Introduction

Genetic and epigenetic alterations are two important mechanisms participating in carcinogenesis that lead to a very high proliferation rates, metastasis and evasion from cell death (Jones et al., 2007; Esteller et al., 2008; Hanahan et al., 2011; Baylin et al., 2016). Genetic and epigenetic alterations were thought to be different sides of a coin for a long period of time. However, the exome sequencing of number of cancers has revealed mutations of genes involved in control of epigenome. At the heart of epigenetic paradigm are highly conserved histone proteins, which have several modifications at their amino-terminal tails including post translational modifications (PTMs) like methylation, acetylation, poly-ADP ribosylation, phosphorylation, ubiquitination, sumoylation, glycosylation and carbonylation (Kouzarides., 1999; Sharma et al., 2010). Alterations in the methylation and acetylation patterns of DNA are of prime importance in carcinogenesis (Feinberg., 2004; Lujambio et al., 2009; Liu et al., 2016). Specific arrangement or code of PTMs is needed at histone tails for the expression of a particular gene. In similarity with genetic code, this phenomenon of spatio-temporal arrangement of PTMs at the histone tails has been referred to as histone code (Jenuwein et al., 2001). Acetylation is one of the important and well studied components of histone code (Durrin et al., 1991; Roth et al., 1992; Fisher et al., 1995). Acetylation of both histones and non-histone proteins at lysine residues is reversible post translational modification which results in accessibility of genetic material for gene expression, DNA replication and DNA damage repair (Marks et al., 2001). Histone acetyl transferase (HATs) and Histone deacetylases (HDACs) are involved in maintaining the dynamic control of protein acetylation levels. So far five families of HAT enzymes have been discovered in humans. Importantly, 18 isoforms of HDACS divided into four classes on the basis of their phylogenetic analysis and homology to yeast enzymes have been discovered in humans (Suzuki et al., 2009). HATs are involved in relaxing nucleosomes and hence activate gene expression and on the contrary, HDACs are involved in condensation of chromatin which favours transcriptional repression. HATs and HDACs association with distinctive DNA binding proteins allows gene specific activation and repression (Zhengke et al., 2014). Interestingly, the unusual or altered expression due to mutation in HDACs is associated with cancer development and its progression making them an important therapeutic drug target (Liu et al., 2018).
Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) where mechanistic involvement of HDACs was established for the first time (Ceccacci and Minucci., 2016). APL is widely used as a model for understanding the role of HDACs in carcinogenesis and the lessons learnt from it could be explored for other cancer (Vlasakova et al., 2007; Ceccacci and Minucci, 2016; Castro et al., 2017). Characteristic features of APL are blocking of myeloid differentiation at the promyelocytic stage and chromosomal translocation (15: 17) which generates the fusion protein PML-RAR, a combination of retinoic acid receptor-a (RAR) and promyelocytic leukaemia protein (PML) (Minucci and Pelicci, 2006). Molecular studies have revealed that the treatment of APL with retinoic acid (RA) is the best example for both transcription therapy and differentiation therapy that reprograms leukaemic cells to terminally differentiated cells (Tallman et al., 1997; Huang et al., 1988).

It has been observed that AML as well as acute lymphoblastic leukemia (ALL) pose increased resistance problem with different chemotherapies (Fraga et al., 2005; Subramanian et al., 2010; Fiskus et al., 2014). H4K16 monoacetylation (ac) and H4K20 trimethylation (3Me) have been established as hallmarks of tumours (Fraga et al., 2005). Loss of acetylation of specific lysine residues of H3 and H4 is one of the key factors thought to be involved in carcinogenesis. Moreover, the loss of H4K16 acetylation has been shown to influence the tumor progression and sensitivity to chemotherapy (Sense et al., 2007; Barbetti et al., 2013). In the last decade, a lot has been revealed regarding the HDAC inhibitors (HDACi) but the mechanistic pathways are still far from actual understanding. In past FDA approved four HDAC inhibitors as drugs against various cancers. Romidepsin and suberoylanilide hydroxamic acid (SAHA) have been approved as drugs against chronic T cell lymphoma while Panobinostat and Belinostat have been approved as drugs against peripheral T cell lymphoma and multiple myeloma respectively (Vlasakova et al., 2007, Castro et al., 2017). Apart from lymphomas and leukemias, many HDAC inhibitors are at advanced stages of clinical trials against a variety of solid and hematological cancers and few HDAC inhibitors in combinatorial therapies have been extended to breast cancer as well, though with limited success. However, in spite of clinical advantages like high specificity towards cancer cells and least drug resistance, HDAC inhibitors are generally associated with fatigue, diarrhea, bone marrow toxicity and
thrombocytopenia (Matthews et al., 2015). Pan-HDAC inhibition is thought to be responsible for toxicity associated with HDAC inhibitors and could be addressable by designing the class and isoform specific HDAC inhibitors (Vlasakova et al., 2007).

Natural products and synthetic compounds have served as indispensable repositories when it comes to cancer drugs. HDAC inhibitors like Apicidin, Trichostatin A, and Romidepsin have been extracted from various natural sources (Kwon et al., 2002; Klisovic et al., 2003; Sawa et al., 2004; Lobera et al., 2013) and some synthesized HDAC inhibitors like SAHA and Belinostat have been great success against different leukemias. But these natural product based HDAC inhibitors have been found to show toxicity and had low antineoplastic activity under preclinical and clinical conditions contrary to the synthetic HDAC inhibitors (Ma et al., 2009). Hence, we used two pronged strategy to overcome this shortcoming. Firstly, β-boswellic acid and l-vasicine derivatives were used to design new potent HDAC inhibitors (Sharma et al., 2014; Ahmed et al., 2017) and both of them showed significant anticancer activity and were found to be quite less toxic under in vivo conditions. Secondly, we used clinically validated pharmacophore of HDAC inhibitors and employed Target Oriented Synthesis (TOS) guided by Structure Activity Relationship (SAR) approach to selectively carry out the modifications in the cap region. We hypothesized that this strategy may enable us to design the isoform specific potent HDAC inhibitors with less toxicity and significant antineoplastic activity. Taking this into account, we started our study to design potent HDAC inhibitors of high specificity with the aim to understand the role of HDACs in cancer biology. The proposed plan of work includes following objectives.

1. To design and screen HDAC inhibitor(s) from natural or semi-synthetic sources based on clinically validated pharmacophore model of Suberoylanilide hydroxamic acid (SAHA).

2. To evaluate the specificity of the identified potent HDAC inhibitor(s) against various human cancer cell lines.

3. To elucidate the molecular mechanistic action of the potent HDAC inhibitor(s) under in vitro with regard to chromatin in human cancer cells.

4. To test the possible strategy of HDAC inhibition and cancer cell death for in vivo tumor regression activity.