DEVELOPING SELECTIVE HISTONE DEACETYLASE 6 INHIBITORS AS POTENTIAL EFFECTIVE ANTICANCER AGENTS

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Cancer

Is a second leading cause of death globally; consequently, massive efforts have been focused on designing of new anticancer medications and embrace new treatment approaches. One of these approaches is inhibiting histone deacetylase

which have a major role in regulation of cellular epigenetic processes. Many histone deacetylase inhibitors have been approved for treatment of cancer, however, some pharmacodynamic and pharmacokinetic limitations have been recorded. One of the

major causes of these limitations is no selectivity of these agents, therefore, recent researches have been oriented toward introducing of selective histone deacetylase inhibitors.

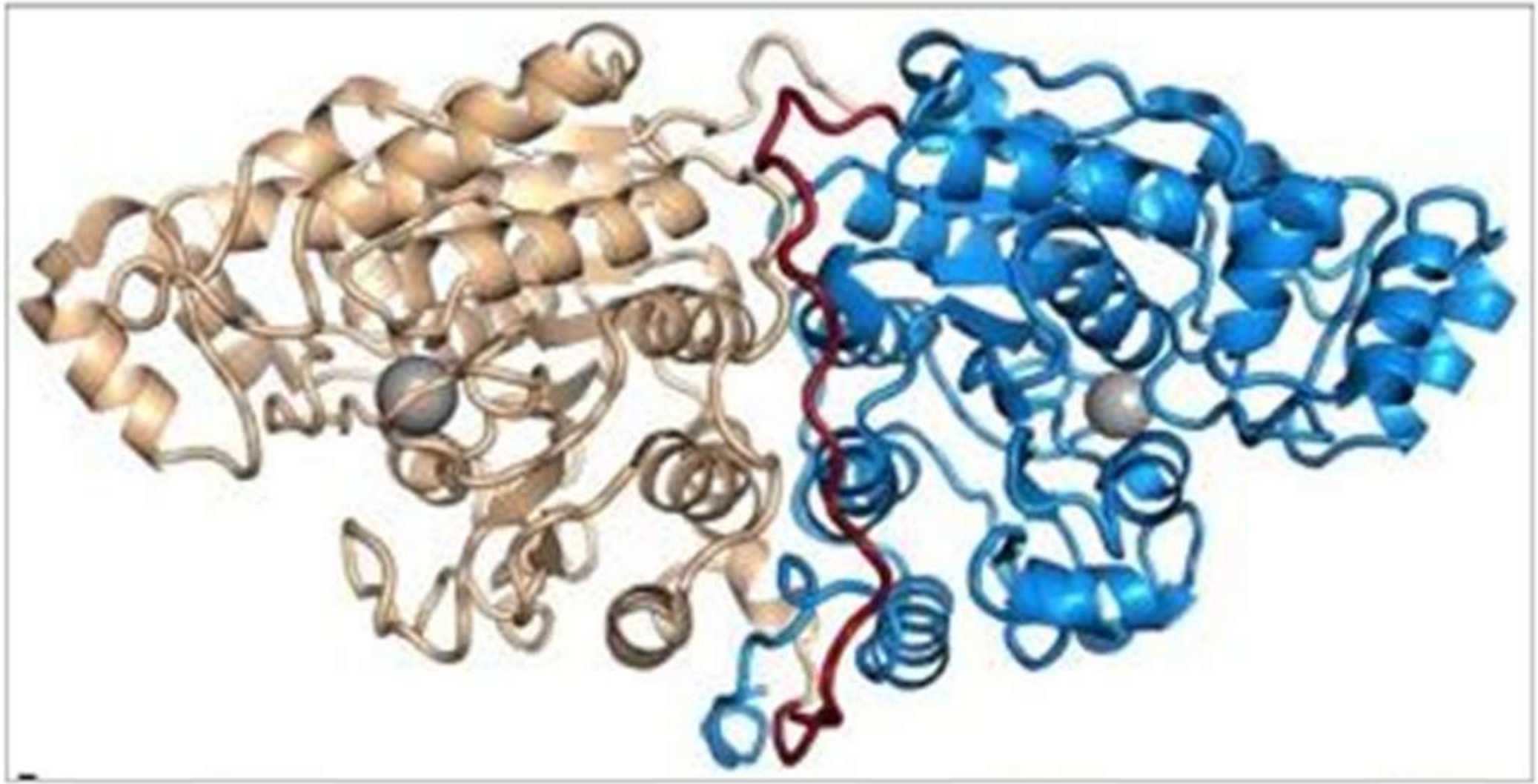
The unique characteristics of histone deacetylase 6 such as, surface shape, location, composition and count of the active site have made it possible to target this enzyme selectively. ACY1215 (Ricolinostat) is one of the investigational selective histone deacetylase 6 inhibitors which may have a promising potential in overcoming the nonselective histone deacetylase inhibitors drawbacks.

Histone Proteins

Histones are highly alkaline proteins that associate with negatively-charged DNA in the nucleus with the purpose to package the DNA into chromatin .Histones can be modified at specific residues by various covalent post-translational reversible modifications (PTMs) that include, among others, acetylation, phosphorylation, phosphoacetylation, methylation, adenylation, ubiquitination, sumoylation, ADP ribosylation and isomerization predominantly at their N- terminal tails These unique chemical changes can affect global chromatin assembly, transcription factor binding, or recruitment of transcriptional cofactors. Histone PTMs also permit chromatin relaxation or compression around genetic loci leading to the promotion or repression of gene transcription. Notably, the majority of histone modifications can occur on the tails of the 4 core histone proteins H2A, H2B, H3, and H4, and can interact with each other, thereby opening a wide array of histone PTM combinations and a complex regulation of gene transcription .

Histone Deacetylase Enzymes

Histone deacetylase enzymes (HDACs) are a family of metalloproteases involved in the regulation of chromosome structure and gene expression .Along with histone acetyltransferases (HATs), both enzymes control the ε-acetylation of histone lysine residues. ε-acetylation is a highly reversible post-translational modification that is regulated by the opposite activities of these two groups of enzymes .At the cellular level, opposing the action of HATs is the most obvious biological activity of HDACs. Therefore, HATs are defined as co-activators and the HDACs are co-repressors as well as their actions are crucial in numerous biological pathways and protein posttranslational modifications such as cellular signaling and metabolism due to the regulation of histones and nonhistone deacetylation by these enzymes which may alter chromatin conformation or modify the activities of transcriptional factors leading to a change in gene expression. Consequently, HDACs have a profound effect on maintaining human disease and health .HDACs play a key role and dysregulated in many diseases such as cancer neurodegenerative ,cardiac diseases ,pulmonary diseases, inflammatory diseases



Crystal structure of the di-domain construct of zebrafish HDAC6 (PDB 5G0J) in which catalytic domain 1 (CD1, tan) and catalytic domain 2 (CD2, blue) are connected by a 23-residue linker (red). Catalytic zinc ions appear as gray spheres

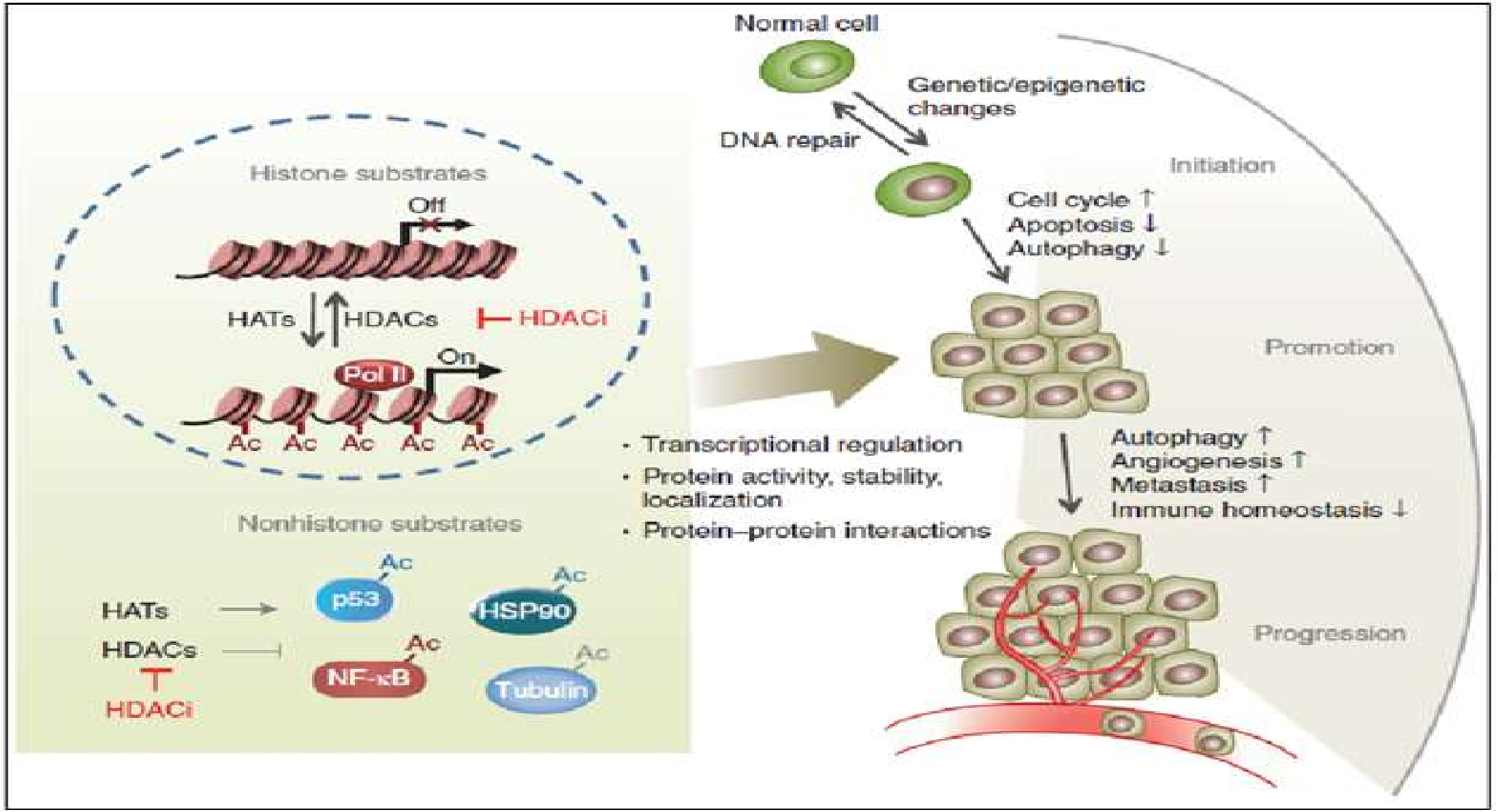


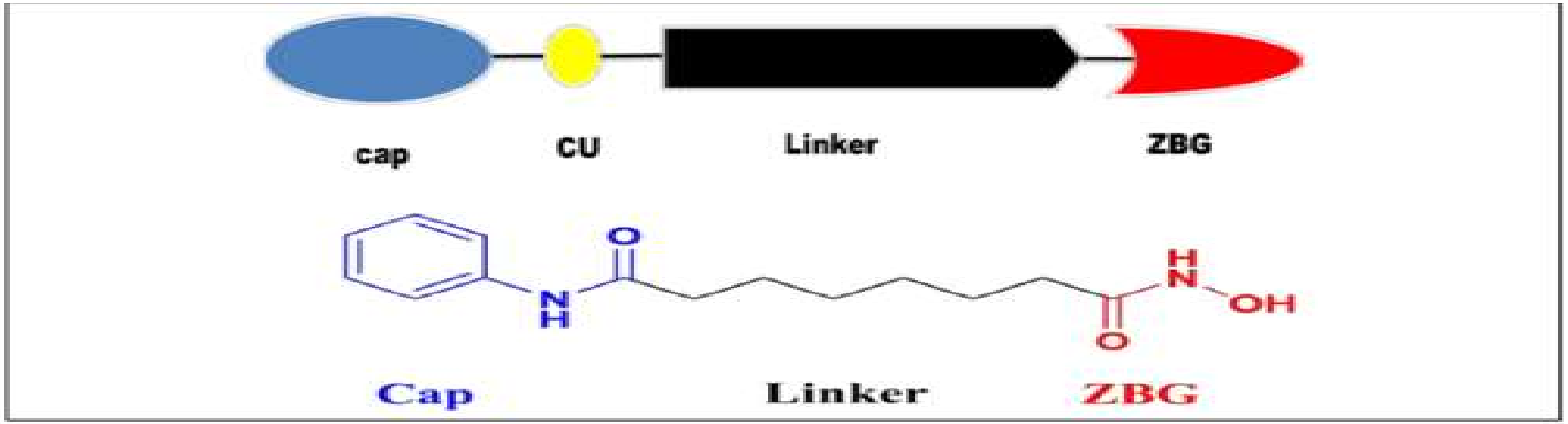
Figure 1. A simplistic illustration of the diverse functions of HDACs and HDACi regulating different stages of cancer through multiple different mechanisms and changing different biological processes. Far-right, ↑ indicates promotion or up-regulation, ↓ indicates repression or down-regulation [21].

Classifications of HDACs

Presently, based on their structural diversity and sequence homologies to yeast and domain organization, eighteen human HDACs were identified and can fall into four classes: Class I Rpd3-like enzymes are comprised of HDAC1, 2, 3, and 8. Class II Hda1-like enzymes are further divided into two subclasses: IIa (HDAC4, 5, 6, 7, and 9) and IIb (HDAC6 and 10). Class III Sir2-like enzymes consist of seven sirtuins. Sirtuins have been shown to regulate many cellular processes including survival, aging, stress response, and metabolism. Class IV contains only HDAC11, which shares sequences similarity to both class I and II proteins. HDACs can also be grouped into either Zn^{2+}-dependent deacetylases (Class I, class II and class IV) that adopt the arginase-deacetylase fold and nicotinamide adenine dinucleotide (NAD+) dependent deacetylases and/or ADP ribosylases (structurally distinct class III HDACs) enzymes that implement an unrelated fold

Overexpression of HDACs has been noticed in many cancers and found to be involved in the mechanism of tumorigenesis, by deacetylating histone and nonhistone proteins (such as p53 which undergoes mutations in more than 50% of all types of cancer), which are involved in the regulation of cell cycle, apoptosis, DNA-damage response, metastasis, angiogenesis, autophagy, and other cellular processes ,High expression levels of these enzymes are correlated with poor patient outcomes and prognosis of many diseases such as multiple myeloma, neuroblastoma, gastric, prostate, and ovarian cancers .The aforementioned data lead to utilizing HDACs as targets in developing new anticancer agents. In October 2006, the first HDAC inhibitor (HDACi) Vorinostat (Zolinza, Suberoylanilide hydroxamic acid, formerly known as SAHA, Merck) was approved by FDA for treating rare cancer cutaneous T-cell lymphoma (CTCL)

Generally, HDACis share three common motifs: a cap group that interacts with the surface of the enzyme also named surface recognition moiety (SRM), a hydrophobic spacer (HS) or a linker group that occupies a hydrophobic channel, and a ZBG that coordinates with the zinc ion (Zn^{2+}) at the bottom of the catalytic pocket, the cap group linked to an HS through a connection unit (CU)



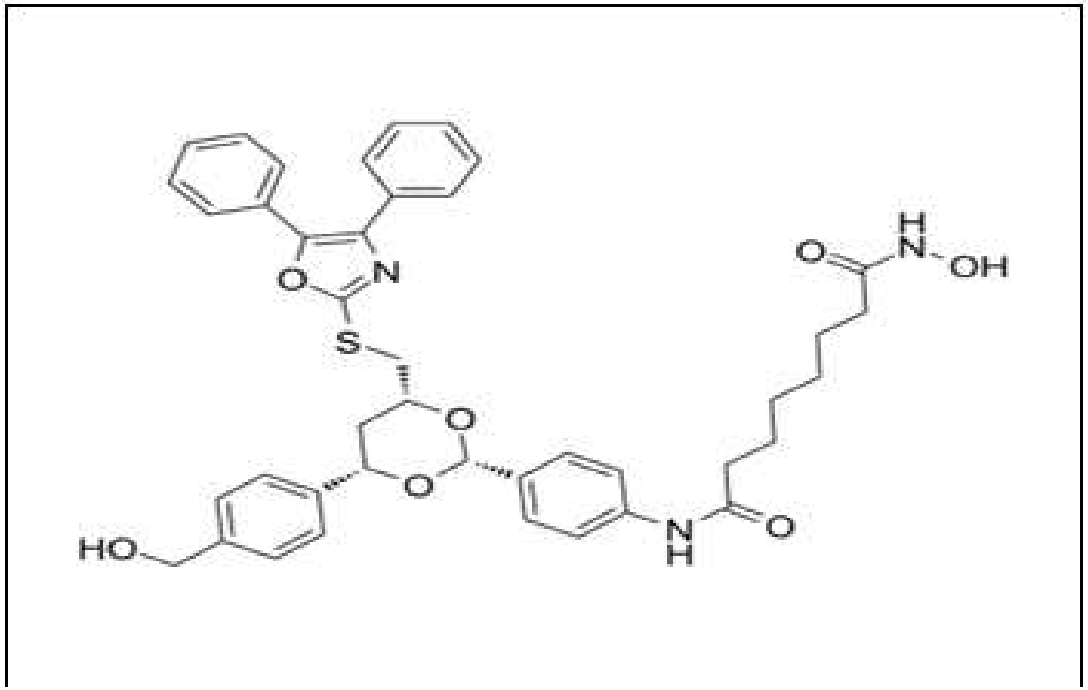
General structural motifs of HDACis and the structure of SAHA

Histone Deacetylase 6 Inhibitors

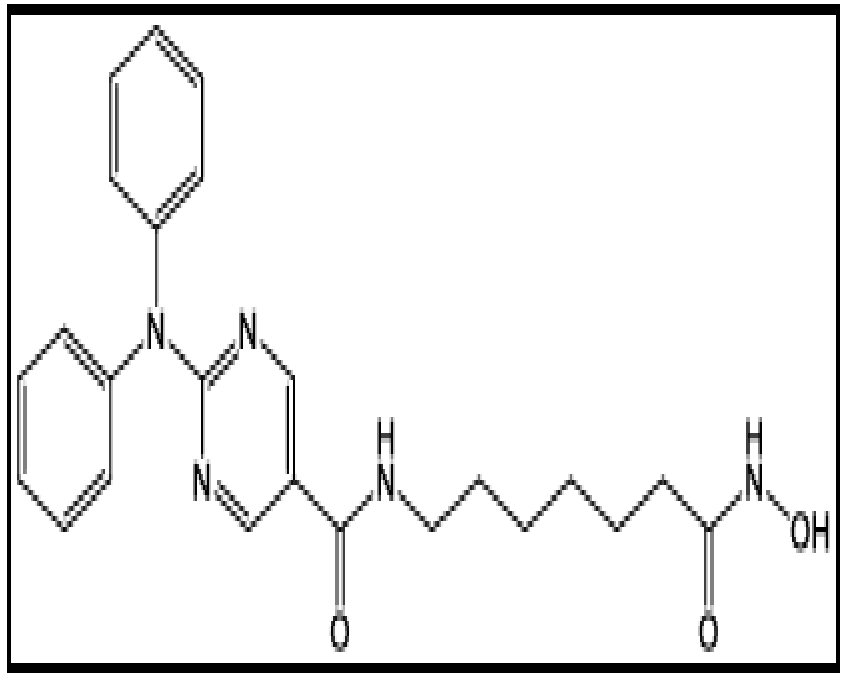
The unique characteristics of HDAC6 such as, surface shape, location, composition and count of the active site have made it possible to target this enzyme selectively. Furthermore, it was found that Knockout of HDAC6 doesn’t affect mice but HDACs1-3 genetic ablation is lethal .Thus, histone deacetylase 6 inhibitors (HDAC 6 inhibitors) may have few side effects and have been widely investigated for a variety of therapeutic purposes, including cancer, neurodegenerative diseases and pathological autoimmune response . In neurodegenerative diseases such as Huntington’s, intracellular axonal transport is increased by tubulin and microtubule acetylation. HDAC6 inhibitors have a blocking effect on the enzymatic function of the C and N-terminal catalytic domains as well as ZnF-UBP domain ,and were found to offer neuroprotection against neurological complications (such as painful peripheral neuropathy) accompany certain anticancer drugs (such as vincristine) without interfering with their anti-cancer efficacy .Therefore, HDAC6 inhibitors could be used effectively in treatment of

cancers by enhancing chemotherapy- trigged cancer cell death as well as preventing the devastating chemotherapy-induced neuropathies .

From a structural point of view, presence of bulky cap groups and/or an aromatic linker has a significant participation in isoform selectivity.Consequently, binding conformation adapts Y-shape during the interaction with HDAC6-binding site .The first selective HDAC6i reported was tubacin .Afterward, researches were focused on improving the very poor druglikeness characteristics of tubacin, such as, a high lipophilicity (Clog P = 6.36) and molecular mass by utilizing information about structure activity relationships (SARs) for the generation of better HDAC6-selective compounds.

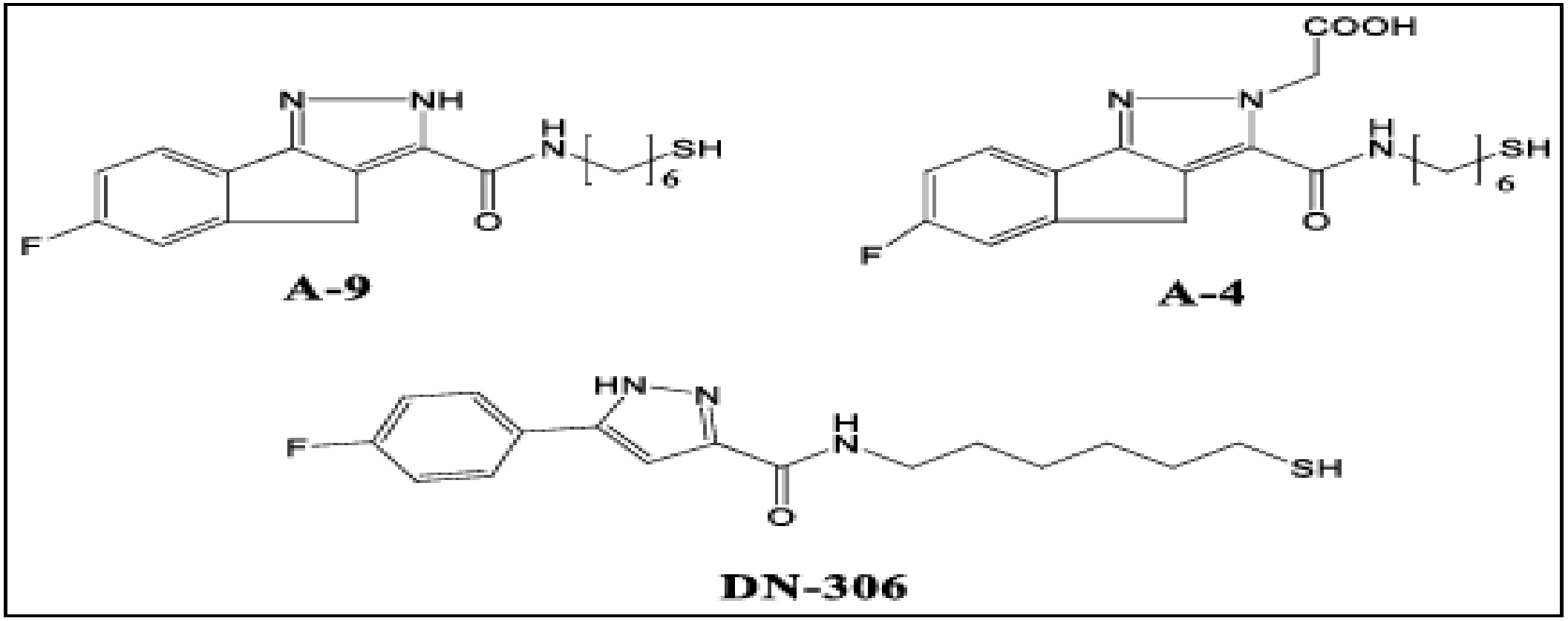


Chemical structure of tubacin



Chemical structure of ricolinostat (ACY-1215)

Recent Examples on Development of New HDAC6 Inhibitors with Anticancer Activity



Chemical structures of selective thiol-based HDAC6 inhibitors

Compound	IC50/(nmol·L^{-1})		Selectivity HDAC1/HDAC6
	HDAC1	HDAC6	
A-4	114	44	2.6
A-9	224	118	1.9
DN-306	580	790	0.8
SAHA	172	41	4.2

Activities of A-4, A-9, DN-306 and SAHA against HDAC1 and HDAC6

Recent Examples on Development of New HDAC6 Inhibitors with Anticancer Activity Up to date, no HDAC6 inhibitor has been approved for treatment of cancer; however, some of them are being investigated in clinical trials. Ricolinostat (ACY-1215), which is a well-known investigational selective HDAC6 inhibitor, has reached advanced stages in some clinical trials .There are extensive efforts for developing new and effectively selective

HDAC6 inhibitors as anticancer agents with few side effects and low toxicity profile. Hopefully, the near future will witness approving and utilizing selective HDAC6 inhibitors for clinical application as anticancer drugs.

A series of bicyclic arylamino/heteroarylamino hydroxamic acids were synthesized and tested for them in vitro HDAC6 inhibitory activity and growth inhibition activity against multiple myeloma cell lines. Among them, compounds 10 and 13

, with a quinoline moiety exhibited the most potent HDAC6 inhibition with IC50 = 0.795 and 0.291nM for compounds 10 and 13 respectively. The screening of 10 and 13 for HDAC isoforms inhibition showed that 10 and 13 have outstanding HDAC6 selectivity over other isoforms, and are between ten and several thousand times more potent than ricolinostat .

The result of cellular assays revealed that 10 and 13 displayed potent antiproliferative activity against multiple myeloma cells such as RPMI 8226, U266, and NCI-H929 cells

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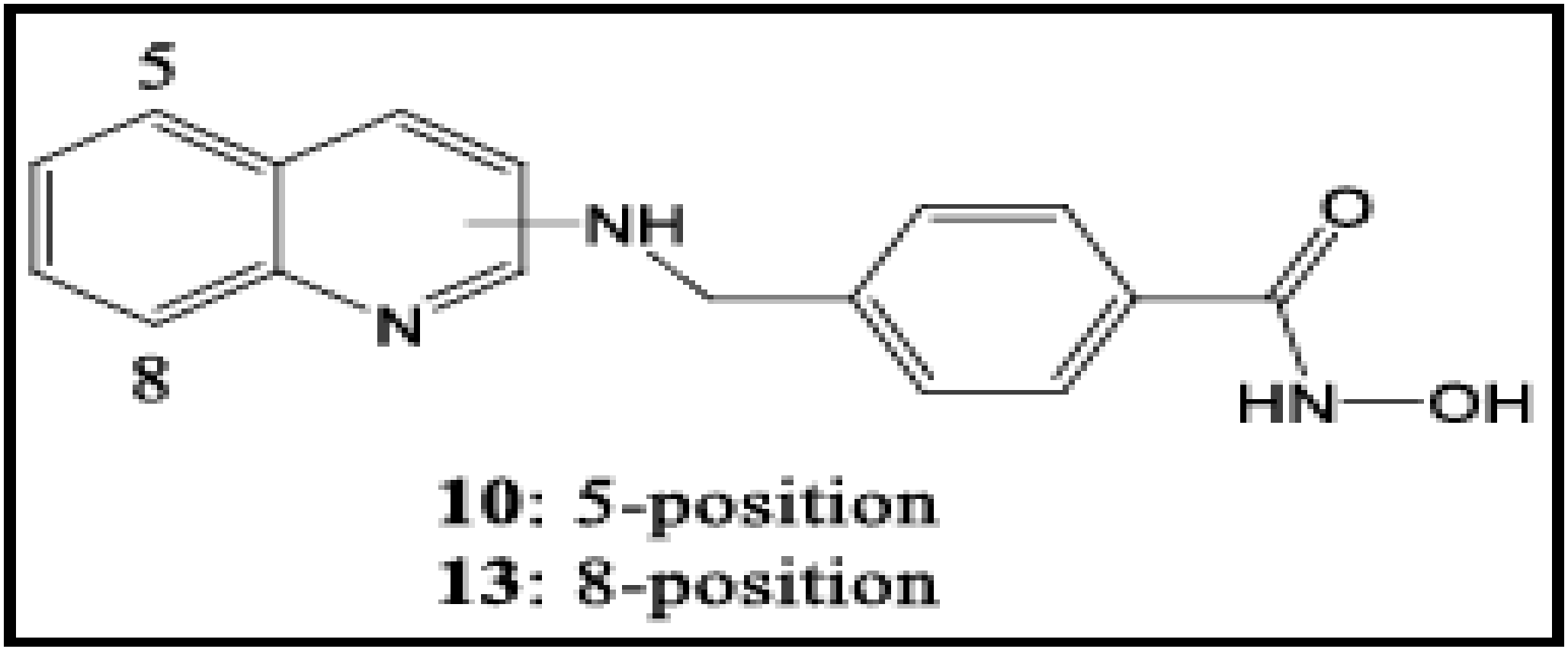

Chemical structure of compounds 10 and 13

Discussion

Cancer imparts a significant concern on human health. Many approaches have been approved for treatment of cancer. However, overcoming cancer cells resistance still occupying a great interest of scientific orientation. HDACs have shown to have an important role in regulation of epigenetic events and consequently, gene transcription. Therefore, it is understood to target these enzymes in fighting cancer. A number of HDAC inhibitors had been approved as anticancer agents (such as SAHA, chidamide and others). Unfortunately, these agents were found to have many pharmacokinetic and pharmacodynamic limitations (rapid metabolism, low intracellular concertation in cancer cells of solid tumors, low oral bioavailability and undesirable side effects). Undesirable side effects were shown to be caused majorly by the lack of specificity of HDAC inhibitors. Therefore, resent efforts have focused on introducing selective HDAC inhibitors. As forementioned, HDAC 6 has a vital role in cancer mechanism, therefore, many selective HDAC 6 have been developed as novel anticancer agents. ACY-1215 (Ricolinostat) is a selective HDAC 6 which is under investigation and hopefully will get FDA approval in the near future for treatment of different types of cancer.

Conclusion

This review focuses on the recent scientific current for introducing selective HDAC 6 inhibitors. These inhibitors were designed mainly to overcome the critical limitation of pan HDAC inhibitors (lack of specificity) which leads to undesirable side effects and may have a potential therapeutic applications as anticancer agents in the future. However, these agents still under investigation in clinical trials and no selective HADC 6 inhibitor has been approved for treatment of cancer yet.


Thank
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