RECENT PROGRESS IN DEVELOPING SELECTIVE HISTONE DEACETYLASE 6 INHIBITORS AS POTENTIAL EFFECTIVE ANTICANCER AGENTS: REVIEW

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Abstract

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. ACY-1215 (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.

Keywords: *Cancer, Epigenetic process, Selective HDAC 6 inhibitors, ACY-1215 (Ricolinostat)*

اخر المستجدات في تطوير مثبطات هيستون ديستيلاز 6 الانتقائية كعوامل محتملة فعالة مضادة للسرطان: مراجعة

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السرطان هو السبب الرئيسي الثاني للوفاة على مستوى العالم. لذلك، تم تركيز جهود ضخمة على تصميم أدوية جديدة مضادة للسرطان وتبني أساليب علاجية جديدة. أحد هذه الأساليب هو تثبيط إنزيمات هيستون ديستيلاز (HDAC) التي لها دور رئيسي في تنظيم العمليات اللاجينية الخلوية. تمت الموافقة على العديد من مثبطات HDAC لعلاج السرطان، ومع ذلك، تم تسجيل بعض القيود الديناميكية والحركية الدوائية. أحد الأسباب الرئيسية لهذه القيود هو عدم انتقائية هذه العوامل، لذلك تم توجيه الأبحاث الحديثة نحو إدخال مثبطات HDAC الانتقائية. جعلت الخصائص الفريدة لـ HDAC6، مثل شكل السطح والموقع والتكوين وعدد الموقع النشط، من الممكن استهداف هذا الإنزيم بشكل انتقائي. ACY-1215 (Ricolinostat) هو أحد مثبطات HDAC الانتقائية التي قد يكون لها إمكانات واعدة في التغلب على عيوب مثبطات HDAC 6 غير الانتقائية.

الكلمات المفتاحية: السرطان، عملية التخلق، HDAC، مثبطات HDAC 6 الانتقائية، ACY-1215 (Ricolinostat)

1. INTRODUCTION

Despite recent major advances in our understanding of cancer and the development of novel treatments, cancer is classified as a second leading cause of death worldwide, and is responsible for about 10 million deaths per year. Globally, one sixth of deaths is due to cancer [1]. The design of anticancer agents became a complicated process [2]. So far, there is no anticancer treatment that is completely effective against dispersed cancer. Over the last three decades, there has been substantial progress in treatment techniques including surgery, radiation, and chemotherapeutics, however, the overall survival rates for cancer patients have not considerably improved due to the lack of markers for its early diagnosis, aggressive behavior, high toxicity and, even more importantly evolving resistance to radio- and chemotherapy [3–5]. Unfortunately, the complete aspects of cancer resistance remain poorly understood. It is thought to be driven by irreversible genetic mutations; emerging evidence also implicates reversible proteomic and epigenetic mechanisms in the development of drug resistance. Tumor microenvironment-mediated mechanisms and tumor heterogeneity can significantly contribute to cancer treatment resistance [6]. With few exceptions, gene changes are difficult to be targeted therapeutically [7]. There have been continuous advances in epigenetic mechanisms with increasing evidence suggests that the epigenetic dysregulation of gene expression interacts broadly with the tumor initiation and progression [8], Which can be widely investigated as an innovative antitumor drug development strategy [9].

The epigenetic process can be defined as the inheritable modifications in phenotype without alterations in a gene sequence, which is important in controlling gene expression [10]. There are three major epigenetic changes: DNA methylation, histone modifications, and microRNAs [11].

1.1 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 [12]. 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 [13]. 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 [14,15].

1.2 Histone Deacetylase Enzymes

Histone deacetylase enzymes (HDACs) are a family of metalloproteases involved in the regulation of chromosome structure and gene expression [16]. 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 [17]. 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[18] as well as their actions are crucial in numerous biological pathways and protein posttranslational modifications such as cellular signaling and metabolism[19] 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 [20]. HDACs play a key role and dysregulated in many diseases such as cancer[21], neurodegenerative [22], cardiac diseases [23], pulmonary diseases [24], inflammatory diseases [25],

and metabolic disorders [26]. HDACs attracted a great attention and interest in the development of anti-cancer agents over the past few decades compared to HATs due to their fundamental role in cell proliferation, cell cycle, and apoptosis of cancer cells (Figure 1) [27–29].

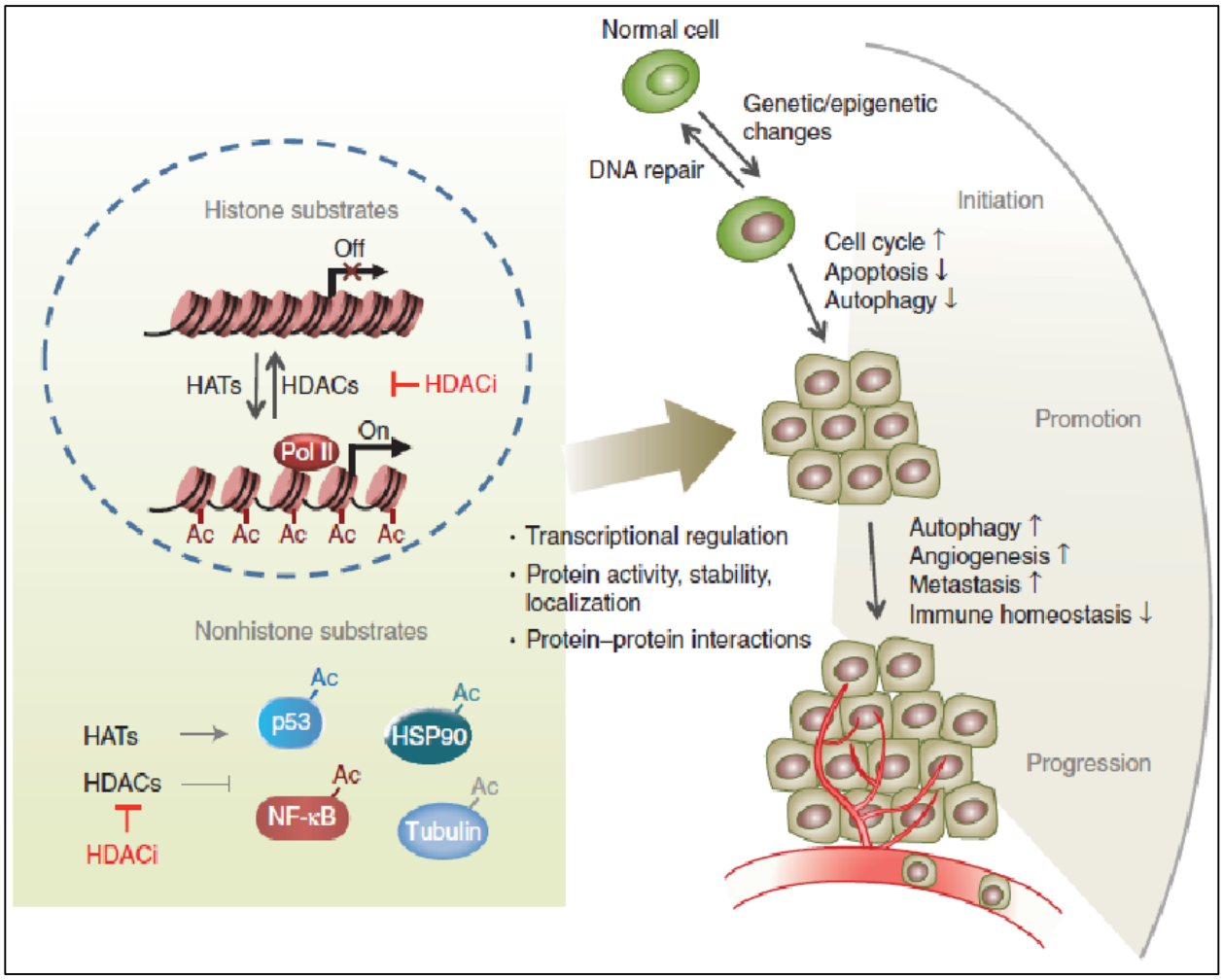


Figure 1. A simplistic illustration of the diverse functions of HDACs and HDACi regulating different stages of cancer through multiple different mechanisms and changing different biological processes. Far-right, ↑ indicates promotion or up-regulation, ↓ indicates repression or down-regulation [21].

1.3 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[21,29]. HDACs can also be grouped into either Zn2+-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 [30,31].

Overexpression of HDACs has been noticed in many cancers and found to be involved in the mechanism of tumorigenesis [32], 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 [21,33]. 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 [34–36]. 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) [37].

1.4 Histone Deacetylase 6 Enzyme

1.4.1 Biology and Structure

Human Histone deacetylase 6 enzyme (HDAC6) (belongs to class IIb) is the largest member of the zinc-dependent HDAC family. It consists of 1215 amino acids (Uniprot Q9UBN7) (Figure 2)[38,39]. The main cellular location of HDAC6 is in the cytosol which is different from other HDACs that are localized in the nucleus due to the presence of a nuclear export sequence (NES) and the SE14 motif, which is required for cytoplasmic retention[40,41]. Additionally, its biological function is unique in that it contains two catalytic domains, designated CD1 and CD2, which are homologous and functionally independent of the overall activity of HDAC6. As well as C-terminal end of HDAC6 contains a Zinc finger-ubiquitin binding protein domain (ZnF-UBP domain, also known as the PAZ, BUZ, or DAUP domain), through which polyubiquitinated misfolded protein cargo is recruited to dynein motors for transport to aggresomes the N-terminal microtubule-binding domain.[42–44]. Moreover, CD1 shows E3 ubiquitin ligase activity[45], while CD2 is the tubulin deacetylase. Enzymological measurements using purified protein constructs corresponding to CD1 and CD2 reveal that CD2 exhibits broad substrate specificity, whereas CD1 exhibits much narrower specificity for peptide substrates bearing C-terminal acetyllysine residues[45]. Finally, the shape of HDAC6 enzyme surface seems different and the channel appears shallower and wider than other HDAC isoforms[46].

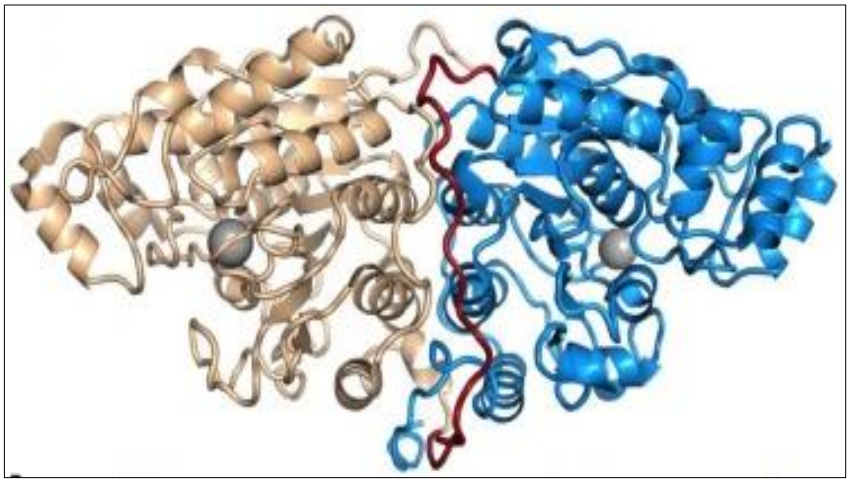


Figure 2. Crystal structure of the di-domain construct of zebrafish HDAC6 (PDB 5G0J) in which catalytic domain 1 (CD1, tan) and catalytic domain 2 (CD2, blue) are connected by a 23-residue linker (red). Catalytic zinc ions appear as gray spheres [32].

1.4.2 The Physiological Function of HDAC6

HDAC6 is in charge of maintaining the acetylation balance of particular cytosolic nonhistone proteins, including α-tubulin, HSP-90, peroxiredoxin, cortactin, and HSF-1 [47]. It acts as a primary tubulin deacetylase in vitro and in vivo [48]. HDAC6 can affect microtubules (MTs) assembly by regulating α-tubulin stabilization and function which are key regulators of cell movement [49]. Moreover, α-tubulin acetylation participates in mitotic events by affecting intracellular trafficking events through the protein encoded by the cylindromatosis gene (CYLD), which is essential for cell cycle progression [50]. HDAC6 can influence actin-dependent cell motility by acting on another nonhistone substrate, cortactin [51]. On the other hand, cortactin is necessary for auto phagosomes and lysosomes, therefore, recruiting and deacetylating cortactin by HDAC6 can promote autophagy[52]. HDAC6 directly interacts with HSP90 through its two catalytic domains and the ubiquitin zinc finger domain, leading to the deacetylation of HSP90 and interfering with its biological function, producing a continued increase of its substrate proteins [53]. On the other hand, HDAC6 forms complexes with HSP90 and HSF1, which participate in activating the heat shock transcription factor 1 (HSF1), stimulating the expression of HSP25 and HSP70, managing of protein folding, and participating in the repair and degradation of misfolded proteins[40]. Furthermore, HDAC6 has a role in cell response to protein misfolding by functioning as a regulator of the ubiquitin and proteasome system (UPS) through binding to ubiquitin-protein via its ZnF-UBP domain [54].

HDAC6 was found to modulate PD-L1 expression via the STAT3 signaling pathway, suggesting its participation in immunoregulation [55]. In addition to deacetylase activity, HDAC6 was also reported to ubiquitinate MSH2, indicating its role as an E3 ubiquitin ligase [56].

As soon as the activity of HDAC6 increases or its expression changes, it becomes a key factor in oncogenic cell transformation and tumor cell proliferation, invasion, metastasis, and mitosis [53,57]. This could partly due to the inactivation of MAPK/ERK pathways by HDACs [58]. Moreover, HDAC6 may be involved in the regulation of p53 function, and directly or indirectly plays a role via HSP90 to stabilize hypoxia-inducing factor 1-α (HIF-1α), a transcriptional factor involved in tumor angiogenesis, Bcr-Abl, FLT-3, c-Raf and AKT, and the breast cancer metastasis suppressor 1, BRMS1. HDAC6 also regulates the androgen receptor (AR) in cancer by modulating HSP9(Figure 3)[59]. Overexpression of HDAC6 is linked to an activated K- ras mutant in colon cancer patients. Moreover, expression of HDAC6 and c-myc are elevated in fibroblasts transformed with activated K-ras and mutant K-ras induces HDAC6 expression by a MAP kinase-dependent pathway[60]. Overexpression of HDAC6 has been found in many cancer and tumor cells such as cutaneous T-cell lymphoma, acute myeloid leukemia (AML) blasts (61), pancreatic adenocarcinoma [62], colon cancer [58], and in mammary tumors [63].

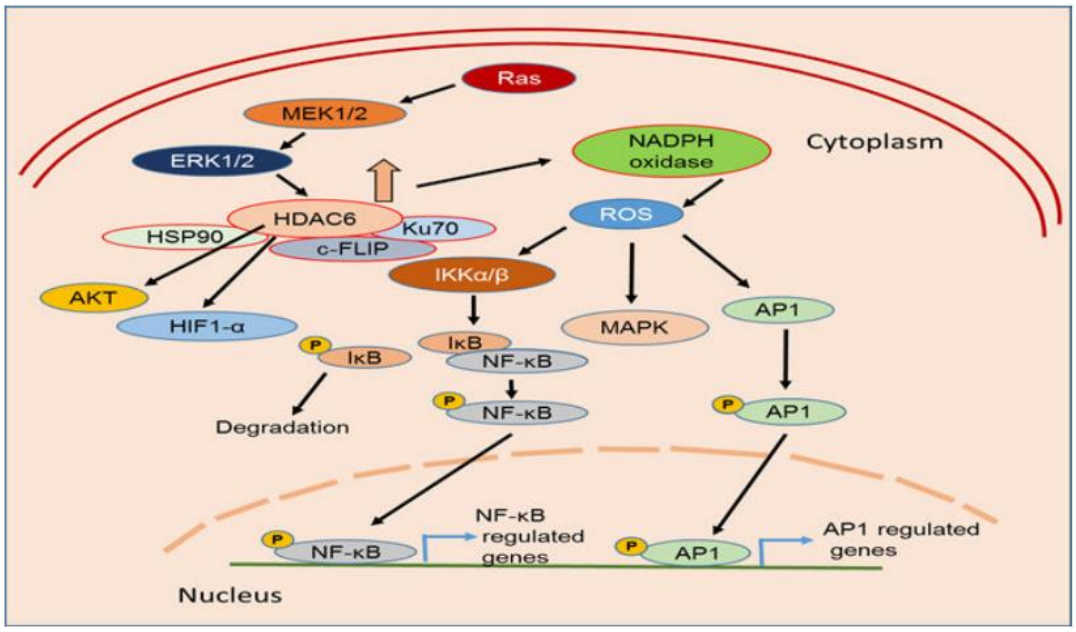


Figure 3. HDAC6 signaling pathways. HDAC6 are involved in the survival of cancer cells by the interaction with various substrates known to regulate several cytoprotective and anti-apoptotic signaling pathways. (Cited from ref 58).

1.5 HDACIs as Anticancer Agents

HDACis are cytostatic agents that enhance the acetylation of cellular proteins and modulating gene expression by blocking HADC activity [64]. Structurally, HDACis are classified into six classes: hydroxamates [e.g. suberoylanilide hydroxamic acid (SAHA, Zolinza™)], benzamides (e.g. chidamide, Epidaza™), tetrapeptides/depsipeptides (e.g. romidepsin, Istodax™), aliphatic acids (e.g. valproic acid), sirtuin inhibitors (e.g. cambinol)(Figure 4) [65] and miscellaneous class which involves diverse zinc binding groups (ZBGs) such as thiols[66], disulfides [67], thioester [68], acylthiourea [69], electrophilic ketones (70] and hydroxypropyl amide group and hydroxyethylamino group [71].

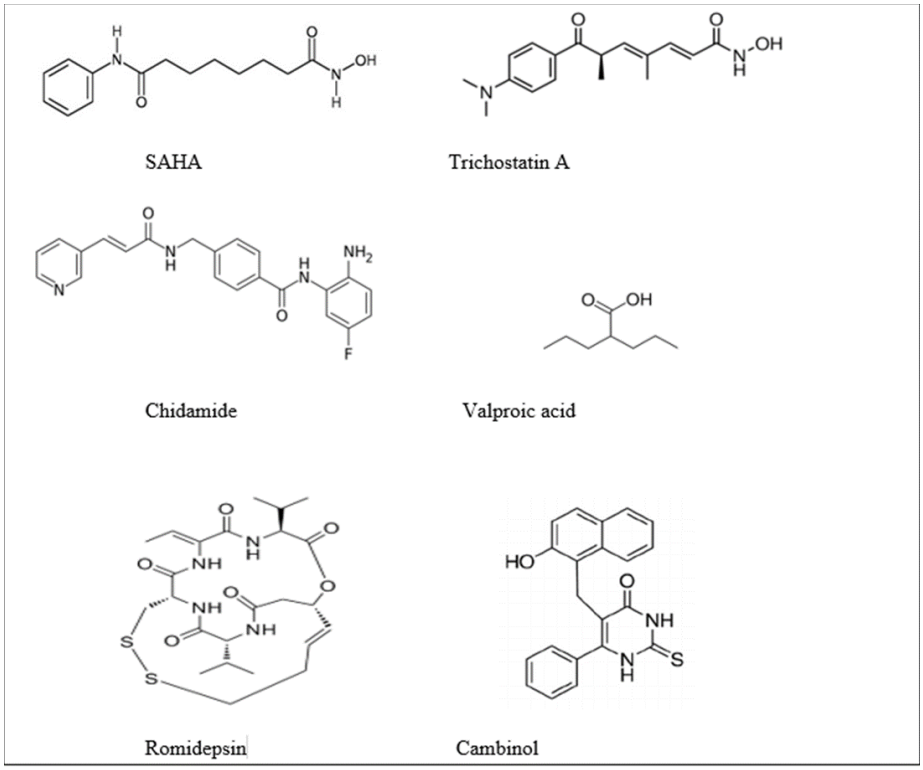


Figure 4. Examples of HDACis classes

Alternatively, HDAC inhibitors can be classified by their specificity for HDAC subtypes or classes. For instance, SAHA and trichostatin A are pan-HDAC inhibitors, while chidamide and romidepsin inhibit class I and valproic acid inhibits class I and IIa HDACs [72]. Histone deacetylases inhibitors proved their potent effects on the reversal of resistance in many cancer cell lines by regulating the expression of many tumor suppressor genes that are involved in apoptosis of cancer cells [73].

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 (Zn2+) at the bottom of the catalytic pocket, the cap group linked to an HS through a connection unit (CU) (Figure 5) [74,75].

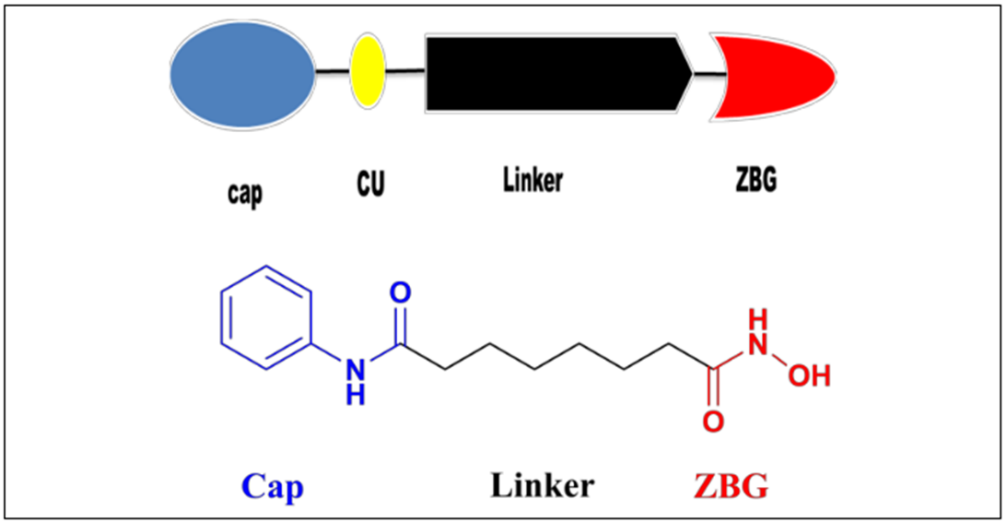


Figure 5. General structural motifs of HDACis and the structure of SAHA (53)

However, the current HDACis have serious limitations including metabolism, ineffectively low concentrations in solid tumors and undesirable side effects such as, fatigue, diarrhea, nausea, thrombocytopenia, and cardiotoxicity [76,77], which are hindering their progress in the clinic. Consequently, efforts have been focused on overcoming these hindrances, including HDAC isoform selectivity, localized administration, and targeting cap groups to achieve selective tissue and cell type distribution [78]. Selective class I HDACis have been investigated but the narrow therapeutic widow of these agents has limited their application [79].

1.6 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 [80]. Furthermore, it was found that Knockout of HDAC6 doesn't affect mice but HDACs1-3 genetic ablation is lethal [81]. 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 [81]. In neurodegenerative diseases such as Huntington's, intracellular axonal transport is increased by tubulin and microtubule acetylation [82,83]. HDAC6 inhibitors have a blocking effect on the enzymatic function of the C and N-terminal catalytic domains as well as ZnF-UBP domain [84,85], 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 [86]. 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 [59].

From a structural point of view, presence of bulky cap groups and/or an aromatic linker has a significant participation in isoform selectivity[87]. Consequently, binding conformation adapts Y-shape during the interaction with HDAC6-binding site [88]. The first selective HDAC6i reported was tubacin (Figure 6). Afterward, researches were focused on improving the very poor drug-likeness characteristics of tubacin, such as, a high lipophilicity (Clog P = 6.36 14) and molecular mass by utilizing information about structure activity relationships (SARs) for the generation of better HDAC6-selective compounds [89–91]. Several HDAC6

inhibitors co-inhibit other HDACs and cause fatal effects rather than inhibition of HDAC6 guanine. The co-inhibition of other HDAC-isozymes has led to misconceptions concerning the biological roles of HDAC6 [92], and preventing a proper understanding of HDAC6 functions in vital cells by the consequent cytotoxic events. While several agents target HDAC6 in the nanomolar range *in vitro*, biological effects are often only described for micromolar and even higher doses, which may also block other HDACs [93,94] . However, promising effects against pancreatic cancer cells have been achieved with drugs targeting HDAC6 and HDAC3 in the nanomolar range[95].

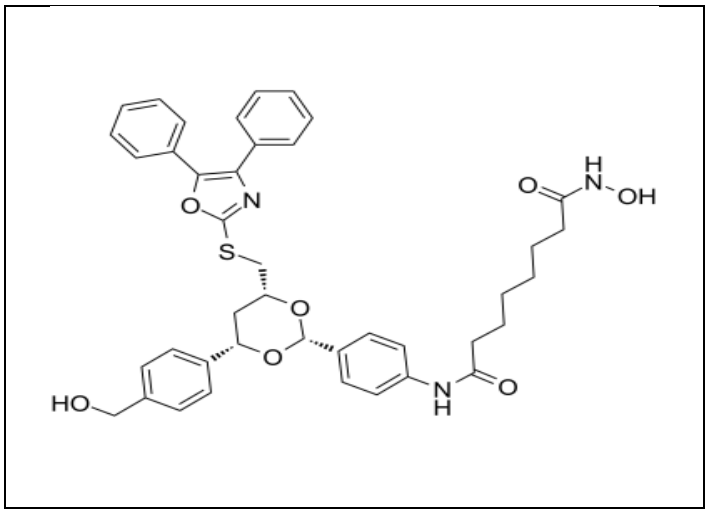


Figure 6. Chemical structure of tubacin

1.7 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 (Table 1). Ricolinostat (ACY-1215) (Figure 7), which is a well-known investigational selective HDAC6 inhibitor, has reached advanced stages in some clinical trials [96]. 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.

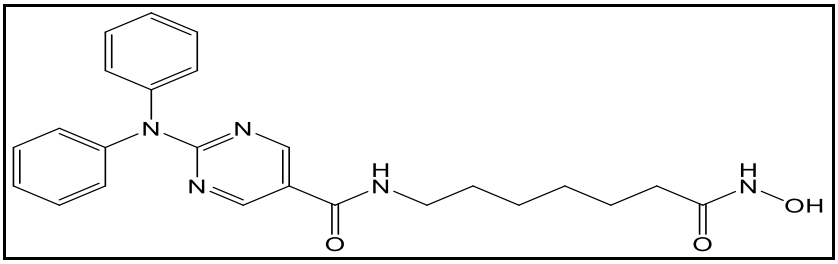


Figure 7. Chemical structure of ricolinostat (ACY-1215)

Table 1. Cancer clinical trials of some selective HDAC6 inhibitors [96]

	Status	Study Title	Conditions	Interventions	Locations
1	Active, not recruiting	A study of selective HDAC6 inhibition with KA2507 in advanced biliary tract cancer	biliary tract cancer	Drug: KA2507	#Cancer Research UK and UCL Cancer Trials Centre London, United Kingdom
2	Recruiting	Safety tolerability and MTD KA2507 (HDAC6 inhibitor) in patients with solid tumours	solid tumor, adult	Drug: KA2507	#M D Anderson Houston, Texas, United States
3	Active, not recruiting	Selective HDAC6 inhibitor ACY 241 in combination with nivolumab in patients with unresectable non small cell lung cancer	non small cell lung cancer	Drug: ACY-241 Drug: Nivolumab	#University of Southern California Los Angeles, California, United States #Dana-Farber Cancer Institute Boston, Massachusetts, United States #Virginia Cancer Specialists, PC Fairfax, Virginia, United States
4	Completed	ACY-1215 for relapsed/refractory lymphoid malignancies	lymphoma lymphoid malignancies	Drug: ACY-1215	#Moffit Cancer Center Tampa, Florida, United States #Columbia University Medical Center New York, New York, United States

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 (**Figure 8**)**, 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 **(**Table 2**)[**97**]**.

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 **(**Table 3**) [**97**]**.

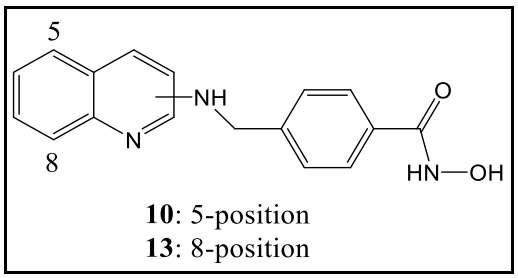


Figure 8. Chemical structure of compounds **10** and **13**

Table 2. Inhibitory activity (IC50, nM) of test compounds 10, 13, and reference Ricolinostat [97**].**

IC50 (nM)			
	10	**13**	**Ricolinostat**
HDAC subtype	selectivity ratio	selectivity ratio	selectivity ratio
HDAC1	7050 (8868)	9550 (32817)	58 (12)
HDAC2	8610 (10830)	12500 (42955)	48 (10)
HDAC3	5480 (6893)	7750 (26632)	51 (11)
HDAC4	4817 (6059)	4438 (15250)	7000 (1500)
HDAC5	2251 (2831)	3112 (10694)	5000 (1100)
HDAC6	0.795 (-)	0.291 (-)	4.7 (-)
HDAC7	766 (964)	709 (2436)	1400 (300)
HDAC8	636 (800)	1190 (4089)	100 (21)
HDAC9	1915 (2409)	1530 (5258)	>10000 (> 2100)
HDAC10	1256 (1580)	9791 (33646)	NA (-)
HDAC11	1004 (1263)	376 (1292)	>10000 (> 2100)

Table 3. Growth inhibition activity (GI50, µM) of 10, 13, and reference 3 against multiple myeloma cell lines(97)**.**

	Multiple myeloma cells			Normal bone marrow cells
Compound	RPMI 8226	U266	NCI-H929	HS-5
10	10.96±2.52	28.40±1.07	6.20±1.41	>100
13	7.49±3.63	40.61±9.84	9.14±2.33	>100
Ricolinostat	6.40±2.68	>100	>100	>100

Benzylic linker has a superior effect on potency and selectivity compared to phenyl and phenylethyl linkers[89]. Consequently, a series of benzylic linker with different phenothiazine cap group including numerous substituents or the oxidation state of the sulphur atom showed excellent selectivity and potency (Table 4). Nitrogen atom was introduced into the phenothiazine system which leads to production of new compounds showing superb potency and selectivity for HDAC6 with 1- azaphenothiazine **7i** as the most selective and potent compound among them. The high selectivity of the phenothiazine-based hydroxamates and their aromatic as a-analogues was rationalized by docking studies for hHDAC1, HDAC6, and HDAC8 [98].

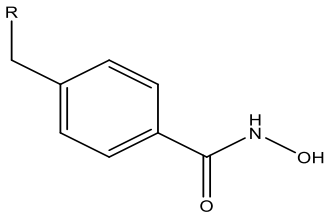
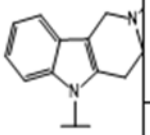
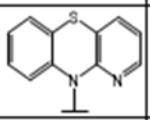
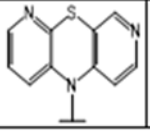


Table 4. In Vitro Inhibition of HDAC1, HDAC6, and HDAC8 (98)

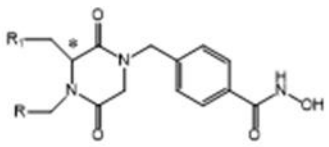
Compound	R	hHDAC1[a] IC50[µM]	hHDAC6[a] IC50[µM]	hHDAC8[a] IC50[µM]	SF 1/6[b]	SF 1/6[c]
SAHA		0.117±0.006	0.104±0.009	0.400±0.100	1	4
Tubastatin A		1.91± 0.42	0.030±0.002	0.695±0.060	64	23
		16.4±2.6[24]	0.015±0.001[24]	0.854±0.040[24]	1093	57
7i		2.69±1.04	0.005±0.001	3.10±0.09	538	620
7n		3.34±0.21	0.012±0.003	2.00±0.03	278	167

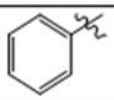
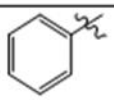
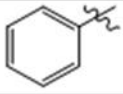
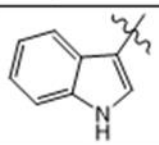
[a]IC50 values are the mean of at least two experiments. [b]SF1/6: SF for HDAC6 over HDAC1 (SF1/6 = IC50(HDAC1)/IC50(HCAC6)). [c]SF8/6: SF for HDAC6 over HDAC8 (SF8/6 = IC50(HDAC8)/IC50(HCAC6)). n.d. not determined

A series of novel HDAC6 inhibitors bearing natural diketoperazine (DKP) scaffold was synthesized and evaluated for their *in vitro* HDACs inhibitory activities and antitumor effects against various cell lines [99]. Most of these derivatives exhibited distinct inhibitory activities and selectivity toward HDAC6[99]. **21b** was found to be the most potent compound against HDAC6 with IC50 value of 0.73 nM and 10941-fold selectivity over HDAC1 (Table 5). Compound **18a** showed weaker enzymatic activity, however, it displayed better activity than **21b** in the NCI antitumor screening toward 59 cell lines (Table 6)[99]. Compared to ricolinostat, **18a**, **18b** and **18d** exhibited greater or equivalent antitumor activities against two multiple myeloma cells with IC50 values in the low micromolar range. Furthermore, the combining **18a** and adriamycin gave better cytotoxic effects than either compound alone (Table 7). **18a** and **18b** also had favorable *In vitro* metabolic stability in HLM (Table 8). These results demonstrated that the synthesized DKP derivatives require further structural modifications and *in vivo* pharmacological evaluation [99].

Table 5. Inhibitory profile of DKP derivatives and trichostatin A (TSA) against human HDAC1, -6, and -8 (IC50, nM) [99**].**

Table 6. *In vitro* antiproliferative screening against 60 cell lines of 21b and 18a (growth percents at 10 µM concentration) [99**].**



Compound	R	R_1	Isomer	IC50		
				HDAC1	HDAC6	HDAC8
18a	Ph		R	9640.00±154.02	9.83± 0.21	145. 00± 2.10
18b	2-Cl-Ph		R	8310.00±126.33	10.10± 0.11	162.00± 1.03
18d	3-Cl-Ph		R	8550.00±193.67	10.50± 0.32	150.00± 2.14
21b	H		R	8020.00± 36.73	0.73± 0.02	513.00±13.17
TSA	/	/	/	8.41± 0.25	5.56± 0.05	1160.00±49.90
HPOB	/	/	/	1780.00±67.20	33.78± 0.05	1990.00±98.50
Tubastatin A	/	/	/	16400	15	854

Origin of cancer	Cell line	**21b**	**18a**
Non-Small Cell Lung Cancer	NCI-H23	101.09	86.72
	NCI-H322M	99.12	89.50
	NCI-H226	97.56	/
	NCI-H460	104.28	81.58
	NCI-H522	96.68	69.76

Table 7. Combined cytotoxic effect of 18a and adriamycin on A549 cell line(99)**.**

Compound	IC50 for individual use	IC50 for combination
18a	100 μM	8.17 μM
adriamycin	255 nM	152 nM
CI50	0.676	

Table 8. In vitro stabilities of compounds 18a, 18b and ACY-1215 toward HLM(99)**.**

Compound	HLM Concentration	Substrate Concentration	T1/2
18a	0.8 mg/mL	0.5 μM	9.7 h
18b	0.8 mg/mL	0.5 μM	10.1 h
Ricolinostat	0.8 mg/mL	0.5 μM	7.5 h

13 novel selective thiol-based HDAC6 inhibitors bearing indeno pyrazole and benzoindazole scaffold were synthesized, and evaluated for their *in vitro* HDAC inhibitory activities. **A-4** (figure 9) was the best of the synthesized compounds with highest selectivity for HDAC6 (IC50=44 nmol/L). Its inhibitory activity was superior to that of DN-306, comparable to that of SAHA(IC50=41 nmol/L)(100). Based on the results of docking study, it was proposed that most of tricyclic compounds after cyclization have superior selectivity against HDAC6 to that of the carbon-carbon bond of DN-306. Besides, taking **A-4** and **A-9** as examples, the compounds with substituted pyrazole ring were superior to the compounds without substituents. Ultimately, the enzyme activity of HDAC1, and the selectivity against HDAC6 were found to be increased in some degrees when compared with DN-30 (Table 9)[100].

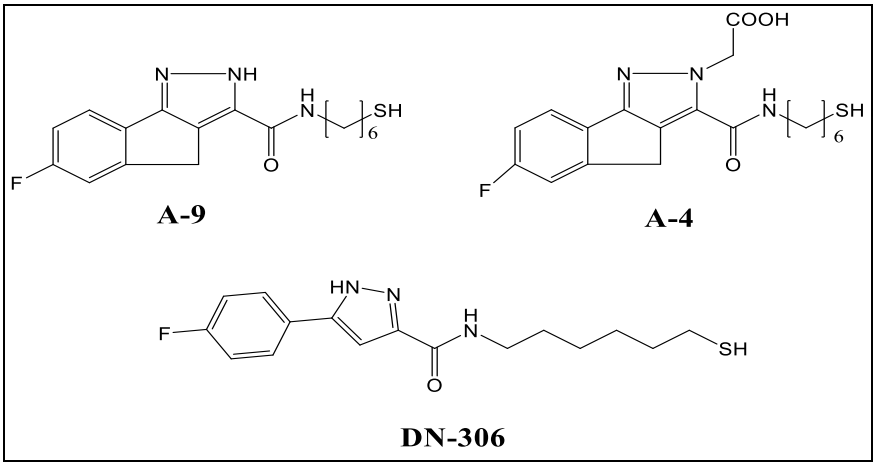


Figure 9. Chemical structures of selective thiol-based HDAC6 inhibitors

Table 9. Activities of A-4, A-9, DN-306 and SAHA against HDAC1 and HDAC6(100)

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

2. 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.

3. 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.

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