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**Review Article**

# HDAC INHIBITORS: A NEW ARMOUR IN ANTI-CANCER THERAPEUTICS

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## ABSTRACT

Histone deacetylase inhibitors (HDACi) are one of the new anti-cancer agents and in fact vorinostat has been approved by the FDA for the treatment of cutaneous T-cell lymphoma. Several classes of HDACi have been identified including organic hydroxamic acids like TSA and SAHA, Short-chain fatty acids like butyrates and valproic acid, benzamides like MS-275, cyclic tetrapeptides like trapoxin and sulfonamide anilides and others. Histone deacetylase inhibitors (HDACi) de-repress genes that results in growth inhibition, differentiation and apoptosis of cancer cells. Vorinostat (SAHA), romidepsin (depsipeptide, FK-228), belinostat (PXD101) and LAQ824 have confirmed therapeutic benefits in cutaneous T-cell lymphoma (CTCL) without combination therapy and have also demonstrated some therapeutic benefit in other malignancies. Beyond cancer, there may be several novel therapeutic areas where HDACi may provide therapeutic benefits like in *Inflammation*, *Polycythemia vera*, essential *Thrombocythemia*, *Myelofibrosis* and *Neurodegenerative* diseases such as *Alzheimer's* disease and *Huntington's* disease. Belinostat appears to be promising for treating low malignant potential ovarian tumor. The combination of azacitidine, valproic acid, and all trans-retinoic acid has significant clinical activity in leukemia. Epigenetic agents in combination regimens for cancer therapy are being actively studied.

**Keywords:** HDACi, SAHA, Hydromates, Cutaneous T-cell Lymphoma, Apoptosis.

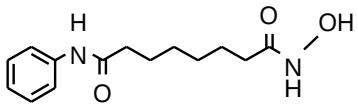
## INTRODUCTION

Enzyme plays a vital role in living organism, in mediating and regulating various biochemical processes including metabolism, catabolism, cellular transduction, cell cycling and development. However, various disorders in human are caused by the dysfunction of enzyme as well as their hyper expression. Histone

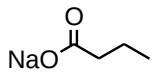
deacetylase (HDACs) is an important class of enzyme which plays an important role in transcriptional regulation.[1] The HDAC family is divided into the Zn-dependent (Class I and Class II) and NAD-dependent (Class III) enzymes. The Zn-dependent enzymes have been the focus of intense research, while the Sir2 family recently implicated in acetylation and regulation of key cell cycle proteins such as p53.[2] Till date, eleven HDAC family members in Classes I and II have been characterized i.e. HDACs 1, 2, 3, 8 are Class I and HDACs 4, to 7, 9 and 10 are Class II, a grouping based on sequence similarity (Table1).[3] The most recently identified member of HDAC family is HDAC 11, most likely Class I, although its similarity to HDAC 1 is weak. Inhibitors of these enzymes are known to induce cell cycle arrest, p53 independent induction of cyclin dependent kinase inhibitor p21, tumor selective apoptosis, and differentiation of normal and malignant cells. So, this direct and indirect effect of HDAC enzyme on tumour cells and metastasis makes histone deacetylase inhibitors (HDACi) as a potential class of anti-cancer agent. SAHA is the first HDACi to meet the FDA Approval for the treatment of cutaneous T cell lymphoma. Several other small molecules which inhibit the HDAC are under clinical trial for cancer.[4]

**Table 1:** Classification of HDAC family

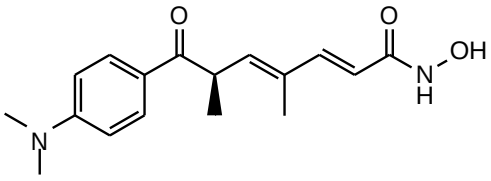
| Zn -Dependant HDAC | | NAD- Dependant HDAC |
|---|---|---|
| Inhibited by TSA | | Inhibited by Nicotinamide |
| Class I | Class II | Class III |
| HDAC 1 | HDAC 4-7 | SIRT 1-7 |
| HDAC 2 | HDAC 9 | |
| HDAC 3 | HDAC 10 | |
| HDAC 8 | | |
| HDAC 11 | | |



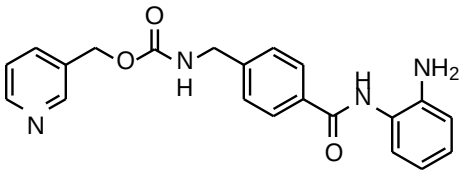
SAHA



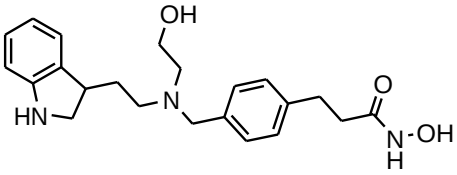
Sodium butyrte



Trichostatin A



MS-275



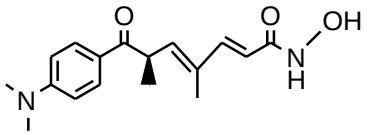
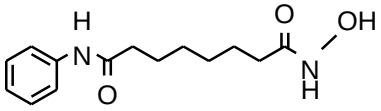
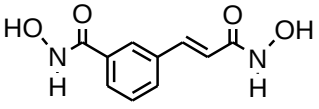
LAQ-824

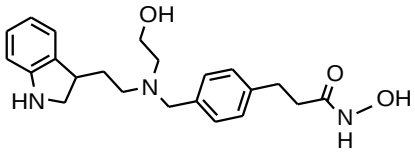
**Figure1:** Some important HDAC inhibitors

## Classification

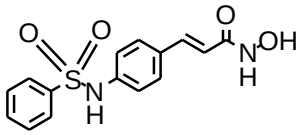
The Classical HDACi acts entirely on class I and class II HDACs by binding to the zinc-containing catalytic domain of the HDACs. Several classes of HDACi have been identified, including (a) organic hydroxamic acids e.g.Trichostatin A (TSA) and suberoyl anilide hydroxamic acid (SAHA), (b) short-chain fatty acids e.g., butyrates and valproic acid (VPA), (c) benzamides e.g. MS-275, (d) cyclic tetrapeptides[5] e.g., trapoxin and (e) sulfonamide anilides and others (Table 2).

**Table 2:** Classification of HDACi based on Chemical Structure

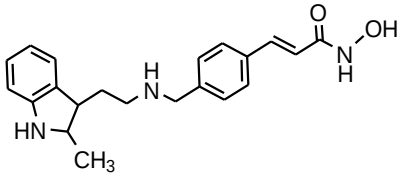
| Class | Compound | Chemical Structure |
|---|---|---|
| Hydroxamates | TSA (Trichostatin) |  |
| | SAHA (Vorinostat) |  |
| CBHA | |  |



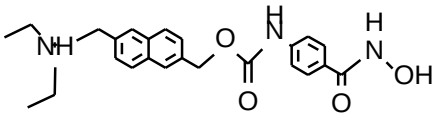
LAQ-824(Dacinostat)



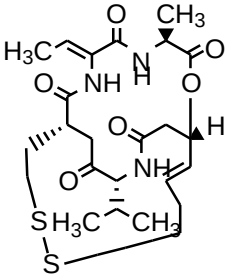
PDX-101(Belinostat)



LBH-589(Panobinostat)

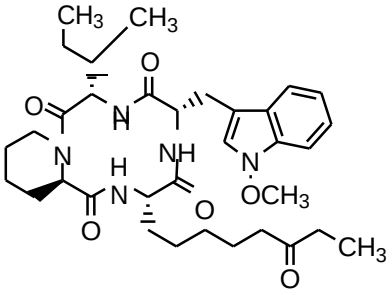


ITF-2357(Givinostat)

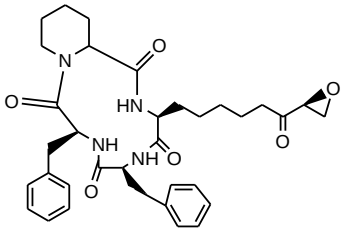


Cyclic peptide

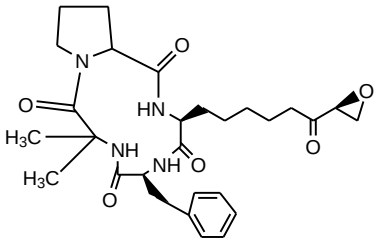
Depsipeptide(Romidepsin)



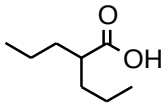
Apicidin



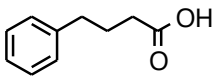
Trapoxins



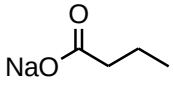
Chlamydocin



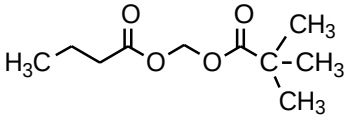
Aliphatic acids — Valproic acid



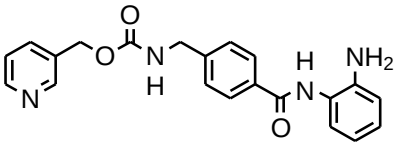
Phenylbutyrate



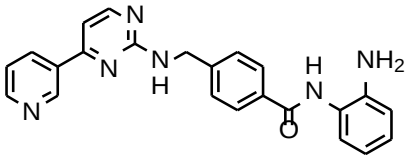
Butyrate



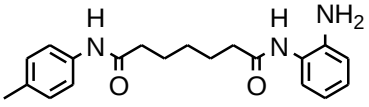
AN-9



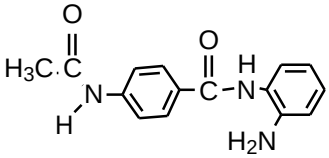
Benzamides — MS-275 (Entinostat)



MGCD0103



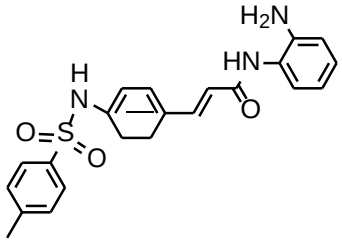
Pimelic diphenylamide

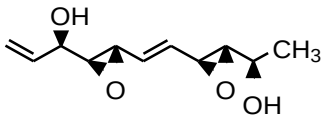


N-acetyldinaline

Sulphonamide anilides

N-2-aminophenyl-3-[4-(4-methylbenzenesulfonylamino)-phenyl]-2-propenamide



| | | |
|---|---|---|
| Others | Depudecin |  |

## Mechanism of Action of HDAC Inhibitors

HDAC enzymes remove the acetyl group from histones using a charge-relay mechanism consisting of two adjacent histidine residues, two aspartate residues and one tyrosine residue, and crucial for this charge-relay system is a $Zn^{2+}$ ion, which binds deep in the pocket of the enzyme.[6] There are a large number of literatures which indicates that HDAC Inhibitors blocks the cell cycle and induce differentiation depending on the cell type and environmental factors.[7] HDAC Inhibitors such as trichostatin A (TSA), SAHA and PXD101, acts by displacing the zinc atom. There are two major pathways of apoptosis, first „„extrinsic" or death-receptor pathway and second the „„intrinsic" or mitochondrial pathway.

All HDAC inhibitors have been reported to follow either one or both of these cell death pathways in many cancer models.[8] The proposed mechanisms of cancer cell death resulting from HDAC inhibitor treatment are as following.

I. Death receptor (extrinsic) pathway of apoptosis
II. Mitochondrial (intrinsic) pathway of apoptosis
III. Inhibition of angiogenesis
IV. Generation of reactive oxygen species (ROS)
V. Autophagy etc.

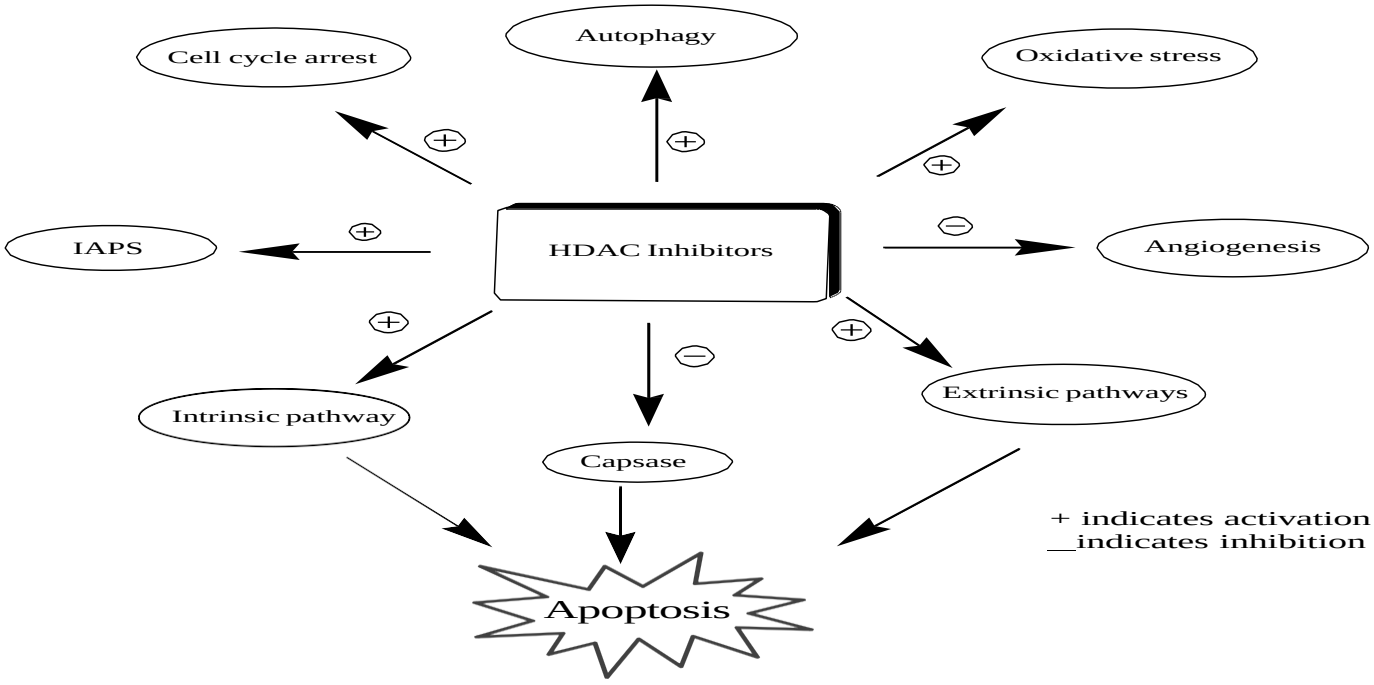



**Figure2:** Mechanisms of HDAC inhibitor-induced cell death

HDAC inhibitors have a multiple effects on cancer cells. HDAC inhibitors also induce apoptosis via both the extrinsic and intrinsic pathway. Apoptosis stimulated by HDAC inhibitors is associated with increased expression of pro-apoptotic genes and decreased expression of anti-apoptotic genes, thus shifting the balance toward cell death. Due to this effect, HDAC inhibitors enhance the efficacy of many pro-apoptotic conventional anticancer agents. Another significant effect associated with HDAC inhibitor-mediated cell death is the generation of reactive oxygen species, ROS. ROS play an important role in activating the intrinsic pathway

of apoptosis. In addition to promoting apoptotic cell death, HDAC inhibitors have also been reported to inhibit tumor angiogenesis and induce autophagy, which may also contribute to their mechanism of action. However, recent evidence suggests that the stimulation of autophagy by HDAC inhibitors may promote survival and thus reduce therapeutic efficacy since its inhibition greatly potentiates HDAC inhibitor-induced cell death.

## Chemistry of Novel HDAC Inhibitors

The classic pharmacophore of HDAC inhibitors consists of three distinct structural parts (a) the zinc-binding group (b) hydrophobic Linker and (c) recognition cap group.

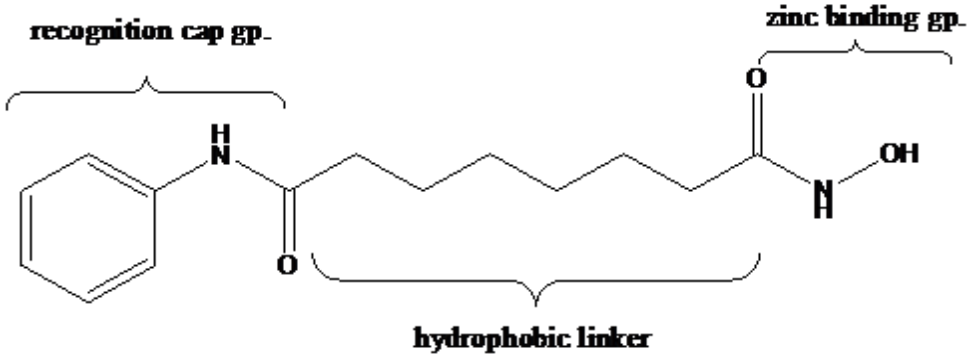



**Figure3:** FDA approved HDAC inhibitor, SAHA

HDACi can be divided into several structural classes including hydroxamates, cyclic peptides, aliphatic acids, and benzamides.[9] TSA was the first natural hydroxamate discovered to inhibit HDACs.[10] Vorinostat is structurally similar to TSA. A series of aminosuberoyl hydroxamic acids have recently been discovered to inhibit HDACs and transform cell proliferation at nanomolar concentrations. Vorinostat is the first HDACi to be approved for clinical use by the Food and Drug Administration.[11] Vorinostat is a pan-inhibitor of class I and class II HDAC proteins. M-Carboxycinnamic acid bishydroxamate is a potent HDACi [12] and is the structural basis for several derivatives including LAQ-824, LBH-589 and a sulfonamide derivative, PXD-101 (Belinostat). These HDACi inhibit class I and class II HDACs. Panobinostat (LBH-589) is a cinnamic hydroxamic acid analogue of M-carboxycinnamic acid bishydroxamate. IF2357 (Italfarmaco) is an HDACi that contains a hydroxamic acid moiety linked to an aromatic ring. The cyclic peptide class is a structurally complex group of HDACi, which includes the natural product depsipeptide (Romidepsin, FK-228), apicidine and the cyclic hydroxamic acid containing peptide group of molecules, all are active at nanomolar concentrations.[13] The aliphatic acids like butyrate, phenylbutyrate and valproic acid are relatively weak inhibitors of the HDACs, with activity at millimolar concentration.[14] Both valproic acid and phenylbutyrate are drugs that are in the market for non-oncological uses like in neurology and psychiatry and were recently shown to have activity as HDACi. MGCD0103 is dihydrobromide salt of a substituted 2-aminophenyl benzamide.[15] Two novel synthetic compounds, SK7041 and SK7068 preferentially target HDAC1 and HDAC2. A small molecule, tubacin, selectively inhibits HDAC6 activity and causes accumulation of acetylated tubulin, but does not affect acetylation of histones and does not inhibit cell cycle progression.[16] It remains to be determined whether selective inhibition of HDACs would be advantageous over pan-inhibition of HDACs in cancer treatment.[17] In prototypical HDAC inhibitor, the capping group is solvent-exposed and interacts with amino acids near the entrance of the active site. The metal binding group resides in the protein interior and complexes the metal ion involved in

catalysis. The linker serves to position the capping and metal binding groups approximately for high-affinity interactions with proteins. The incorporation of substituent on the linker adjacent to the metal binding moiety has a variable influence on inhibitory activity.

## Other Disease That May Be Treated With HDACI

Beyond cancer there may be several novel therapeutic areas where HDACi may provide therapeutic benefits. Early in the investigations on HDAC inhibitors it was recognized that one of the effects was the activation of latent viruses (e.g., HIV). As this may emproblematic in the development of HDACIs, this mechanism can be utilized to reactivate a latent virus thus making it susceptible to subsequent treatment with targeted anti-viral therapy.[18] This concept has been validated in humans with valproic acid, the HDACi and highly active antiretroviral therapy where resting cell infection declined significantly in three out of four patients treated with this combination.[19] HDACi can reduce graft-versus-host disease following bone marrow transplantation by suppressing pro-inflammatory cytokines such as TNFa.[20] Also, HDACi have a long history of use in psychiatry and neurology as mood stabilzers and anti-epileptics. For example valproic acid, marketed as a drug under the trade names Depakene, Depakote, and Divalproex. In more current times, HDACi are being studied as a drug for neurodegenerative diseases such as Alzheimer's disease and Huntington's disease.[21] Enhancement of memory formation is increased in mice with HDACi like sodium butyrate or SAHA, is also observed.[22] Trichostatin A (TSA) and others are being investigated as anti-inflammatory agents.[23] ITF2357 (Givinostat) is under investigation for treatment of polycythemia vera (PV), Essential thrombocythemia (ET) and Myelofibrosis (MF).[24] AR-42 has started clinical trials in 2010 for various cancers like relapsed or treatment-resistant multiple myeloma, chronic lymphocytic leukemia or lymphoma.[25]

## FDA Approved and Under Clinical Trial HDACI

Vorinostat (SAHA, Zolinza®), a hydroxamate-based inhibitor was the first HDACi to be approved by the Food and Drug Administration (FDA) in 2006 for the treatment of CTCL for patients who had already received two or more prior systemic therapies. It has long been considered to inhibit all zinc-dependent HDACs in the nanomolar conc. Recent studies suggest that it has only weak inhibitory affect on class IIa enzymes.[26] Romidepsin (Istodax®), a structurally different cyclic peptide (depsipeptide or FK-228), was second HDAC inhibitor that approved by the FDA at the end of 2009. Romidepsin was isolated from *Chromobacterium violaceum* and inhibits the activity of HDACs at nanomolar conc. This compound inhibits if at all possible class I HDACs and is therefore called a class-selective inhibitor in compare to Vorinostat which also acts robustly on HDAC6. Besides its approval for CTCL, Romidepsin is investigated as a treatment option in other cancer types as monotherapy as well as in combination therapy.[27] Panobinostat (LBH589) is in clinical trials for various cancers including a phase III trial for cutaneous T cell lymphoma (CTCL).[28] Valproic acid, as Mg valproate is in phase III trials for cervical cancer and ovarian cancer. Mocetinostat (MGCD0103) is a benzamide histone deacelytase inhibitor undergoing clinical trial in phase II for treatment of various cancers including follicular lymphoma, Hodgkin‟s lymphoma and acute myeloid leukemia. Givinostat (ITF2357) is a histone deacelytase inhibitor with potential anti-inflamatory, anti-angiogenic, and anti-neoplastic activities.[29] Belinostat is in phase II trial with NDA submission planned for 2011. Entinostat (MS-275) is in phase II for Hodgkin lymphoma, lung cancer and breast cancer.[30]

## CONCLUSION

In this review we summarize studies on classification, mechanism of action, chemistry and the use of HDACi. Many questions are currently still unveiled with respect to HDACi mechanism of action, specificities for definite tumor and the regulation mechanisms of the specific gene expression. Also, it is important to distinguish the HDAC specificity of HDACi for the development of selective therapy on the molecular level. Epigenetic agents in combination regimens for cancer therapy are being actively studied. Volinostat and Romidepsin are two potent HDACi that got FDA approval for the treatment of cutaneous T cell leukoma. Panobinostat, Mocetinostat, Givinostat, Belinostat and Entinostat are some other HDACi that are under clinical trial for cancer.

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