Natural HDAC Inhibitors: Nature's Answer to the Cancer

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ABSTRACT

Post translational modifications of histones play a significant role in regulation of physiological and pathological processes in the body. Acetylation is one of the most important chromatin modifying mechanisms being controlled by histone acetyl transferases and histone deacetylases. The histone deacetylases (HDAC) are basically responsible for silencing of gene transcription. Cancer involves silencing of a large number of genes. Inhibition of such gene silencing could possibly lead us to alleviatin or control of cancer progression. Natural HDAC inhibitors, a highly researched topic thus seems an attractive method to conquer the kingdom of cancer and thus prove to be the 'bull's eye' like target for cancer treatment.

Keywords: Cancer, Epigenetics, Histone deacetylase, Natural HDAC inhibitors

Abbreviation:

Histone deacetylases (HDAC), Histone deacetylases inhibitor (HDACi), Histone acetyl transferases (HAT), Short chain fatty acid (SCFA), Cyclin-dependent kinase (CDK), signal transducer and activator of transcription 5 (STAT5), Androgen receptor (AR), Trichostatin A (TSA), Suberoylanilide hydroxamic acid (SAHA)

1. Introduction

The chromatin configuration is a dynamic feature being controlled by myriad signals and inputs which modulate the covalent modifications so as to facilitate or hinder the binding of promoters to the binding site by opening or closing the chromatin network.

These post translational modifications occur generally at the N terminal amino acid functional groups in the tail region of the histones [1-3]. The well-known modifications are acetylation, methylation, phosphorlyation, ubiquitylation, sumoylation, ADP-ribosylation, deimination and proline isomerization [4, 5]. Acetylation, occurring majorly on lysine residues alters the electrostatic charges in the nucleotide and thereby regulates gene expression [6, 7]. Histone acetyltranferases (HAT) transfers acetyl group from acetyl-CoA to ε-amino group of lysine residues on N-terminal region histones in tail regions. This relaxes the chromatin structure and allows transcription to proceed [8]. While deacetylation catalyzed by histone deacetylases (HDAC) restores positive charge on histone coupling negatively charged DNA to it [9]. In general, histone and other proteins' acetylation promotes gene transcription and deacetylation leads to repression or gene silencing [10, 11].

HDAC plays an important role in all walks of life, may it be cell cycle progression,

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inflammatory response, cell death or any other aspect of cell functioning. The wide application of the enzyme in physiological and pathological conditions makes it a hit in the research community, especially due its role in cancer development. HDAC inhibitors, which inhibit the gene silencing phenomenon facilitated by HDAC, have been found to decelerate the rate of tumor development in various *in vivo, in vitro* models as well as in clinical trials. Both synthetic and natural HDAC inhibitors are promising anticancer agents, but the inhibitors from nature's store have proved their worth and win the race by a significant margin over the synthetic ones. This review discusses the natural HDAC inhibitors, their mechanism of action their uses and stages in drug development.

2. HDAC and its role

2.1. HDAC classification

Till date, eighteen human HDAC enzymes are well recognized and categorized in to 4 classes based on their structural similarity with yeast proteins as Classes I, II, III and IV. Classes I, II and IV are Zinc (Zn^{2+}) dependent for deacetylase activity and Classes III are NAD-dependent subfamily and are structurally related to yeast sirtuins, Sir2 [9, 12]. These have sequence homology to yeast transcriptional proteins and each class are grouped based on their domains, Class I are homologous to Rpd3 yeast proteins and subdivided in to HDAC1, HDAC2, HDAC3, and HDAC8. Class I HDACs are abundantly available in the nuclear region and are expressed throughout the body [13]. Class II resembles closely the yeast Hda1. It can be further divided into subclasses IIA and IIB, IIA is constituted by HDAC4, HDAC5, HDAC7, and HDAC9 while HDAC6 and HDAC8 belong to sub class IIB. Class II HDAC is abundant, tissue specific and is localized both in cytosol as well as nucleus [14]. Class IV contains HDAC11 and has structural similarity with class I and II HDAC's. Although not much is known about its function and regulation, assumptions are made as to its possible role in immune responses [15]. The expression of genes depends upon the degree of compaction of packaging of nucleotide, because as the DNA becomes more tightly wrapped around the histones, the exposure of promoter region to the transcription factors. Acetylation adds the negative charge to the histone and reduces the interaction of DNA with histones, to loosen the complex and promote transcription. The site responsible for enzymatic activity is constituted by a sequence of 390 conserved amino acids forms a slightly curved pocket
with wider bottom (16). Deacetylation occurs from two adjacent histidine molecules, two aspartic residues and one tyrosine (17). Zinc ion forms the essential component of the charge relay system as it gets bound to the zinc binding site in the HDAC structure.

2.2. HDAC in health and diseases

HDACs play a deciding role in development, proliferation, differentiation, and cell death [18]. HDAC1 knockout mice show embryonic lethal phenotype, with severe growth and proliferation defects [19] which may be attributed to increased expression of CDK inhibitors, p21 and p27 [20]. The knockout of HDAC2, HDAC3, HDAC5, and HDAC9 has been found to be associated with severe heart complications that culminate in death within 24 hours of birth [19]. An up regulation of ligand induced drug storage in heart occurs due to HDAC3 ablation which decreases their life span to 2-3 months [19]. The ablation of either HDAC5 or HDAC9 alone is not lethal whereas simultaneous deletion of both leads to lethal ventricular septal defects and a thin-walled myocardium [18]. HDAC4 regulates RUNX2 and MEF2C expression and modulates chondrocyte hypertrophy and leads to death of excessive ossification [21]. HDAC7 and HDAC8 knockouts lead to impaired vascular development and craniofacial features respectively [18]. HDAC6 knockout mice do not show any defects other than hyperacetylation of tubulin [22]. HDAC10 and HDAC11 genes' knockout have not been

explored much. HDAC8 has been found to play a significant role in skull morphogenesis [23].

Owing to their global presence,HDAC's are implicated in gene regulation of cell growth, survival, and proliferation and aberrant changes in epigenetic regulation of chromatin by acetylation/deacetylation leading to many diseases such as cancer [2]. HDAC and HAT form multiprotein complexes as co-activators and co-repressors in the process of gene regulation [24]. The process of acetylation and deacetylation affects more than 50 non-histone protein substrates like transcription regulators, signal transducers, DNA repairing enzymes, nuclear import regulators, nuclear transcription factors p53, E2F, c-Myc, nuclear factor κB (NF-κB), hypoxia-inducible factor 1 alpha HIF-1α [25-27].

3. HDAC inhibitors and their mechanism of action

3.1. HDAC inhibitors

Different chemical compounds from synthetic and natural sources have been discovered. These may be different structural background like acyclic molecules, cyclic peptides, aliphatic compounds and benzamides [28, 29]. HDAC inhibitors show inhibitory activity on different HDACs. MS-275, also known as Entinostat inhibits HDAC1 and HADC3 (IC_{50} 0. 3mM and 8 mM, respectively) whereas in case of HDAC6 and HDAC8no inhibition was observed [30]. The molecules, SK7041 and SK7068 exhibit growth inhibibitory activity owing to their capacity to target HDAC1 and HDAC2 in *in vitro* and *in vivo* cancer models. A small molecular weight molecule, tubacin is a cell permeable molecule with specific HDAC6 inhibitory characteristics which does not affect histone acetylation and associated changes in cell cycle progression. It inhibits second deacetylase domain (DD2) and induces expression of the transcription factor like DDIT3 (CHOP/GADD153) (www.abcam.com/Tubacin-ab142183.pdf) [22]. Treatment with HDACi has been found to show profound effect on gene expression pattern by affecting the gene promoter directly or the other secondary and downstream effectors [31]. TSA alters about 2% of the 340 genes, as shown by differential display experiments [32, 33]. Based upon the microarray analysis it was discovered that expression of about 7–10% of the genes get altered in various cancer cell lines with butyrate, TSA, MS-275, vorinostat or FK228 (depsipeptide) [31, 34, 35].

3.2. Natural HDAC Inhibitors: Classification and mechanism of action

3.2.1. Acyclic molecules

3.2.1.1. Hydroxamic acid derivatives

These are early compounds that are recognized for potent histone deacetylase activity, with antitumor and cell differentiation effects [36, 37]. This class of compounds has general formula R'-C(=O)NROH with a hydroxylamine derivative substituted to carboxylic acid moiety, where R and R' are substituted organic residues [38]. Trichostatin A (TSA) fungal antibiotic, is a well-known histone deacetylase inhibitor belonging to this class [39]. It is metabolite isolated from *Streptomyces hygroscopicus* bacteria with antifungal activity against *Trichophyton* [40]. The structure and function of deacetylase is determined from binding studies with TSA and suberoylanilide hydroxamic acid (SAHA) [16]. Finnin and colleagues were first to elaborate the structure of deacetylase enzyme based on crystal structure of HDLP (structural homologue of HDAC in bacterium *Aquifex aeolicus*), that HDAC1 have 31% structural homology to HDLP, with similarity of active site. Crystallographic studies revealed open pocket like structure leading to tunnel of ~7 Å narrowing to ~4.5 Å by 5.5 Å, at end of tunnel, Zn^{2+} cation is coordinated by two aspartic acid residues, one histidine residue and a water molecule [16].

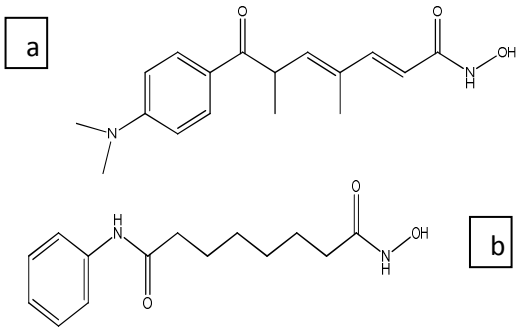

Figure 1. Chemical structures of natural HDAC inhibitors (a) Trichostatin A (TSA) (b) SAHA

Structure of SAHA bound to an HDAC-like protein

The postulated mechanism of deacetylase enzyme is, acetylated lysine coordinates with zinc cation forming a tetrahedral intermediate complex, followed by nucleophilic reaction with water liberating acetic acid and free deacetylated lysine [41]. Moreover, catalytic activity of deacetylase is inhibited due to false substrate complex formed from interaction of TSA with zinc cation regarded to be active site, preventing the access of acetylated lysine residues on histone tails. It is assumed that hydroxamic moiety chelates zinc at the active site, long aliphatic chain span over hydrophobic tunnel from hydrophilic sites to surface, blocking entrance to pocket. Further, from these interactions, a pharmacophore for inhibiting deacetylase activity has been developed. The key features include a metal binding site to interact with zinc at active site, a hydrophobic recognition domain to interact with surface amino acid residues and a linker connecting both (n=6 optimal chain length) (16, 42-44).

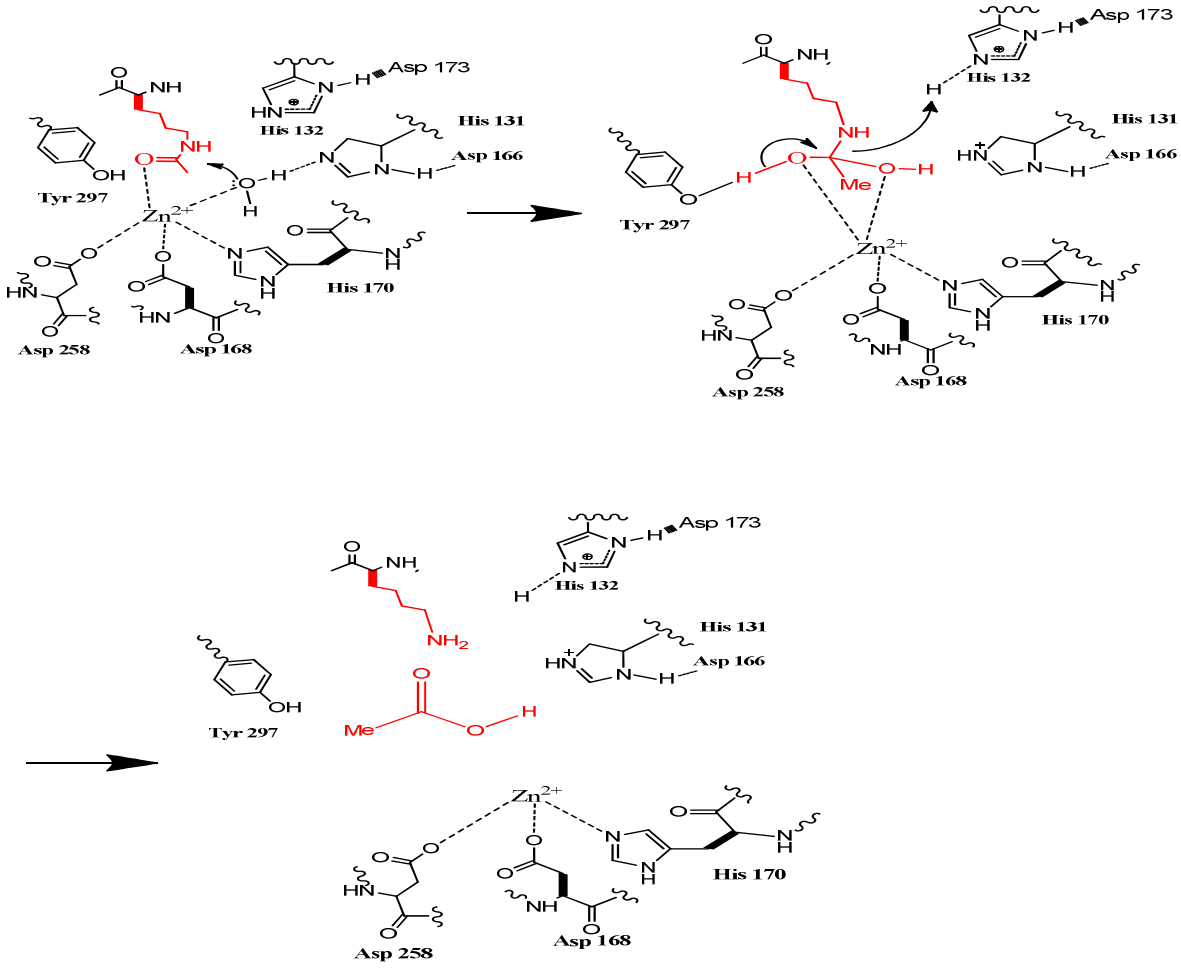

Figure 2. Proposed mechanism of action of Zn^{2+}dependent HDACs and TSA binding model with zinc ion in HDAC8 active site.

Vorinostat (SAHA), the only HDACi approved by FDI from this class is marketed by Merck Pharmaceuticals in the trade name Zolinza for the treatment of cutaneous T cell lymphoma.. Although it is not naturally occurring, it's worth mentioning that based on its structural similarity with TSA, it found to inhibit histone deacetylases [45]. Though TSA and SAHA were potent in sub micro and nano molar range, these are comparatively short lived, non-specific, with solubility, bioavailability, pharmacokinetic discrepancies and toxicities especially cardiac toxicity due non-specific activity on pan HDAC classes [44, 46].

3.2. Aliphatic acids

Carboxylate butyric acid is the first molecule to be discovered in inhibiting histone deacetylase, although later it was observed to induce differentiation in Friend leukemia cells [47]. Butyric acid is a short chain fatty acid (SCFA) produced from intestinal micro flora. Other fermentation metabolites from intestine like acetate, propionate and valerate were also identified as HDAC inhibitors albeit with low potency [48-51]. Although butyrate's effect in preventing colorectal cancer is dubious yet it was shown to have exceptional HDAC inhibiting activity [48]. Butyrate acts on both class I and IIA. It is seen that butyrate act on cell proliferation, differentiation and induces apoptosis, a positive correlation is also observed with gene expression, due to intracellular butyrate concentration affecting gene regulation at colonic epithelium [52-54].

In 1882, Burton synthesized valproic acid (2-propylvaleric acid, 2-propylpentanoic acid or n-dipropylacetic acid), a synthetic derivative of valeric acid that occurs in plant *Valerian* species (*Valeriana officinalis*) [55]. The use of valproic acid (marketed as Convulex, Pfizer and Byk Madaus), Depakene (Abbott Laboratories), Depakine (Sanofi Aventis), Epival (Abbott Laboratories, U.S. & Canada), Stavzor (Noven Pharmaceuticals) as antiepileptic and mood stabilizer is well established since the 1960's. Yet its HDAC inhibition (in millimolar range) was highlighted in the past decade only [56-58]. Although anticonvulsant and mood stabilizing properties are not attributed to HDAC inhibition, teratogenic defects are assumed due to HDAC inhibition, early in embryonic development [56,57,59]. It inhibits both class I and IIA enzymes [60, 61] and affects proliferation, differentiation, and apoptosis [62].

It is reported that induction of peroxisomal proliferation, activation of a glucocorticoid receptor and altered gene expression and from inhibiting co-repressors complexes of HDAC it relieves transcriptional repression [57, 63]. It is seldom used alone in treatment of cancer yet combination therapies are reported to have additive effect and seem to be promising in chemoresistant cancers [64-66].

3.3. Bromotyrosine derivatives

Bromotyrosine derivatives are natural products isolated from different marine sponges, mostly belonging to the order Verongida [67]. Psammaplin A is the first identified member of bisulphide bromotyrosine derivatives from numerous marine sponges, containing both oxime functional groups and carbon–sulphur bonds [67-69]. 3-bromo-4-hydroxyphenylacetonitrile, also known to be Psammaplin A is isolated from *psammaplysilla sp* [70]. It inhibits class I HDAC's at nanomolar range, and has DNA methyltransferase inhibitory activity [71, 72].

Although mechanism of action is unclear, free thiol's formed by reduction with glutathione(reducing agent) are assumed to be responsible for zinc chelation at active binding site of enzyme [71].

NVP-LAQ824 a synthetic analogue of psammaplin A is made to overcome stability issues of Psammplin A,with

potent activity (IC50 =32 nM) against class I and class II HDAC's but dropped in phase II clinical trials in 2005 [73-76].

3.2. Mechanism of action

HDAC inhibitors function by removing the zinc ion, which is an important component of charge relaying system from the enzyme's structure to make it non-functional. TSA the most potent reversible non selective HDACi, [29] with an IC50 in low nanomolar range [39] gets attached to the active site using its hydroxamic acid group and the 5-C linker to phenyl [16].

The mechanism through which HDAC modulates cancer development is not completely understood.

However, various propositions have been made regarding the mechanism involved in regulation of cell death by HDAC mediated gene expression causing histone hyperacetylation at promoters of apoptosis-inducing genes such as TNFSF10 which codes for TRAILand BMF; transcription factors such as inhibition of SP1 and C/EBPα, leading to down regulation of the anti-apoptotic protein Bcl-2, following HDACi treatment. HDAC induced cell death promotion proceeds by two mechanisms- (i) alteration of JAK/STAT pathway (ii) human RAD23 homolog B (HR23B) [47].

The correlation between HR23B expression and clinical response to vorinostat [48], and the interaction between Hsp90 and HDAC6 were found to be crucial factors to decide sensitivity to HDACi-induced apoptosis [49]. Various mechanisms involved in antitumor activity of HDAC inhibitors are shown in the figure 3.

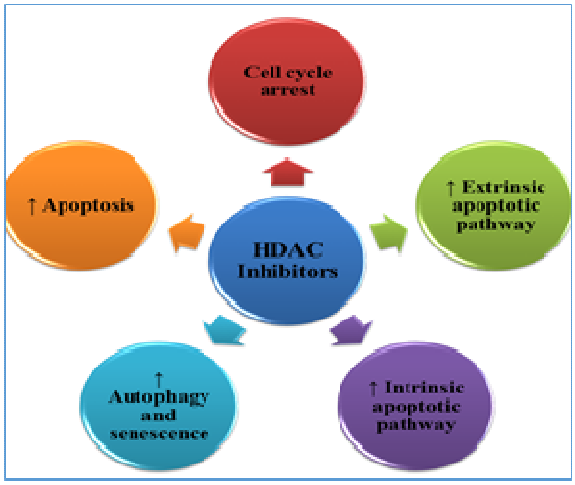

Figure 3: Various mechanisms involved in antitumor activity of HDAC inhibitors

HDAC inhibitors lead to alterations in cell cycle progression by inducing the expression of the cyclin dependent kinase inhibitor p21 (WAF1/CIP1) [11]. These alterations are independent of role of p53 protein [77]. The drug, vorinostat leads to post translational histone modifications like acetylation and methylation at lysine in histone H3 and H4 associated with the p21 promoter region in ARP-1 cells. It also leads to a marked reduction in non-nuclear HDAC1 and Myc, and recruitment of RNA polymerase II, with insignificant alterations in HDAC2 or other proteins in the complex [78]. The activation of promoter gene of p21 gene leads to increased expression of HDAC1, HDAC2, Myc, BAF155,Brg-1, GCN5, p300 and Sp1 [78]. The alteration of gene activity by HDACi depends upon the composition and configuration of proteins. HDAC activity is required for transcriptional activation mediated by signal transducer and activator of transcription 5 (STAT5) [79], thus HDACi results in the repression of genes like androgen receptor (AR) [80].

4. Conclusion

Based on the above discussions, it can be concluded that natural HDAC inhibitors have surfaced as one of the most successful HDAC inhibitors. Though in a very nascent stage of drug development, these molecules appear to

be alluring anticancer agents. Further research prodding the application of these molecules to combat cancer is necessary to confirm their utility.

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5. Conflict of interest

No conflict of interest between the authors.

References:

[1] Cremer T, Cremer C. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet 2001;2(4):292-301.

[2] Grunstein M. Histone acetylation in chromatin structure and transcription. Nature. 1997;389(6649):349-52.

[3] Cheung WL, Briggs SD, Allis CD. Acetylation and chromosomal functions. Curr Opin Cell Biol. 2000;12(3):326-33.

[4] Kouzarides T. Chromatin modifications and their function. Cell. 2007;128(4):693-705.

[5] Sterner DE, Berger SL. Acetylation of histones and transcription-related factors. Microbiol Mol Biol Rev. 2000;64(2):435-59.

[6] Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, et al. Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science. 2009;325(5942):834-40.

[7] Clayton AL, Hazzalin CA, Mahadevan LC. Enhanced histone acetylation and transcription: a dynamic perspective. Mol cell. 2006;23(3):289-96.

[8] Loidl P. Histone acetylation: facts and questions. Chromosoma. 1994;103(7):441-9.

[9] Ruthenburg AJ, Li H, Patel DJ, Allis CD. Multivalent engagement of chromatin modifications by linked binding modules. Nat Rev Mol Cell Bio. 2007;8(12):983-94.

[10] Wolffe AP. Histone Deacetylase--A Regulator of Transcription. Science. 1996;272(5260):371-.

[11] Xu W, Parmigiani R, Marks P. Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene. 2007;26(37):5541-52.

[12] Blander G, Guarente L. The Sir2 family of protein deacetylases. Annu Rev Biochem. 2004;73(1):417-35.

[13] Gray SG, Ekström TJ. The human histone deacetylase family. Exp Cell Res. 2001;262(2):75-83.

[14] Verdin E, Dequiedt F, Kasler HG. Class II histone deacetylases: versatile regulators. Trends Genet. 2003;19(5):286-93.

[15] Yang X-J, Seto E. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. Nat Rev Mol Cell Bio. 2008;9(3):206-18.

[16] Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, Marks PA, et al. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. Nature. 1999;401(6749):188-93.

[17] Somoza JR, Skene RJ, Katz BA, Mol C, Ho JD, Jennings AJ, et al. Structural snapshots of human HDAC8 provide insights into the class I histone deacetylases. Structure. 2004;12(7):1325-34.

[18] Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nat Rev Genet. 2009;10(1):32-42.

[19] Montgomery RL, Davis CA, Potthoff MJ, Haberland M, Fielitz J, Qi X, et al. Histone deacetylases 1 and 2 redundantly regulate cardiac morphogenesis, growth, and contractility. Gene dev. 2007;21(14):1790-802.

[20] Yamaguchi T, Cubizolles F, Zhang Y, Reichert N, Kohler H, Seiser C, et al. Histone deacetylases 1 and 2 act in concert to promote the G1-to-S progression. Gene dev. 2010;24(5):455-69.

[21] Vega RB, Matsuda K, Oh J, Barbosa AC, Yang X, Meadows E, et al. Histone deacetylase 4 controls chondrocyte hypertrophy during skeletogenesis. Cell. 2004;119(4):555-66.

[22] Li X. Epigenetics and autosomal dominant polycystic kidney disease. Biochim Biophys Acta - Molecular Basis of Disease. 2011;1812(10):1213-8.

[23] Haberland M, Mokalled MH, Montgomery RL, Olson EN. Epigenetic control of skull morphogenesis by histone deacetylase 8. Gene dev. 2009;23(14):1625-30.

[24] Sengupta N, Seto E. Regulation of histone deacetylase activities. J Cell Biochem. 2004;93(1):57-67.

[25] Glozak MA, Sengupta N, Zhang X, Seto E. Acetylation and deacetylation of non-histone proteins. Gene. 2005;363:15-23.

[26] Marmorstein R. Structure of Histone Deacetylases: Insights into Substrate Recognition and Catalysis. Structure. 2001;9(12):1127-33.

[27] Johnson CA, White DA, Lavender JS, O'Neill LP, Turner BM. Human class I histone deacetylase complexes show enhanced catalytic activity in the presence of ATP and co-immunoprecipitate with the ATP-dependent chaperone protein Hsp70. J Biol Chem. 2002;277(11):9590-7.

[28] Marks PA. Histone deacetylase inhibitors: A chemical genetics approach to understanding cellular functions. Bba-Gene Regul Mech. 2010;1799(10–12):717-25.

[29] Marks PA, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer. 2001;1(3):194-202.

[30] [30]Monneret C. Histone deacetylase inhibitors. Eur J Med Chem. 2005;40(1):1-13.

[31] Sasakawa Y, Naoe Y, Sogo N, Inoue T, Sasakawa T, Matsuo M, et al. Marker genes to predict sensitivity to FK228, a histone deacetylase inhibitor. Biochem pharmacol. 2005;69(4):603-16.

[32] Emiliani S, Fischle W, Van Lint C, Al-Abed Y, Verdin E. Characterization of a human RPD3 ortholog, HDAC3. P Natl Acad Sci-Biol. 1998;95(6):2795-800.

[33] Van Lint C, Emiliani S, Ott M, Verdin E. Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. EMBO J. 1996;15(5):1112.

[34] Chambers A, Banerjee S, Chaplin T, Dunne J, Debernardi S, Joel S, et al. Histone acetylation-mediated regulation of genes in leukaemic cells. Eur J Cancer. 2003;39(8):1165-75.

[35] Glaser KB, Staver MJ, Waring JF, Stender J, Ulrich RG, Davidsen SK. Gene expression profiling of multiple histone deacetylase (HDAC) inhibitors: defining a common gene set produced by HDAC inhibition in T24 and MDA carcinoma cell lines. Mol Cancer Ther. 2003;2(2):151-63.

[36] Luong QT, O'Kelly J, Braunstein GD, Hershman JM, Koeffler HP. Antitumor activity of suberoylanilide hydroxamic acid against thyroid cancer cell lines in vitro and in vivo. Clin Cancer Res. 2006;12(18):5570-7.

[37] Munster PN, Troso-Sandoval T, Rosen N, Rifkind R, Marks PA, Richon VM. The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces

differentiation of human breast cancer cells. Cancer Res. 2001;61(23):8492-7.

[38] Rajwade RP, Pande R, Mishra K, Kumar A, Pandey B. Quantitative Structure-ActivityRelationship (QSAR) of N-Arylsubstituted Hydroxamic Acids as Inhibitors of Human Adenocarinoma Cells A431. Med Chem. 2008;4(3):237-43.

[39] Yoshida M, Kijima M, Akita M, Beppu T. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J Biol Chem. 1990;265(28):17174-9.

[40] Tsuji N, Kobayashi M, Nagashima K, Wakisaka Y, Koizumi K. A new antifungal antibiotic, trichostatin. Journal Antibiot. 1976;29(1):1-6.

[41] Vanommeslaeghe K, Proft FD, Loverix S, Tourwe D, Geerlings P. Theoretical study revealing the functioning of a novel combination of catalytic motifs in histone deacetylase. Bioorgan Med Chem. 2005;13(12):3987-92.

[42] Remiszewski SW, Sambucetti LC, Atadja P, Bair KW, Cornell WD, Green MA, et al. Inhibitors of human histone deacetylase: synthesis and enzyme and cellular activity of straight chain hydroxamates. J Medicinal Chem. 2002;45(4):753-7.

[43] Jung M, Hoffmann K, Brosch G, Loidl P. Analogues of trichosтatin a and trapoxin B as histone deacetylase inhibitors. Bioorg Med Chem Lett. 1997;7(13):1655-8.

[44] Miller TA, Witter DJ, Belvedere S. Histone deacetylase inhibitors. J Med Chem. 2003;46(24):5097-116.

[45] Marks PA, Breslow R. Dimethyl sulfoxide to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. Nat Biotechnol. 2007;25(1):84-90.

[46] Gallinari P, Di Marco S, Jones P, Pallaoro M, Steinkühler C. HDACs, histone deacetylation and gene transcription: from molecular biology to cancer therapeutics. Cell Res. 2007;17(3):195-211.

[47] Leder A, Leder P. Butyric acid, a potent inducer of erythroid differentiation in cultured erythroleukemic cells. Cell. 1975;5(3):319-22.

[48] Boffa LC, Vidali G, Mann R, Allfrey V. Suppression of histone deacetylation in vivo and in vitro by sodium butyrate. J Biol Chem. 1978;253(10):3364-6.

[49] Reeves R, Candido E. Turnover of histone acetyl groups in cultured cells is inhibited by sodium butyrate. FEBS Lett. 1978;91(1):117-20.

[50] Sealy L, Chalkley R. The effect of sodium butyrate on histone modification. Cell. 1978;14(1):115-21.

[51] Kiefer J, Beyer-Sehlmeyer G, Pool-Zobel BL. Mixtures of SCFA, composed according to physiologically available concentrations in the gut lumen, modulate histone acetylation in human HT29 colon cancer cells. Brit J Nutr. 2006;96(05):803-10.

[52] Siavoshian S, Segain J, Kornprobst M, Bonnet C, Cherbut C, Galmiche J, et al. Butyrate and trichostatin A effects on the proliferation/differentiation of human intestinal epithelial cells: induction of cyclin D3 and p21 expression. Gut. 2000;46(4):507-14.

[53] Hague A, Manning A, Hanlon K, Hart D, Paraskeva C, Huschtscha L. Sodium butyrate induces apoptosis in human colonic tumour cell lines in a p53-independent pathway: implications for the possible role of dietary fibre in the prevention of large-bowel cancer. Int J Cancer. 1993;55(3):498-505.

[54] Daly K, Shirazi-Beechey SP. Microarray analysis of butyrate regulated genes in colonic epithelial cells. DNA Cell Biol. 2006;25(1):49-62.

[55] Burton B. On the propyl derivatives and decomposition products of

ethylacetoacetate. Am Chem J. 1882;3:385-95.

[56] Göttlicher M, Minucci S, Zhu P, Krämer OH, Schimpf A, Giavara S, et al. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. EMBO J. 2001;20(24):6969-78.

[57] Phiel CJ, Zhang F, Huang EY, Guenther MG, Lazar MA, Klein PS. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. J Biol Chem. 2001;276(39):36734-41.

[58] Göttlicher M. Valproic acid: an old drug newly discovered as inhibitor of histone deacetylases. Ann Hematol. 2003;83:S91-2.

[59] Eikel D, Lampen A, Nau H. Teratogenic effects mediated by inhibition of histone deacetylases: evidence from quantitative structure activity relationships of 20 valproic acid derivatives. Chem Research Toxicol. 2006;19(2):272-8.

[60] Gurvich N, Tsygankova OM, Meinkoth JL, Klein PS. Histone deacetylase is a target of valproic acid-mediated cellular differentiation. Cancer Res. 2004;64(3):1079-86.

[61] Krämer OH, Zhu P, Ostendorff HP, Golebiewski M, Tiefenbach J, Peters MA, et al. The histone deacetylase inhibitor valproic acid selectively induces proteasomal degradation of HDAC2. EMBO J. 2003;22(13):3411-20.

[62] Blaheta RA, Michaelis M, Driever PH, Cinatl J. Evolving anticancer drug valproic acid: insights into the mechanism and clinical studies. Med Res Rev. 2005;25(4):383-97.

[63] Werling U, Siehler S, Litfin M, Nau H, Göttlicher M. Induction of differentiation in F9 cells and activation of peroxisome proliferator-activated receptor δ by valproic acid and its teratogenic derivatives. Mol Pharmacol. 2001;59(5):1269-76.

[64] Kuendgen A, Strupp C, Aivado M, Bernhardt A, Hildebrandt B, Haas R, et al. Treatment of myelodysplastic syndromes with valproic acid alone or in combination with all-trans retinoic acid. Blood. 2004;104(5):1266-9.

[65] Cortes J. Treatment of myelodysplastic syndromes with valproic acid alone or in combination with all-trans retinoic acid. Curr Hematol Rep. 2005;4(3):173-4.

[66] Soriano AO, Yang H, Faderl S, Estrov Z, Giles F, Ravandi F, et al. Safety and clinical activity of the combination of 5-azacytidine, valproic acid, and all-trans retinoic acid in acute myeloid leukemia and myelodysplastic syndrome. Blood. 2007;110(7):2302-8.

[67] Cordell GA. The alkaloids: chemistry and biology: Academic Press; 2008.

[68] Arabshahi L, Schmitz FJ. Brominated tyrosine metabolites from an unidentified sponge. J Org Chem. 1987;52(16):3584-6.

[69] Rodriguez A, Akee RK, Scheuer PJ. Two bromotyrosine-cysteine derived metabolites from a sponge. Tetrahedron Lett. 1987;28(42):4989-92.

[70] Quiñoà E, Crews P. Phenolic constituents of Psammaplysilla. Tetrahedron Lett. 1987;28(28):3229-32.

[71] Kim DH, Shin J, Kwon HJ. Psammaplin A is a natural prodrug that inhibits class I histone deacetylase. Exp Mol Med. 2007;39(1):47-55.

[72] Piña IC, Gautschi JT, Wang G-Y-S, Sanders ML, Schmitz FJ, France D, et al. Psammaplins from the Sponge Pseudoceratina purpurea: Inhibition of Both Histone Deacetylase and DNA Methyltransferase. J Org Chem. 2003;68(10):3866-73.

[73] Catley L, Weisberg E, Tai Y-T, Atadja P, Remiszewski S, Hideshima T, et al. NVP-LAQ824 is a potent novel histone deacetylase inhibitor with significant activity against multiple myeloma. Blood. 2003;102(7):2615-22.

[74] Weisberg E, Catley L, Kujawa J, Atadja P, Remiszewski S, Fuerst P, et al. Histone deacetylase inhibitor NVP-LAQ824 has significant activity against myeloid leukemia cells in vitro and in vivo. Leukemia. 2004;18(12):1951-63.

[75] Wagner JM, Hackanson B, Lübbert M, Jung M. Histone deacetylase (HDAC) inhibitors in recent clinical trials for cancer therapy. Clin Epigenetics. 2010;1(3-4):117-36.

[76] Remiszewski SW. The discovery of NVP-LAQ824: from concept to clinic. Curr Med Chem. 2003;10(22):2393-402.

[77] Acharya MR, Sparreboom A, Venitz J, Figg WD. Rational development of histone deacetylase inhibitors as anticancer agents: a review. Mol Pharmacol. 2005;68(4):917-32.

[78] Gui C-Y, Ngo L, Xu W, Richon V, Marks P. Histone deacetylase (HDAC) inhibitor activation of p21WAF1 involves changes in promoter-associated proteins, including HDAC1. Proc Natl Acad Sci USA. 2004;101(5):1241-6.

[79] Rascle A, Johnston JA, Amati B. Deacetylase activity is required for recruitment of the basal transcription machinery and transactivation by STAT5. Mol Cell Biol. 2003;23(12):4162-73.

[80] Wang X, Wei X, Pang Q, Yi F. Histone deacetylases and their inhibitors: molecular mechanisms and therapeutic implications in diabetes mellitus. Acta Pharm Sin B. 2012;2(4):387-95.