Research Article

Design and Synthesis of Novel Spirohydanto in Derivatives with Potential Anticancer Activity

Al-Shimaa M. Ahmed, Mai E. Morsi, Eman A. Mahmoud and Omar M. Aly
Department of Medicinal Chemistry, Faculty of Pharmacy, Minia University.

Abstract
A series of novel *Spiro[imidazolidine-4,1'(2'H)-naphthalene]-2,5-dione* **4a-d** were designed and synthesized as Histone deacetylase inhibitors (HDACIs) by applying a novel Cap group (Spirohydantoin) that act as a surface recognition moiety, and a different zinc binding group (carboxylic group) aiming at increase the stability and improve the pharmacokinetic profile of the hydroxamic derivatives. The chemical structures of the synthesized compounds were elucidated on the basis of their IR, ^{1}H NMR, as well as high resolution mass spectra, and they were evaluated for their anti-proliferative activities against two cancer cell lines HepG2, and MCF7. Results revealed that compounds **4a-d** exhibit significantly low cytotoxicity in cancer cells, but they still being simple structures that are easily synthesized and can serve as lead compounds to further investigate more active entities exhibiting good stability and improved potency.

Key words: Histone deacetylase, Histone deacetylase inhibitors, hydroxamic acid, Spirohydantoins

Introduction
Cancer is mainly a disease of tissue growth regulation failure resulted from alterations of genes which regulate cell growth and differentiation. These alterations of tumor suppressor genes or oncogenes are not always due to mutation, they may also be due to epigenetic deregulation of gene expression which was found to be implicated in cancer pathogenesis.[1]Among these epigenetic regulations are histone acetylation/ deacetylation which are regulated by equilibrium between the two enzymes Histone acetyl transferase (HAT) and Histone deacetylase (HDAC).[2]

Histone deacetylase (HDAC) proteins are enzymes that influence gene expression by altering the acetylation status of lysine residues on nucleosomal histones. The HDAC family consists of 18 members and is divided into four classes.[2, 3]

Conventional HDAC enzymes involve Classes I, II, and IV which belong to zinc dependent amino hydrolases, and all require a zinc molecule (Zn^{2+}) as an essential cofactor in their active site, and they are inhibited by Zn^{2+}-binding histone deacetylase inhibitors.[4] However, non-conventional HDACs or class III, including 7 members, called Sirtuins and they require NAD^+ as a cofactor instead of Zn^{2+}.[5]

HDAC proteins are over-expressed in many cancers,[6] making them attractive targets of anti-cancer drugs.[7-9] In fact, two HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA, Vorinostat) and Romidepsin, **Fig. 1** were approved for treatment of cutaneous T-cell lymphoma. [10-12] In addition to lymphoma, HDAC activity has been associated with a variety of other cancers. Altered HDAC2 activity in gastric cancers,[13] mutations of HDAC1 and HDAC3 in lung cancers,[14] over expression of HDAC2 in aggressive cutaneous T-cell lymphoma (CTCL),[15] and abnormal HDAC 8 activity in acute myeloid leukemia tissue have been reported for class I HDACs, over expression of class I HDAC was also observed in ovarian cancer, and over-production of class II HDAC 6 was observed in breast cancer tissues. Inhibition of HDACs

has been shown to lead to cell differentiation, apoptosis and cell-cycle arrest in several cancer cell lines.[16]

Histone deacetylase (HDAC) inhibitors have become promising anticancer agents in recent years.[17] They have shown ability to block angiogenesis and cell cycling, as well as initiate differentiation and apoptosis. [18-20] HDAC inhibition has recently been clinically validated as a new therapeutic strategy for cancer treatment. Intense research activities are ongoing toward improving the pharma-cokinetic and therapeutic indices of current HDAC inhibitors. The classical pharma-cophore for HDAC inhibitors consists of three distinct structural motifs:[6, 21] the zinc-binding group (ZBG), a hydrophobic linker, and a recognition cap group **Fig. 2**. The X-ray structure of human HDAC8, bound to SAHA or Trichostatin A (TSA), confirmed that the ZBG interacts with a Zn^{2+} ion at the base of a channel-like active site.[22, 23]

The hydrophobic linker efficiently presents the ZBG to the active site by filling the channel while the cap group at the other end of the linker makes contacts with amino acid residues at the rim of the channel. The cap group could be linked to the aliphatic linker group through either hydrogen-bond accepting or donating groups such as keto- and amide-groups.[24-26] the common ZBG of HDAC inhibitors is the hydroxamate moiety.[27-29] Because of the short half-life and poor bioavailability associated with the hydroxamic acid functional group, it became desirable to find replacement groups that would possess nano molar activity against HDACs.

The structural modifications of the hydroxamate ZBG have been modestly successful; yielding isosteres such as benzamide, α-ketoesters, electrophilic ketones, mercaptoamide and phosphor-nates.[30, 31]

In the current study we introduce a different ZBG which is a carboxylic group aiming at providing better stability and more favorable pharmacokinetic profile to the designed compounds.

Moreover, based on the fact that the cap group presents an alternative opportunity to discover potent and more selective HDAC inhibitors, a novel cap group is introduced in the present work presenting an opportunity to improve binding of the compounds to the enzyme active site.

Materials and methods

Chemistry

Melting points were determined on Stuart electro-thermal melting point apparatus and are uncorrected. IR spectra were recorded on Nicolet iS5 FT-IR spectrometer at Minia University.

^{1}H NMR spectra were carried out using Bruker apparatus 400 MHz spectrometer, using TMS as internal reference. Chemical shifts (δ) values are given in parts per million (ppm) relative to TMS using DMSO-d_6 (2.5) as a solvent and coupling constants (J) in Hertz. Splitting patterns are designated as follows: s, singlet; br.s, broad singlet; d, doublet; t, triplet; q, quartet; p, pentet; dd, doublet of doublet; m, multiplet.

High resolution mass spectra (HRMS) were obtained on a Thermo Scientific Q Exactive™ Orbitrap mass spectrometer at The University of British Columbia Canada.

Reactions were routinely monitored by thin-layer chromatography (TLC) using Merck 9385 pre-coated aluminum plate silica gel (Kieselgel 60) 5 x 20 cm plates with a layer thickness of 0.2 mm, and spots were visualized by exposure to UV-lamp at λ = 254 nm.

Biological evaluation (*in vitro* anti-proliferative activity)

Cell culture

Cancer cells from different cancer cell lines human breast adenocarcinoma (MCF7), and human hepatocellular carcinoma (HEPG2), were purchased from American type Cell Culture collection (ATCC, Manassas, USA) and grown on the appropriate growth medium Dulbecco's modified Eagle's medium (DMEM) or Roswell Park Memorial Institute medium (RPMI 1640) supplemented with 100 mg/

mL of streptomycin, 100 units/mL of penicillin and 10% of heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO_2 atmosphere at 37ºC

Cytotoxicity assay by 3-[4,5-dimethylthia -zole-2-yl]-2,5-diphenyltetrazolium bromide (MTT)

Exponentially growing cells from the two cancer cell lines were trypsinized (for detachment), counted and seeded at the appropriate densities (2000-1000 cells/0.33 cm^2 well) into 96-well microtiter plates. Cells then were incubated in a humidified atmosphere at 37˚C for 24 hours. Then, cells were exposed to different concentrations of compounds (0.1,1, 10,100,1000 µM) for 72 hours. The viability of treated cells was determined using MTT technique as follow. Media were removed; cells were incubated with 200 µl of 5% MTT solution/well (Sigma Aldrich, MO) and were allowed to metabolize the dye into a colored-insoluble formazan crystals for 2 hours. The remaining MTT solution were discarded from the wells and the formazan crystals were dissolved in 200 µl/well acidified isopropanol for 30 min, covered with aluminum foil and with continuous shaking using a MaxQ 2000 plate shaker (Thermo Fisher Scientific Inc, MI) at room temperature. Absorbance were measured at 570 nm using a Stat Fax^R 4200 plate reader (Awareness Technology, Inc.**,** FL). The cell viability were expressed as percentage of control and the concentration that induces 50% of maximum inhibition of cell proliferation (IC_{50}) were determined using Graph Pad Prism version 5 software (Graph Pad software Inc, CA). [32, 33]

Results

Chemistry

Synthesis of ε-(2,5-dioxo-3',4'-dihydro-2'*H*-spiro[imidazolidine-4,1'-naphthalen]-1-yl) alkanoic acids (4a-d)

The parent spirohydantoin (3',4'-dihydro-2'*H*-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione) **2** was prepared in a good yield as reported,[34] via Bucherer-Bergs reaction,[35] by heating α-tetralone **1** with potassium cyanide and anhydrous ammonium carbonate. **(Scheme 1)**

The intermediate ethyl ester derivatives **3a-d** was prepared in a moderate yield by reacting **2** with various ethylbromo esters, and anhydrous potassium carbonate in dry acetone. The synthesis of the acid derivatives **4a-d** was achieved in a moderate yield by ester **3** hydrolysis with 20% potassium hydroxide, then acidification with a mineral acid to yield the free acid derivatives, according to **Scheme 1**.

Synthesis of 5-(2,5-dioxo-3',4'-dihydro-2'*H*-spiro[imidazolidine-4,1'-naphthalen]-1-yl) pentanoic acid 4d

Yellowish white powder in (4.02 g, 50.88% yield); mp 233°C; IR (cm^{-1}): 3270 (OH), 3400 (NH), 1766 (C=O), 1711 (C=O), 1697 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.26 (t, 2H, CH_2), 1.53 (m, 2H, CH_2), 1.60 (m, 2H, CH_2), 3.43 (t, 2H, CH_2), 2.79 (t, 2H, J = 6.80 Hz, CH_2), 1.91 (m, 2H, J = 6.80 Hz, CH_2), 2.07 (t, 2H, J = 6.80 Hz, CH_2), 6.99 (dd, 1H, J = 8.00 Hz, Ar-H), 7.18 (d, 1H, J = 8.00 Hz, Ar-H), 7.23 (dd, 1H, J = 8.00 Hz, Ar-H), 7.25 (d, 1H, J = 8.00 Hz, Ar-H), 8.83 (s, 1H, NH), 12.03 (br. s, 1H, OH); HRMS: m/z calculated for $C_{17}H_{20}N_2O_4$: 315.13503, found: 315.13531.

Biological evaluation

Evaluation of *in vitro* anti-proliferative activity

The *in vitro* anti-proliferative activities of the compounds were investigated against two cancer cell lines including human breast adenocarcinoma (MCF-7), and human hepatocellular carcinoma (HepG2) using MTT assay. The IC_{50} values are presented in **Table 1**.

Tables

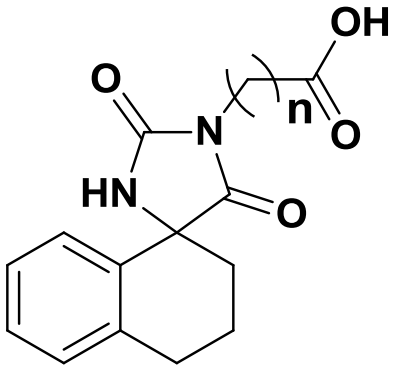
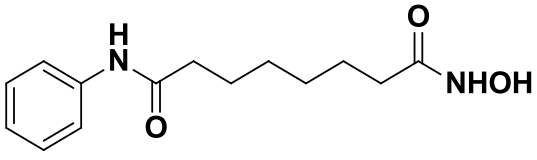


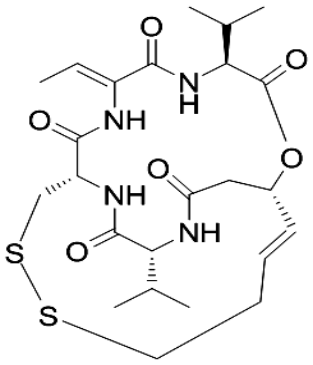
Table 1: *in vitro* anti-proliferative activity of compounds 4 a-d against two cancer cell lines

Compound		IC50 (µM)[a]/ cell line[b]	
Number	n	MCF-7	HepG2
4a	**1**	**1000**	**1000**
4b	**2**	**1000**	**1000**
4c	**3**	**676.08**	**1000**
4d	**4**	**1000**	**1000**

[a] The concentration (µM) that produce 50% reduction in cell growth, the number represent the average results from triplicate experiments with deviation of less than 10%
[b] Cell lines: MCF-7, human breast adenocarcinoma; HepG2, human hepatocellular carcinoma

Figures and schemes





A **B**

Fig. 1: **A** (suberoylanilide hydroxamic acid (SAHA, Vorinostat)) and **B** (Romidepsin)

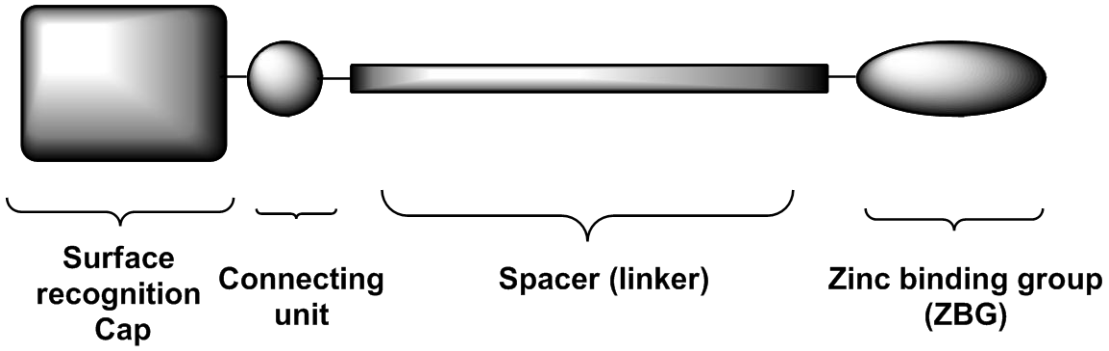
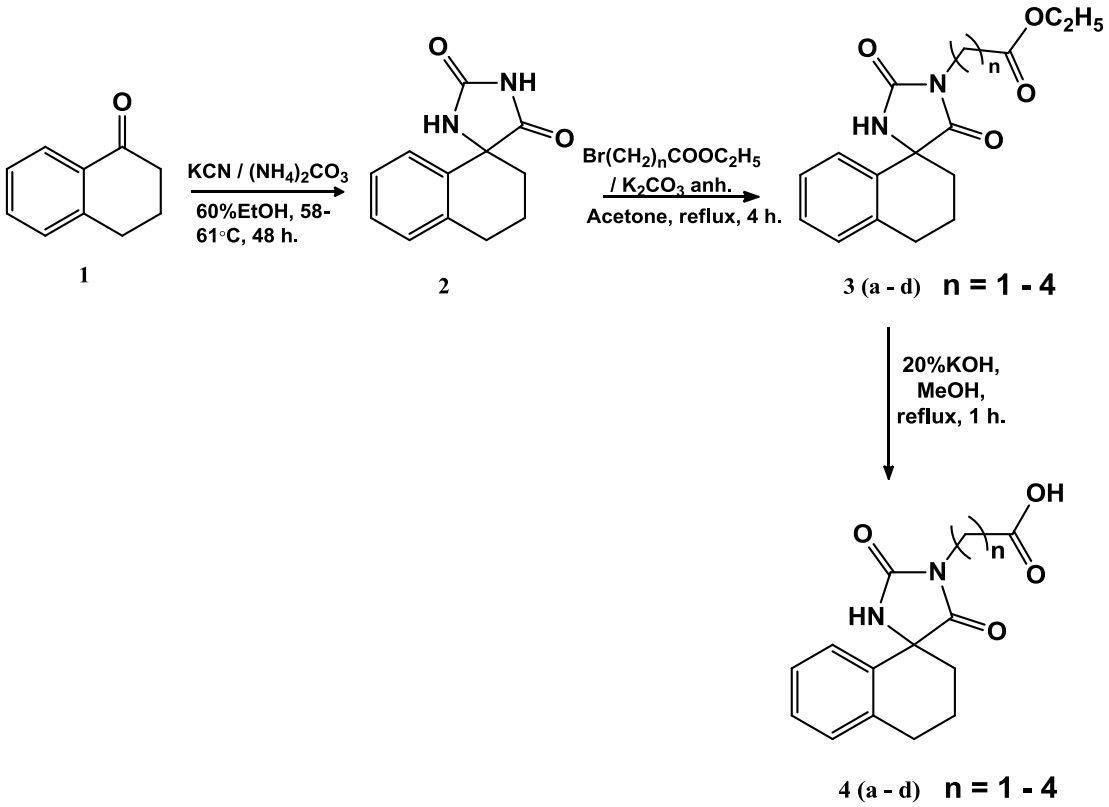


Fig. 2: Classical pharmacophore for HDAC inhibitors



Scheme 1: Synthesis of ε-(2,5-dioxo-3',4'-dihydro-2'*H*-spiro[imidazolidine-4,1'-naphthalen]-1-yl)alkanoic acids **4 (a-d)**

Discussion

The effect of the exposure of synthesized compounds was tested on the viability of MCF-7 human breast adenocarcinoma cells, and HepG2 human hepatocellular carcinoma cells. While it is true that compounds **4 a-d** exhibit significantly low cytotoxicity in cancer cells, they are simple structures that are easily synthesized and may possess good stability, although it remains to be confirmed. They can serve as lead compounds to discover more active entities acting as HDAC inhibitors, that exhibit good stability and improved pharmacokinetic profile.

Acknowledgments

We gratefully acknowledge Dr. Tamer Abdelghany at Faculty of Pharmacy Al-Azhar University for biological analysis, Dr. Safwat Rabea at University of British Columbia for spectral analysis is also gratefully acknowledged.

References

1. Toyota M, Issa J-PJ, editors. Epigenetic changes in solid and hematopoietic tumors. Seminars in oncology; 2005: Elsevier.
2. De Ruijter A, Van Gennip A, Caron H, Kemp S, van Kuilenburg A. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J. 2003;370:737-49.
3. Blander G, Guarente L. The Sir2 family of protein deacetylases. Annual review of biochemistry. 2004;73(1):417-35.
4. Gregoretti I, Lee Y-M, Goodson HV. Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. Journal of molecular biology. 2004; 338(1):17-31.
5. Luo J, Nikolaev AY, Imai S-i, Chen D, Su F, Shiloh A, et al. Negative control of p53 by Sir2α promotes cell survival under stress. Cell. 2001;107(2):137-48.
6. Firestein R, Blander G, Michan S, Oberdoerffer P, Ogino S, Campbell J, et al. The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. PloS one. 2008;3(4): e2020-e.
7. Batty N, Malouf GG, Issa JPJ. Histone deacetylase inhibitors as anti-neoplastic agents. Cancer letters. 2009;280(2):192-200.
8. Marks PA, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. Nature Reviews Cancer. 2001; 1(3):194-202.
9. Li J, Li G, Xu W. Histone deacetylase inhibitors: an attractive strategy for cancer therapy. Current medicinal chemistry. 2013;20(14):1858-86.
10. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. Nature reviews Drug discovery. 2006;5(9):769-84.
11. Mann BS, Johnson JR, Cohen MH, Justice R, Pazdur R. FDA approval summary: vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. The oncologist. 2007; 12 (10):1247-52.
12. Grant S, Easley C, Kirkpatrick P. Vorinostat. Nature Reviews Drug Discovery. 2007;6(1):21-2.
13. Grant C, Rahman F, Piekarz R, Peer C, Frye R, Robey RW, et al. Romidepsin: a new therapy for cutaneous T-cell lymphoma and a potential therapy for solid tumors. 2010.
14. Song J, Noh JH, Lee JH, Eun JW, Ahn YM, Kim SY, et al. Increased expression of histone deacetylase 2 is found in human gastric cancer. Apmis. 2005;113(4):264-8.
15. Bartling B, Hofmann H-S, Boettger T, Hansen G, Burdach S, Silber R-E, et al. Comparative application of antibody and gene array for expression profiling in human squamous cell lung carcinoma. Lung Cancer. 2005;49(2):145-54.
16. Marquard L, Gjerdrum L, Christensen IJ, Jensen P, Sehested M, Ralfkiaer E. Prognostic significance of the therapeutic targets histone deacetylase 1, 2, 6 and acetylated histone H4 in cutaneous T-cell lymphoma. Histo-pathology. 2008;53(3):267-77.
17. KrennHrubec K, Marshall BL, Hedglin M, Verdin E, Ulrich SM. Design and evaluation of 'Linkerless' hydroxamic acids as selective HDAC8 inhibitors.

Bioorganic & medicinal chemistry letters. 2007;17(10):2874-8.
18. Khabele D, Son D-S, Parl AK, Goldberg GL, Augenlicht LH, Mariadason JM, et al. Drug-induced inactivation or gene silencing of class I histone deacetylases suppresses ovarian cancer cell growth: implications for therapy. Cancer biology & therapy. 2007;6(5):795-801.
19. Saji S, Kawakami M, Hayashi S-i, Yoshida N, Hirose M, Horiguchi S-i, et al. Significance of HDAC6 regulation via estrogen signaling for cell motility and prognosis in estrogen receptor-positive breast cancer. Oncogene. 2005;24(28):4531-9.
20. Dokmanovic M, Clarke C, Marks PA. Histone deacetylase inhibitors: overview and perspectives. Molecular cancer research. 2007;5(10):981-9.
21. Oki Y, Issa J-PJ. Review: recent clinical trials in epigenetic therapy. Reviews on Recent Clinical Trials. 2006;1(2):169-82.
22. Saito A, Yamashita T, Mariko Y, Nosaka Y, Tsuchiya K, Ando T, et al. A synthetic inhibitor of histone deace-tylase, MS-27-275, with marked in vivo antitumor activity against human tumors. Proceedings of the National Academy of Sciences. 1999;96(8):4592-7.
23. Glick RD, Swendeman SL, Coffey DC, Rifkind RA, Marks PA, Richon VM, et al. Hybrid polar histone deacetylase inhibitor induces apoptosis and CD95/CD95 ligand expression in human neuroblastoma. Cancer research. 1999; 59(17):4392-9.
24. Butler LM, Agus DB, Scher HI, Higgins B, Rose A, Cordon-Cardo C, et al. Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, supp-resses the growth of prostate cancer cells in vitro and in vivo. Cancer research. 2000;60(18):5165-70.
25. Oyelere AK, Chen PC, Guerrant W, Mwakwari SC, Hood R, Zhang Y, et al. Non-peptide macrocyclic histone deacetylase inhibitors. Journal of medicinal chemistry. 2008;52(2):456-68.
26. Monneret C. Histone deacetylase inhibitors. European journal of medicinal chemistry. 2005;40(1):1-13.
27. Bottomley MJ, Surdo PL, Di Giovine P, Cirillo A, Scarpelli R, Ferrigno F, et al. Structural and functional analysis of the human HDAC4 catalytic domain reveals a regulatory structural zinc-binding domain. Journal of Biological Chemistry. 2008;283(39):26694-704.
28. Finnin M, Donigian J, Cohen A, Richon V, Rifkind R, Marks P, et al. Nature401, 188-193.Finnin188401Nature1999.
29. Nielsen TK, Hildmann C, Dickmanns A, Schwienhorst A, Ficner R. Crystal structure of a bacterial class 2 histone deacetylase homologue. Journal of molecular biology. 2005;354(1):107-20.
30. Schuetz A, Min J, Allali-Hassani A, Schapira M, Shuen M, Loppnau P, et al. Human HDAC7 harbors a class IIa histone deacetylase-specific zinc binding motif and cryptic deacetylase activity. Journal of Biological Chemistry. 2008; 283(17):11355-63.
31. Vannini A, Volpari C, Filocamo G, Casavola EC, Brunetti M, Renzoni D, et al. Crystal structure of a eukaryotic zinc-dependent histone deacetylase, human HDAC8, complexed with a hydroxamic acid inhibitor. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(42):15064-9.
32. 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.
33. 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.
34. Watson PJ, Fairall L, Santos GM, Schwabe JW. Structure of HDAC3 bound to co-repressor and inositol tetraphosphate. Nature. 2012;481 (7381):335-40.

35. Bressi JC, Jennings AJ, Skene R, Wu Y, Melkus R, De Jong R, et al. Exploration of the HDAC2 foot pocket: Synthesis and SAR of substituted N-(2-aminophenyl) benzamides. Bioorganic & medicinal chemistry letters. 2010; 20(10):3142-5.
36. Lee H-Z, Kwitkowski VE, Del Valle PL, Ricci MS, Saber H, Habtemariam BA, et al. FDA Approval: Belinostat for the Treatment of Patients with Relapsed or Refractory Peripheral T-cell Lymphoma. Clinical Cancer Research. 2015: clin-canres. 3119. 2015.
37. Oanh DTK, Van Hai H, Park SH, Kim H-J, Han B-W, Kim H-S, et al. Benzothiazole-containing hydroxamic acids as histone deacetylase inhibitors and antitumor agents. Bioorganic & medicinal chemistry letters. 2011;21 (24):7509-12.
38. Pabba C, Gregg BT, Kitchen DB, Chen ZJ, Judkins A. Design and synthesis of aryl ether and sulfone hydroxamic acids as potent histone deacetylase (HDAC) inhibitors. Bioorganic & medicinal chemistry letters. 2011;21(1):324-8.
39. El-Beltagi H, Martens A, Lelieveld P, Haroun E, Hagenbeek A. Acetyl-dinaline: a new oral cytostatic drug with impressive differential activity against leukemic cells and normal stem cells—preclinical studies in a relevant rat model for human acute myelocytic leukemia. Cancer research. 1993;53 (13):3008-14.
40. Rajak H, Kumar P, Parmar P, Thakur BS, Veerasamy R, Sharma PC, et al. Appraisal of GABA and PABA as linker: design and synthesis of novel benzamide based histone deacetylase inhibitors. European journal of medicinal chemistry. 2012;53:390-7.
41. Cai J, Wei H, Hong KH, Wu X, Cao M, Zong X, et al. Discovery and prelimi-nary evaluation of 2-amino-benzamide and hydroxamate derivatives containing 1, 2, 4-oxadiazole moiety as potent histone deacetylase inhibitors. European journal of medicinal chemistry. 2015;96:1-13.
42. Wada CK, Frey RR, Ji Z, Curtin ML, Garland RB, Holms JH, et al. α-Keto amides as inhibitors of histone deacetylase. Bioorganic & medicinal chemistry letters. 2003;13(19):3331-5.
43. Ueda H, Nakajima H, Hori Y, Fujita T, Nishimura M, Goto T, et al. FR901228, a novel antitumor bicyclic depsipeptide produced by Chromobacterium violaceum No. 968. I. Taxonomy, fermentation, isolation, physico-chemical and biological properties, and antitumor activity. The Journal of antibiotics. 1994;47(3):301-10.
44. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immuno-logical methods. 1983;65(1):55-63.
45. Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH, et al. Evaluation of a soluble tetrazolium/ formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Research. 1988;48(17):4827-33.
46. GOODSON LH, HONIGBERG IL, Lehman J, Burton W. Potential Growth Antagonists. I. Hydantoins and Disubstituted Glycines1, 2. The Journal of Organic Chemistry. 1960; 25(11): 1920-4.
47. Li JJ. Bucherer—Bergs reaction. Name Reactions. 2009:76-7.