Natural Products Impacting DNA Methyltransferases and Histone Deacetylases

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Epigenetics refers to heritable changes in gene expression and chromatin structure without change in a DNA sequence. Several epigenetic modifications and respective regulators have been reported. These include DNA methylation, chromatin remodeling, histone post-translational modifications, and non-coding RNAs. Emerging evidence has revealed that epigenetic dysregulations are involved in a wide range of diseases including cancers. Therefore, the reversible nature of epigenetic modifications concerning activation or inhibition of enzymes involved could be promising targets and useful tools for the elucidation of cellular and biological phenomena. In this review, emphasis is laid on natural products that inhibit DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) making them promising candidates for the development of lead structures for anticancer-drugs targeting epigenetic modifications. However, most of the natural products targeting HDAC and/or DNMT lack isoform selectivity, which is important for determining their potential use as therapeutic agents. Nevertheless, the structures presented in this review offer the well-founded basis that screening and chemical modifications of natural products will in future provide not only leads to the identification of more specific inhibitors with fewer side effects, but also important features for the elucidation of HDAC and DNMT function with respect to cancer treatment.

Keywords: epigenetics, natural products, inhibition, DNA methyltransferases, histone deacetylases, cancer

INTRODUCTION

Natural products originating from diverse sources including plants, microorganisms, and marine sponges are capable to influence epigenetic modifications (Deng et al., 2018; Lascano et al., 2018). Epigenetics refers to heritable changes in gene expression as well as chromatin structure without change in a DNA sequence (Dawson, 2017). Enzymes involved in these modifications were already
identified and include DNA methyltransferases, histones acetyltransferases/deacetylases, histone lysine as well as arginine methyltransferases and histone demethylases (Deng et al., 2018). The most common epigenetic modifications include DNA methylation, chromatin remodeling, histone post-translational modifications, and silencing of gene expression through non-coding RNAs (Allis and Jenuwein, 2016). Epigenetic dysregulations have been shown to be involved in several diseases such as cancers, neurodegenerative, or parasitic diseases and obesity (Duraisingh and Skillman, 2018; Ferioli et al., 2019). Cancer is a disease strongly tied to epigenetic changes which lead to silencing of tumor suppressor genes and thus promote tumor formation and proliferation (Jones and Baylin, 2007; Esteller, 2008). Therefore, due to the reversible nature of epigenetic modifications with respect to activation or inhibition, the genes and proteins representing key epigenetic players are considered as prime targets for the treatment and prevention of cancers (Ellis et al., 2009). In this review, we focus on natural products from plants, sponges, bacteria, and fungi that may serve as leads for drug discovery and possible further development for the treatment of cancers. We describe natural compounds of all biosynthetic classes inhibiting enzymes involved in the major epigenetic modifications regulating gene expression which include DNA methyltransferases (DNMTs) and histone deacetylases (HDACs). Emphasis will be on their biological properties and mode of action. Most of the natural products seem to display an indirect effect on HDACs and DNMTs and we aim to provide in this review all the data reported in the literature. It is worth to mention that currently, modifiers of DNMTs and HDACs are the only two classes of epigenetic drugs under investigation in the clinical setting.

DNA METHYLTRANSFERASES INHIBITORS (FIGURE 1)

The transfer of methyl groups to DNA is performed by DNA methyltransferases (DNMTs). These enzymes create 5-methylcytosines (5mCs) which lead to gene repression. The development of novel drugs targeting cancer and other diseases involve DNMTs as epigenetic targets (Medina-Franco et al., 2015). DNMT1, DNMT3a, and DNMT3b were identified as the three catalytically active DNMTs in mammals. DNMT1 is described as the maintenance methyltransferase, while DNMT3a and DNMT3b are de novo methyltransferases (Auclair and

![FIGURE 1 | Structures of DNA methyltransferases inhibitors.](image-url)
A crucial epigenetic modification is the modulation of the activity of DNA methyltransferases which affect DNA repair mechanisms in the cells or gene expression. The pathogenesis of human cancer is partly due to aberrant modifications in the activity of DNMTs (Jasek et al., 2019). DNMTs modulate DNA methylation and in general perform methylation of nitrogen (N-methylation), oxygen (O-methylation), and carbon (C-methylation). Those events are universal processes critical to all organisms. The O-methylation patterns of polyhydroxylated small molecules in plants, are essential using the same or similar intermediates and substrates to generate final product distribution through multiple branched biosynthetic pathways (Okano et al., 1999; Zubieta et al., 2001). In any organism, methylation is essential in the management of normal biological activities (Esteller, 2007). Methylation can cause direct suppression of gene expression even though it is an heritable change in the DNA without a modification of the sequence (Das and Singal, 2004). Hypomethylation and hypermethylation of DNA are seen in cancer cells (Qi and Xiong, 2018). The expression of pro-metastatic genes and quiescent proto-oncogenes is assisted by hypomethylation, and thus increase the progression of tumors. Silencing of genes influencing important cellular signaling pathways can be due to hypermethylation of the promoter regions of tumor-suppressor genes which play an important role in neoplastic transformation of cells (Qi and Xiong, 2018).

There are few drugs targeting DNA methyltransferase as inhibitors (DNMTIs), such as azacytidine and decitabine which are the most outstanding epigenetic drugs extensively utilized as epigenetic modulators. However, their application for oncological diseases is restricted by their relative toxicity and poor chemical stability (Gnyszka et al., 2013). Promising results in cancer treatment via influencing DNA methylation are expected from natural products isolated from plant, animals, and microorganisms. Indeed, bioactive phytochemicals that are widely available and exhibit less systemic toxicity have shown potential use in clinical development. (Jasek et al., 2019).

| Compound name | IC_{50} Values | DNMTIs | Reference |
|---------------|---------------|--------|-----------|
| (-)-Epigallocatechin-3-gallate | 0.47 μM | DNMT1 | Lee et al., 2005 |
| Curcumin | 0.30 μM | DNMT1 | Kwon et al., 1998 |
| Kazinol Q | 7.00 μM | DNMT1 | Weng et al., 2014 |
| Nanaomycin A | 0.50 μM | DNMT3b | Kuck et al., 2010 |
| Parthenolide | 3.50 μM | DNMT1 | Liu et al., 2009a |
| Antroquinonol D | 5.00 μM | DNMT1 | Wang et al., 2014 |

TABLE 1 | Selected DNMT inhibitors.
prostate cancer LNCaP cells and androgen insensitive PC-3 cells (Srivastava et al., 2007). Curcumin was reported to covalently block the catalytic thiolate of DNMT1 with an IC50 value of 30 nM after 72 h of treatment and leading to an inhibitory effect on DNA methylation (Liu et al., 2009b). Yu et al. (2013), showed a down-regulation in DNMT1 expression caused by curcumin in acute myeloid leukemia (AML) cell lines, both in vitro and in vivo (Yu et al., 2013). The same study highlighted reduction in the expression of positive regulators of DNMT1, Sp1 and p65 by curcumin. In AML cell lines, the latter results correlated with a decrease in binding of these transcription factors to the DNMT1 promoter. Curcumin displayed an inhibitory effect on DNMT in three colorectal cells lines including CT116, HT29 and RKO (Link et al., 2013). The DNA methylation changes in the same study occurred only in a subset of primarily partially methylated genes, and in a time-dependent manner. As mentioned earlier, the DNMT inhibition activity of curcumin has also been questioned (Medina-Franco et al., 2011).

**Quercetin**

Quercetin (3), an ubiquitous dietary flavonoid, is commonly found in fruits, vegetables, and beverages. It has attracted considerable attention owing to its potent antioxidant and antiproliferative activities. The induction of apoptosis and cell cycle arrest by quercetin in several cancer cell lines among which breast carcinoma, human esophageal squamous cell carcinoma, and prostate cancer cell lines has been demonstrated (Nair et al., 2004; Jeong et al., 2009; Zhang et al., 2009). Recently, it was reported that the pro-apoptotic effect of quercetin was mediated by inhibition of DNMT, especially DNMT1 and DNMT3a in vitro and human xenograft models (Alvarez et al., 2018). Another study displayed a concentration-dependent effect on hypermethylation of the tumor suppressor gene, p16INK4a when the human colon carcinoma RKO cells were treated by quercetin (Tan et al., 2009). This led to the reversal of the abnormal hypermethylation status of this gene after 120 h exposure to quercetin. Quercetin is currently undergoing phase I clinical trials in combination with green tea extracts (Zwergel et al., 2013). The combination of resveratrol with pterostilbene, another stilbene found in the plant, revealed a decrease of the activity of DNMT3b in HCC1806 triple-negative cancer cells without affecting the control MCF10A breast epithelial cell line (Kala et al., 2015). Another study showed that resveratrol can down-regulate the activity of DNMTs, as well as other proteins including HDAC1 and MeCP2 in MDA-MB-231 and MCF7 breast cancer cell lines (Mirza et al., 2013). However, the positive effects of resveratrol have been largely demonstrated in vitro or animals studies, with only limited effects reported in clinical studies (Shih et al., 2019).

Furthermore, a lower DNMT inhibition of resveratrol has been observed when compared to other dietary bioactive compounds such as EGCG.

**Nanaomycin A**

Nanaomycin A (6) is a quinone isolated from Streptomyces rosa var. notoensis and first reported as an antibiotic (Tanaka et al., 1975). The treatment of three different human tumor cell lines including HCT116 (colon), A549 (lung), and HL60 (bone marrow) cells with nanaomycin A resulted in growth inhibition of all three cell lines and caused induction of apoptosis (Kuck et al., 2010). Furthermore, an enzymatic assay revealed that the antiproliferative activity of nanaomycin A was due to its specific inhibition of DNMT3b with an IC50 value of 500 nM. This result was confirmed further by docking studies, which established the non-covalent binding of nanaomycin A to the active site of DNMT3b (see Molecular Modeling and Docking as a Tool for a Mode of Action Prediction of Natural Product DNMTs and HDACs Inhibitors) (Kuck et al., 2010). Furthermore, Nakamae and co-workers, recently suggested that, DNMT3b inhibition by nanaomycin A can promote the hepatoblast differentiation (Nakamae et al., 2018). The later result should assist in the future generation of functional hepatocyte-like cells for pharmaceutical research. Moreover, the induction of genomic demethylation is caused by nanaomycin A which is the first selective inhibitor of DNMT3b (Caulfield and Medina-Franco, 2011). It has been hypothesized that the inhibition arises from the nucleophilic attack of the catalytic cysteine residue within the active site of DNMT to the α,β-unsaturated carbonyl of nanaomycin A (Caulfield and Medina-Franco, 2011). While the further characterization is needed, the reactivity of the Michael
acceptor moiety and toxicity of nanaomycin A may lead to challenges in such characterization in the future.

Genistein

Genistein (7) is an isoflavone originally isolated from the dyer’s broom Genista tinctoria (Mukund et al., 2017). It was reported to be a potent inhibitor of cell proliferation on pancreatic cancer cell lines through apoptosis induction, regulation of the signal transducer and activator of transcription 3 (STAT3) signaling pathway, and G2/G1 cell cycle arrest (Bi et al., 2018). Ralhan and co-workers showed that genistein is able to induce a significant decrease in the transcript levels of all the DNMTs including DNMT1, DNMT3a, and DNMT3b, in breast cancer (Mirza et al., 2013). Another study confirmed this antiproliferative activity on the human breast cancer lines MCF-7 and MDA-MB-231 (Xie et al., 2014). The latter activity was found to be due to the inhibition of DNMT1 and molecular modeling indicated the direct interaction of genistein with the catalytic domain of this enzyme (Xie et al., 2014). However, only 40% inhibition of DNMT activity was observed at 100 μM contradicting the modeling data and thus indicating a low potential of genistein for DNMT inhibition. This weak effect suggests an indirect pathway of inhibition of DNA methylation, based on the identification of multiple targets for genistein including HDAC, tyrosine specific protein kinase, topoisomerase I and II, and NF-kB (Akiyama et al., 1987; Okura et al., 1988; Li and Sarkar, 2002; Sundaram et al., 2018). Despite the preclinical data reported for genistein showing its remarkable efficacy against prostate cancer in vitro with diverse molecular targets, there is no convincing clinical proof or evidence that genistein might be useful in prostate cancer therapy. Moreover, genistein is a multi-target compound limiting, therefore, its potential clinical use (Majid et al., 2009; Parker et al., 2009).

Sulforaphane

The isothiocyanate sulforaphane (SFN) (8) is found in cruciferous vegetables, such as broccoli sprouts and broccoli (Fahey et al., 1997). The strong inhibition of the growth of the human breast cancer cells lines MCF-7, MDA-MB-231, and SK-BR-3 by sulforaphane with IC50 values of 14.05, 19.35, and 16.64 μM respectively has been reported. Inhibition of cell growth was accompanied by cell cycle arrest, elevation in the levels of the tumor suppressors p21 and p27 and cellular senescence as well as induction of apoptosis. The anticancer effects of sulforaphane were found to be mediated by a global DNA hypomethylation, decreased levels of DNMT1 and DNMT3b and changes in the microRNA profiles of the three breast cancer cell lines (Lewinska et al., 2017). A further study showed that combination of sulforaphane and withaferin A, another natural compound, significantly causes down-regulation of overexpressed DNMT3a, DNMT3b, and HDAC1 and breast cancer cell death (Royston et al., 2017). Recently, it has been demonstrated that sulforaphane can suppress the growth of NPC cells via the inhibition of DNMT1 and the restoration of the expression of Wnt inhibitory factor 1 (WIF1) (Chen et al., 2019b).

Na and co-workers reported that sulforaphane up-regulates NrF2 expression and promotes its nuclear translocation through decreasing levels of DNA methylation of the NrF2 promoter in a cellular model of Alzheimer’s disease (Zhao et al., 2018). Furthermore, sulforaphane was shown to exert its chemopreventive effect in lung cancer A549 cells partly through the down-regulation of the activity of DNMT3a (Gao et al., 2018). Reduction of the toxicity of the chemotherapeutic drug cadmium selenide by sulforaphane in human hepatocytes through induction of glutathione synthesis was shown, thereby protecting the liver against toxicity and allowing the use of higher doses (Wang et al., 2015). However, there is no proof that a higher concentration could be achieved clinically using either diet-derived or supplemented SFN. The use of phytochemicals including SFN for patients with diagnosed cancers still need deep studies whether or not the patient is undergoing chemotherapy (Houghton, 2019). Furthermore, the presence of a highly electrophilic chemical functionality will probably cause several off-target effects despite that the downstream epigenetic effects observed upon the use of sulforaphane are promising and may improve chemopreventive activity (Cherblanc et al., 2013).

Boswellic Acids

Boswellic acids (BA) (9) are pentacyclic terpenoids extracted from Boswellia serrata; a plant used traditionally to treat inflammatory diseases (Zhou et al., 2017). Inhibition of cell proliferation and apoptosis induction in colorectal cancer (CRC) cell line through up-regulation of miR-34a and down-regulation of miR-27a by the most active boswellic acid, acetyl-11-keto-β-boswellic acid (AKBA), is well established (Takahashi et al., 2012; Toden et al., 2015). AKBA induces demethylation and concurrent up-regulation of tumor suppressor genes including SAMD14 and SMPD3 in CRC cells (Shen et al., 2012). Moreover, AKBA was reported inhibiting DNMT in SW48 and SW480 CRC cell lines at a concentration of 40 μM. BA has been established as a multitargeting agent involved in the treatment of diverse chronic diseases including cancers (Roy et al., 2019). Indeed, modulation by boswellic acids of diverse molecular targets, such as kinases, enzymes, growth factors, receptors, transcription factors, and others related to the proliferation and survival of cells is possible (Roy et al., 2016). However, the possible development of boswellic acids as an effective drug has been tumbled down due to concerns regarding the pharmacokinetic properties.

Z-Ligustilide

Z-ligustilide (10) is the most potent bioactive component found in Angelica sinensis, a herb from traditional Chinese medicine (TCM) used in the treatment of breast cancer (Ma et al., 2017). This compound has been reported to induce apoptotic cell death in human ovarian cancer cells (Lang et al., 2018). Z-ligustilide was reported to inhibit the growth of murine prostate cancer TRAMP C1 cells. The same study showed that Z-ligustilide reduced the methylation level of the first five CpGs of the NrF2 promoter. An enzymatic assay showed that Z-ligustilide...
blocks DNA methyltransferase activity of the CpG methylase M.SssI \textit{in vitro} (Su et al., 2013). The latter is structurally significantly similar to the DNMT. This result suggests that Z-ligustilide acts through an indirect mechanism of DNA methylation inhibition limiting its potential use in clinical trials.

### Parthenolide

Parthenolide (11) is a germacrane sesquiterpene lactone isolated from the plant \textit{Tanacetum parthenium} (Freund et al., 2019). This plant is commonly used for its inflammatory properties and suggested to be used in epigenetic cancer therapy (Akihisa et al., 2003; Ghantous et al., 2012). Parthenolide inhibits the detyrosination of microtubules and accelerates neuronal growth (Freund et al., 2019). A recent report showed that parthenolide induces apoptosis and inhibits proliferation of human 786-O kidney cancer cells \textit{in vitro} (Dong et al., 2019). The compound is also known to induce apoptosis in primary acute myeloid leukemia (AML) cells, including the stem and progenitor cell compartment through inhibition of NF-κB and HSP70 (Pei et al., 2009). Parthenolide has been reported to inhibit DNMT1 with an IC\textsubscript{50} value of 3.5 μM. This inhibition is thought to be due to its gamma methylene lactone which probably alkylates the proximal thiolate of Cys\textsuperscript{1226} of the catalytic domain (Li et al., 2009a). Recently, a new parthenolide derivative, dimethylamino-parthenolide, has been reported to inhibit the Nuclear chain factor kappa-light-chain enhancer of activated B cells (NF-κB) pathway and causes depletion of glutathione levels; the latter causing cancer cells to be more susceptible to oxidative stress-induced cell death (Pei et al., 2009; Lamture et al., 2018). This result highlights the potential role of this drug as a chemopreventive agent and in epigenetic cancer therapy. Recently, it has been demonstrated that combination of the anticancer drug actinomycin-D, which functions by intercalating into DNA, and dimethylamino-parthenolide results in a synergistic inhibition of Panc-1 pancreatic cancer cell growth (Lamture et al., 2018). However, the potential clinical use of parthenolide is still not clear. Indeed, analysis of parthenolide activity at Cancer Research Technologies, using a fluorescence intensity assay for DNMT1 did not find DNMT1 activity (Cherblanc et al., 2013). Moreover, the presence of several off-target effects complicates the analysis of cell-based assays.

### Antroquinonol D

Antroquinonol D (12) is an ubiquinone derivative isolated from the mycelium of \textit{Antrodia camphorata} (Wang et al., 2014). This compound inhibited the growth of MCF7, T47D, and MDA-MB-231 breast cancer cells without harming normal MCF10A and IMR-90 31 cells with IC\textsubscript{50} values of 8.01, 3.57, and 25.08 μM respectively (Wang et al., 2014). The authors reported that antroquinonol D can inhibit the activity of DNMT1 in MDA-MB-231 breast cancer cells with an IC\textsubscript{50} value lower than 5 μM. This result was confirmed by molecular modeling which revealed that antroquinonol D binds to the catalytic subunit of DNMT1 and competes for the same binding pocket in the DNMT1 enzyme as the cofactor SAM (S-adenosylmethionin) (Wang et al., 2014). Antroquinonol D was also found to reverse the silencing of multiple tumor suppressor genes in the same study.

### HDAC INHIBITORS

Histone deacetylases (HDACs) also called lysine deacetylases (KDAC) are a family of hydrolases catalyzing removal of acetyl groups from lysine residues on histone tails (Glozak and Seto, 2007). This removal of acetyl groups allows compacted chromatin to reform and this process is associated with transcriptional repression (Glozak and Seto, 2007). HDACs are classified in four distinct subtypes: class I (HDACs 1, 2, 3, and 8 localized in the nucleus), class II (IIa: HDACs 4, 5, 7, and 9; IIb: HDACs 6 and 10, found in both nucleus and cytoplasm), class III (nicotinamide adenine dinucleotide-dependent SIRT [sirtuin] enzymes [Sirt 1–7]), and class IV (HDAC 11). The latter shares structural similarities with both class I and II HDACs as they are Zn-dependent enzymes (Haberland et al., 2009; McKinsey, 2012). Class I HDACs play an important role in cell survival and proliferation, while class II may have tissue-specific roles (Li et al., 2015). It has been reported that functional dysregulation of HDACs affects the expression of numerous genes that have an impact on apoptosis and the cell cycle (Hauser and Jung, 2008). Indeed, HDACI is overexpressed in prostate cancer cells, while gastric carcinomas, colorectal carcinomas, and cervical dysplasias overexpress HDAC2 (Halkidou et al., 2004; Huang et al., 2005; Song et al., 2005).

HDAC inhibitors (HDACI) are emerging therapeutic agents, since their targets play an important role in cancer initiation and progression (Lee et al., 2017). Indeed, histone deacetylation plays a key role in tumor suppressor genes silencing (TGSs) in several cancers, thus the restoration of the acetylation of lysine residues by HDAC inhibitors will oppose the frequent HDAC overexpression in cancer (Perri et al., 2017). HDACs are also known to regulate non-histone proteins involved in cancer development such as p53, and NF-κB and can affect their function leading to modified expression of cancer-related genes (Saunders and Verdin, 2007; Wilcox, 2016). HDACs can repress the expression of receptors for growth-restraining signaling molecules such as TGFβ receptor (transforming growth factor beta receptor), leading to unhindered cell growth, which is preventable by HDAC inhibition (Glozak and Seto, 2007).

The well-known effect of HDACI is to lead to cell cycle arrest and induction of cell senescence (Golabek et al., 2015). Since the approval of suberanilohydroxamic acid (SAHA) by the US Food and Drug Administration for the treatment of T-cell lymphoma in 2006, the relevance of HDACI in cancer therapy has been strongly pointed out. HDACI are usually classified in two main groups: group I which displays the zinc-binding mode of action includes linear inhibitors, cyclic tetrapeptides, and cyclic depsipeptides; and group II with a non-zinc-binding mode of action includes miscellaneous inhibitors (Lascano et al., 2018). It is worth mentioning that most HDACI share the same overall structure including a cap terminus, a linker region, and a zinc-binding group (ZBG) (Figure 2). As shown in Table 2, nature is a promising source of HDACI that may inspire the development of lead structures for the potential treatment of
and this compound was first reported to display antifungal activity (Tsuij et al., 1976; Fleming et al., 1983). Trichostatin A (TSA) (13) is the first natural product derived HDAC inhibitor and was isolated from the bacterium Streptomyces hygroscopicus (Tsuij et al., 1976; Yoshiida et al., 1990). Its structure consists of an aromatic group, a conjugated diene linker region, and a hydroxamic tail. The first racemic synthesis of TSA was reported by Krebs and co-workers in 1983 and this compound was first reported to display antifungal activity (Tsuij et al., 1976; Fleming et al., 1983). Trichostatin A was reported to reversibly inhibit mammalian HDAC and accordingly was found to induce accumulation of acetylated histones in a variety of mammalian cell lines when applied in nanomolar concentration (Yoshida et al., 1990; Yoshiida et al., 1995). The crystal structure showed that trichostatin A non-covalently binds to the active site of HDACs through the terminal hydroxamic acid group which chelates the Zn$^{2+}$ in a bidentate fashion (see Natural Product HDAC Inhibitor in the PDB) (Finnin et al., 1999). Kinetic studies revealed that trichostatin A is a competitive inhibitor (Sekhavat et al., 2007). Yokoyama and co-workers reported that trichostatin A inhibits HDAC in a dose-dependent manner in vascular smooth muscle cells (VSMCs) with an IC$_{50}$ value of 0.08 μM (Okamoto et al., 2006). Trichostatin A is frequently used as a positive control and a reference when compared to other HDAC inhibitors and considered today as one of the most potent HDAC inhibitors (Ying et al., 2008; Benelkebir et al., 2011). However, the observed mutagenicity of the hydroxamic moiety along with lack of HDAC isoform selectivity limits its clinical use (Khan et al., 2008; Shen and Kozikowski, 2016).

Depudecin

Depudecin (14) is a linear polyketide containing two epoxide groups isolated from the culture broth of the soil fungus Alternaria brassicicola (Matsumoto et al., 1992). The structure of depudecin consists of a bis-trans-epoxide moiety and six asymmetric centers. Depudecin has been reported to display an in vitro inhibitory effect against HDAC1 in a dose-dependent manner with an IC$_{50}$ value of 4.7 μM (Kwon et al., 1998). The relatively low potency of depudecin precluded further work on this compound. Depudecin showed also the ability to induce morphological reversion in NIH 3T3 fibroblast cells to their normal flat phenotype (Kwon et al., 1998).

Psammaplin A

Psammaplin A (PsA) (15) is a monobrominated tyrosine derived oxime containing cystamine isolated from several marine sponges including Psammaplysilla sp. (Quiñoà and Crews, 1987; Tabudravu et al., 2002). Psammaplin A is one of the members of the psammaplin class and was reported to suppress carcinogenic properties of several human cancer cell lines including lung, breast, colon, and ovarian cancer in vitro (Park et al., 2003). Psammaplin A inhibited in vivo tumor growth in the A549 lung xenograph mouse model while maintaining low toxicity (Piña et al., 2003). Moreover, psammaplin A was reported to inhibit the activity of HDAC and DNMT at nanomolar levels in vitro (Piña et al., 2003). Hyperacetylation of histone H3 by psammaplin A demonstrated that the compound is a specific and potent inhibitor of class I HDAC rather than class II (Kim et al., 2007). Moreover, psammaplin A was found to be a natural prodrug. Its activation is caused by reduction of the disulfide bond leading to a thiol. The latter thiol chelates the Zn$^{2+}$ ion in the active site of HDAC and precludes access to the natural substrate (Kim et al., 2007). Indeed, the reduced form of psammaplin A highly selectively inhibited HDAC1 with an IC$_{50}$ value of 45 nM (Baud et al., 2012). The hyperacetylation of histone by psammaplin A was correlated
cancer. However, the lack of HDAC isoform selectivity for most of the natural products may limit their clinical use.

Zinc-Binding Inhibitors

Linear HDAC Inhibitors (Figure 3)

Trichostatin A

Trichostatin A (TSA) (13) is the first natural product derived HDAC inhibitor and was isolated from the bacterium Streptomyces hygroscopicus (Tsuij et al., 1976; Yoshiida et al., 1990). Its structure consists of an aromatic group, a conjugated diene linker region, and a hydroxamic tail. The first racemic synthesis of TSA was reported by Krebs and co-workers in 1983 and this compound was first reported to display antifungal activity (Tsuij et al., 1976; Fleming et al., 1983). Trichostatin A

![FIGURE 2 Common features of HDAC inhibitors.](Image)

### TABLE 2 | Selected HDAC inhibitors.

| Compound name | IC$_{50}$ Values | HDAC Isoforms | Reference |
|---------------|------------------|---------------|-----------|
| Trichostatin A | 0.08 μM | NT | Okamoto et al., 2006 |
| Depudecin | 4.70 μM | HDAC1 | Kwon et al., 1998 |
| Psammaplin A | 0.042 μM | HDAC1 | Baud et al., 2012 |
| Sulforaphane | 36.00 μM | HDAC2 | Choi et al., 2018 |
| Bis(4-hydroxybenzyl)sulfide | 0.60 μM | HDAC9 | Sasamura et al., 2010 |
| Azumamide E | 0.05 μM | HDAC1 | Maulucci et al., 2007 |
| Apicidin | 0.10 μM | HDAC2 | |
| Apicidin | 0.08 μM | HDAC3 | |
| Apicidin A | 0.001 μM | NT | Singh et al., 2002 |
| Apicidin D$_1$ | 0.004 μM | NT | |
| Apicidin B | 0.01 μM | NT | Singh et al., 2001 |
| Apicidin C | 0.006 μM | NT | |
| FR285222 | 0.017 μM | NT | Sasamura et al., 2010 |
| AS1387392 | 0.022 μM | NT | |
| Chalmydonic | 0.00015 μM | HDAC1 | Furumai et al., 2001 |
| 1-alaninechlamydonic | 0.0064 μM | NT | Du et al., 2014 |
| Trapoxin A | 0.00082 μM | HDAC1 | Furumai et al., 2001 |
| Microsporin A | 0.524 μM | HDAC6 | |
| Romidepsin | 0.036 μM | HDAC1 | Furumai et al., 2002 |
| Largazole | 0.047 μM | HDAC2 | |
| Epicocconigrone A | 4.6 μM | HDAC6 | El Amrani et al., 2014 |
| | 1.6 μM | HDAC8 | |
| | 8.4 μM | HDAC10 | |

NT: Not Tested.
with an up-regulation of tumor suppressors such as p21WAF1 and gelsolin (Kim et al., 2007; Ahn et al., 2008). Psammaplin A was also reported to induce an increase of apoptosis, most likely by inducing expression of p21WAF1. It is worth to mention that the physiologic instability of the psammaplin class has precluded further clinical investigations. However, an analogue of psammaplin class, NVP-LAQ824, which induces apoptosis, has entered phase I clinical trials (Remiszewski, 2003; Cuneo et al., 2007).

**Diallyl Disulfide (DADS)**

Diallyl disulfide compounds are organosulfur compounds (OSC) which are released by plants of the Allium genus including onion, garlic, scallion, and leek (Bae et al., 2019). In general, organosulfur compounds are known to modulate the activity of several enzymes involved in the activation or detoxification of carcinogens and inhibit the formation of DNA adducts in diverse target tissues (Omar and Al-Wabel, 2010). DADS are known to induce cell cycle arrest, differentiation, and apoptosis in several cancer cell lines (Herman-Antosiewicz and Singh, 2004). In fact, DADS belong to the group of dietary HDAC inhibitors. Allyl mercaptan (16), a well-known DADS, induced histone acetylation in the liver and this compound was also recognized as the active HDAC inhibitor rather than the parent compound DADS (Druesne et al., 2004). Indeed, the authors reported that allyl mercaptan inhibits 92% of HDAC activity while the parent compound, inhibited only 29% at the concentration of 200 μM. DADS is also known to induce the hyperacetylation of histone H4, the activation of caspase-3, and the modulation of the anti-apoptotic paralogues including Bcl2, BAX, and Bcl-xL in human leukemia, lung cancer, and breast cancer (Herman-Antosiewicz and Singh, 2004; Zhao et al., 2006). The latter result suggested that the Bcl-2 family is targeted by DADS. Besides, DADS was reported to cause hyperacetylation of H3 and H4 leading to an increase of the expression of the tumor suppressor p21WAF1 in human acute myeloid leukemia HL-60 cells and colon cancer cells in vitro and in vivo.

**S-allyl-mercapto-L-cysteine (SAMC)** is another organosulfur compound which was also reported to induce hyperacetylation of H3 and H4 in human colon and breast cancer cells (Lea et al., 2002). The same study reported that SAMC inhibits cell proliferation of DS19 mouse erythroleukemia cells with an IC50 value of 0.5 μM (Lea et al., 2002). SAMC has also been reported to inhibit the growth of the breast cancer cell lines MCF-7 and MDA-MB-231 through cell cycle arrest in the G0/G1 phase (Zhang et al., 2014). These findings support the continue investigation of SAMC as an alternative agent in the chemoprevention and chemotherapy of human breast cancer. Despite the lack of direct inhibition of HDAC by SAMC, it can be assumed that this compound displays this activity with the same mechanism as other organosulfur compounds. However, the stability of DADS causes difficulties in reproducibility of findings.

**Sulforaphane (SFN)**

The isothiocyanate sulforaphane (SFN) (8), as mentioned earlier, is found in cruciferous vegetables, including broccoli sprouts and broccoli (Fahey et al., 1997). Isothiocyanates result from the hydrolysis of glucoraphanin by the plant enzymes myrosinases (Kim and Park, 2016). Isothiocyanates are a family of compounds including sulforaphane, allyl isothiocyanate, benzyl isothiocyanate, phenetyl isothiocyanate, etc. (Kim and Park, 2016). Sulforaphanes have been reported to possess anticancer activities in xenograft models of prostate cancer and in induced animal models (Zhang et al., 1994; Fahey et al., 1997). Myzak and co-workers reported that sulforaphane possesses anticancer activity through the inhibition of HDAC activity and increase in the histone acetylation in HCT116 human colorectal cancer cells (Myzak et al., 2004). The same study showed that the metabolite sulforaphane-cysteine which displayed greater HDAC inhibitory effect than SFN at a concentration of 15 μM.
is the active form of SFN and other isothiocyanates. This observation was further confirmed by molecular studies which revealed a plausible interaction for sulforaphane-cysteine within the active site of the HDAC-like protein (see Natural Product HDAC Inhibitor in the PDB) (Myzak et al., 2004). Moreover, 40% of the growth inhibition of xenografts of human PC-3 prostate cancer cell in mice by SFN was observed at a concentration of 15 μM, accompanied by a significant down-regulation of HDAC activity in the xenografts (Myzak et al., 2007). Recently, SFN has been reported to selectively inhibit HDAC2 and 9 with IC₅₀ values of 36 and 0.6 μM respectively (Choi et al., 2018). In silico studies confirmed that isothiocyanates can bind to allosteric active sites of HDAC based on their similar structural features with other HDAC inhibitors (Furumai et al., 2001; Ho et al., 2009; Rajendran et al., 2013). However, isothiocyanates are known to react preferably with diverse thiol and amine (bio)nucleophiles (Jacob, 2006), which strongly suggest non-specific and likely indirect effects on a given epigenetic target.

**Bis(4-Hydroxybenzyl)sulfide**

Bis(4-hydroxybenzyl)sulfide (18) is a sulfur compound isolated from the root extract of the Chinese medicinal plant *Pleuropterus ciliinervis* (Son et al., 2007). *In vitro*, it displayed inhibitory activity against HDAC in HeLa cells with an IC₅₀ value of 1.43 μM (Son et al., 2007). Besides, this compound inhibited also the growth of several cancer cell lines among which the prostate PC-3 and breast MDA-MB-231 cell line with IC₅₀ values of 7.86 and 1.45 μM respectively (Son et al., 2007). Like other organosulfur compounds, stability is a big issue that may affect the reproducibility of these findings.

**Cyclic Tetrapeptides (Figure 4)**

**Azumamide E**

The cyclic tetrapeptide azumamide E (19) was isolated from the marine sponge *Mycale izuensis* along with other azumamides (A, B, C, and D) (Nakao et al., 2006). Structurally, azumamide E include four D-α-amino acids (D-Phe, D-Tyr, D-Ala, D-Val) and...
respectively, while the other apidicins including apidicin D1 possess a strong inhibition of HDACs in K562 human leukemia cells, with an IC50 value of 0.064 μM (Nakao et al., 2006). Another study reported that azumamide E selectively inhibited class I HDAC in HeLa nuclear extracts, particularly HDACs 1-3 with IC50 values of 0.05, 0.1, and 0.08 μM respectively (Maulucci et al., 2007). Docking studies showed that azumamide E inserts the Amnaa side chain into the active site of HDACs, where the carboxylic acid will chelate the Zn2+ in a bidentate fashion (Maulucci et al., 2007). Total synthesis of azumamide E revealed that switching of the carboxylic acid group to a hydroxamic acid leads to an increase in potency for HDAC inhibition (Wen et al., 2007). A subsequent synthesis of azumamide E by Villadsen et al., revealed that 19 is also a potent inhibitor of HDAC10 and 11 evidencing its lack of isoform specificity (Villadsen et al., 2013).

**Apidicins**

Apidicin (20) and apidicin A (21) are a family of natural products first isolated from the endophytic fungus *Fusarium pallidoroseum* (Singh et al., 1996). Structurally, all apidicins contain N-methoxy-L-tryptophan, except for apidicin A. Moreover, all apidicins contain D-pipeolic acid, except for apidicin B (22) which contains D-proline. Furthermore, apidicin C (23) and F (28) contain L-valine and L-phenylalanine, respectively, while the other apidicins including apidicin D1 (24), D2 (25), D3 (26), and E (27) consist of L-isoleucine at the corresponding position. Another common feature of all the apidicins is the presence of (2S)-amino-8-oxo-decanoic acid (Aoda) or its derivative. These compounds displayed a high inhibitory activity of protozoal HDAC and in HeLa cell extracts in nanomolar range (Singh et al., 2001; Singh et al., 2002). A selective inhibition of class I HDAC2, 3, and 8 in nanomolar concentrations by apidicin A and D1 (24) has been reported as well as the inhibition of class IIa HDAC 4 and 7 in ovarian cancer cells (Khan et al., 2008; Ahn et al., 2012). The binding of apidicin to the active site of HDAC is enabled through the insertion of the acetylated lysine mimic. Indeed, the carbonyl group can chelate the Zn2+ at the bottom of the active site precluding the binding of the natural substrate as demonstrated by the lack of activity of apidicin D2 (25) and D3 (26) (Colletti et al., 2001; Singh et al., 2001). Apidicin inhibited the proliferation of diverse cancer cell lines through the induction of transcriptional activation of p21WAF1Cip1 and gelsolin (Han et al., 2000; Kim et al., 2001). The reversion of morphological changes of HeLa cells as well as hyperacetylation of histone H4 accompanied antiproliferative activity of apidicin on those cells (Han et al., 2000). Apidicin-mediated growth inhibition of the promyelocytic leukemia cell line HL60 was also reported to be due to a transiently increased expression of Fas/Fas ligand. The latter resulted in activation of caspase-3 and 9 and execution of apoptotic cell death (Kwon et al., 2001).

**Helminthosporium carbonum (HC) Toxin**

HC-Toxin (29) is a cyclic tetrapeptide isolated from the fungal culture of *Helminthosporium carbonum* (Liesch et al., 1982). The structure of HC-toxin consists of D-proline, D-alanine, L-alanine, and (2S)-amino-8-oxo-9,10-epoxydecanoic acid (Aoe). HC-Toxin is an inhibitor of HDAC in several organisms including plants, insects, and mammals (Walton et al., 2006). Its Aoe moiety has been reported to be relevant for HDAC inhibition as well as the α-keto epoxide moiety (Walton and Earle, 1983). Indeed, the latter binds covalently into the active site of HDAC through epoxide opening by nucleophilic residues of the active site (Walton and Earle, 1983). HC-toxin was reported to induce G0/G1-cell arrest and apoptosis in neuroblastoma (NB) cell lines and primary cell cultures in the nanomolar range (Deubzer et al., 2008). In other studies, HC-toxin displayed antiproliferative activity against intrahepatic cholangiocarcinoma (ICC) cells and breast cancers cells lines (Joung et al., 2004; Zhou et al., 2016).

**FR235222**

FR235222 (30) was initially reported from the fermentation broth of the soil fungus *Acremonium* sp. No. 27082 (Mori et al., 2003). The structure of FR235222 consists of L-Phe and three unusual α-amino acids including (2R,4S)-4-methylproline (4-MePro), and (2S)-isovaline (iva), and (2S,9R)-2-amino-8-oxo-9-hydroxydecanoic acid (Aoh). This natural compound showed potent inhibition of partially purified HDAC fractions mammalian lymphoid cell lines (IC50 value of 17 nM) and selective immunosuppressive activity. Indeed, the authors found that FR235222 selectively inhibits both lymphocyte proliferation and lymphokine production; the target was identified as HDAC in T cells. The authors reported that FR235222 caused G1 cycle arrest accompanied by an increase of p21 and down-regulation of cyclin E, antiproliferative effects, and accumulation of acetylated histone H4. Another study, in which the prostate cancer cell line LNCaP was treated with FR235222 at a concentration of 0.5 μM, revealed that the increase in histone H4 acetylation is accompanied by caspase-3-dependent induction of apoptosis (D’Acunto et al., 2010). Besides, the authors demonstrated that FR235222 can increase the level of the endogenous anti-inflammatory protein ANXA1 involved in apoptosis. It is worth mentioning that, the restoration of ANXA1 expression in the prostate cancer cell line LNCaP reduced cell viability and proliferative response and induced caspase-mediated apoptosis (Hsiang et al., 2006). FR235222, with an IC50 value of 60 nM, was also reported to inhibit HeLa cell HDACs and HDAC3 was identified as its main target in *Toxoplasma* tachyzoites (Yoshida et al., 1990; Bougdour et al., 2009).

AS1837392 (31), a synthetic analogue of FR235222, in which the MePro of FR235222 is replaced by proline, was also isolated from the same fungus *Acremonium* sp. This compound showed similar HDAC inhibitory effects as FR235222 with IC50 an value of 22 nM (Sasamura et al., 2010). AS1837392 also displayed
potent inhibitory activity against splenocyte proliferation, with an IC\textsubscript{50} value of 4.6 nM (Sasamura et al., 2010).

**Chlamydocin**

Chlamydocin (32) was first isolated from the fungus *Dictyostelium chlamydocarpum* and is a cyclic tetrapeptide containing 2-aminoisobutyric acid (Alb), L-phenylalanine, D-proline, and L-2-amino-8-oxo-9,10-epoxydecanoic acid (Closes and Huguenin, 1974). Chlamydocin exhibited cytotoxicity against mouse P-815 mastocytoma cells in vitro with a 10–100 times higher activity than clinical agents including actinomycin D, vinblastine, vincristine, amethopterin, and colchicine in the same assay (Stähelin and Trippmacher, 1974; Walton et al., 1985). Chlamydocin has also been reported to inhibit the proliferation of diverse cancer cell lines with IC\textsubscript{50} ranging from 0.36 to 45 nM. In the same study, it was reported that chlamydocin inhibits HDAC with an IC\textsubscript{50} value of 1.3 nM and the antiproliferative activity was found to be accompanied by an increase in the accumulation of the acetylated histones H3 and H4, the induction of p21\textsuperscript{Waf1/Cip1}, and cell cycle arrest in the G\textsubscript{2}/M phase (Schepper et al., 2003). Chlamydocin selectively inhibited HDAC1 with an IC\textsubscript{50} value of 0.15 nM (Furumai et al., 2001). Chlamydocin may share some mode of action as the aforementioned epoxide-based HDAC inhibitors such as HC-Toxin.

The fungal culture *Tolypocladium* sp. produced a closely related compound 1-alaninechlamydocin (33), which displayed strong antiproliferative effects in a human pancreatic carcinoma cell line MIA PaCa-2 in a nanomolar range (Du et al., 2014). Besides, 1-alaninechlamydocin also inhibited the growth of another human pancreatic carcinoma cell line (Panc-1) and the immortalized pancreatic duct cell line hTERT-HPNE at low-nanomolar concentration without induction of cytotoxicity in both cell lines at a concentration up to 10 μM. It was found that 1-alaninechlamydocin induces apoptosis and G2/M cell cycle arrest by inhibiting HDAC activity with an IC\textsubscript{50} value of 6.4 nM (Du et al., 2014).

**Trapoxin (TPX)**

Trapoxin A (34) and B (35) are cyclotetrapeptides originally isolated from the fungal culture of *Helicoma ambiens* and caused the increase of highly acetylated core histones in diverse mammalian cell lines (Itazaki et al., 1990; Kijima et al., 1993). Trapoxin A (34) (also known as trapoxin) induced cell differentiation, cell cycle arrest, and reversal of transformed cells morphology (Yoshida et al., 1995). Trapoxin (34) was first reported to be an irreversible HDAC inhibitors (Taunton et al., 1996). However, Horinouchi and co-workers demonstrated later that trapoxin reversibly inhibits only HDAC6 through the ketone moiety. The latter undergoes a nucleophilic attack to form a zinc-bound tetrahedral gem-diolate without affecting the epoxide moiety (Furumai et al., 2001). This mechanism is in contrast to other epoxyketone that are commonly thought to be irreversible HDAC inhibitors (Taunton et al., 1996). In the same study, 34 displayed selective inhibition of HDAC1 and 6 with IC\textsubscript{50} values of 0.82 and 524.0 nM respectively while 35 showed similar potency (Furumai et al., 2001). Also, trapoxin B (35) was reported to be more potent than trichostatin A in the respective inhibition of H1299 and HCT116 cell proliferation (Remiszewski et al., 2002). It has been reported that, trapoxin A (34) binds to the active site of HDAC8 through the same mode of action already known by its effect on HDAC6 (Porter and Christianson, 2017).

**Microsporin A**

Microsporin A (36) which is closely related to the trapoxins was produced by the fungus *Microsporum cf. gypseum* along with an analogue Microsporin B (37) (Gu et al., 2007). Both natural products harbor a nonproteinogenic moiety made of (2S)-amino-8-oxodecanoic acid (Aoda) and (2S)-amino-8-hydroxydecanoic acid respectively along with other units including D-pipecolin, L-phenylalanine, and L-alanine (Gu et al., 2007). Microsporin A (36) showed potent in vitro cytotoxicity against human colon adenocarcinoma HCT-116 and a mean IC\textsubscript{50} value of 2.7 μM in the National Cancer Institute's diverse 60-cell line panel. Microsporin A (36) in the same study showed greater in vitro inhibition against both a mixture of HDACs and HDAC8 than the reference antitumor agent HDAC inhibitor SAHA with IC\textsubscript{50} values of 0.14 and 0.55 μM, respectively (Gu et al., 2007).

**Cyclic Depsipetides (Figure 5)**

**Romidepsin**

The bicyclic depsipetide romidepsin (38) was originally produced by *Chromobacterium violaceum* that displayed antitumor activity (Ueda et al., 1994). This natural product was initially isolated under the name FR901228 and is now adays known as romidepsin or Istodax® (trade name), or FK228 (Vandermolen et al., 2011). In 2009, the prodrug romidepsin (38) was approved for the treatment of cutaneous and/or peripheral T-cell lymphoma by the US Food and Drug Administration (FDA) (Prince et al., 2013). The chemical structure of 38 consists of two valine units with opposite configurations, D-cysteine, (Z)-dehydrobutyryl, and (3S)-hydroxy-7-mercapo-4-heptenoic acid (Shigematsu et al., 1994). Compound 38 acts as a potent and selective HDAC inhibitor of class I HDACs in particular HDAC1, 2, and 4 with IC\textsubscript{50} values of 36, 47, and 510 nM respectively (Furumai et al., 2002). This compound has an internal disulfide bond of 38 that is reduced in the presence of cellular glutathione to generate a sulfhydryl (thiol) moiety, which is the active form of Romidepsin behaving as a Zn\textsuperscript{2+} binding group in the active site.

**Largazole**

Largazole (39) was isolated through a bioassay-guided fractionation of the crude extract of the cyanobacterium *Symploca* sp. (Taori et al., 2008). Its structure consists of uncommon features including a thiazole ring linearly fused to a substituted (4R)-methylthiazoline and a (3S)-hydroxy-7-mercaptohept-4-enolic acid like in FK228. Largazole (39) selectively inhibited, at nanomolar concentration, the growth of the human epithelial cancer cells MDA-MB-231 in a dose-dependent manner. Indeed, non-transformed murine epithelial cells NmuMG remained unsusceptible to 39 in this study (Taori
et al., 2008). The authors reported the same selectivity for transformed fibroblastic osteosarcoma U2OS cells (IC50 55 nM) over nontransformed fibroblast NIH3T3 (IC50 480 nM). It has been reported that 39 selectively inhibits class I HDAC1 over class II HDAC4 with IC50 values of 11.4 nM and 3 μM respectively (Souto et al., 2010). Largazole induced an increase of H3 and α-tubulin acetylation, and an up-regulation of p21WAF1/CIP1 in NB4 cells (Souto et al., 2010). Increased acetylation of H3 and up-regulation of p21WAF1/CIP1 was also observed in another study, in which 39 induced cell cycle arrest at G1 phase at nanomolar range concentration in HCT116 cells (Liu et al., 2010). Compound 39 is a prodrug that requires activation through hydrolysis to form the thiol 40 as the active form exhibiting high isozyme-selective HDAC inhibition activity (Hong and Luesch, 2012).

**Spiruchostatins**

Spiruchostatin A (41) and B (42), both sharing several structural features with romidepsin, were isolated from *Pseudomonas* sp. in 2001 (Masuoka et al., 2001). Another analogue, spiruchostatin C (43), was isolated from *Burkholderia thailandensis* (Masuoka et al., 2001; Klausmeyer et al., 2011). Spiruchostatin A–C (41–43) demonstrated selective inhibition of class I HDACs, in particular HDAC1, with low nanomolar IC50 values (Narita et al., 2009; Narita et al., 2013). Moreover, 41 was reported inhibiting 14 cancer cell lines at nanomolar range (Shindoh et al., 2008). In the latter study, it was found that 41 induces selective accumulation of acetylated histones in tumor tissues, p21WAF1/Cip1 expression and cell cycle arrest. Another study reported that a higher increase in the formation of intracellular reactive oxygen species accompanied induction of apoptosis in human lymphoma U937 cells by 41 and 42 (Rehman et al., 2014).

**Non Zinc-Binding Inhibitors (Figure 6)**

**Ursolic Acid**

The pentacyclic ursolic acid (44) can be found in fruits such as blueberries, apple peels, olive, and cranberries as well as in diverse herbs (Ikeda et al., 2008). Compound 44 was reported to inhibit the growth of HL60 cells resulting from an increase in the accumulation of acetylated histone H3 (Chen et al., 2009). The increased acetylation of H3 was found to be induced by the inhibition of HDAC 1, 3, 4, 5, and 6 (Chen et al., 2009). The binding of 44 to the active site of class I HDAC and HDAC7 isoforms has been recently confirmed through docking studies (Ishola and Adewole, 2019). Moreover, the same study showed 44 fulfills oral druggability of Lipinski rule five.

**Epicocconigrones**

Epicocconigrones A (45) and B (46) are polyketides isolated from an endophytic fungus identified as *Epicoccum nigrum* in 2013 (El Amrani et al., 2014). Compound 45 displayed strong inhibition of HDAC with an IC50 value of 9.8 μM (El Amrani et al., 2014). In vitro test of 45 showed that this compound inhibits several HDACs (1, 2, 3, 8, 6, 10, and 11) with IC50 values between 1.6 and 12.9 μM. It is worth to mention that 45, selectively inhibited HDAC8 with higher potency in comparison to the reference compound SAHA in the same study. Compound 45 also inhibited proliferation of the human lymphoma cell line RAJI by 50% at a concentration of 5 μM after
72 h of treatment. Moreover, 32% of the U937 cell line were also inhibited at the same concentration (El Amrani et al., 2014).

Curcumin
The polyphenol curcumin (2), as mentioned earlier, was isolated from the rhizome Curcuma longa (Soleimani et al., 2018). Curcumin (2) is known to possess HDAC inhibitory activity in different cancer cell lines (Soflaei et al., 2018). Indeed, induction by curcumin of cell cycle arrest at G2/M phase, apoptosis, and increase in tubulin acetylation in medulloblastoma cells, through the inhibition of HDAC, in particular HDAC4 was reported (Lee et al., 2011). It has been reported that 2 may be of considerable value in synergistic therapy of cancer in a manner that the drug dose level could be strongly minimized to reduce the associated toxicity (Roy et al., 2011). Indeed, 2 combined with other HDAC inhibitors such as vorinostat resulted in a marked enhancement of the antiproliferative activity of the associated drug, and sensitization to apoptosis (Giommarelli et al., 2010). A similar observation was made when 2 was combined with cyclophosphamide and paclitaxel (Roy et al., 2011). Nevertheless, curcumin is known to show pan-activities, which is why any reported specificity needs to be seen with caution.

n-Butyric Acid
n-Butyric acid (47) is a short-chain fatty acid reported as metabolite of Staphylococcus epidermidis, a skin probiotic bacterium (Claudel et al., 2019; Traisaeng et al., 2019). n-butyric acid has been reported to inhibit HDAC, DNA synthesis, and cell growth in colon tumor cell lines (Andriamihaja et al., 2009; Zhang et al., 2010; Traisaeng et al., 2019). Its poor pharmacological properties are due to a rapid metabolism. Along with the multigram doses required to achieve therapeutic concentrations in vivo, they precluded its use in cancer therapy and other medical disorder (Miller et al., 1987; Steliou et al., 2012). To overcome this limitation, butyric acid prodrugs have been synthesized including pivaloyloxyethyl butyrate (AN-9) (48) and butyroyloxymethyl butyrate (AN-1) (49), which showed antineoplastic activity and radiosensitizing capacity in the treatment of malignant gliomas (Entin-Meer et al., 2005). However, these prodrugs did not succeed as viable drugs (Steliou et al., 2012). Interestingly, the arginine salt of butyrate gave successful results in clinical studies and is used for the treatment of diseases such as thalassemia and sickle-cell disease (Steliou et al., 2012). Other short-chain fatty acids such as 4-phenylbutyrate (50), 2,2-dimethylbutyric acid (51), and valproic acid (52) have been synthesized and also displayed inhibition of...
HDAC (Steliou et al., 2012). Valproic acid (52) is currently used in the treatment of epilepsy (Georgoff et al., 2018). Thus, butyrate-based epigenetic compounds represent a promising route for the development of new HDADi.

**Aceroside VIII**

Aceroside VIII (53) is a diarylheptanoid isolated from the Japanese white birch *Betula platyphylla*. This compound weakly but selectively inhibited the activity of HDAC6 in HT29 CRC cells (Ryu et al., 2015). However, combination of this compound with the well-known selective HDAC6 inhibitor γ-lactame A452 led to a significant increase of the levels of acetylated α-tubulin. Furthermore, the treatment of HT29 CRC cells with 10 μM aceroside VIII associated with 0.1 μM A452 led to a significant decrease of cell growth up to 84% (Ryu et al., 2015). The same study showed that cell death caused by aceroside is partly dependent on caspase activation. This study highlighted a synergistic effect of natural products and selective HDAC6 inhibitors.

**Molecular Modeling and Docking as a Tool for a Mode of Action Prediction of Natural Product DNMTs and HDACs Inhibitors**

Molecular docking is a computational method employed for understanding, the interaction between a small molecule (e.g. a potential drug) and its macromolecular target, e.g. a protein or receptor. Docking simulations are often used to elucidate the key binding interactions and binding modes of small molecules and their drug targets (Kellenberger et al., 2008). Scoring functions are mathematical/statistical methods implemented in docking algorithms for quantifying the interactions, hence the putative binding of a drug molecule to its target (Chen, 2015). When properly trained, a scoring function could be used as a criterion for selecting a subset of (best-scoring) ligands or small molecules, which have been stored in electronic databases (often several thousands or even millions). During a virtual screening experiment via docking, a large electronic database of ligands is docked into the binding site of a protein and putative binding is characterized using a scoring method. For each ligand, several conformers are stored as “docking poses” and the top-scoring poses are chosen as potential binders. This selected subset of compounds (called docking “hits”) are then tested biologically. Thus, the number of compounds to be tested is drastically reduced, hence cutting down the cost of identification of a lead compound.

Structure-based molecular modeling (e.g. molecular docking, molecular dynamics, structure-based quantitative structure-activity relations) and ligand-based modeling (e.g. pharmacophore modeling, similarity searching) have assisted the identification of novel natural product inhibitors and modulators of DNMTs and HDACs and in explaining their inhibitory effect. Some of these results have been summarized in recent reviews (Medina-Franco and Caulfield, 2011; Saldivar-González et al., 2018).

**Interactions of Natural Compounds Within the Binding Site of DNMT1**

The anthraquinone derivative Nanaomycin A (6), from the National Cancer Institute (NCI)/Developmental Therapeutics Program Open Chemical Repository screening program (http://dtp.cancer.gov), displayed potent antiproliferative effects on HCT116, A549, and HL60 cell lines as mentioned earlier in this review (Kuck et al., 2010). A study on the identification of DNMT1 inhibitors through a virtual screening showed that 6 induces antiproliferative effects in three different tumor cell lines (Chen et al., 2007). Furthermore, biochemical *in vitro* assay using DNMT1 or DNMT3b showed that nanaomycin A selectively inhibits DNMT3b. A docking study of nanaomycin A towards a homology model of the catalytic site of DNMT3b was conducted in order to rationalize the biochemical activity at the molecular level (Kuck et al., 2010). Indeed, docking studies confirmed that nanaomycin A binds in the active site of DNMT3b in which its carboxylic acid is capable of forming hydrogen bonds with the side chain of arginine residues. Moreover, its carbonyl oxygen atom and adjacent hydroxyl group were predicted to form an extensive hydrogen bond network with the side chain of two arginine residues. Besides, the side chain of a glutamic acid residue forms a hydrogen bond with the hydroxyl. Furthermore, a possible explanation of the selectivity of nanaomycin A for DNMT3b was suggested. Indeed, docking studies of nanaomycin A with a previously validated homology model for the catalytic site of human DNMT1 did not show similar H-bonds with the equivalent glutamic acid and arginine residues (Siedlecki et al., 2003). Thus, the anticancer effects of nanaomycin A could be attributed to its ability to selectively inhibit DNMT3b. Thus, the anthracene group of nanaomycin A represents a valuable scaffold for the development of future selective DNMT isoform inhibitors. However, some lasting cardiotoxicity may prevent its clinical use. It is worth to mention that nanaomycin A is the first non-SAH (S-adenosylhomocysteine) DNMT3b-selective compound.

Based on the anticancer activity of the isoflavone genistein toward MDA-MB-231 human breast cancer cells and MCF-7, associated with the resulting decrease in the level of global methylation, a docking study, similar to the aforementioned nanaomycin A, was performed. Indeed, molecular modeling of the interaction between genistein and the DNMT1 binding site, as shown in Figure 7, revealed potential H-bond interactions (Xie et al., 2014). This study demonstrated that genistein might inhibit the binding of hemimethylated DNA, competitively to the catalytic domain of DNMT1. Moreover, the authors also demonstrated that genistein has a demethylating effect in the region of multiple tumor suppressor genes (TSG) including Adenomatous polyposis coli (APC), ataxia telangiectasia mutated (ATM), phosphatase and tensin homolog (PTEN), and increased the mRNA expression of these genes. It is worth to mention that, silencing of the expression of TSGs in cancer cells is mainly due to hypermethylation of CpG islands in the promoter region (Xie et al., 2014). These results suggested that...
genistein could increase the expression of certain TSGs in human breast cancer cells by reducing the activity of DNMTs and mRNA expression of DNMT1. Thus, genistein or its structural analogs could be potentially used as demethylation agents.

Similarly, molecular docking has been used to illustrate why the phenolic derivative curcumin (2) inhibits the enzymatic activity of an analogue of DNMT1 (M. SssI) at the lower nM range, meanwhile a close natural analogue, hexahydrocurcumin (54), showed no inhibition of the same enzyme up to 100 μM (Liu et al., 2009b). This finding could be explained by docking of curcumin and its tetrahydro analogue towards the catalytic domain of DNMT1. Since it was suggested experimentally that curcumin blocks the catalytic thiolate of Cys1226 of DNMT1 covalently to exert its inhibitory effect, docking validation showed the absence of such interactions in docking pose with the other docked curcumin analogues, including demethoxycurcumin (55), bisdemethoxycurcumin (56), tetrahydrocurcumin (57) (Figure 8, Liu et al., 2009b). Compounds 2, 55, and 56 showed similar inhibitory effects, indicating that either of bis-α,β-unsaturated ketones is required for the observation of the activity. However, as mentioned earlier, the bioactivity of curcumin should be analyzed with care.

Discovery of Novel DNMT Inhibitors by Docking-Based Virtual Screening

Several isoforms of DNMTs occur in mammals, e.g. DNMT1, DNMT3a, and DNMT3b as mentioned earlier in this review (Chen et al., 2014). These are attractive targets in cancer chemotherapy and several crystal structures are available in the protein databank (Berman et al., 2000) for structure-based virtual screening projects (e.g. PDB ID: 4DA4) (Song et al., 2012). Several successful virtual screening campaigns via docking have been conducted for the discovery of DNA methyltransferase inhibitors (Kuck et al., 2010; Medina-Franco et al., 2011; Medina-Franco and Yoo, 2013; Chen et al., 2014). These include, for example, 44 natural germacrolides docked against the homology model of the human DNMT1 (Liu et al., 2009b), and the lead-like subset of ~89,425 natural products from the ZINC database (Irwin and Shoichet, 2005) which were docked against the homology model of the human DNMT1 (Medina-Franco et al., 2011). Indeed, from the docking-based screening performed by Liu and co-workers, it was observed that γ-methylactone compounds could be effective DNMT inhibitors. Moreover, the same study resulted in the discovery of parthenolide and curcumin mentioned earlier in this review. This result was confirmed by Yoo and coworkers who demonstrated that the binding models of compounds such as curcumin and parthenolide suggest that these natural products are covalent blockers of the catalytic site of DNMT (Yoo and Medina-Franco, 2011). Thus, compounds such as parthenolide are potential blockers of DNMT1.

Natural Product HDAC Inhibitor in the PDB

Based on the evidence of HDAC inhibitory effects along with tumor-suppressing activities of SFN (8), Myzak et al. (2004) carried out a molecular modeling study of the SFN-Cys HDAC binding site on the HDAC crystal structure. It was shown that the buried cysteine amino group is positioned to make a single H-bond with His132 when 8 was made to interact with this binding site (Myzak et al., 2004). The same study reported that a combination of SFN (8) with trichostain A (13) led to an increase in the inhibition of the HDAC activity. It is worth to mention that 8 is metabolized into its major active form sulforaphane-cystein. Indeed, studies with SFN and media treated from SFN-treated cells indicated that the parent compound was not responsible for the HDAC inhibition activity, and this was
proven through the use of glutathione S-transferase that blocked the first step in the metabolism of SFN (Myzak et al., 2004). Therefore, SFN may be an effective stand alone chemotherapeutic agent or work in synergy with other HDAC inhibitors. However, the lack of isoform selectivity inhibition may limit its potential clinical use.

Based on the known inhibitory activity of trichostatin A (TSA) (13), Finnin et al. (1999) carried out a molecular modeling study of this compound to establish the mechanism of HDAC inhibition (Finnin et al., 1999). This study showed that the binding of TSA proceeds by the insertion of its long aliphatic chain into the HDLP pocket, thus making multiple tube-like contacts to the hydrophobic portion of the pocket. Furthermore, it was revealed that the hydroxamic acid group of TSA coordinates the active-site zinc in a bidentate fashion using its carbonyl and hydroxyl group. It is worth to mention that other natural HDAC inhibitors such as HC-toxin (29) and trapoxin (34) mentioned earlier in this review, contain groups that are analogous to the active-site/zinc-binding groups and the cap aliphatic chain of TSA. However, they have an epoxysketone group instead of a hydroxamic acid group. It has been suggested that the epoxy group may crosslink to an active site nucleophile (Yoshida et al., 1995). Furthermore, interaction of their ketone group with polar residues and possibly the zinc, at the bottom of the active-site pocket might be possible (Finnin et al., 1999). Indeed, the reduction of the carbonyl to a hydroxyl group, or its elimination, that led to a high decrease in the activity of 29, supported this assumption (Shute et al., 1987). This mode of action could be assumed to be identical for other HDAC inhibitors including chlamydocin (32), 1-alaninechlamydocin (32). Besides, the authors suggested that the larger size of the macrocycle (cap group) of 34 and 29 compared to that of 13 could allow more extensive contacts at the rim of the pocket and in the shallow grooves surrounding the pocket entrance.

**HDAC Inhibitors Inspired From the Natural Product Psammaplin A (PsA)**

Inspired by the NP PsA (15), Baud et al. (2013) designed a new set of picolinamide-based histone deacetylase inhibitors, i.e. designing a focused library, which is based on the PsA core (Baud et al., 2013). Based on the HDAC inhibitory and antitumor (Garcia et al., 2011) activities of this marine metabolite, the authors proceeded by probing the features of this molecule, which are responsible for its activity (Baud et al., 2012). In searching for a molecular replacement for the oxime unit of psammaplin A, Baud and coworker were able to discover a new...
set easily synthesizable, isoform-selective, fragment-sized, and highly ligand efficient $N$-2-(thioethyl)picolinamide HDAC inhibitors bearing a chloropyridine motif, with low-nanomolar potencies (Baud et al., 2013). The synthesized compounds selectively inhibited HDAC1 with low-nanomolar potencies. Because selective HDAC1 inhibition has been suggested to be an effective anticancer strategy, this study showed that compounds with the chloropyridine motif will be a valuable design criterion for lead compound development of new and chemical probes that target HDAC1.

Further molecular modeling of the compound PsA and its most potent designed analogs as cytotoxic agent that act by histone deacetylase inhibition (Wen et al., 2016) was conducted against the HDAC1 binding site (PDB ID: 4BKX) (Millard et al., 2013). A comparison of the binding interactions was carried out, for example, of the synthetic analogue; $(2E,2'E)-N,N'-$(disulfanediylbis(ethane-2,1-diyl))bis(2-(hydroxyimino)-3-(2,4-dichlorophenyl)propanamide, which showed better HDAC inhibitory activity than PsA and comparable antiproliferative activity with psammaplin A (15) against all four tested cancer cells (Wen et al., 2016). Figure 9 shows that psammaplin A binds to HDAC1, forming key interactions with the protein in several areas. For example, the thiol group in PsA chelates the $Zn^{2+}$ ion, while the oxime group forms an H-bond with Asp99 bridged by a water molecule. On the other hand, the 3-bromo-4-hydroxy phenyl group in PsA forms a few hydrophobic contacts to His178, Tyr204, and Phe205 around the surface recognition motif, while the hydroxyl group is optically attached to the
para-position of benzene. This enhances interaction with Glu203 at the entrance to the active site tunnel (Figure 9). Besides, it was observed from molecular dynamics studies that the non-covalent interactions between the inhibitor and target protein were quite stable through 2.5 ns. The more potent synthetic analogue of this NP (shown in Figure 9B) shared a similar binding mode as PsA, with similar and stable interactions. However, as mentioned earlier, the physiologic instability of PsA has precluded further clinical investigations.

CONCLUSION

Despite intensive research efforts, cancer is yet one of the primary global causes of death. Although numerous potent anti-cancer drugs have been developed in recent decades, there is still a huge need for specific agents with low side effects (Bray et al., 2018). DNA methylation and histone acetylation are important physiological mechanisms that maintain genome integrity. Altered DNA methylation and/or histone acetylation patterns have consistently been documented as the earliest molecular changes occurring upon tumor establishment. Since the presence of HDACs are important in both the development and progression of cancer, addressing the related epigenetic processes with HDAC inhibitors (HDACi) is a promising starting point for developing new and potent anticancer drugs (Li and Seto, 2016). HDAC inhibitors have already proven their fundamental efficacy against cancer in preclinical and clinical studies, which has led to the FDA approval of vorinostat (SAHA), romidepsin (FK288), panobinostat (LBH589), and belinostat (PXD101) for cancer therapy (Nguyen et al., 2013; Suraweera et al., 2018; Mehdiratta et al., 2020). Besides, a double-digit number of other HDAC inhibitors are currently under investigation in Phase II or III trials (Suraweera et al., 2018). Nevertheless, it should be noted that many HDAC inhibitors fail in clinical development due to lack of efficacy or too many side effects (Slingerland et al., 2014). Another major drawback is the fact that the majority of the already established HDAC inhibitors are only approved for the treatment of T-cell lymphomas, due to insufficient efficacy against solid tumors (Slingerland et al., 2014). Consequently, the identification of new HDAC inhibitors that are both potent and specific is of great importance to adequately address the pathophysiological importance of epigenetic mechanisms in modern cancer therapy. Many compounds are made chemically in academia and industry for the development of new HDACi. However, nature also offers an almost inexhaustible pool of new bioactive substances, often exhibiting novel and unexpected chemical scaffolds (Herrmann et al., 2017; Chen et al., 2018). Countless drugs based on natural products are impressive proof of the healing power hidden in nature (Dias et al., 2012). For these reasons, it seems highly advisable to screen newly discovered natural products for HDAC inhibitory activity to identify new and superior lead compounds for the development of a new generation of HDAC inhibitors. Moreover, emerging reports show that intelligently designed combination therapies with common cytostatic drugs can synergistically increase the efficacy of the inhibitors (Suraweera et al., 2018).

Many of the points discussed for HDACi apply in a similar or even the same way to DNA methyltransferase inhibitors (DNMTIs). For example, DNMTIs are most effective in hematological diseases such as myelodysplastic syndromes, whereas treatment success in solid tumors is limited (Gurion et al., 2010). Similar to HDACi, the aim is therefore to use wisely designed combination treatments to synergistically enhance the effect of well-established anti-cancer drugs through HDACi (Gnyszka et al., 2013). This approach is certainly functional, as shown by the combination with oxaliplatin or doxorubicin (Flis et al., 2009; Vijayaraghavalu et al., 2013). One problem associated with the clinical use of classical DNMTIs is that they often exhibit mutagenic effects, such as 5-azacytidine or zebularine (Amacher and Turner, 1987; Lee et al., 2004). Here, natural product-based DNMTIs such as epigallocatechin-3-gallate appear to be more advantageous, since the latter is not incorporated into the DNA, but binds directly to the catalytic region of DNMTs (Fang et al., 2003).

Natural products endowed with DNMTs and HDACs inhibition functions were reviewed in this article. Indeed, a wide range of natural compounds from plants, microorganisms, and marine sponges was presented and their potential to inhibit DNMTs and HDACs discussed. Furthermore, this study showed that compounds possessing a strong zinc-binding group are more promising HDAC inhibitors even though the presence of the latter is not a prerequisite for HDAC inhibition. Molecular modeling and docking increasingly shows to be a powerful tool for studying the interactions between the drug target and its potential inhibitors, paving the way towards the further development of novel HDAC and DNMT inhibitors as anti-tumor agents which could be natural product-inspired. However, most of the natural products presented showed an indirect effect and lack isoform selectivity, which may limit their development into clinical use. The capability to selectively inhibit single HDAC or DNMT isoforms currently represents a major challenge in the design of HDAC and DNMT inhibitors. This approach could represent an opportunity to derive improved agents which could target specific types of cancer. It is worth to mention that, combination of HDACs or DNMTs inhibitors with anti-EZH2 could increase their efficacy without overlapping toxicity (Shahabipour et al., 2017). This implies the identification of natural products that could target other epigenetic regulatory enzymes which is beyond the scope of this review.

HDACi are important for innate defense function, macrophage differentiation, and polarization (Han and Lee, 2009). Indeed, treatment of B16/F10 murine melanoma cells by the natural HDACi romidepsin showed that HDACi are promising agents in the human melanoma immunotherapy pretreatment (Murakami et al., 2008). A study by Cabanel et al. (2015) showed the importance of TsA in the regulation of macrophage differentiation and elongation (Cabanel et al., 2015). Another study also highlighted that TsA treatment inhibits inflammatory cytokine secretion and improves both CD1d and class II MHC-mediated antigen presentation. Thus,
this treatment may enhance the suppression of antitumor NK T cell responses (Tiper and Webb, 2016). The same study showed that the restoration of antitumor responses to mantle cell lymphoma could be improved by treatment with HDACi. Based on these observations and despite the need of additional preclinical data to access the efficacy and toxicity of HDACi, several clinical investigations have started by associating HDACi with immunotherapeutics for patients with advanced prostate, renal, or urothelial cell carcinoma (Mazzone et al., 2017). Another study suggested that SAHA might improve the activity of the immunotherapeuticavelumab in both tumor and NK cells (Hicks et al., 2018). It is worth to mention that SAHA possesses also a hydroxamic acid function such as trichostatin A indicating that a possible derivatization of the latter could improve its efficacy. Thus, these studies suggest that combination of natural HDAC inhibitors with immunotherapeutics could improve the treatment of cancer. However, preclinical studies to access the efficacy and toxicity of this combination are still needed. A particular interest should be given on class I-specific HDACi, which are believed to provide a promising future in the cancer treatment (Mazzone et al., 2017). Indeed, combination of natural selective HDAC inhibitors might increase the anticancer drug efficacy as demonstrated in the case of the natural HDAC6-selective inhibitor acesoride VIII (Ryu et al., 2015).

However, the multitarget effects of natural products is a serious limitation of their use in the area of epigenetic drugs. Thus, chemical derivatization and molecular studies could improve their effects for a better understanding of their mechanism of action. On the other hand, the multi-target property of natural products could be utilized for the treatment of diseases including cancer, Alzheimer’s disease, and diabetic cardiomyopathy. Indeed, promising multi-target molecules have been studied for the aforementioned diseases (Musso et al., 2015; Badal et al., 2017; Karuppagounder et al., 2017). In addition, only few virtual screenings studies have been performed yet and their increase will provide hope for the discovery of potential DNMT and HDAC inhibitors. Nonetheless, the structures presented in this review offer the well-founded basis that screening and chemical modifications of natural products will in future provide not only leads to the identification of more specific inhibitors with fewer side effects, but also important features for the elucidation of HDAC and DNMT function for cancer treatment.

AUTHOR CONTRIBUTIONS

SA and RM edited and reviewed the manuscript. SA wrote the first draft of the manuscript. SA, FN-K, FS, ME, AN, and SM wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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