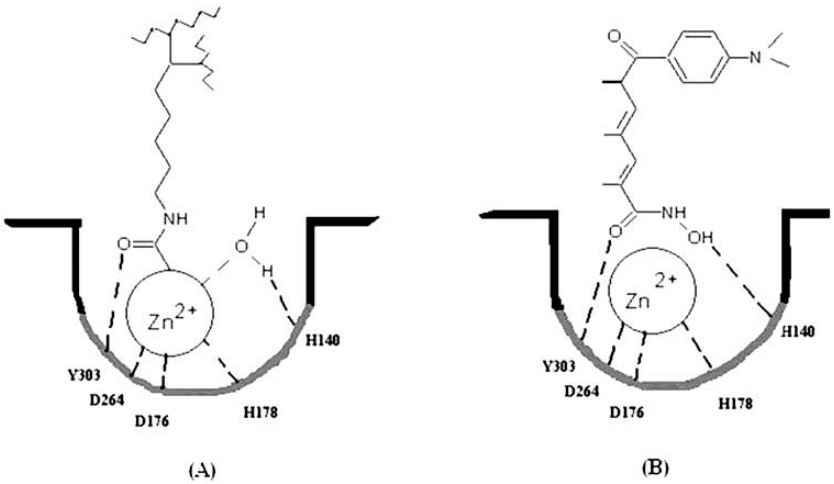


Fig. (2). Diagrams of the HDAC1 catalytic site, based on the data of Finnin, thick black lines represent hydrophobic surfaces and thick gray lines indicate areas rich in charged amino acids. (**A**) Interaction of an acetyl-lysine residue and a water molecule with the zinc cation. (**B**) Trichostatin A (TSA) chelating $Zn2^{+}$ and thus, inhibiting the activity of HDAC enzymes.

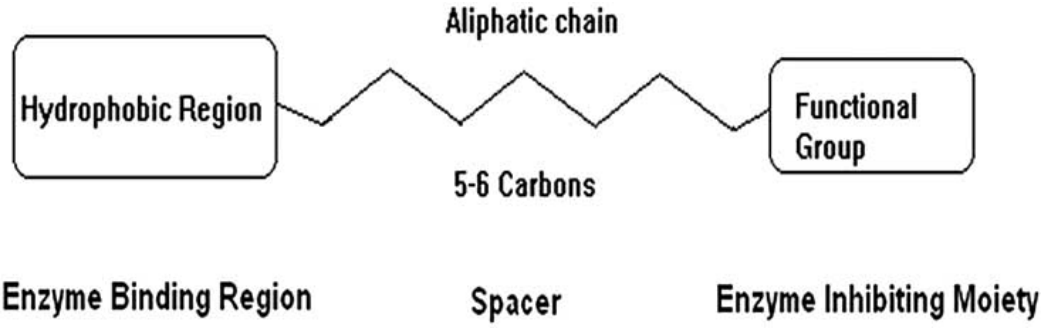


Fig. (3). Structural requirements for HDAC inhibitor.

2. Cyclic tetrapeptides containing a 2-amino-8-oxo-9,10-epoxy-decanoyl (AOE) moiety (e.g., trapoxin A) [30]) and Cyclic peptides not containing the AOE moiety (e.g., FR901228 and apicidin [31])
3. Benzamides (e.g., MS-27-275 [32])
4. Short-chain fatty acids (e.g., butyrates [33])
5. Miscellaneons

6.1. Hydroxamates

Trichostatin A (TSA) and Trichostatin C were initially isolated as fungistatic antibiotics from *Streptomyces hygroscopicus*, but later studies demonstrated that TSA is a potent and specific inhibitor of the HDAC. SAHA inhibits HDAC by binding to a zinc ion in the catalytic domain of the enzyme, thereby preventing the deacetylation of histones, and it is postulated that other hydroxamates work in similar fashion. In late 2006, Vorinostat or suberoylanilide hydroxamic acid (Zolinza^{TM}) (Fig. (**4**)) became the first HDAC inhibitor to gain US FDA approval and is used for the treatment of the cutaneous manifestations of T-cell lymphoma (CTCL), [34-36].

Oxamflatin was found to inhibit HDAC *in vitro* and *in vivo*, improving the expression of gelsolin, cyclin E and cyclin-dependent kinase (CDK) inhibitors.

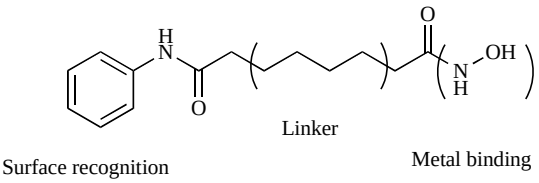
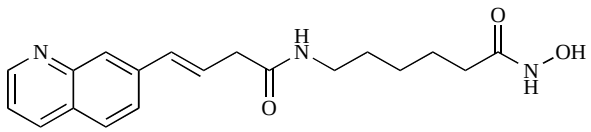


Fig. (4). Vorinostat (ZolinzaTM).

Victor Andrianov and collegues [37] designed and synthesized series of arylamide hydroxamates. This approach yielded compound such as (E)-N-[6-(hydroxyamino)-6-oxohexyl]-3-(7-quinolinyl)-2-propenamide (HDAC IC_{50} 8 nM) which showed potent *in vivo* activity in the P388 mouse leukemia syngeneic model. Based upon this study structure activity relationship (SAR) was established (Fig. (**5**)) as follows:

1. Linking section between the hydroxamic acid and amide must be at least five carbon atoms in length.
2. Compounds with unsaturated carbon adjacent to the amide, shows excellent potency. Strong potency was also maintained using an alkynyl analogue.
3. The nature of the sp3 or sp2 hybridized carbon atom next to the amide linker determines the orientation of the terminal aryl group. The entrance of the narrow tunnel leading to the surface pocket is lipophilic and



E)-N-[6-(hydroxyamino)-6-oxohexyl]-3-(7-quinolinyl)-2-propenamide

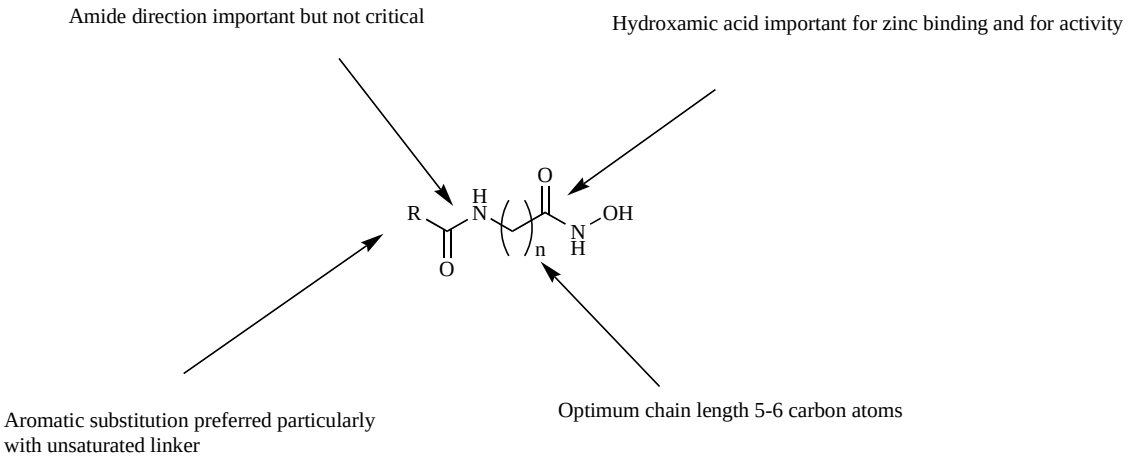


Fig. (5). Active molecule and SAR summary of Victor *et al.* work.

small variations in the contact distances governed by the nature of the hybridized carbon atom may change the lipophilic and electrostatic binding contributions.

4. Bulkier head groups revealed that there were some steric constraints to activity.

Medicinal chemist like Po C. Chen and Coworkers reported the suitability of a 1,2,3-triazole ring as a surface recognition cap group-linking moiety [38] in SAHA-like HDAC inhibitors [39]. Using ''click'' chemistry [40, 41], several triazole-linked SAHA-like hydroxamates were synthesized (Fig. (**6**)). Structure–activity relationship revealed that the position of the triazole moiety as well as the identity of the cap group markedly affected the *in vitro* HDAC inhibition and cell growth inhibitory activities of these compounds.

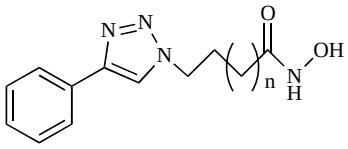


Fig. (6). General structure of aryltriazolylhydroxamates.

Synthesis of a series of sulfonamides (Fig. (**7**)) and their biological evaluation as HDAC inhibitors was also carried out [42].

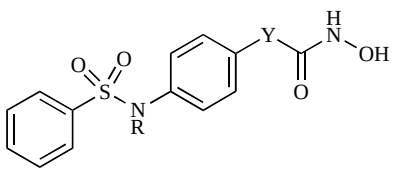


Fig. (7). General structure of sulphonamide hydroxamates.

Based upon the molecular structure of known HDAC inhibitors, several research groups have designed and synthesized new inhibitors. Few of these molecules are reported in the following Table **1**:

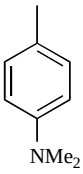
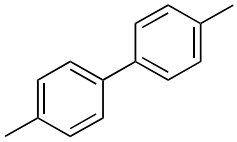
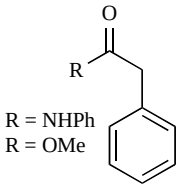
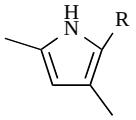
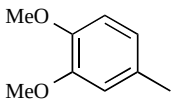
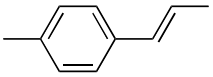
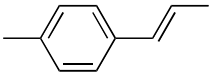
As depicted in Table **1**, these target compounds essentially differ by the nature of the hydrophobic group which binds to the enzyme. It is also observed that the connection unit is usually an amide group, which provides optimum activity.

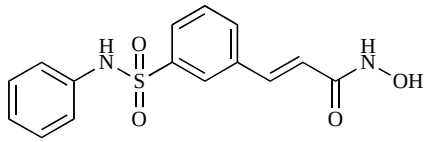
Other hydroxamic acid derivatives are NVP-LAQ824 (formerly LAQ824), PDX 101. NVP-LAQ824 and LDH589 are among many HDAC inhibitors developed by Novartis. Both of these compounds are in their Phase I trials. LAQ824 induces acetylation of the heat shock protein (HSP)-90 [50], inhibiting the binding to ATP and the chaperone association with Bcr-Abl, promoting proteosomal degradation of the latter. PDX 101 is an hydroxamate HDAC inhibitor belonging to the class of sulfonates developed by TopoTarget Prolifix in UK [51, 52], which is also under phase I study. Few examples of HDAC inhibitors containing hydroxamic acid moiety are shown in Fig. (**8**).

6.2. Cyclic Tetrapeptides/Epoxide

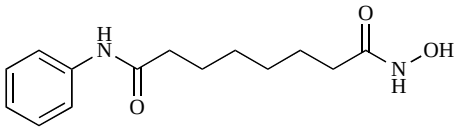
Depsipeptide FK228 (Fig. (**9**)) is a naturally occurring polypeptide which has been isolated from *Chromobacterium violaceum*. It is a stable prodrug. After the uptake by the cell, FK228 is reduced by glutathione into its active form called 'redFK' [53]. This reduced disulfide bond, releases a free thiol moiety that can bind to the zinc ion present at the bottom of a narrow binding pocket in the HDACs. FK228 is under phase II clinical trials for cutaneous T-cell lymphoma [54].

Table 1. Hydroxamic Acid Derivatives HDACIs Containing a Linear and Cyclic Spacer

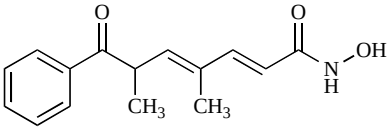
Comp. No.	Enzyme binding region	linker	Spacer-CO-NHOH	Reference No.
1		CONH	$(CH_2)_{5\text{-}6}$	[43, 44]
2	Phenyl, Naphthyl, biphenyl 	CO or C = NOH	$(CH_2)_6$	[45]
3	 R = NHPh R = OMe	NHCO	$(CH_2)_6$	[46]
4		CONH	$(CH_2)_6$	[47]
5		SO_2NH		[48]
6		$NHSO_2$		[49]



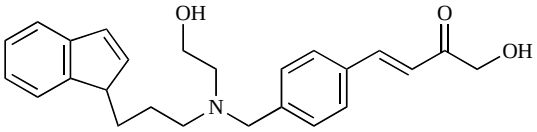
PDX 101



SAHA



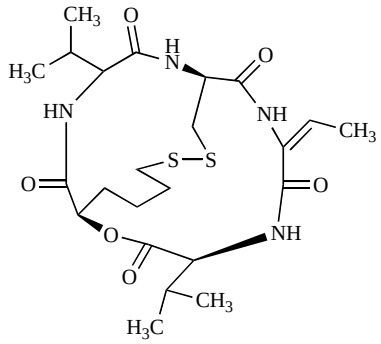
Trichostatin A



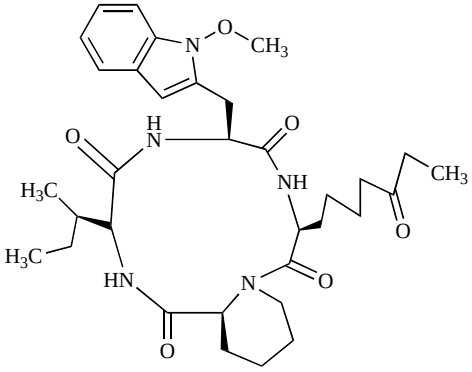
NVP-LAQ 824

Fig. (8). Structures of hydroxamic acid derived HDACIs.

Belonging to the same class of cyclic tetrapeptides but lacking the terminal α-keto epoxide, Apicidin (Fig. (**9**)) was initially isolated by Merck's researchers from two Fusarium species (ATCC 74289 and ATCC 74322) as a novel antifungal metabolite. It inhibits proliferation of tumor cells and subsequently induces apoptosis through selective induction of Fas/Fas ligand in human acute promyelocytic leukemia cells [55]. Apicidin contains an ethyl ketone as potential zinc binding group (ZBG). Its unique feature is that it lacks the epoxide moiety which is an important part in the structure of other cyclic tetrapeptide HDACI such as Trapoxin [56]. A series of Apicidin derivatives (Fig. **10**) were synthesized by Philip Jone & co. and evaluated for HDACI activity [57].



Depsipeptide - FK228



Apicidin

Fig. (9). Structure of cyclic tetrapeptides.

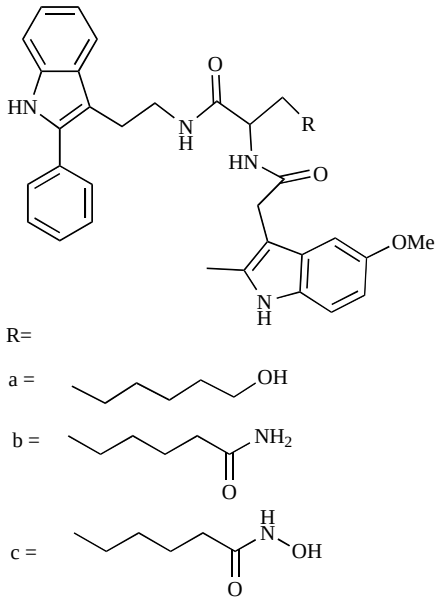
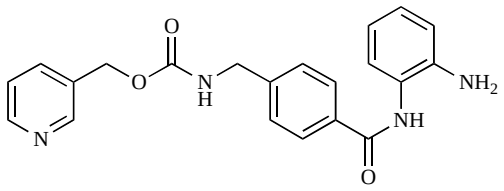


Fig. (10). General structure of Apicidin related compound.

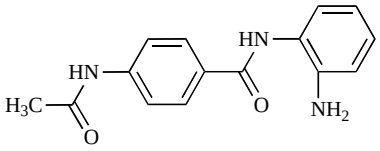
Investigations revealed that ketone was one of the most promising compounds as some alternative structures either lost HDAC activity, such as alcohol (Fig. (**10a**)) or amide (Fig. (**10b**)). It was also observed that when ketone was replaced by the strong zinc chelator, like hydroxamic acid (Fig. (**10c**)), HDAC inhibitory activity increased by many folds.

6.3. Benzamide Containing HDACIs

Benzamides are not structurally similar to other HDACIs but some derivatives have found to inhibit HDAC enzyme *in vivo* and *in vitro*. One of the most active derivatives, MS-275 (Fig. (**11**)), induces hyperacetylation of nuclear histone in various tumor cell lines. It was found to be an inhibitor of class I enzymes but relatively weak inhibition of HDAC 8 [58]. p-N-Acetyl dinaline (or CI-994) which is the 4-acetylamino-N-(2′- aminophenyl) benzamide was discovered as effective against mouse, rat and human tumor cells [59]. CI-994 (Fig. (**11**)) has been introduced in clinical trials for a number of tumor diseases. The clinical potential of this drug has been also established against colorectal cancer [60].



MS - 275



Cl - 994

Fig. (11). Benzamide containing HDACIs.

Many researchers are taking efforts to develop a HDACI with greater selectivity, tolerability and improved efficacy. SAR focused on benzamide lead and structural diversification around the pyridine core of nicotinamide (Fig. (**12**)), showed an HDAC inhibitory activity of 12.4 μM [61].

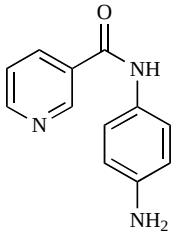
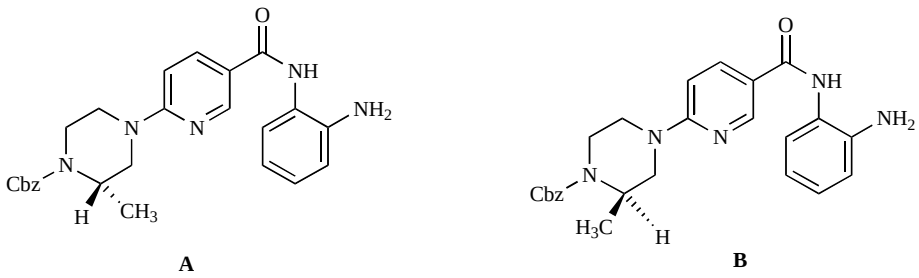


Fig. (12). N-(2-aminophenyl)nicotinamide as a benzamide lead.

It was further determined that the 6th position of the pyridine ring could be easily functionalized and that derivatization with cyclic amines, such as N-substituted piperazines, led to a significant enhancement in HDAC inhibitory activity. The two most potent inhibitors to emerge from the SAR were nicotinamides **A** with an HDAC 1 IC_{50} of 73 nM and **B** with an HDAC1 IC_{50} of 40 nM (Fig. (**13**)).



A **B**

Fig. (13). Potent HDAC inhibitors derivatives of nicotinamides.

N-Hydroxy- 4-(3-phenylpropanamido) benzamide (HPPB), another HDACI which contains N-hydroxybenzamide as zinc-chelating moiety [62] was explored further and it is used as molecular framework for the design of novel HPPB-like HDACIs (Fig. **14**).

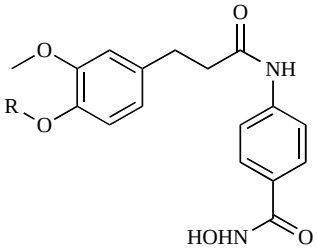
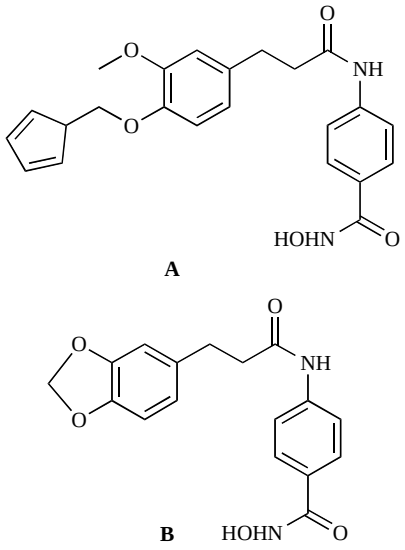


Fig. (14). Molecular framework for the novel HPPB-like HDACIs.

Several HPPB derivatives exhibited HDAC inhibitory activities with IC_{50} values below 1 μM. In this series, the thiophene substituted derivative **A** (IC_{50} 0.3 μM) and benzo[d][1,3]dioxole derivative **B** (IC_{50} 0.4 μM) were found to be the most active and showed growth inhibition in human carcinoma cells HCT116 and A549 (Fig. **15**).



A **B**

Fig. (15). HPPB derivatives containing hydroxyl benzamide.

6.4. Short Chain Fatty Acid

Generally short chain fatty acids (SCFAs) are not very potent in inhibiting HDACs as they are required in millimolar concentration. Drugs depicted in Fig. (**16**) are considered as important tool to study the structure and mechanism of the HDACIs.

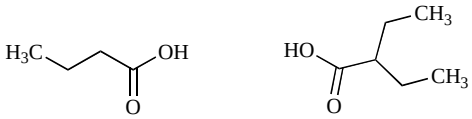


Fig. (16). Short chain fatty acid as HDACIs.

6.5. Miscellaneous HDACIs

Various SAHA-based non-hydroximates inhibitors were recently reported. Among them, a semi-carbazide and two bromoacetamides displayed anti-HADC activity (Fig. (**17A**)) [63]. Other potential HDACIs include trifluoromethyl ketone containing HDAC inhibitor property as shown in the Fig. (**17**) [64]. Based on this study, other electrophilic ketones such as heterocyclic ketones were investigated [65]. Among them, (Fig. (**17C**)) was found to be the most potent inhibitor of HDAC. Using similar approach, a series of ether-linked compounds were synthesized, eg. α-keto amide (Fig. (**17D**)) which exhibited potent activity against HDAC [66].

7. INHIBITORS OF SIRTUINS: SIR2-RELATED NAD+-DEPENDENT PROTEIN DEACETYLASES

A handful of organic molecules that modulate the enzymatic activities of sirtuins are known. The vitamin nicotinamide acts as a non-competitive sirtuin inhibitor. Grozinger identified three small molecules viz. sirtinol, A3 and M15, as sirtuin inhibitors from a cell based screening [67]. In particular, HR 73, the analogue of splitomycin, was found to inhibit human SIRT 1 as well as induce p53 hyperacetylation and decrease HIV transcription. These sirtuin modulators can be useful to dissect the physiological functions of sirtuin proteins and may have values in fighting cancer, viral infections and age-related diseases [68].

Structures of few Inhibitors of Sirtuins are depicted in the following Fig. (**18**):

8. CLASS-SELECTIVE AND ISOFORM-SELECTIVE HDAC INHIBITORS

The majority of HDACI drugs which inhibits all HDAC isoforms non-selectively are known as pan-inhibitors. SAHA and TSA are the pan-inhibitors, since they affect the activity of HDAC 1–9 [69] with almost equivalent potency. Selective HDAC inhibitors, which affect either a single HDAC isoform, are termed as isoform-selective HDACI or those from

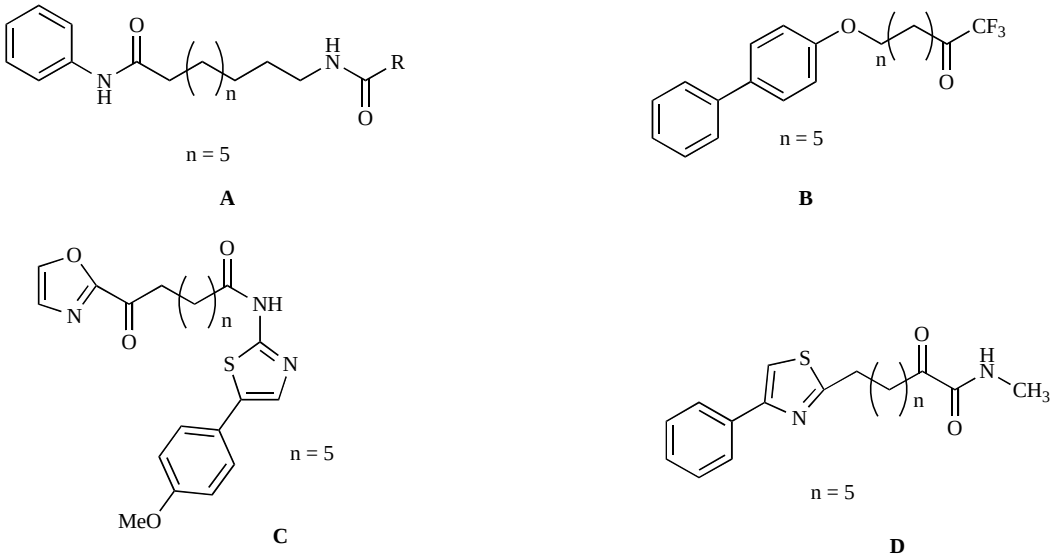


Fig. (17). HDAC inhibitors containing various electrophilic moieties.

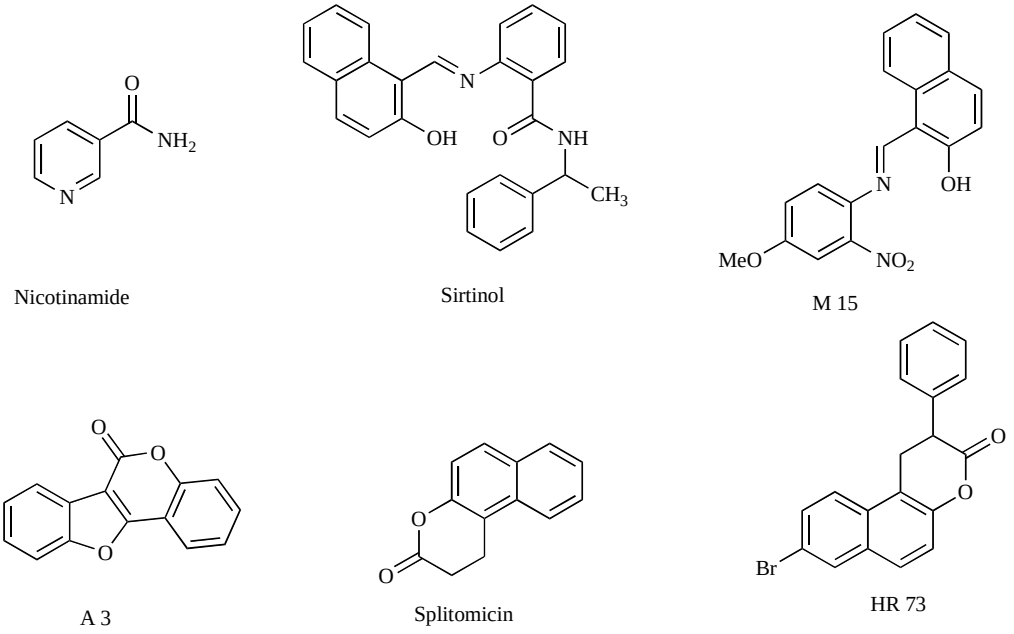


Fig. (18). Chemical structures of SIRT-2 inhibitors.

a single class are called as class-selective HDACI. These selective HDACIs can be helpful in defining the molecular mechanism involving HDAC activity [70]. In addition, it is possible that a class-selective or isoform-selective HDAC inhibitor would provide a more effective and specific way of treatment compared to pan-inhibitors.

The high sequence similarity within the active sites of the isoforms makes inhibitor design challenging. Also, slight differences among the active sites of each human isoform are not well characterized due to the limited crystallographic analysis. Despite these challenges, several class-selective and isoform-selective HDAC inhibitors are reported in the past decade.

HDAC inhibitors with cyclic peptide moieties at the capping group region display class I selectivity. For example, natural products trapoxin A (TPX), trapoxin B, chlamydocin, and Cyl-2 displayed selectivity against HDAC 1 [71]. Tubacin is more selective against HDAC 6 by 4-fold over HDAC 1. Hence, tubacin is considered a selective HDAC 6 inhibitor i.e. isoform 6 selective [72]. In total, the trends observed with capping group derivatives suggest that compounds containing smaller capping groups have generally less class I selectivity compared to most cyclic peptide derivatives. Therefore, the size of the capping group is very important for governing class selectivity.

Many class I-selective HDAC inhibitors contain benzamides as metal-binding groups. Most notably, MS-275 displayed class I selectivity with at least 135-fold preference for HDAC1 and 3 [73]. Similar to MS-275, CI-994 and MGCD0103 showed both class and isoform selectivity when tested against HDAC 1, 3, 6, and 8 [73, 74].

The pan-HDACI TSA was modified to create compounds SK-7041 and SK-7068 (Fig. **19**). In these cases, an amide bond and phenyl ring are present in the linker, unlike in TSA. SK-7041 and SK-7068 showed preferential inhibition of HDAC 1 and 2 over HDAC 3, 4, 5, and 6 [75]. Because TSA is a pan-inhibitor and maintains an identical metal binding moiety and capping group compared to SK-7041, the data showed that modifications in the linker region govern selectivity.

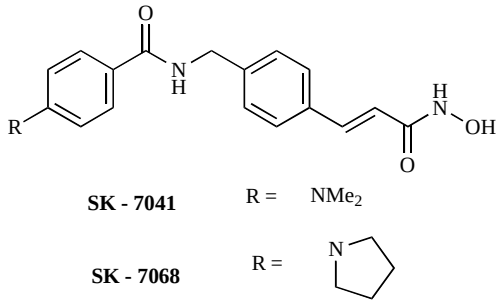


Fig. (19). Modified Pan-inhibitor TSA.

Based on the crystal structure analysis, selective HDAC 8 inhibitors were also designed. Compound **A** (Fig. **20**) displayed potent inhibition of HDAC 8 with a greater than 140- and 115-fold isoform selectivity over HDAC 1 and HDAC 6, respectively (HDAC 8 IC_{50} = 700 nM). Compound **B** (Fig. **20**) displayed greater than 330- fold and 180-fold isoform selectivity for HDAC 8 over HDAC 1 and HDAC 6, respectively (HDAC 8 IC_{50} = 300 nM).

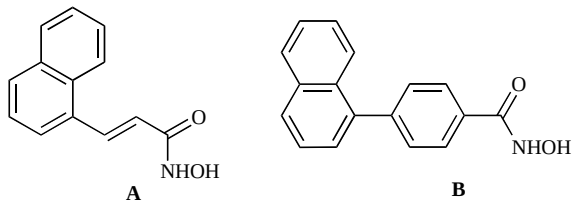


Fig. (20). Selective HDACIs against HDAC 8.

Based on the same design strategies of compounds A and B, PCI-34051 (Fig. **21**) was designed with an indole present in the linking domain. PCI-34051 demonstrated greater than 290-fold selectivity for HDAC 8 over HDAC 1–3, 6, and 10 where HDAC 8 IC_{50} was 10 nM. HDAC 8-selective compounds, SB-379278A (Fig. **21**) displayed roughly a 60-fold preference for HDAC 8 inhibition compared to HDAC 1 and 3, suggesting HDAC 8 isoform selectivity (HDAC8 IC_{50} = 500 nM) [76]. Considering the selectivity of A, B, PCI-34051 and SB-379278A compounds, it can be concluded that the aromatic linking group plays an important role in the HDAC 8 selectivity.

Valproic acid (VPA) is a moderately potent HDACI that contains an unusual branched aliphatic linker region. It is considered as class I-selective inhibitor because this drug does not inhibits HDAC 6 and HDAC 10 isoforms of class IIb [77]. Similarly, butyrate also inhibits the activity of HDAC 1, but not HDAC 6 or HDAC 10 [78]. The data suggested that compounds containing an aliphatic linker, but lacking a distinct capping group, are not tolerated in the hydrophobic active site region of the class IIb HDAC isoforms. It is also notable that VPA and butyrate display carboxylic acid metal binding moieties, which may influence on selectivity.

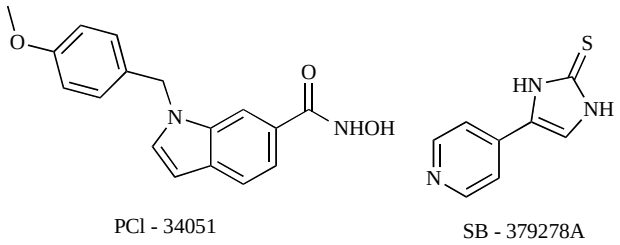


Fig. (21). Selective HDAC 8 inhibitors.

From above data, we can observe that the class I-selective inhibitors, HDAC 1, 2, and 3 are targeted by multiple inhibitors, excluding HDAC 8. In the cases where isoform selectivity is observed, HDAC 1 and HDAC 8 are targeted exclusively. While, very less attention is given for class II-selective HDAC inhibitors including HDAC 4 and HDAC 6 targeted exclusively. Hence, it is noteworthy that lots of efforts have been made to design selective inhibitors, but only a few of the isoforms are targeted by the available compounds. This suggsts that more intensified efforts are needed to create inhibitors to all possible isoforms of HADC enzyme.

9. CLINICAL PHARMACOLOGY

As mentioned earlier, Vorinostat or suberoylanilide hydroxamic acid was the first HDAC inhibitor to gain US FDA approval and is used for the treatment of the cutaneous manifestations of T-cell lymphoma (CTCL). Vorinostat demonstrates potent anti-tumor effects against three well-established CTCL cell lines (MJ [G11], Hut78, and HH) as well as in primary peripheral blood lymphocytes from CTCL patients [79]. Phase I trials with SAHA, administered either intravenously or orally, found that the drug causes accumulation of acetylated histones in peripheral mononuclear cells and tumor cells, is well-tolerated. In phase II, it was observed that SAHA administered orally has good bioavailability. Also, it showed a rapid onset of action and exhibits significant clinical activity against transformed tumors, erythroderma, and nodes in heavily pretreated, refractory CTCL patients.

The mechanism of selective apoptosis produced by vorinostat may potentially involve a variety of pathways, including an increase in p21WAF1, an alteration in bax/bcl-2 ratio, or a decrease in Stat6, etc. [79]. The most common major toxicities reported that were possibly or probably related to oral vorinostat therapy were fatigue and gastrointestinal symptoms, including diarrhoea, altered taste, nausea, and dehydration from not eating/drinking. Thrombocytopenia was dose limiting in patients receiving oral vorinostat at the

higher induction doses of 300 mg twice daily for 14 days. It is also observed that these side effects were dose related and reversible upon cessation of therapy [80]. It is observed that HDACIs are highly effective orally as well as intravenously with little and manageable toxicity to non-malignant cells. With the approval of vorinostat for the treatment of CTCL, the application of epigenetic regulation in the treatment of various therapeutic conditions has expanded, not only for various cancer malignancies, but also to a much broader range of disorders. Besides vorinostat, there are more than 8 other HDAC inhibitors undergoing active clinical investigation. Hence, it would not be impossible to expect an epigenetically novel targeted therapy for the treatment of various disorders.

10. THERAPEUTIC APPLICATION OF HDAC INHIBITORS

Intensive therapeutic development efforts have focused on targeting HDAC with small molecules. Though the therapeutic application of HDACIs have broaden to include various disorders like polyglutamine-repeat disorder, CNS disorders, inflammatory disorders but the major target of HDAC- based therapy is cancer.

10.1. HDAC Inhibitors as Anticancer Agent

Several evidences suggested that inappropriate transcriptional activation and repression mediated by HATs and HDACs is a common cause of formation of various types of cancer. These enzymes thus, represent newer molecular targets in cancer management, hence there exists a need to develop inhibitors that could reprogram transcription and inhibit tumor cell growth and progression. HDACIs have shown activity against diverse cancer types and notable effects on tumor cell proliferation, programmed cell death, differentiation and angiogenesis *in vitro* and *in vivo*. Currently, more than a dozen of phase I and II clinical trials are in progress, involving the use of HDACIs for hematological and solid malignancies.

HDACI-induced cell cycle arrest and growth inhibition is usually correlated with transcriptional activation of genes like, p21WAF1/CIP1, p27KIP1, and/or inhibition of cyclin A, cyclin D and thymidylate synthetase [81]. HDACIs induces apoptosis by targeting both non-histone and chromatin histone proteins. Acetylation of the DNA end-joining Ku70 protein by HDACIs sensitizes cancer cells to apoptotic stimuli [82-84].

They also activate the mitochondrial apoptotic pathway by transcriptional activation of pro-apoptotic proteins: thioredoxin binding protein 2, BAK, Bax, Apaf-1, Bad, Bim, Bid, caspase-3, and caspase-9; and repression of anti-apoptotic proteins: thioredoxin, Bcl-2, Bcl-XL, XIAP, Mcl-1 [85]. Furthermore, the inhibitors like apicidin and CBHA stimulate FAS receptor and its ligand FASL gene transcription [86, 87], while valproic acid up-regulates transcription of TRAIL, FAS and FASL in leukemic cells, leading to selective activation of the death receptor apoptosis pathway [88-90].

HDAC inhibitors are also known to have anti-angiogenic effects [91, 92]. HDAC 1 can be induced by hypoxia. It is also involved in angiogenesis through negative regulation of the tumor suppresser gene p53 and VHL. Trichostatin A was found to inhibit angiogenesis by upregulating these genes and downregulating hypoxia-inducible factor 1 α (HIF-1α), which controls induction of hypoxia [93, 94].

CTCLs encompass a heterogeneous group of rare extranodal lymphoproliferative disorders [95]. US FDA has approved Vorinostat (Zolinza) capsules as second-line therapy for CTCL in patients with progressive, persistent, or recurrent disease following two systemic therapies.

10.2. HDACIs as Anti-Inflammatory Agents

In recent years, HDACIs have emerged as potent candidate for anti-inflammatory drugs, offering new strategy of therapeutic intervention for rheumatoid arthritis or lupus erythematosus. The molecular mode of action of HDACIs is still controversial as it relies on reduced inflammatory mediator production, such as nitric oxide or cytokinase, which implies inhibition of the transcription factor NF-κB. Recent results have indicated that HDACIs can reduce the cytokine and NO production which contribute to various inflammatory diseases. Butyrate and TSA inhibit IL-8 expression in colonic epithelial cells suggested that HDACIs can also be used for the effective treatment of ulcerative colitis [96]. Systemic lupus erythematosus (SLE) is one of the autoimmune diseases characterized by heightened levels of cytokines produced by T-cells, polyclonal B-cell activation, dysregulated autoantibody production and renal inflammation. Interestingly, TSA and SAHA were found to inhibit IL-6, IL-10, IL-12 and IFN-γ production [97]. Thus, HDAC inhibitors can be used in the treatment of SLE.

10.3. HDACIs for Management of Asthma and Chronic Obstructve Pulmonary Disorder (COPD)

Inflammatory lung diseases are characterized by increased expression of multiple inflammatory genes that are regulated by proinflammatory transcription factors, such as nuclear factor-kB. CREB-binding protein, etc. It is observed that in asthma, there is a marked increase in HAT and a small reduction in HDAC activity compared with normal airways, thus favoring increased inflammatory gene expression [98]. However in COPD, there is a marked reduction in HDAC activity in the lung parenchyma, and this decrease is correlated with disease severity. Theophylline is one of the HDAC activators, and can be used in these cases. Understanding the molecular pathways whereby theophylline activates HDACs may lead to novel therapeutic approaches for treating inflammatory diseases in the future [99].

Eosinophils are also important inflammatory cells involved in the pathogenesis of asthma and exacerbations of COPD. Accumulation and activation of neutrophils at the inflamed site is also involved in the pathogenesis of COPD, severe asthma and asthma exacerbations [100]. However, it is known that HDACIs induces apoptosis in a number of tumor cells but not in non-tumor cells [101, 102]. But the promising *in vivo* findings by Choi and coworkers [103] and Kankaanranta H. *et al.* [104] suggests to consider HDACIs as a novel class of drugs to treat various anti-inflammatory, anti-allergic conditions. Their study proved that HDACIs induce apoptosis in human eosinophils and neutrophils. The

mechanism of action in eosinophils involves c-jun-N-terminal kinase and caspases 3 and 6. Thus, HDAC inhibitors have anti-eosinophilic and anti-neutrophilic properties and are possible drug candidates to treat eosinophilic or neutrophilic inflammation.

10.4. HDACIs for CNS Disorders

Progress in the field of chromatin remodeling and transcriptional regulation provides evidences that balance between HAT and HDAC plays an important role in the CNS homeostasis. Various CNS disorders that occur due to mutation in the gene encoding HATs and can be treated with the HDAC inhibitors, includes:

Rubinstein-Taybi Syndrome

Mutation in the CREB binding protein and p300, genes with HAT function, causes the mental retardation that is associated with Rubinstein-Taybi syndrome. Improvement in the long term memory is observed with the HDACI like trichostatin A (TSA).

Psychiatric Disorder

Chromatin remodeling also plays an important role in cognitive impairment that is associated with the psychiatric and neurogenerative disorders. So, targeting histone deacylation may provide benefit for the treatment of depression, schizophrenia, drug addiction and anxiety disorders. HDAC, particularly HDAC 5, seem to mediate antidepressant activity in animal studies. HDAC inhibitor like sodium butyrate exerted antidepressant-like effects in mice, which suggests the broad application of HDACIs in the treatment of cognitive disorders. Other CNS disorders which can be treated with HDACI are Rett syndrome, Friereich's ataxia, Fragile X symdrome, Motor-neuron diseases [105].

10.5. Polyglutamine-Repeat Disease Like Huntington Disease

Huntington's disease (HD) is an autosomal-dominant neurodegenerative disorder characterized by motor dysfunction, psychiatric symptoms, cognitive decline and shortened life span [106, 107]. It belongs to a family of at least nine inherited neurodegenerative diseases caused by an expansion of CAG triplet repeat within the coding region of otherwise-unrelated genes, resulting in an elongated polyQ stretch in the responsible proteins [108]. In each polyQ disease, longer CAG repeat lengths are correlated with increased disease severity and earlier age of onset. The huntingtin (Htt) protein, affected in HD, normally has a stretch of 6-35 glutamines in the amino terminal portion of the protein in normal individuals, whereas, an expansion in the range of 36-121 is observed in patients [109]. Expanded polyQ stretches can inhibit acetylation of transcription cofactors, leading to cellular toxicity. The administration of SAHA and butyrate was found to be effective in HD flies [110, 111]. These observations emphasize that further analysis of target molecules that alter transcriptional activity like HDAC inhibitors might have some efficacy in polyQ disorders.

CONCLUSION

Epigenetic modulation has evolved as a new path for the treatment of various therapeutic complications. It includes methylation of genomic DNA as well as post-translational modification of chromatin-associated proteins, in particular, histones. Acetylation and deacetylation of histone protein by histone deacetylase alters chromatin structure and affects transcriptional regulation. HDAC inhibitors are a new class of targeted therapeutic agents which mediates the regulation of gene expression. HDACIs act on HDAC enzymes and monitors normal cell function. In general, SAR studies revealed that all HDAC inhibitors should possess large hydrophobic region, an aliphatic chain and active functional group in their structure. These structural features are well reflected in some drugs like Vorinostat, which is the first HDAC inhibitor to gain US FDA approval. It is used in the treatment of the cutaneous manifestations of T-cell lymphoma (CTCL). This drug showed good efficacy orally and through i.v. route with few adverse effects. Various small molecule HDACIs are highly effective in up-regulating tumor suppressor gene expression, reducing tumor growth and inducing apoptosis. At the same time, HDAC inhibitors have emerged as strong contenders for anti-inflammatory drugs, offering new lines of therapeutic intervention for rheumatoid arthritis or lupus erythematosus. They also have been recognized as potentially useful therapeutic targets for a broad range of human brain disorders, asthma, COPD, etc. Although mounting data predict a therapeutic benefit for HDAC-based therapy, drug discovery and development of clinical candidates face significant challenges. One of these is the non-selectivity of the HDACIs. The majority of HDACI which inhibits all HDAC isoforms are non-selective. Selective HDAC inhibitors would aid in defining the molecular mechanism connecting HDAC activity in the treatment of various disorders. Hence, more rigorous efforts are needed to create inhibitors to all possible isoforms of HDAC enzyme.

An interesting strategy for the development of future treatment concepts involving HDAC inhibitors may involve combination of basic, clinical, and translational studies which will ultimately determine the clinical utility of these agents and their proper mechanism of action. Currently, efforts are ongoing to develop potent, stable and selective HDAC inhibitors. In future, this might give rise to the tailored use of HDACI in order to treat the complex functions of HDACs in a cell-type-specific manner.

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