



HISTONE DEACETYLASE INHIBITORS IN CANCER THERAPY: AN UPDATE

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ABSTRACT

Histone deacetylase inhibitors (HDACi) are novel class of anti-neoplastic agents and they mostly act by enhancing acetylation of histones, and promotes uncoiling of chromatin and activation of a large number of genes implicated in the regulation of cell survival like proliferation, differentiation and apoptosis. Most of them are therapeutic targets for cancer, neurodegenerative diseases and a number of other disorders. The histone deacetylases (HDACs) can be divided into two families, which include a total of eleven enzymes. The Zn⁺² dependent HDAC family composed of class I (HDACs 1, 2, 3 and 8), class II a/b (HDACs 4, 5, 6, 7, 9 and 10), and class IV (HDAC 11) and (2) Zn⁺² independent NAD-dependent class III SIRT enzymes. Histone deacetylase inhibitors (HDACi) which have been investigated for their antitumor potency are of with different chemical structures i.e Short-chain fatty acids (e.g. sodium butyrate, phenylbutyrate, valproic acid and (AN-9), Hydroxyaminoic acids (SAHA, pyroxamide, TSA, oxamflarin and CHPAs), Synthetic benzamide derivatives (e.g., MS-275 and CI-994), Cyclic tetrapeptides (such as depsipeptide, trapoxin and apicidin), Electrophilic ketones (trifluoromethylketone), and Miscellaneous (depudecin, SNDX-275 and isothiocyanates). HDACi can have multiple mechanisms of inducing transformed cell growth arrest and cell death. These HDAC substrates are directly or indirectly involved in numerous important cell pathways including control of gene expression, regulation of cell proliferation, differentiation, migration, and death. As a consequence, HDACi can have multiple mechanisms of inducing transformed cell growth arrest and cell death. HDACi can be used in combination with radiation therapy, antitubulin agents, topoisomerase I and II inhibitors etc.

Keywords: Histone deacetylase inhibitors, histone deacetylases, cancer, anti-neoplastic agents.

INTRODUCTION

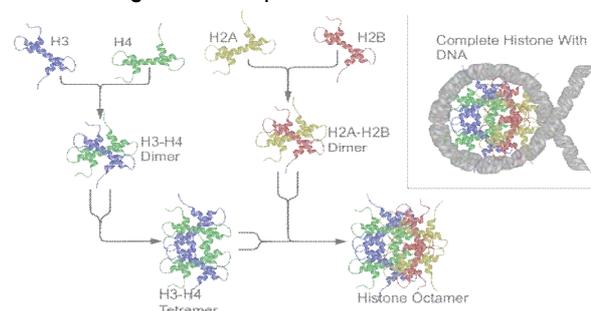
DNA methylation and histone acetylation are two most studied epigenetic modifications, although ethyl, acetyl, phosphoryl, and other modifications of histones have been described^{1,2}. Histone deacetylase inhibitors [HDACi] are a new generation of chemical agents being used to develop therapy against cancer and other diseases³⁻⁵. Cancer is a major health care problem in the world. As of 2004, worldwide cancer caused 13% of all deaths (7.4 million). The leading causes were: lung cancer (1.3 million deaths/year), stomach cancer (803,000 deaths), colorectal cancer (639,000 deaths), liver cancer (610,000 deaths) and breast cancer (519,000 deaths).

The double stranded helical DNA structure was wrapped in to nucleosome by histones. Histones are strong alkaline proteins which pack and order the DNA in to structural units called nucleosomes. The nucleosome core particle represents the chromatin organization and is composed of two couples of each of histones H2A, H2B, H3 and H4 and are assembled in an octameric core^{6,7}. Each core histone consists of the C-terminal globular domain and the N-terminal tail. The N-terminal tails have been a subject of intensive research for last 5 years. The main reason for this interest is that a posttranslational modification of these tails namely acetylation of the lysine residues, has been established to play an important role in regulation of gene activity⁷. There is usually a strong correlation between activity of a particular gene and acetylation of chromatin. The enzymes that promote histone acetylation are called histone acetyltransferases and many of them have been identified as transcription

activators. Conversely, many histone deacetylases, which promote the reverse reaction, turned out to be generally repressors of transcription. Histone acetyl transferases contributes to activation of transcription by converting inactive chromatin into active chromatin via acetylation of histone tails^{8,9}.

In general, increased levels of histone acetylation (hyperacetylation) are associated with increased transcriptional activity, whereas decreased levels of acetylation (hypoacetylation) are associated with repression of gene expression¹⁰⁻¹² (Figure 1).

Figure 1: Complete Histone with DNA



HISTONE ACETYLATION & DEACETYLATION

(HISTONE ACETYL TRANSFERASE AND HISTONE DEACETYL TRANSFERASE)

Acetylation of the lysine residues at the N terminus of histone proteins removes positive charges, thereby reducing the affinity between histones and DNA. This makes RNA polymerase and transcription factors easier

to access the promoter region. Therefore, in most cases, histone acetylation enhances transcription while histone deacetylation represses transcription.

Figure 2: Acetylation and deacetylation of the lysine residue



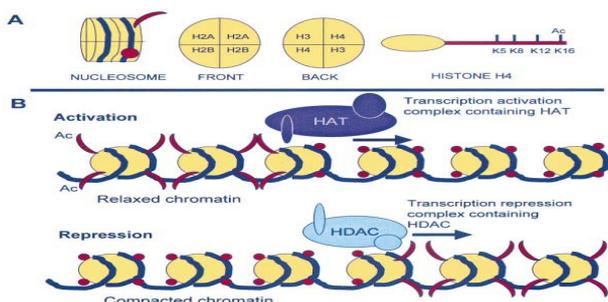
In general, histone acetylation is linked to transcriptional activation and associated with euchromatin. When it was first discovered, it was thought that acetylation of lysine neutralizes the positive charge normally present, thus reducing affinity between histone and (negatively charged) DNA, which renders DNA more accessible to transcription factors. Research has emerged, since, to show that lysine acetylation and other posttranslational modifications of histones generate binding sites for specific protein-protein interaction domains, such as the acetyl-lysine-binding bromodomain.

HISTONE DEACETYLASES (HDACS)

Relative levels of histone acetylation are known to be determined by the enzymatic activities of both histone acetyltransferases (HATs) and histone deacetylases (HDACs).

The HDACs can be divided into two families: (1) the Zn²⁺ dependent HDAC family composed of class I (HDACs 1, 2, 3 and 8), class II a/b (HDACs 4, 5, 6, 7, 9 and 10), and class IV (HDAC 11) and (2) Zn²⁺ independent NAD-dependent class III SIRT enzymes (Table 1). The class I HDACs, apparently the “true” histone deacetylases, are localized to the nucleus of cells. The classes II a/b deacetylases have both histones and non-histone proteins as substrates and are primarily localized to the cytoplasm but are known to shuttle in and out of the nucleus through association with 14-3-3 proteins. The class II enzymes are characterized by either a large N-terminal domain or a second catalytic domain (e.g., HDAC 6 which contains both a histone and a tubulin deacetylase catalytic domain).

Figure 3: Transcription process and its regulation



(A) Representation of a nucleosome. (B) Transcriptional repression and activation in chromatin^{10, 15, 16}.

Table 1: Histone deacetylases

Group	Class	Name
Zn ²⁺ -dependent	I	HDAC1
		HDAC2
		HDAC3
		HDAC8
	II	HDAC4
		HDAC5
		HDAC7
		HDAC9
		HDAC6
	IIb	HDAC10
	IV	HDAC11
NAD ⁺ -dependent	III	SIRT 1-7

The class III SIRT are NAD⁺-dependent deacetylases with non-histone proteins as substrates (in mammalian cells) and have been linked to regulation of caloric utilization of cells (only in yeast are the SIR proteins known to be histone deacetylases)¹³. HDACs do not function independently but rather in concert with multi-protein complexes (e.g., NCoR, SMRT, MEF, MeCP2, Sin3A, etc.¹⁴ that are recruited to specific regions of the genome that in turn generate the unique spectrum of expressed and silenced genes that are characteristic of the expression profile(s) responsible for the malignant phenotype of cancer cells.

HISTONE DEACETYLASE INHIBITORS (HDACi)

Histone deacetylase inhibitors generally have in common three structural characteristics: a zinc binding moiety, an opposite capping group, and a straight chain alkyl, vinyl or aryl linker connecting the two. Based on the co-crystal structures of hydroxamate inhibitors with HDAC4, HDAC 8 and an anaerobic bacterial HDAC like protein (HDLP)¹⁷⁻¹⁹. It is clear that these functional groups interact with three conserved regions of the active site. The first is the zinc ion that facilitates amide hydrolysis and is at the bottom of the narrow catalytic pocket.

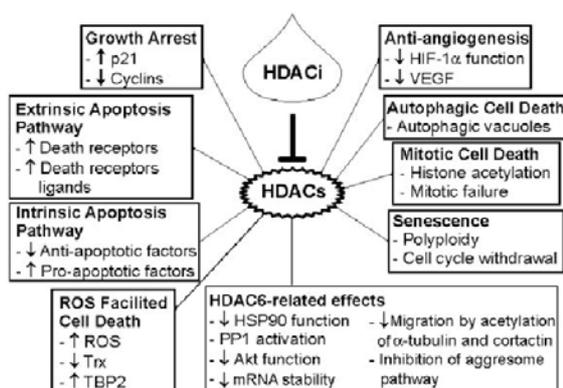
A variety of novel HDAC inhibitors have been isolated and a number of them have entered clinical trials. These compounds include:

1. Short-chain fatty acids (e.g., sodium butyrate, phenylbutyrate, valproic acid and (AN-9)
2. Hydroxyamino acids (e.g., SAHA, pyroxamide, TSA, oxamflarin and CHPAs)
3. Synthetic benzamides derivatives (e.g., MS-275 and CI-994)
4. Cyclic tetrapeptides (such as depsipeptide, trapoxin, apicidin)^{20,21}.
5. Electrophilic ketones (trifluoromethylketone), and
6. Miscellaneous (depudecin, SNDX-275, and isothiocyanates).

MECHANISM OF ACTION

HDACi can selectively suppress the expression of HDAC 7 gene and, to a lesser extent, HDAC 4 gene. An increasing number of proteins are being identified as substrates of HDACs which alters the structure and, as a consequence, the activity of these target proteins. Non-histone protein targets of HDACs include transcription factors, transcription regulators, signal transduction mediators, DNA repair enzymes, nuclear import regulators, chaperone proteins, structural proteins, inflammation mediators, and viral proteins. Acetylation can alter the stability of these proteins and affect protein–protein interactions. These HDAC substrates are directly or indirectly involved in numerous important cell pathways including control of gene expression, regulation of cell proliferation, differentiation, migration, and death. As a consequence, HDACi can have multiple mechanisms of inducing transformed cell growth arrest and cell death. This may be a key factor in the anti-cancer activity of HDACi against a broad spectrum of hematologic and solid neoplasms. Altered expression of HDACs has been reported in association with a number of human cancers. In certain transformed cells, HDAC 1 has been reported to interact directly with transcription repressors, and with pocket proteins, Rb, p107, p130, and with YY1, associated with deregulation of cell proliferation. HDAC 2 and HDAC 3 protein levels have been found increased in some colon cancers. HDAC 1 in gastric cancers and HDAC 5 and HDAC 10 in lung cancers with poor prognosis are reduced. HDAC 1 and HDAC 3 expression is elevated in some breast cancer tumors. Lower HDAC 1 expression has been associated with an invasive esophageal cancer phenotype. Increased expression of HDAC 6 has been reported to correlate with improved disease free and overall survival in patient with hormone sensitive breast cancer.

Figure 4: Mechanism of action of HDACi



HDAC 2 over expression has been found in precancerous lesions and colon cancers associated with abnormal adenomatous polyposis coli tumor suppressor gene. It remains to be established whether these HDAC associations contribute to the malignant phenotype. In addition to altered expression of HDACs, aberrant recruitment of these enzymes to specific loci occurs in certain malignancies. Aberrant recruitment of HDACs to

transcription factors that involve oncogenetic DNA binding fusion proteins resulting from chromosomal translocation or over expression of repressive transcription factors, such as, the oncogenic PML-RAR α , or PLZF-RAR α , and AML1-ETO fusion proteins are found in acute promyelocytic leukemia and acute myeloid leukemia respectively.

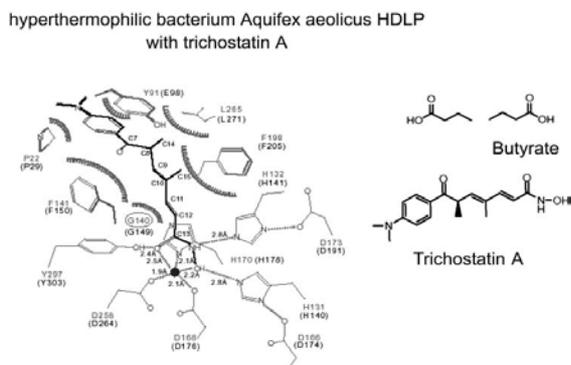
The transcription factor BCL-6 is over expressed in large B cell lymphomas and recruits HDAC 2. Structural mutations in HDACs appear to be rare in cancers. A truncation mutation of HDAC 2 has been discovered in human colon cancer and human endometrial cancer cell lines. A human prostate cell line has been found that does not express HDAC 6, with consequent constitutive accumulation of acetylated tubulin and other acetylated proteins that are targets of this deacetylase²². HDAC inhibitor-mediated effects on cell growth are related to their capacity to alter the expression of genes intimately involved in cell cycle progression. Studies of transcriptional regulation by HDAC inhibitors have demonstrated that only a restricted subset of cellular genes is sensitive to the degree of histone acetylation in chromatin²³. These genes include CDKN1A, which encodes for the cyclin-dependent kinase inhibitor p21, CDKN2A which encodes INK4, also known as p16; genes for cyclin E and thioredoxin binding protein 2 (TBP2); and the putative tumor suppressor gelsolin, which is involved in control of cell shape and tumor invasiveness^{20,21}. HDAC inhibitors, when administered to different cancer cell types, induce to varying degrees cell cycle arrest in G₁ and G₂ phase, apoptosis and/or differentiation²⁴⁻²⁶. Exposure of different cell lines to HDAC inhibitors results in up-regulation, at the transcriptional level, of the endogenous CDK inhibitor p21, which inhibits multiple cyclin/CDK complexes and may also exert direct antiapoptotic actions^{27, 28}. Concurrent with changes in expression of cell cycle-related genes associated with growth arrest, HDAC inhibitors control the expression of genes such as gelsolin involved in morphologic and cytoarchitectural changes associated with cell differentiation²⁹. The mechanism by which HDAC inhibitors induce apoptosis is still not completely understood. Although many actions are common to all HDAC inhibitors, the underlying mechanisms of HDAC inhibitor-induced apoptosis appear to vary with the agent. These may include activation of either caspase-dependent (both receptor and/or the mitochondrial-mediated apoptotic cascades), caspase-independent pathways. In addition to the primary activation of the cell death pathway, it is becoming apparent that once caspase cascades are activated, resulting down-regulation and/or degradation of key molecules may play a critical role in the amplification of the apoptotic signal. Such considerations are also relevant to the case of HDAC inhibitor mediated lethality. In addition, HDAC-inhibitors modulate the expression of proteins involved in the regulation of apoptotic process. On the other hand, HDAC inhibitors may also lead to up regulation of genes that exert anti-apoptotic actions. Several studies have shown that p21 protects cells from apoptosis and attenuates the toxicity

of certain cytotoxic agents in a variety of tumor cell types^{30, 31}. In this way, loss of p21 in conjunction with dysregulation of other cell cycle regulatory proteins e.g., cyclins D1, E, A and pRb), may disrupt the maturation program of cells exposed to HDAC inhibitors, and cause them to engage an alternative, apoptotic program.

Short-chain fatty acids

Among these agents sodium butyrate was highly focused from the early stages of HDAC inhibitors investigators. Sodium butyrate is a non-toxic short-chain fatty acid found naturally in the gastrointestinal tract, and appears to be responsible for the protective effects associated with high-fiber diets³². Butyrate was thought to be important for proper epithelial cell regulation, but was also found to have an antiproliferative and differentiation-inducing activity on various human colon carcinoma cells, normal cells, and neoplastic cells³³⁻³⁵. Butyrate inhibits most HDAC except class III HDAC and class II HDAC6 and -10. During inhibition of HDAC activity, HAT activity continues, which results in histone hyperacetylation. Histones, however, are not the only substrates of these enzymes. High-mobility group proteins are acetylated. This modification has a wide range of effects on the function of the high-mobility group proteins in remodeling chromatin structure and regulating gene expression³⁶⁻³⁸.

Figure 5: Schematic representation of histone deacetylase-like protein (HDLP, gray) interactions with trichostatin A (TSA).



Recently, the crystal structure of an HDAC-like protein from the hyperthermophilic bacterium *Aquifex aeolicus* with the HDAC inhibitor trichostatin A (TSA; Fig. 5) was reported³⁹. The structure shows the position of the essential zinc atom that is involved in catalysis of class I and II HDAC. HDAC-like protein shares 35.2% similarity over a 390-residue region with mammalian HDAC1, this region constitutes the deacetylase core. The aliphatic chain of TSA occupies a hydrophobic cleft on the surface of the enzyme (Fig. 5). Possibly two butyrate molecules also could occupy the hydrophobic pocket and inhibit the enzyme. However, butyrate was found to be a noncompetitive inhibitor of HDAC, which argues that butyrate does not associate with the substrate-binding site⁴⁰. The binding site and mechanism by which butyrate inhibits HDAC activity remain unknown. However, the

short sodium butyrate half-life and difficulties in achieving millimolar plasma concentrations. To overcome these difficulties, other derivatives have been investigated. For example phenylbutyrate also induced growth arrest and differentiation in primary leukemic cells *in vitro*^{41, 42}. At present investigations are going on the clinical usage of phenyl butyrate for both hematological and solid tumor malignancies. Valproic acid (VPA) an anticonvulsant has been shown to have HDACi activity also at millimolar concentrations

Hydroxyamic Acid Derivatives

The first hydroxamate discovered to inhibit HDACs was the natural compound trichostatin A (TSA)⁴³. TSA causes histone hyperacetylation and inhibits cell proliferation at nanomolar concentrations, but its high toxicity precludes its therapeutic use. Vorinostat (SAHA((suberoylanilide hydroxamic acid)) was found to inhibit class I HDACs, HDACs 1, 2, 3, and 8, class II HDACs, HDACs 6 and 10 and HDAC11. Vorinostat does not inhibit HDAC 4, 5, 7, and 9 at concentrations that are clinically relevant. It is a second-generation polar-planar compound that induces growth arrest, differentiation and/or apoptosis. It is the first HDACi approved for cancer therapy-advanced cutaneous T-cell lymphoma⁴⁴. SAHA is approximately 1000-fold more potent in a molar basis than HMBA (hexamethyl-enebisacetamide), a first generation hybrid polar compound, in inducing maturation in murine erythroleukemia cells (MEL)⁴⁵. In studies involving human breast cancer cells, SAHA inhibited clonogenic growth and ultimately induced apoptosis. *In vivo* experiments in rats demonstrated that SAHA, when included in the diet, served as both a chemopreventive and chemotherapeutic agent against carcinogen-induced mammary tumors. Similarly, the HDAC inhibitor SAHA has shown antiproliferative and pro-apoptotic actions in several other cancer cell models including prostate, bladder carcinoma and myeloma⁴⁶⁻⁴⁸. Several new compounds with HDAC inhibitory activity have been developed which are based on the chemical structure of earlier hydroxamic acid HDACs. Recently, a trapoxin analogue has been synthesized in which the epoxyketone group was replaced by a hydroxamic acid. The hybrid compound, designated cyclic hydroxamic-acid-containing peptide 1 (CHAPI), is a reversible inhibitor of HDACs. N-carboxycinnamid acid bishydroxamic is a potent HDAC inhibitor and the structural bases for several derivatives including panobinostat (LBH589), and bleinostant (PXD-101) (Table III). These HDACi target a similar profile of HDACs as vorinostat.

Synthetic benzamides derivatives

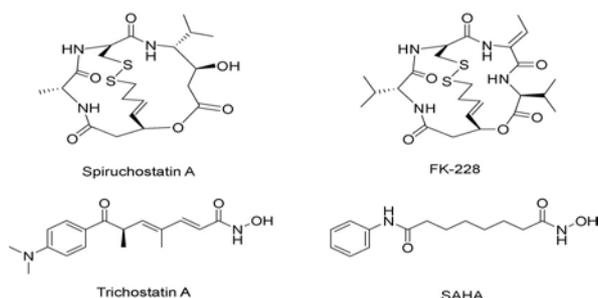
This class of compounds consists of a structurally diverse group of agents that contain a benzamide moiety. This group is postulated to enter the catalytic site and bind the active zinc²⁰. Two compounds have been described as members of this group, MS-275 and CI-994. As in the case of other compounds of this class, MS-275-associated HDAC-inhibitory activity is accompanied by an increase in

expression of the CDKI p21 and accumulation of cells in G₁-phase⁴⁹. MS-275 displays antiproliferative activity toward several human cancer cell lines including breast, colorectal, leukemia, lung, ovary and pancreas. In human breast cancer cell lines, it has been postulated that the antitumor activity of MS-275 may involve induction of TGF- β -receptor expression and as a consequence, potentiation of the tumor suppressor activity of the TGF- β signaling pathway. Similarly, MS-275 activity against pediatric solid tumor cell lines has recently been related to effects on TGF β . The HDAC inhibitory activity of MS-275 occurs at micromolar drug levels. The second compound with a benzamide structure is CI-994. CI-994 is an investigational anticancer drug with a broad spectrum of activity in murine and human tumor xenografts, although its specific mechanism of action remains unknown. Following CI-994 administration, inhibition of both histone deacetylation and cellular proliferation at the G₁ to S transition phase of the cell cycle occur.⁵⁰ MS-275 is a novel benzamide-based HDACi which like other non-hydroxamic acid inhibitors is somewhat selective for the class I HDACs^{13,51,52}.

Cyclic tetrapeptides

The cyclic peptides are a structurally complex group of HDAC inhibitors, such as, romidepsin (depsipeptide). Romidepsin inhibits primarily HDAC 1 and, more weakly, HDACs 2 and 3. Depsipeptide (FK228, FK901228) is a novel HDAC inhibitor isolated from *Chromobacterium violaceum* that possesses potent antitumor activity against human cancer cell lines and inhibits the growth of tumor generated in mice.⁵³ In human leukemia cells (U937), depsipeptide was a strong inhibitor of cell growth with IC₅₀ at nanomolar concentrations and proved very active in inducing apoptosis in cells from patients with chronic lymphocytic leukemia.⁵⁴ In addition, FK228 has been shown to act as an antiangiogenic agent by modulating the expression of genes implicated in this process. Mechanistic studies in non-small-cell lung cancer cells showed that FK228-induced cell growth arrest and apoptosis.⁵⁵ Phase I-II trials involving depsipeptide are currently in progress.

Spiruchostatin A is a natural bicyclic depsipeptide from the organism *Pseudomonas sp.* with structural similarities to FK228 and was identified in a screen for activators of transforming growth factor β -mediated signalling⁵⁶. Both compounds share the intramolecular disulfide bond that, in FK228.



Apicidin is another novel cyclic tetrapeptide compound

whose structure is related to that of trapoxin. It has a potent broad spectrum of antiprotozoal activity against apicomplexan parasites, which appears to involve HDAC inhibition. Apicidin displayed marked antiproliferative effects in a wide variety of human cancer cell lines including breast, osteosarcoma, stomach and v-ras-transformed NIH3T3 cells⁵². Interestingly, aside from effects on cell proliferation, apicidin-induced hyperacetylation of histones resulted in potent stimulation of fetal haemoglobin expression in erythroid cells, similar to the actions of valproic acid. These observations provide a possible basis for the use of HDAC inhibitors in the treatment of sickle cell anemia and/or thalassemia.

Electrophilic ketones

Trifluoromethyl ketones are potent inhibitors of aspartyl⁵⁷ and serine⁵⁸ proteases, by formation of stabilized hemiketals and hemithioketals at the active site. A recent report on HDAC inhibitors showed that trifluoromethyl ketone attached to aromatic amides has micromolar inhibitory activity for HDAC⁵⁹.

USES OF HDACI WITH OTHER DRUGS

Based upon the preclinical findings of HDACi *in vitro* and in animal models *in vivo*, a number of HDACi have been used in clinical trials for treating hemopoietic and solid tumors^{13,60}. HDACi have been shown to cooperate with radiation therapy, antitubulin agents, topoisomerase I and II inhibitors, cisplatin, the kinase inhibitor imatinib, proteasome inhibitors such as bortezomide, the heat shock protein-90 inhibitor 17-N-allylamino-17-demethoxygeldanamycin, the Her2 receptor inhibitor trastuzumab, retinoids, inhibitors of DNA methylation, estrogen receptor antagonists, dexamethasone, etc.

CONCLUSION

HDACi have a potential role in treating the malignancies and solid tumours. They can be used along with other antineoplastic agents but the specific action of HDACi responsible for antitumor activity was not clearly known. So further studies were required on these HDACi for their recognition as antitumour agents.

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