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Epigenetic Control Using Natural Products and Synthetic Molecules

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Abstract: The term "epigenetics" is defined as "heritable changes in gene expression that occur without changes in DNA sequence". Recently, it has been revealed that DNA methylation and histone modifications such as acetylation, methylation and phosphorylation are epigenetic mechanisms according to this definition. In other words, these posttranslational modifications are important factors in determining when and where a gene will be expressed. To date, several enzymes that catalyze DNA or histone modifications have been identified, such as DNA methyltransferases and histone deacetylases. Inhibitors and activators of enzymes controlling epigenetic modifications are considered useful not only as tools for the elucidation of cellular and biological phenomena, but also as therapeutic agents, since disruption of the balance of epigenetic networks is known to cause some disease states such as cancer. In this review, we present natural products and synthetic molecules that inhibit or activate enzymes catalyzing DNA methylation or histone modifications, and discuss the potential of epigenetic therapy.

Keywords: Epigenetics, DNA methylation, histone modification, inhibitor, activator, epigenetic therapy.

INTRODUCTION

According to the "Central Dogma of Molecular Biology" [1], DNA is the only source of genetic information, which flows linearly from DNA to RNA to protein. However, a number of phenomena cannot be explained by this Central Dogma. For example, the function and morphology of brain cells are completely different from those of liver cells although these cells contain the same set of genes. It is because the kinds of genes expressed in brain cells are different from those expressed in liver cells. Therefore, it is important to understand when and where a gene will be expressed.

The term "epigenetics" was defined as "heritable changes in gene expression that occur without changes in DNA sequence" by Dr Alan Wolffe in 1999 [2]. Recent studies have revealed that DNA methylation and histone modifications such as acetylation and methylation are epigenetic mechanisms according to this definition [3, 4]. Thus far, several enzymes that methylate DNA or posttranslationally modify histones have been identified. For instance, DNA methyltransferases add a methyl group at the 5-position of cytosine residues in DNA and histone acetyltransferases transfer an acetyl group to lysine residues of histones [5, 6]. Inhibitors and activators of these enzymes are considered useful as tools for the understanding of the role of these modifications in genome function and regulation. In addition, since disruption of the balance of epigenetic networks is known to cause some disease states such as cancer [7, 8], these enzyme inhibitors or activators could be therapeutic agents. In this review, we present natural products and synthetic molecules that inhibit or activate enzymes catalyzing DNA methylation or histone modifications, and discuss the potential of epigenetic therapy.

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DNA METHYLATION INHIBITORS

DNA Methylation

DNA methylation at the 5-position of cytosine in CpG dinucleotides is an important mechanism for the epigenetic regulation of gene expression. Hypermethylation occurs in the CpG-rich sequence, so called CpG islands, where core promoters and transcription initiation sites are located. The hypermethylation in the CpG islands leads to the silencing of genes [9-11]. CpG island-specific hypermethylation is a common characteristic of cancer cells. This causes the silencing of tumor suppressor genes such as $p16^{INK4a}$ and *human mutL homologue 1* (*hMLH1*) which are involved in the tumorigenic process including DNA repair, cell cycle regulation and apoptosis [12-15]. Therefore, DNA methylation inhibitors may work against cancer.

DNA Methyltransferases

DNA methylation is catalyzed by DNA methyltransferases (DNMTs) using *S*-adenosyl-L-methionine (SAM) as the methyl donor (Fig. **1**). At present, four mammalian DNMTs, namely, DNMT1 [16], DNMT2 [17], DNMT3A and DNMT3B [18], have been identified. DNMT1 is regarded as a maintenance methyltransferase because it has a preference for hemi-methylated DNA substances that are methylated in one strand and unmethylated in the other. The primary function of DNMT1 might be the copying of the methylation patterns from the parental DNA strand to the newly replicated daughter strand during the DNA replication process [19]. The function of DNMT2 is still unknown. Direct evidence of catalytic activity has not been provided yet, thus it has been suggested that DNMT2 might not function as a DNA methyltransferase [20, 21]. However, according to a more recent report by Liu *et al*, weak DNA methyltransferase activity of DNMT2 was observed in mouse and human cells [22]. In contrast to

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DNMT1, DNMT3A and DNMT3B add a methyl group to unmethylated CpG base pairs. Such *de novo* methylation by these enzymes is responsible for the establishment of DNA methylation patterns during embryogenesis [14, 18, 23, 24].

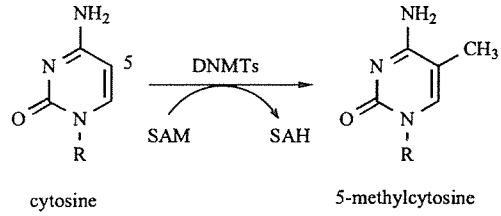


Fig. (1). DNA methylation at the 5-position of cytosine by DNMTs. SAH: *S*-adenosyl-L-homocysteine.

The catalytic mechanism for the methylation of cytosine-5 has been studied extensively and is well understood [25-27]. As depicted in Fig. **2**, a thiol of the cysteine residue in the active site of DNMTs serves as a nucleophile that attacks the 6-position of cytosine to generate a covalent DNA-protein intermediate which possesses nucleophilic properties at the 5-position. This reactive intermediate accepts a methyl group from SAM to form the 5-methyl covalent adduct and *S*-adenosyl-L-homocysteine (SAH). Following the methyl transfer, the proton at the 5-position is abstracted by a basic residue in the active site of the enzyme which is removed from the 6-position by β-elimination to generate the methylated cytosine and free enzyme.

DNA Methylation Inhibitors

To date, several DNA methylation inhibitors have been developed. Cytidine analogues such as 5-azacytidine (5-aza-CR) **1** and 5-aza-2'-deoxycytidine (5-aza-CdR) **2** (Fig. **3**) have long been known to have activity to inhibit DNA methylation [28]. It has also been reported that inhibition of methylation induced by 5-aza-CR **1** and 5-aza-CdR **2** reactivates the expression of genes that have been repressed by DNA methylation [29-31]. 5-Aza-CR **1** and 5-aza-CdR **2** have antiproliferative activity against cancer cells and are used for the clinical treatment of acute myeloid leukemia and myelodysplastic syndrome [32-34]. However, 5-aza-CR **1** and 5-aza-CdR **2** have problems such as instability in aqueous media, toxicity and poor bioavailability [35]. To overcome these problems, the novel cytidine analogues 5-fluoro-2'-deoxycytidine (FCDR) **3** and zebularine **4** were developed [28]. In particular, zebularine **4** is a promising compound [36]. Zebularine **4** is stable and minimally toxic both *in vitro* and *in vivo* [37]. Furthermore, zebularine **4** was orally administered to achieve the reactivation and demethylation of a silenced and hypermethylated *p16* gene in human bladder tumor cells grown in nude mice [37]. In addition, it has also been shown that the continuous treatment of cultured cancer cells with zebularine **4** effectively sustains demethylation of the *p16* 5' region and prevents gene resilencing [38].

Cytidine analogues **1**–**4** are mechanism-based inhibitors of DNMTs. Their inhibitory mechanisms are well investigated. As mentioned above, the catalytic mechanism of DNMTs involves the addition of a thiol to the 6-position of the target cytosine, which activates the carbon at the 5-position allowing a nucleophilic reaction with SAM (Fig. **4a**). When 5-aza-CR **1** or 5-aza-CdR **2**, in which the carbon at the 5-position of the cytosine is replaced with a nitrogen, is incorporated into a DNA sequence, nucleophilic attack is facilitated at the 6-position and a slow methyl transfer takes place, but there is no hydrogen at the 5-position to abstract and the covalent complex persists [39-41] (Fig. **4b**). FCDR **3** inhibits DNMTs in a similar manner. Following the formation of a covalent DNA-enzyme complex at the 6-position and methylation at the 5-position, the analogue remains bound to the active site, because the abstraction of fluorine can not be achieved [26, 42] (Fig. **4c**). The X-ray crystal structure of a bacterial DNMT from *Haemophilus haemolyticus* with an oligodeoxynucleotide duplex containing zebularine **4** made clear the inhibitory mechanism of zebularine **4** [43]. As with 5-aza-CR **1**, 5-aza-CdR **2** and

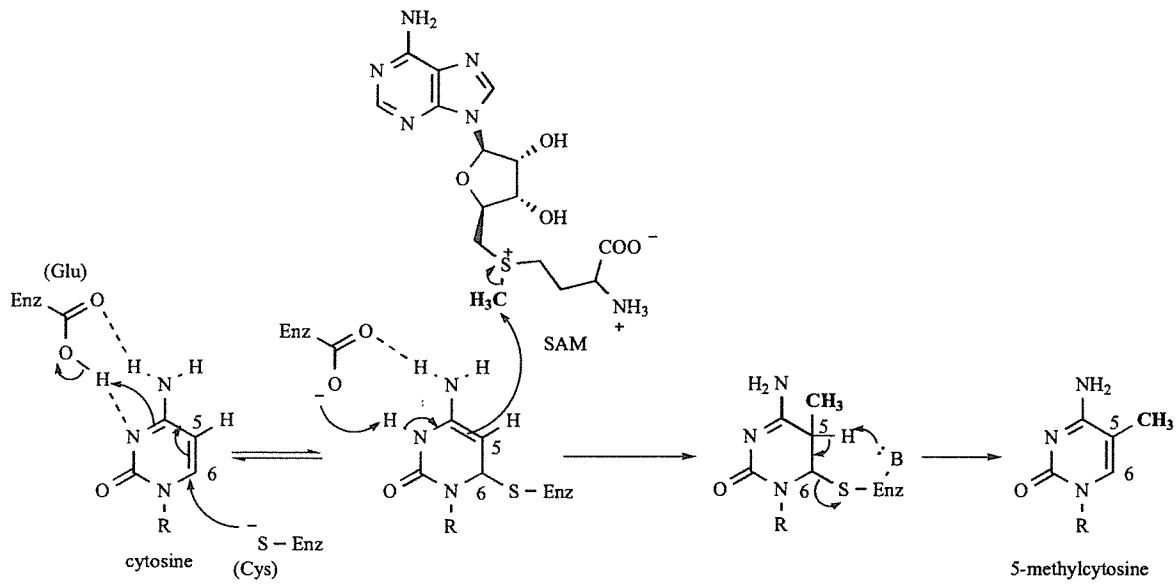


Fig. (2). Proposed catalytic mechanism for the methylation of cytosine by DNMTs [25-27].

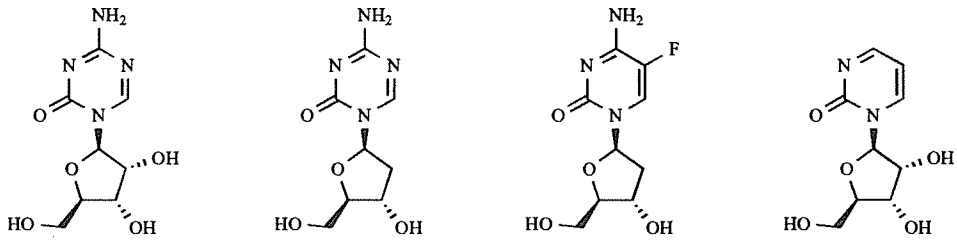


Fig. (3). Cytidine analogues as DNA methylation inhibitors.

FCDR **3**, following nucleophilic attack at the 6-position, proton transfer occurs at the 5-position instead of methyl transfer to form a stable covalent complex (Fig. **4d**).

Non-nucleoside DNA methylation inhibitors have also been reported (Fig. **5**). Procainamide **5**, approved by the FDA for the treatment of cardiac arrhythmias, has been

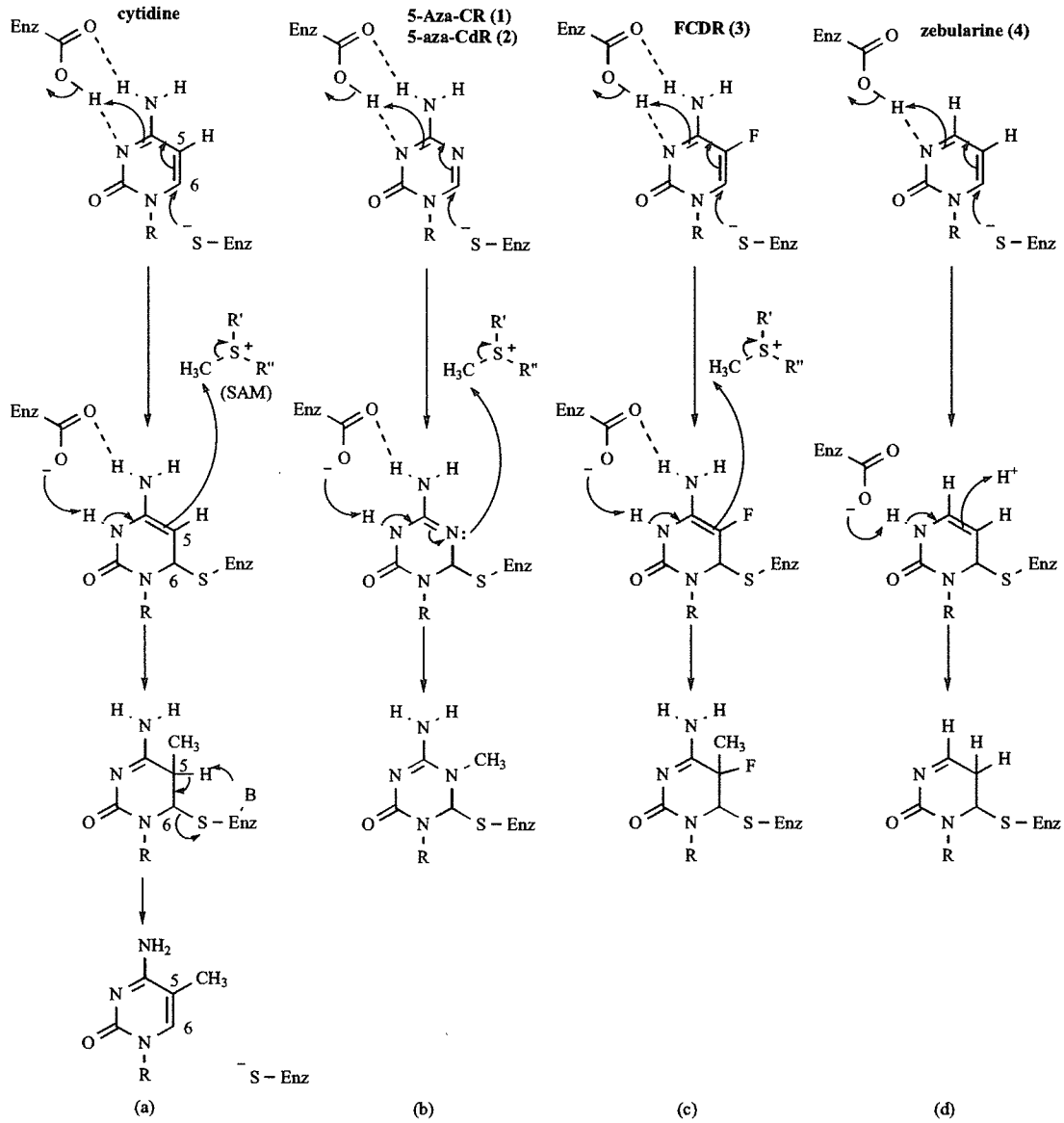


Fig. (4). Catalytic mechanism for the methylation of cytosine by DNMTs (a) and inhibitory mechanism of 5-Aza-CR **1** (b), 5-aza-CdR **2** (b), ECDR **3** (c) and zebularine **4** (d) [26, 39-43].

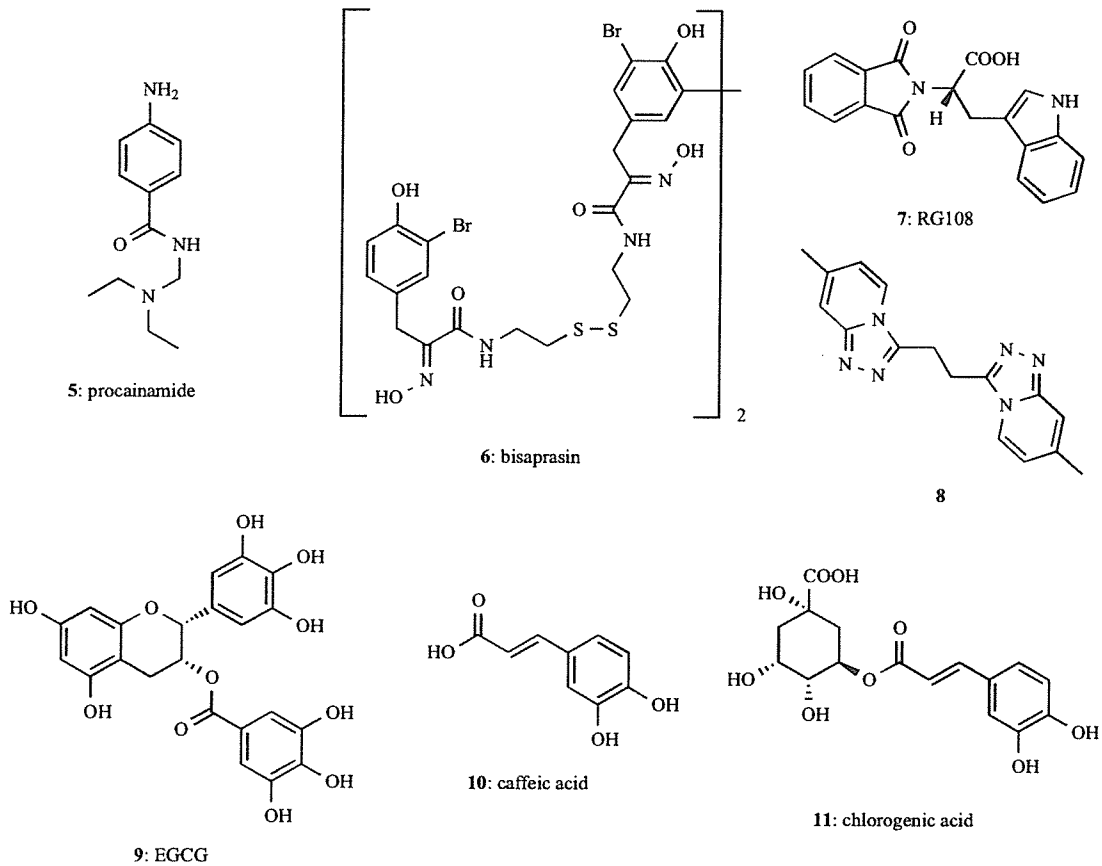


Fig. (5). Non-nucleoside DNA methylation inhibitors.

reported to be a DNA-demethylating agent with antiproliferative activity against human cancer cells [44-46]. The DNA-demethylating action of procainamide **5** is thought to be mediated by its binding to GC-rich DNA sequences [47, 48]. Crews and co-workers isolated disulfide bromotyrosine derivatives such as bisaprasin **6** from the sponge *Pseudoceratina purpurea*, which were found to be highly potent DNMT1 inhibitors (IC_{50} of **6** = 3.4 nM) [49]. Lyko and co-workers identified RG108 **7** as a DNMT inhibitor by screening in silico a small-molecule data base using a three-dimensional model of the human DNMT1 catalytic domain [50]. RG108 **7** efficiently inhibited DNMTs *in vitro* without forming a covalent complex with the enzymes. Low micromolar concentrations of RG108 **7** induced significant demethylation of genomic DNA without any detectable toxicity in HCT116 cells. Interestingly, RG108 **7** caused demethylation and reactivation of silenced tumor suppressor genes, but it did not affect the methylation of centromeric satellite sequences. The same group has recently reported another small-molecule non-nucleoside DNA methylation inhibitor **8**, which was also identified using a three-dimensional model of the human DNMT1 and a modified docking and scoring procedure [51]. A recent study by Yang and co-workers showed that (-)-epigarllocatechin-3-gallate (EGCG) **9**, a major polyphenol from green tea, can inhibit DNMT activity competitively (K_i = 6.89 μM) and reactivate silenced genes such as $p16^{INK4a}$ and *hMLH1* in cancer cells [52]. More recently, two common polyphenols, caffeic acid **10** and chlorogenic acid **11**, were found to inhibit DNMT1 with IC_{50}s of 2.3 and 0.9 μM, respectively [53]. Kinetic analyses showed that **10** and **11** inhibit DNA methylation predominantly through a noncompetitive mechanism. This inhibition was largely due to increased formation of SAH, a feedback inhibitor, resulting from the catechol-*O*-methyltransferase (COMT)-mediated *O*-methylation of the dietary catechols **10** and **11** (Fig. **6**).

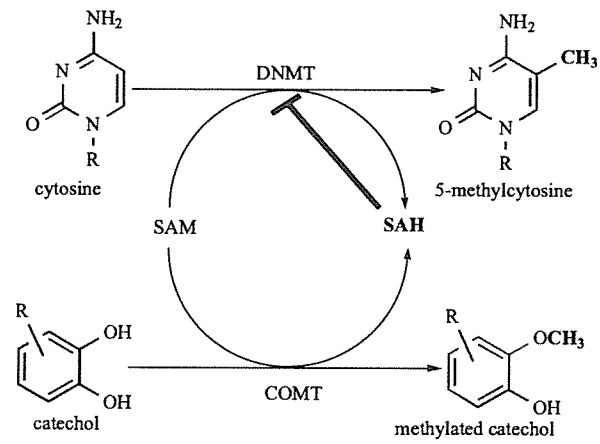


Fig. (6). Inhibitory mechanism of caffeic acid **10** and chlorogenic acid **11** [53].

HISTONE ACETYLATION MODULATORS

Histone Acetylation and Deacetylation

Histone acetylation is one of the most widely studied epigenetic mechanisms of gene expression. The steady state of nucleosomal histone acetylation is established by a dynamic equilibrium between competing histone acetyltransferases (HATs) and histone deacetylases (HDACs). The acetylation of specific histone lysine residues is catalyzed by HATs, while deacetylation is catalyzed by HDACs (Fig. **7**). In general, hyperacetylation of histone lysine residues facilitates transcriptional activation whereas deacetylation causes transcriptional silencing [54-57].

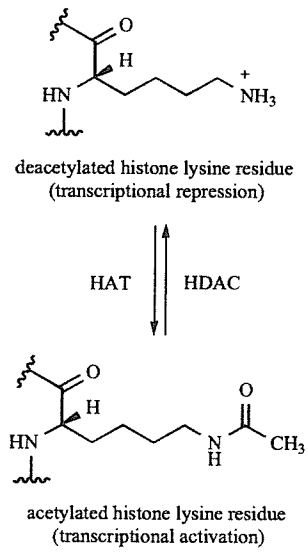


Fig. (7). Reversible acetylation of histone lysine residues.

Histone Acetyltransferases (HATs)

The recent discovery that a number of transcriptional activators have HAT activity supports the idea that histone acetylation is correlated with transcriptional activation. These activators include the GNAT, MYST, p300/CBP, SRC and TAFII250 families [58]. Each of the human HATs shows specificity and has different functions (Table **1**) [59]. For example, GCN5 acetylates histone H3 specifically and activates transcription, while Tip60 acetylates histone H4 and functions in DNA repair and the induction of apoptosis.

Denu and co-workers carried out a detailed kinetic analysis to elucidate the molecular mechanism of histone acetylation by GCN5 [60]. Their study indicated that the acetylation by GCN5 proceeds *via* a ternary complex formed by acetyl-CoA, histone H3 and GCN5. The X-ray crystal structure of *Tetrahymena* GCN5 (tGCN5) bound to CoA and a histone H3 peptide was published by Rojas *et al.* [61]. This crystal structure shed light on how histone H3 is recognized and on the catalytic mechanism of GCN5. The structural data revealed that GCN5 has a recognition motif for the Gly-Lys-Xxx-Pro sequence in histone H3, and showed that CoA is essential for rearranging the enzyme for the binding of a histone. Additionally, the X-ray crystal structure of human PCAF bound to CoA indicated that PCAF catalyzes the histone acetylation by a mechanism similar to GCN5 [62]. The proposed catalytic mechanism is illustrated in Fig. **8**. First, a proton is shuttled from the substrate lysine to Glu 122, whose basicity is increased by the vicinity of several non-polar residues (Tyr 115, Phe 120, Leu 158, Tyr 160, Trp 185, Ile 189 and Leu 197) [63], *via* a water molecule held in place by hydrogen bonds from the backbone carbonyl of Val 123 and the backbone amide of Tyr 160. Next, the nucleophilic amine of the deprotonated lysine attacks the carbonyl of acetyl-CoA whose electrophilicity is increased by a hydrogen bond with the backbone amide of Leu 126. The nucleophilic attack results in a tetrahedral transition state that is stabilized by a hydrogen bond to the backbone amide of Leu 126. Finally, the acetylated lysine and CoA are released.

Table 1. Classification of HATs [58, 59]

HAT	Histone Specificity	Function
GNAT family		
GCN5	H3 >> H4	transcriptional activation
PCAF	H3 >> H4	transcriptional activation
Elp3	H2A, H2B, H3, H4	transcriptional elongation
ATF-2	H2B > H4	gene-specific transcriptional activation
HAT-1	H4 >> H2A	histone neogenesis
MYST family		
Tip60	H4 >> H3 > H2A	DNA repair, apoptosis
MOZ	H3, H4, H2A	leukemogenesis
MORF	H4 > H3 > H2A	unknown
HBO1	H3, H4	DNA replication
p300/CBP family		
p300/CBP	H3, H4 > H2A, H2B	transcriptional activation
SRC family		
SRC-1	H3 > H4	transcriptional activation
ACTR	H3, H4 > H2B	transcriptional activation
TAFII250 family		
TAFII250	H3 > H4	cell-cycle progression

HAT Modulators

To date, several types of HAT modulators have been reported (Fig. **9**). The first reported HAT inhibitors were substrate analogues in which a peptide substrate was covalently linked to an acetyl-CoA [64]. Further study of the structure-activity relationship led to the identification of a potent p300 inhibitor **12** (IC_{50} = 0.7 μM) [65]. Recently, Cole and co-workers have reported a CoA analogue conjugated to a oligoArg peptide *via* disulfide linkage as the first potent and selective cell-permeable p300 inhibitor [66]. Several natural products have been reported to inhibit HAT activity. Kundu and co-workers identified anacardic acid **13** from cashew nut shell liquid, known to have antitumor activity [67], as an inhibitor of p300 and PCAF (IC_{50}s = 8.5 μM and 5 μM, respectively) [68]. Interestingly, CTPB **14**, the amide derivative of **13**, enhanced p300 HAT activity (4-fold activation of p300 at 200 μM). However, cells were

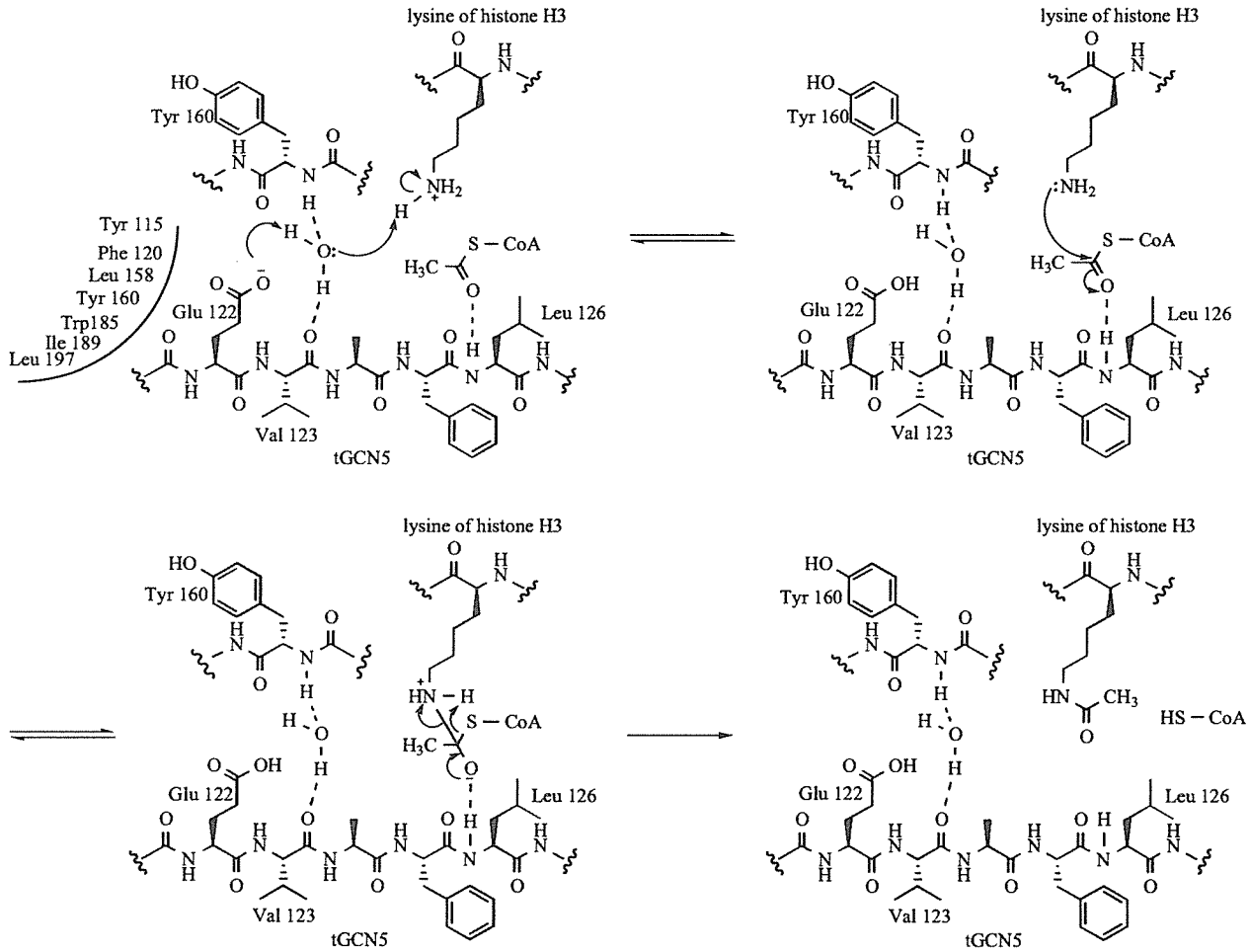


Fig. (8). Proposed reaction mechanism for the acetylation of histone H3 by tGCN5 [60-62].

impermeable or poorly permeable to both compounds **13** and **14**. The same group reported garcinol **15**, a polyisoprenylated benzophenone derivative from *Garcinia indica* fruit rind, as the first cell-permeable inhibitor of p300 and PCAF [69]. Curcumin **16**, a major curcumanoid in the spice turmeric, has been reported to be a specific inhibitor of p300/CBP [70]. Interestingly, curcumin **16** also inhibited the acetylation of HIV-Tat protein *in vitro* by p300 as well as proliferation of the virus. Thus, p300 may be a new target in the treatment of HIV, and non-toxic curcumin **16** may serve as a lead compound. A group led by Giannis identified the γ-butyrolactone derivative MB-3 **17** which was designed based on the proposed catalytic mechanism of GCN5 [71]. MB-3 **17** showed only weak inhibition of CBP (IC_{50} = 0.5 mM), but it inhibited GCN5 with an IC_{50} of 100 μM. Since MB-3 **17** is a small molecule and cell-permeable inhibitor of GCN5, it may be a lead structure from which more potent GCN5 inhibitors can be developed.

Class I and Class II Histone Deacetylases (HDACs)

In 1996, Schreiber and co-workers reported the isolation and cloning of the first human HDAC (HDAC1), which was very similar to the yeast transcriptional regulator Rpd3p [72]. Since then, 18 HDACs have been identified. As shown in Table **2**, they are divided into two categories, zinc-dependent enzymes (Class I and Class II) and NAD^+-dependent enzymes (Class III) (see the section Class III HDACs: Sirtuins) [54, 73-75]. Class I HDACs include HDAC1, HDAC2, HDAC3 and HDAC8, and show homology to the yeast protein RPD3. Class II HDACs include HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10, and are homologous to the yeast enzyme HDA1. HDAC11, the most recently identified member, is classified to the new Class IV of HDACs [76]. Class I HDACs are expressed in the nuclei of most cell lines and are involved in the transcriptional repression of a number of genes [77-79]. In contrast, the localization of Class II enzymes is not limited to the nucleus. They are also expressed in cytoplasm [80] and are associated with muscle differentiation block [81] and microtubule stability. Since Class I and Class II HDACs have been reported to be linked with carcinogenesis [82-85], these enzymes are promising targets in the treatment of cancer.

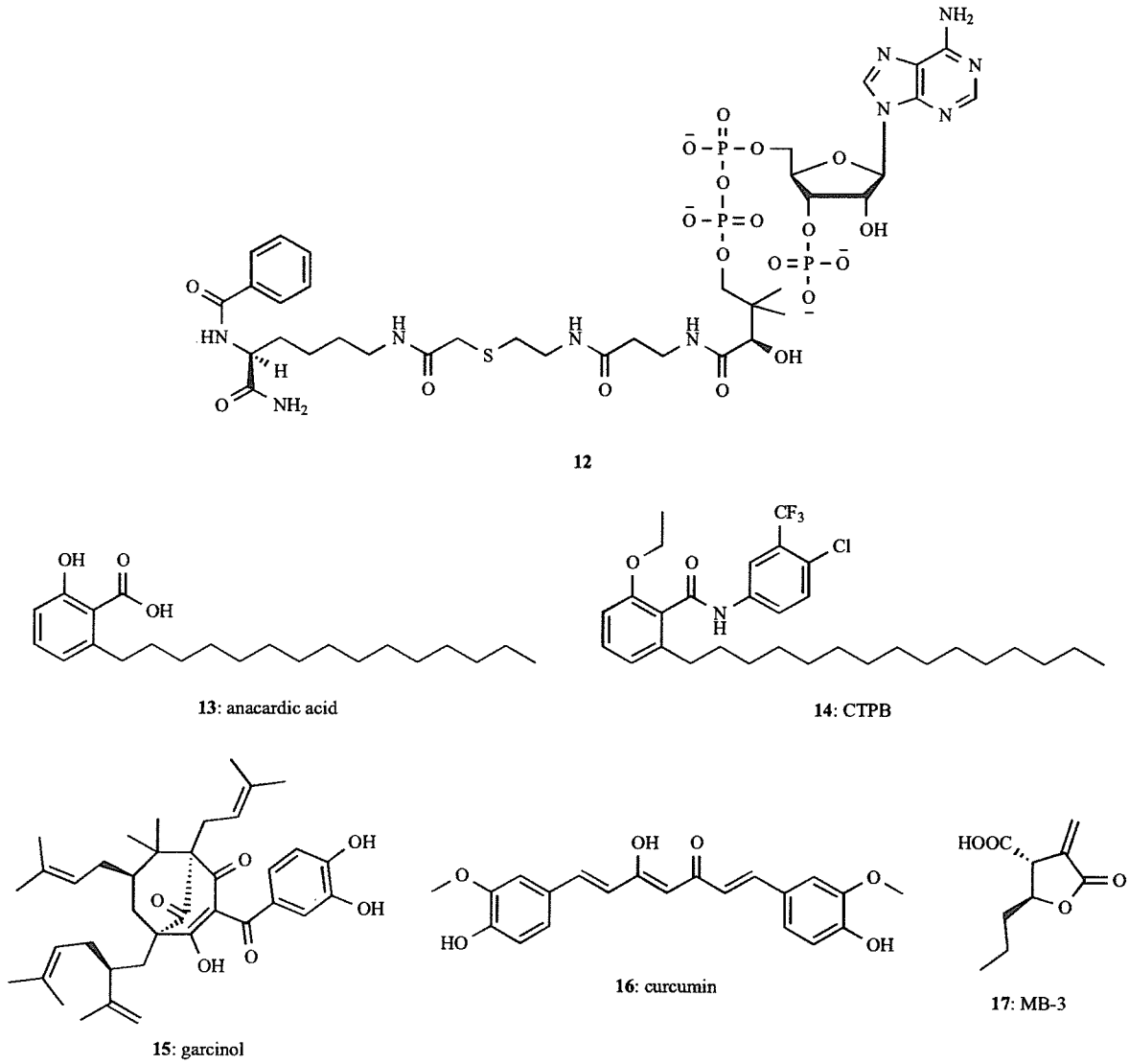


Fig. (9). Structures of HAT modulators.

In recent years, substantial progress has been made in the structural study of HDAC. In 1999, Finnin *et al.* published the X-ray crystal structures of an archaebacterial HDAC homologue (HDAC-like protein, HDLP) complexed with inhibitors [86], and in 2004, the crystal structures of human HDAC8 complexed with inhibitors were reported independently by two research groups [87, 88]. It was revealed that HDACs contain a zinc ion at the bottom of the active site and that the active center consists of a tyrosine, two asparatic acids and three histidines. The X-ray structures have led to a solid understanding of not only the three-dimensional structure of the active site of HDACs but also yielded insights into the catalytic mechanism for the deacetylation of acetylated lysine substrates. The proposed mechanism is depicted in Fig. **10**. In this proposal, the carbonyl oxygen of the substrate binds the zinc and is located adjacent to a water molecule that coordinates the zinc ion. The carbonyl carbon, which becomes a better electrophile through its coordination to the zinc ion, is attacked by the water molecule activated by His 140 (HDAC1 numbering) and the zinc ion. The nucleophilic attack results in a tetrahedral transition state, which is stabilized by two zinc-oxygen interactions and by a hydrogen bond with the Tyr 303 hydroxyl group. In the final step, the transfer of a proton from His 141 to the nitrogen of the intermediate triggers the scission of the carbon-nitrogen bond and yield the acetate and lysine products. Recent computational calculations of the chemical properties of the active site of HDAC have suggested the possibility of a different mechanism of deacetylation [89]. The calculations supported the novel mechanism shown in Fig. **11**. Upon the binding of an acetylated lysine, the carbonyl is attacked by hydroxide ion bound to the zinc and Tyr 303, in analogous with carbonic anhydrase [90]. The attack results in a tetrahedral transition state, in which an excess of negative

Table 2. HDAC Family [54, 73-81]

HDAC	Localization	Function
Zinc-dependent HDACs		
Class I (RPD3 homologue)		
HDAC1	nucleus	transcriptional repression
HDAC2	nucleus	transcriptional repression
HDAC3	nucleus, cytoplasm	transcriptional repression
HDAC8	nucleus	transcriptional repression
Class II (HDA1 homologue)		
HDAC4	nucleus, cytoplasm	transcriptional repression muscle differentiation block
HDAC5	nucleus, cytoplasm	transcriptional repression muscle differentiation block
HDAC6	cytoplasm	regulation of microtubule stability and function
HDAC7	nucleus, cytoplasm	transcriptional repression muscle differentiation block
HDAC9	nucleus, cytoplasm	unknown
HDAC10	cytoplasm	unknown
Class IV		
HDAC11	nucleus	unknown
NAD^+-dependent HDACs		
Class III (Sir2 homologue)		
SIRT1-7	nucleus	functional regulation of p53

charge is expected between His 141 N^{ε} and the amide nitrogen, but this energetically unfavorable situation is resolved by proton transfer from His 140 to His 141. The higher acidity of His 141 causes the protonation of the amide nitrogen, which results in the cleavage of the amide bond.

Class I and Class II HDAC Inhibitors

The inhibition of HDACs causes histone hyperacetylation and leads to the transcriptional activation of genes such as *p21*$^{WAF1/CIP1}$ [91], *FAS* and *caspase-3* [92] which are associated with cell cycle progression, differentiation and tumorigenesis. In addition, there is evidence that HDAC inhibitors are drug candidates for other diseases such as inflammation [93]. Therefore, HDACs have emerged as attractive targets in drug development [94-102].

To date, a number of HDAC inhibitors have been developed. As shown in Fig. **12**, HDAC inhibitors typically possess a zinc-binding group (ZBG), which coordinates the zinc ion in the active site, a cap substructure, which interacts with amino acids at the entrance of the *N*-acetylated lysine binding channel, and a linker connecting the cap and the ZBG at a proper distance.

The largest class of HDAC inhibitors have a hydroxamic acid as a ZBG. The crystal structures of the HDLP/hydroxamate and HDAC8/hydroxamate complexes have made it clear that the enzyme contains a zinc ion at the bottom of the active site and that the hydroxamic acid group coordinates the zinc ion through its two oxygen atoms and also forms three hydrogen bonds between its CO, NH and

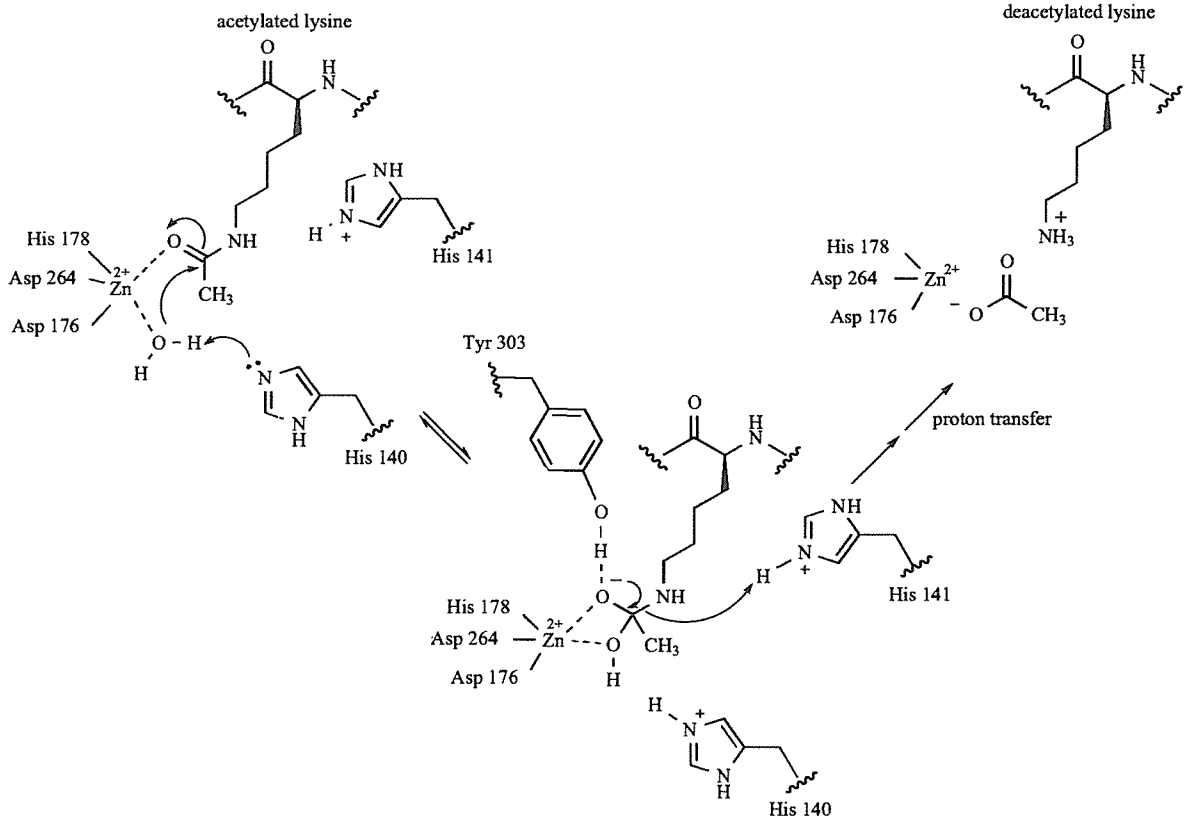


Fig. (10). Catalytic mechanism for the deacetylation of acetylated lysine proposed by Finnin *et al.* [86].

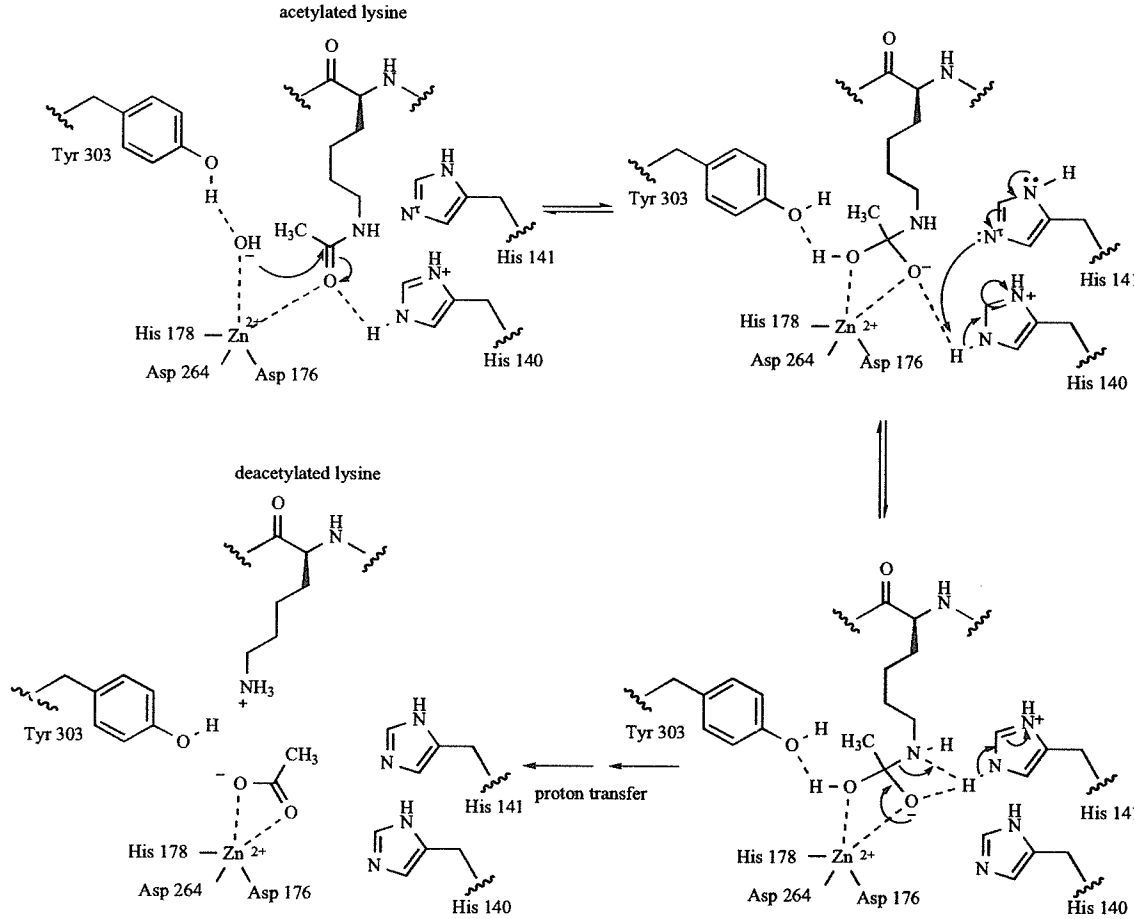


Fig. (11). Catalytic mechanism for the deacetylation of acetylated lysine proposed by Vanommeslaeghe *et al.* [89].

OH groups and Tyr 297, His 132 and His 131 (HDLP numbering), respectively (Fig. **13**) [86-88]. These excellent HDAC-binding properties are responsible for the fact that the majority of known HDAC inhibitors are hydroxamates. Representative hydroxamate inhibitors are shown in Fig. **14**. The first reported hydroxamate inhibitor was the natural product Trichostatin A (TSA) **18** which was isolated from *Streptomyces hygroscopicus* [103, 104]. TSA **18** inhibited Class I and Class II HDACs in the nanomolar range and induced terminal cell differentiation, apoptosis and cell cycle arrest, but it had no antitumor activity in nude mice bearing xenografts of human melanoma cells [105], which is probably a consequence of its rapid metabolic inactivation. Suberoylanilde hydroxamic acid (SAHA) **19** is one of the second generation synthetic hydroxamate inhibitors. SAHA **19** has been reported to inhibit cell growth, induce terminal differentiation in tumor cells, and prevent the formation of tumors in animal models [106-108]. Replacement of the cap and linker part of TSA **18** and SAHA **19** produced a number of structurally diverse inhibitors with improved potency or toxicity. SAHA-related straight chain hydroxamates include the 3-pyridinyl analogue pyroxamide **20** [109], phenylalanine derivative M232 **21** [110], cyclic tetrapeptide CHAP31 **22** (a trapoxin analogue, see below) [111, 112], 1,3-dioxane analogue tubacin **23** [113, 114] and so on. Notably, tubacin **23**, identified by a combinatorial approach [115], showed selective inhibition of HDAC6 in cells, and thus proved useful for elucidation of the function of HDAC6 [116-118]. Cinnamyl hydroxamic acid derivatives such as PXD101 **24** [119], LAQ 824 **25** [120-122] and phthalimide analogue **26** [123] tend to have a high HDAC inhibitory potency. Among these analogues, PXD101 **24** and LAQ 824 **25** had significant activity and low toxicity in tumor xenograft assays. *N*-Hydroxybenzamide derivatives such as **27** [124-126] and **28** [127], in which the linker contains a phenyl ring, are also known to inhibit HDACs. Compound **28**, a hybrid hydroxamate with valproic and phenylbutyric acid (see below), is one of the most potent derivatives with an IC_{50} of 16 nM. At present, some of the hydroxamate HDAC inhibitors are undergoing clinical trials for the treatment of cancer. In fact, SAHA **19** is currently in phase III clinical trials [128].

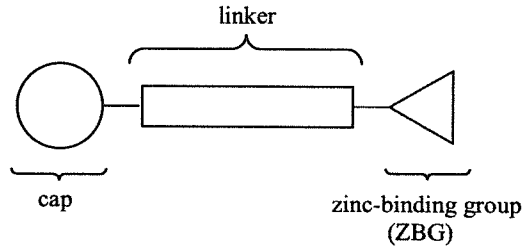


Fig. (12). Pharmacophoric summary of the structural characteristics of HDAC inhibitors.

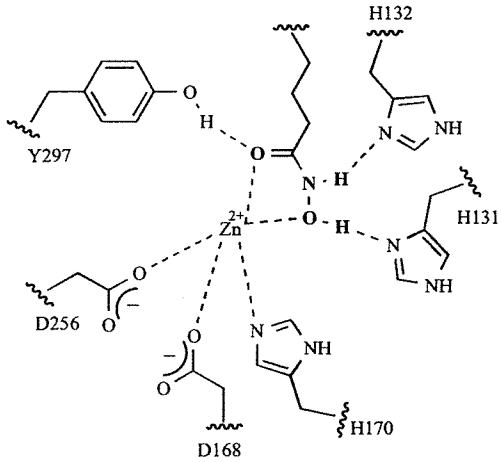


Fig. (13). Hydroxamic acid in the catalytic core of HDLP [86].

Although hydroxamic acids are frequently employed as ZBGs, they often have metabolic and pharmacokinetic problems such as glucoronidation and sulfation that result in a short half-life *in vivo*. Many hydroxamates are unstable *in vivo*, and are prone to hydrolysis. Such concerns over metabolic stability and the toxicity [129] associated with hydoxamic acids have triggered research activities to find replacement groups that possess strong inhibitory action against HDACs [130]. Examples of non-hydroxamate inhibitors of Class I and Class II HDACs are shown in Fig. **15**.

Small fatty acids including sodium butyrate **29**, sodium phenylbutyrate **30** and valproic acid **31** are one of the most important classes of non-hydroxamate inhibitors. Although their HDAC inhibitory activity is weak (IC_{50} > 0.1 mM), they have antiproliferative and differentiating activity against several types of cancer cells [131-134]. They most probably

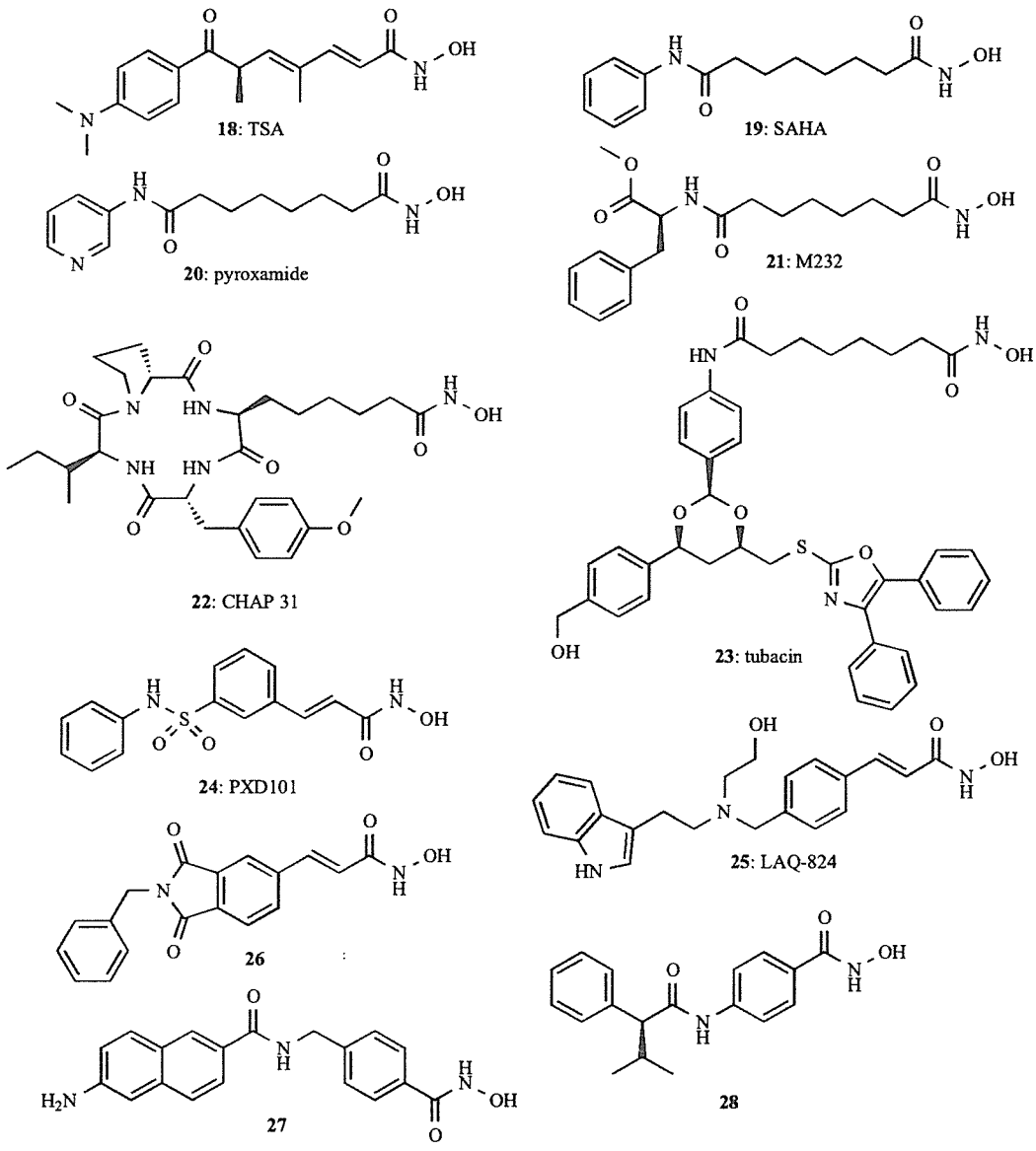


Fig. (14). Examples of hydroxamate inhibitors of Class I and Class II HDACs.

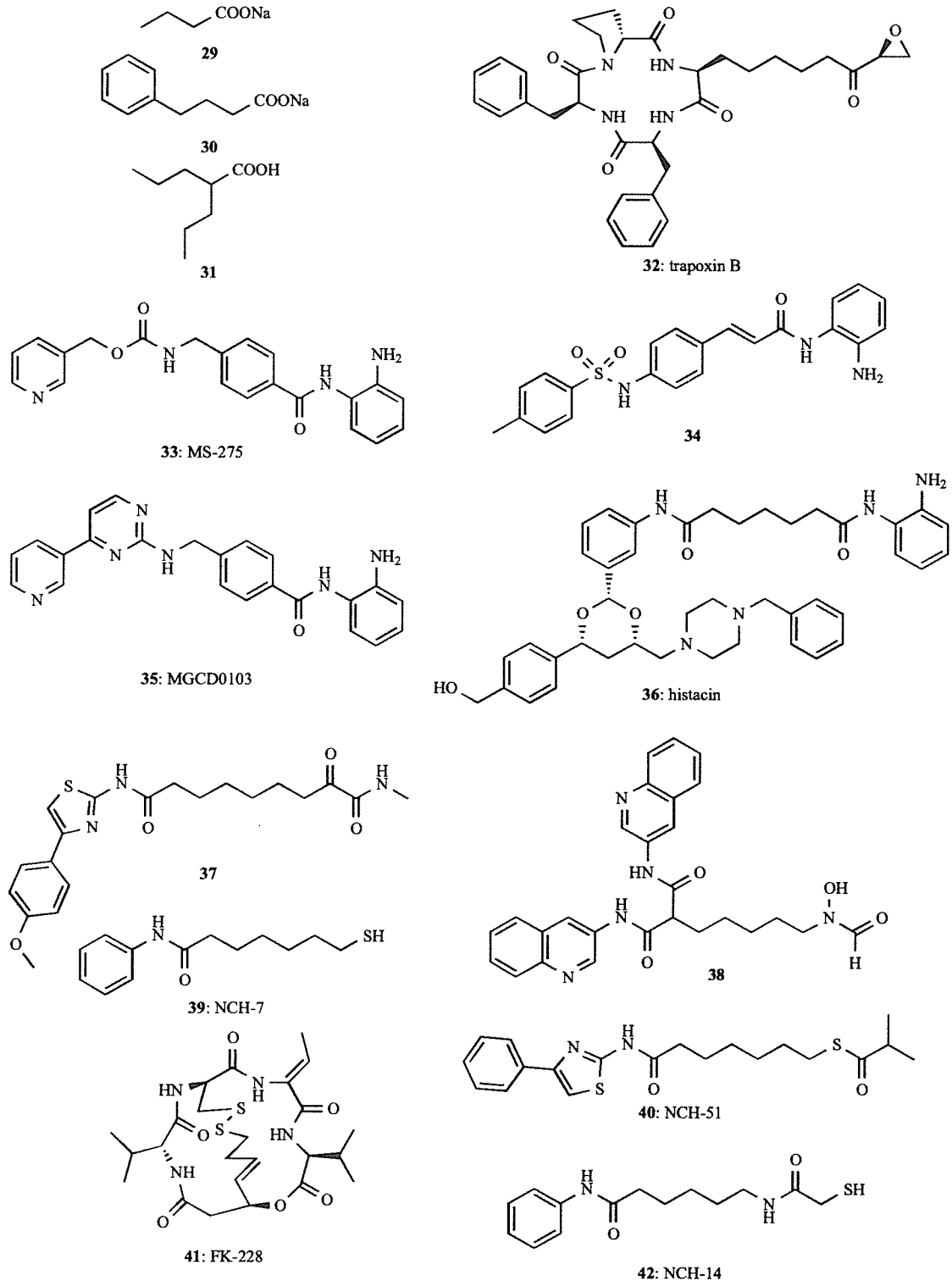


Fig. (15). Examples of non-hydroxamate inhibitors of Class I and Class II HDACs.

inhibit HDACs by binding to their catalytic center. Some of the small fatty acid analogues such as **29** and **31** are currently in Phase I or Phase I/II trials for the treatment of cancer [135].

Cyclic tetrapeptides bearing epoxyketone such as trapoxin B **32** were originally discovered by the screening of natural products for antiparasitic or antiproliferative activity [136-138]. These compounds were also found to be HDAC inhibitors [139, 140]. Experiments *in vitro* using mouse

histone deacetylase revealed that **32** irreversibly inhibits histone deacetylase [140]. It is speculated that the epoxide of **32** is attacked by an active site nucleophile and forms a covalent complex with the HDAC. Subsequently, a matrix derived trapoxin analogue was used to isolate a nuclear protein, HDAC1, for the first time, using affinity chromatography [72].

o-Aminoanilide derivatives are another class of non-hydroxamate inhibitors, and some of them are in clinical development. The first reported *o*-aminoanilide HDAC inhibitor was MS-275 **33**, which was identified from a set of synthetic benzamide derivatives [141]. MS-275 **33** inhibited HDACs with an IC_{50} of 4.8 μM and showed significant oral anticancer activity without severe side effects in animal models [142]. MS-275 **33** is currently in phase II clinical trials for the treatment of cancer. Bouchain *et al.* at Methyl-Gene Inc. identified sulfonamide anilides such as **34** as inhibitors of HDACs [143, 144]. Compound **34** exhibited antitumor activity *in vitro* and *in vivo*. Its antitumor activity correlated well with its ability to alter the expression of cell cycle regulators such as $p21^{WAF1/CIP1}$ and cyclin A and to arrest the cell cycle. In addition, MGCD0103 **35**, another *o*-aminoanilide compound from MethylGene Inc., is in clinical trials for the treatment of cancer [145]. Schreiber and co-workers prepared approximately 2,400 *o*-aminoanilides using a combinatorial approach in order to find isozyme-selective HDAC inhibitors [115]. Interestingly, this effort led to the identification of histacin **36**, an inhibitor inactive toward HDAC6 in cells [113, 114]. Presently, it is unclear whether *o*-aminoanilide derivatives interact with the zinc ion in the active site or bind at an allosteric site. However, the similarity in structure-activity relationships between *o*-aminoanilides and hydroxamates indicates that *o*-aminoanilides inhibit HDACs in a binding mode similar to hydroxamates [144, 146-148].

Electrophilic ketones have been described as non-hydroxamate HDAC inhibitors [149-154]. In aqueous medium, these exist in equilibrium with their respective hydrates, which could chelate the zinc ion in the active site of HDACs. Among several types of electrophilic ketone inhibitors, α-ketoamides such as compound **37** showed potent inhibition of HDAC (IC_{50} < 10 nM) and excellent antiproliferative activity against HT1080 and MDA435 cell lines (IC_{50} < 1 μM) [152]. Notably, compound **37** exhibited significant antitumor effects in an *in vivo* tumor model.

Compounds bearing *N*-formyl hydroxylamine could inhibit HDACs by forming a bidentate chelate with zinc ion in the active site of Class I and Class II HDACs. Schultz and co-workers synthesized a series of *N*-formyl hydroxylamines and evaluated their biological activity [155]. Among these compounds, **38** was the most potent HDAC inhibitor (IC_{50} < 1 μM). In addition, the antiproliferative activity of compound **38** was comparable to that of SAHA **19**.

We conducted a systematic study of ZBGs for Class I and Clas II HDAC inhibitors [156-161], and identified several new lead structures. Notably, thiol was found to be a ZBG with inhibitory activity comparable to hydroxamic acid [157, 159]. NCH-7 **39**, in which the hydroxamic acid of SAHA **19** is replaced by a thiol, was as potent as SAHA **19** in an enzyme assay using HeLa nuclear extracts (IC_{50} of SAHA **19** = 0.28 μM, IC_{50} of **39** = 0.21 μM). The results of an enzyme kinetic assay (Lineweaver-Burk plot) established that thiol **39** engages in competitive inhibition with acetylated lysine substrate, which suggests that a thiol interacts with a zinc ion in the active site. Optimization of the linker and aromatic cap of **39** and a prodrug-based approach led to a highly potent cancer cell growth inhibitor, NCH-51 **40**. NCH-51 **40** displayed potent antiproliferative activity against various human cancer cells, with EC_{50} values ranging from 1 to 10 μM, and these activities were comparable to those of SAHA **19** (average EC_{50} of **40** 3.8 μM, SAHA **19** 3.7 μM). In addition, treatment of HCT 116 cells with compound **40** gave rise to elevated and dose-dependent levels of acetylated histone H4 and $p21^{WAF1/CIP1}$. These results suggest that the antiproliferative activity of compound **40** significantly is caused by the inhibition of HDACs. As for a thiol as ZBG, it was demonstrated that the disulfide bond of FK228 **41**, a depsipeptide HDAC inhibitor, is reduced in the cellular environment, releasing the free thiol analogue as the active species [162].

We and others reported mercaptoacetamide as an alternative to hydroxamic acid [158, 163, 164]. NCH-14 **42** had an IC_{50} of 0.39 μM and exhibited competitive inhibition versus acetylated lysine substrate, which indicates that a mercaptoacetamide interacts with a zinc ion in the active site. Computational analysis suggests that **42** inhibits HDACs by chelating the zinc ion in a bidentate fashion through its sulfur and oxygen atoms. Since mercaptoacetamides are reported as potent, long-lasting, and less toxic matrix metalloproteinase inhibitors [165, 166], they could be improved HDAC inhibitors. In addition, recent reports on the theoretical study of non-hydroxamate ZBGs supports the findings discussed here [167].

Interestingly, several recent studies revealed that many non-hydroxamate HDAC inhibitors are inactive against HDAC6 [114, 130], indicating that non-hydroxamates exhibit a significant selectivity. Consequently, further study of non-hydroxamate HDAC inhibitors may offer a basis for the design of isozyme-selective inhibitors, and may at the same time surmount the possible toxicological and metabolic problems associated with hydroxamates.

Class III HDACs: Sirtuins

Sirtuins including human SIRT1-7 form their own separate class of HDACs (Class III) (Table **2**) [74]. They share homology with the yeast enzyme Sir2 (silent information regulator-2) [168]. The yeast Sir2 was originally described as a factor required for maintenance of gene silencing at the mating type loci, telomeres and rDNA [169-172], and was subsequently shown to be associated with the extension of lifespan conferred by caloric restriction [173]. In addition, the function of sirtuins has been suggested to correlate with some cancers. SIRT1, one of the human sirtuins, has been reported to exert a regulatory effect on the non-histone protein p53 *via* the deacetylation of Lys 382 [174-176]. Very recently, it has been shown that SIRT1 regulates HIV transcription by deacetylating the HIV Tat protein [177].

The deacetylation of histone and non-histone proteins catalyzed by sirtuins has been shown to be NAD^+-dependent, releasing nicotinamide and *O*-acetyl-ADP-ribose [178-183]. Recently, Marmorstein and co-workers reported the high-

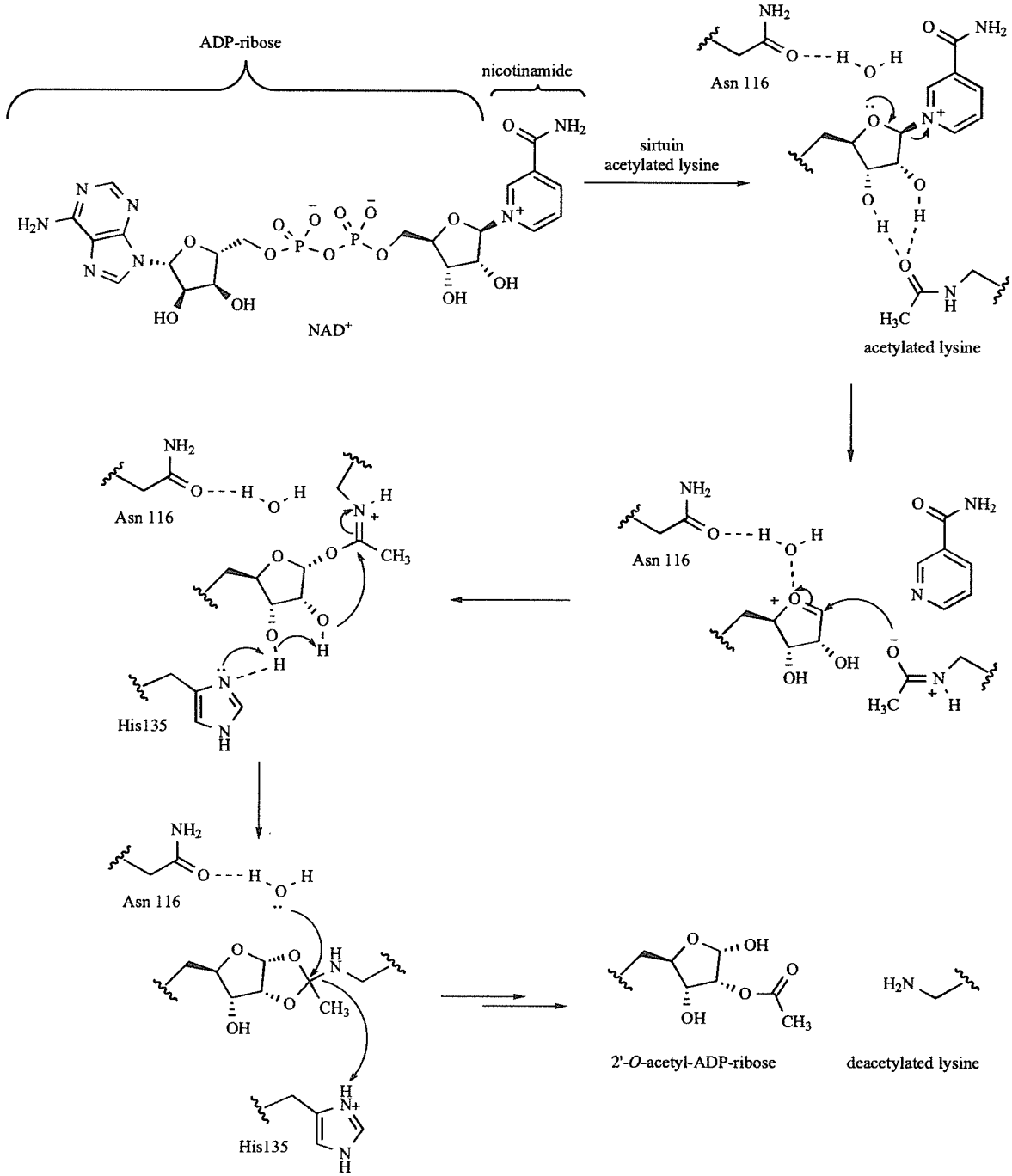


Fig. (16). Proposed catalytic mechanism for the deacetylation of acetylated lysine by sirtuins [184].

resolution ternary structure of yeast Hst2 (homologue of Sir2) with an acetylated histone H4 peptide and a nonhydrolyzable NAD^+ analogue, carba-NAD^+, as well as a ternary complex with ADP-ribose, a reaction intermediate analog formed immediately after NAD^+ hydrolysis [184]. These crystal structures have led to a solid understanding of the catalytic mechanism for the deacetylation of acetylated lysine substrates by sirtuins (Fig. **16**). First, nicotinamide is released from NAD^+ to yield an oxonium cation intermediate that is stabilized by a water molecule coordinated with Asn 116. Next, the oxygen of the acetylated lysine substrate undergoes nucleophilic attack to the oxonium cation intermediate. The resulting imido ester intermediate is attacked by the 2'-OH group of the ADP ribose indirectly activated by His 135. Finally, the cleavage of the acetal analogue by the water coordinated to Asn 116 and the protonated His 135 affords deacetylated lysine and 2'-*O*-acetyl-ADP-ribose. This proposed mechanism may have implications for the design of sirtuin inhibitors or activators.

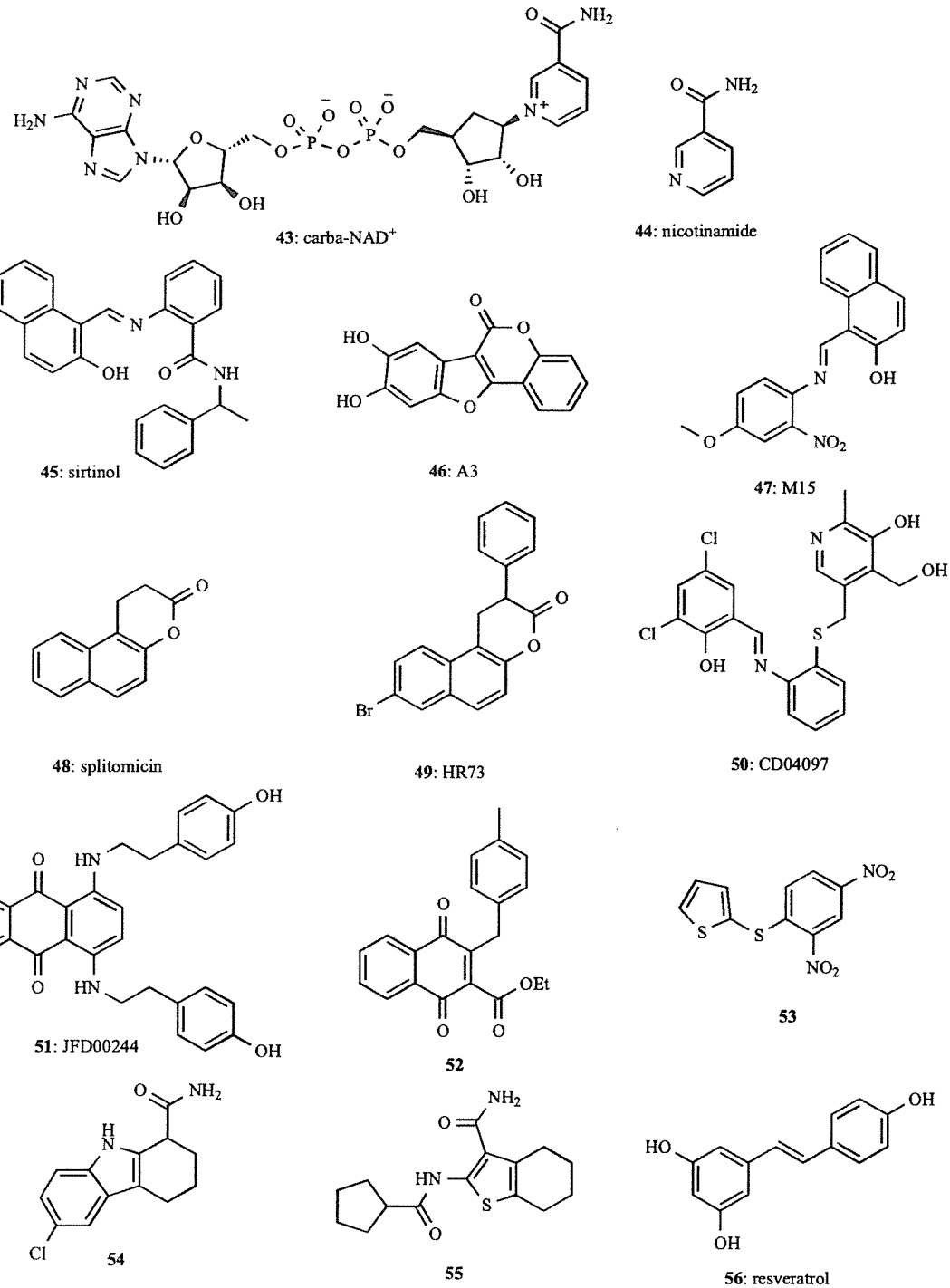


Fig. (17). Structures of sirtuin modulators.

Sirtuin Modulators

Sirtuin modulators are needed to elucidate the biological functions of these enzymes and to prevent or treat cancer and age-related diseases. The first reported inhibitors of sirtuins were carba-NAD^+ **43** and nicotinamide **44** (Fig. **17**) [180]. As expected from its structure, carba-NAD^+ **43**, a non-hydrolyzable NAD^+ analogue, inhibited a Sir2 homologue (HST2) by competing with NAD^+ (K_i = 200 μM). In contrast to carba-NAD^+ **43**, nicotinamide **44** was a non-competitive inhibitor of HST2. It is likely that nicotinamide **44** inhibits the enzyme through product inhibition, by shifting the equilibrium in the reaction, enzyme + NAD^+ ↔ enzyme-ADP-ribose + nicotinamide, towards the reactants.

Nicotinamide **44** also inhibited yeast Sir2 and human SIRT1 (IC_{50} < 50 μM), and it strongly inhibited yeast silencing, increased rDNA recombination and shortened replicative lifespan to that of a *sir2* mutant [185]. Schreiber and co-workers identified sirtinol **45**, A3 **46** and M15 **47** as sirtuin inhibitors by a high throughput phenotypic screening in cells [186]. These three compounds inhibited yeast Sir2 transcriptional silencing activity in cells, and yeast Sir2 and human SIRT2 deacetylase activity *in vitro* (IC_{50}s = 30-70 μM). Recently, Mai and co-workers studied the structure-activity relationships of sirtinol analogues and found that 4-[(2-hydroxy-1-naphthalenylmethylene)amino]-*N*-(1-phenylethyl)benzamide (*para*-sirtinol) was 10-fold more potent than sitinol against SIRT1 enzyme [187]. Splitomicin **48** was discovered by the screening of a 6000-compound library as a yeast Sir2 inhibitor (IC_{50} = 60 μM) [188]. However, splitomicin **48** did not inhibit human SIRT1. Studies of the structure-activity relationship and structural optimization of splitomicin analogues [189, 190] led to the identification of HR73 **49** which inhibits human SIRT1 with an IC_{50} of 5 μM [177]. Since HR73 **49** also inhibited the deacetylation of HIV Tat protein and HIV transcription, this compound may serve as a lead compound in the treatment of HIV-1 infection. Tervo and co-workers discovered novel human SIRT2 inhibitors using an in silico approach [191]. Molecular modeling, virtual screening and subsequent experimental tests resulted in the identification of two novel inhibitors, CD04097 **50** and JFD00244 **51**, which inhibit SIRT2 with IC_{50}s of 74 μM and 57 μM, respectively. The compounds, structurally related to **51**, have been presented by Guilford Pharmaceuticals Inc. A naphthoquinone derivative **52** inhibited human SIRT2 with an IC_{50} of 3.6 μM [192]. The same group also identified 2-(2',4'-dinitrophenylthio)thiophene **53** as a new sirtuin inhibitor. Compound **53** had an IC_{50} of 3.0 μM in a SIRT2 assay [193]. Indole derivatives bearing amide such as **54** have been reported as selective SIRT1 inhibitors for potential use in diseases such as cancer and metabolic disorders [193, 194]. A high level of activity was exhibited by compound **54** (IC_{50} = 0.098 μM), the most potent inhibitor of sirtuins reported so far. Tetrahydro benzothiophene derivatives having amide such as **55** have also been found to be potent SIRT1 inhibitors (IC_{50} of **55** = 0.20 μM) [195].

Plant polyphenols such as resveratrol **56** and butein were reported as sirtuin activating compounds (STACs) [196]. Of these STACs, resveratrol **56** increased deacetylation of a modified p53 peptide substrate approximately 13-fold for human SIRT1 and 2-fold for yeast Sir2. STACs have also been reported to increase the lifespan of yeast [196], flies and worms [197]. However, it is unclear whether STACs really activate the deacetylation of internal proteins. Bedalov and co-workers showed that *in vitro*, resveratrol **56** enhances the binding and deacetylation of peptide substrates that contain a non-physiological fluorescent moiety which is used for assaying sirtuin fluorescent activity, but has no effect on the binding and deacetylation of acetylated peptides lacking the fluorophore [198]. Additionally, they also found that resveratrol **56** has no detectable effect on sirtuin activity *in vivo*. These findings suggested that the longevity effects of resveratrol **56** are not due to the activation of sirtuins, and there must be other mechanisms that account for the effects of resveratrol **56**.

HISTONE METHYLATION MODULATORS

Histone Lysine Methylation and Demethylation

It has been reported that the methylation of histone (H) lysine (K) residues occurs at H1K26, H3K4, H3K9, H3K27, H3K36, H3K79 and H4K20, and is responsible for transcriptional activation as well as silencing (Table **3**) [199, 200]. In addition, the ε-amino group of lysine residues can be methylated in the form of mono-, di- or trimethylation, and this differential methylation gives functional diversity to each site of lysine methylation. For example, di-methylation at H3K4 occurs at both inactive and active genes, whereas tri-methylation is exclusive to active genes [201].

Table 3. Specificity and Function of HKMTs [199, 200]

Substrate	HKMT	Function
H3K4	ySET1	transcriptional activation
	SET7/9	transcriptional activation
	MLL	transcriptional activation
	Ash1	transcriptional activation
	SMYD3	transcriptional activation
H3K9	SUV39H1	transcriptional repression
	SUV39h2	transcriptional repression
	Clr4	transcriptional repression
	Dim5	transcriptional repression
	Kryptonite	transcriptional repression
	G9a	transcriptional repression
	Eu-HMTase	transcriptional repression
	ESET/SETDB1	transcriptional repression
	E(Z)/EZH2	transcriptional repression
	Ash1	transcriptional activation
H3K27	E(Z)/EZH2	transcriptional repression
	Ezh2	X-chromosome inactivation
H3K36	Set2	transcriptional activation
	NSD1	unknown
H3K79	Dot1/DOT1L	transcriptional repression
H4K20	SET9	DNA damage
	Pr-SET7/Set8	transcriptional repression
	SUV4-20	transcriptional repression
	Ash1	transcriptional activation
	NSD1	unknown
H1K26	EZH2	unknown

To date, a number of histone lysine methyltransferases (HKMTs) have been identified, and many of them display substrate specificity [198, 199] (Table **3**). With the exception of Dot1/Dot1L [202, 203], HKMTs have the evolutionally conserved SET domain that is necessary for HKMT activities [204]. The high resolution crystal structure of a ternary complex of human SET7/9 (a mono-methylase) with

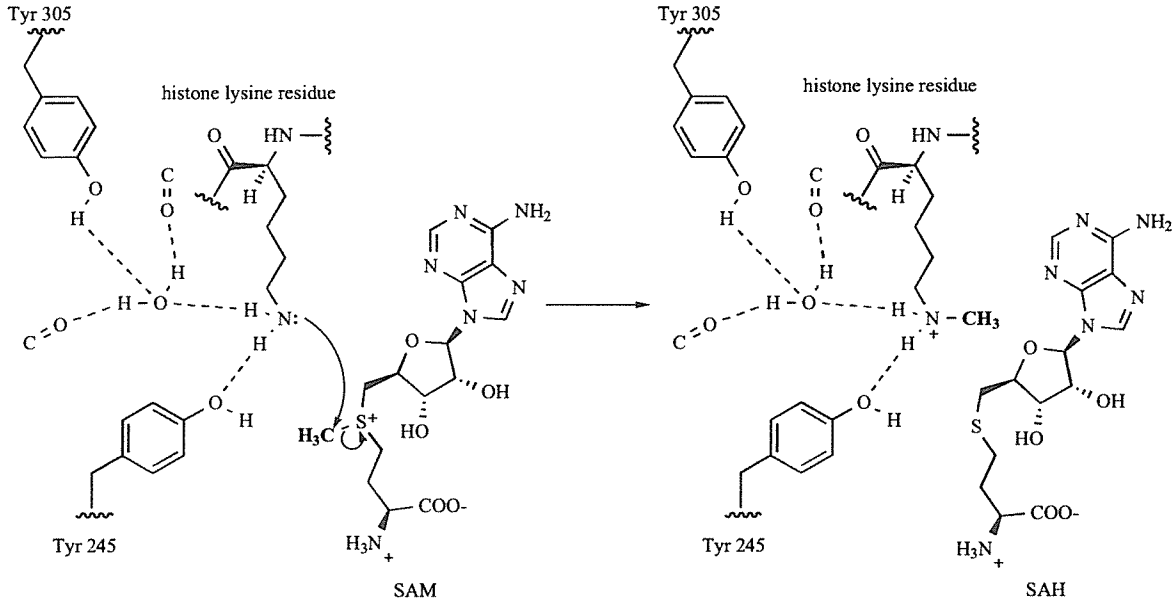


Fig. (18). Proposed catalytic mechanism for the methylation of lysine by SET7/9 [205].

a histone peptide and SAH has provided insights into the catalytic mechanism for the histone lysine methylation (Fig. **18**) [205]. The crystal structure showed that the substrate lysine is stripped of all solvent molecules except for the one water molecule and this desolvation will lower the p*K*a of the lysine amino group as well as enhance its nucleophilicity. The nucleophilic nitrogen of the lysine attacks the methyl group of SAM to produce methylated lysine and SAH. The resulting protonated methyl lysine is stabilized by Tyr 245 and the water molecule coordinated with Tyr 305 and the two main chain carbonyls. Inspection of the sequence of other HKMTs such as SUV39H1 (a tri-methylase) [206, 207] and G9a (a di-methylase) [208] suggests why these enzymes catalyze the di- or tri-methylation of their lysine targets. Tyr 305 is substituted for a valine in SUV39H1 and a proline in G9a. These substitutions would seem likely to produce a cavity in the active site that could accommodate an existing methyl group on the lysine.

Lysine-specific demethylase 1 (LSD1) has recently been identified as an enzyme which is able to demethylate a specific lysine (H3K4) and is associated with transcriptional repression [209]. More recently, it has been reported that the androgen receptor appears to change the specificity of LSD1 from H3K4 to H3K9, which activates the transcription of androgen receptor target genes [210]. Demethylation by LSD1 is considered to proceed *via* an amine oxidase reaction as shown in Fig. **19** [209]. As described above, LSD1 demethylates methylated H3K4 or H3K9 specifically. However, as can be expected from the mechanism involved, demethylation by LSD1 is limited to mono- or di-methylated lysine. LSD1 can not demethylate tri-methylated lysine. Thus far, the enzyme that is able to demethylate tri-methylated lysine has not been identified.

Histone Lysine Methylation Modulators

Recently, a group led by Imhof reported the first HKMT inhibitor [211]. To find small molecules that inhibit the activity of recombinant *Drosophila melanogaster* SUV39 protein, they screened 2,976 compounds. Strong inhibition was observed with chaetocin **57** (Fig. **20**), which was initially isolated from the fermentation broth of *Chaetomium minutum* and belongs to the class of 3-6 epi-dithio-

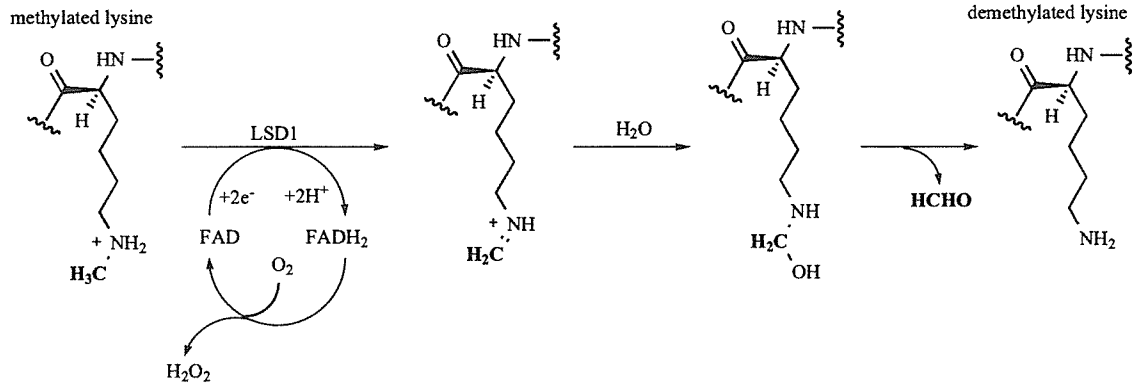
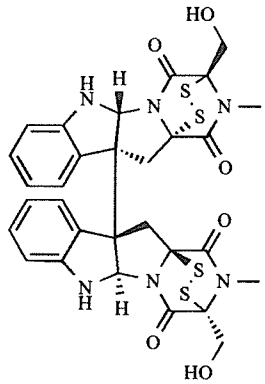


Fig. (19). Reaction mechanism for methyl group removal by LSD1 [209].

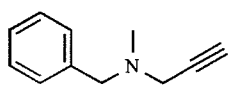
diketopiperazines. Chaetocin **57** had an IC_{50} of 0.6 μM and acted as a competitive inhibitor for SAM. Furthermore, chaetocin **57** inhibited the human ortholog of SUV39, mouse G9a and *Neurospora crassa* Dim5 with IC_{50}s of 0.8 μM, 2.5 μM and 3 μM, respectively, whereas it did not exhibit strong inhibition of E(Z), Pr-SET7 and SET9 (IC_{50} > 90 μM). These results suggest that chaetocin **57** is a specific inhibitor of enzymes belonging to the SUV39 family. Since chaetocin **57** also shows inhibition of SUV39 *in vivo*, it may be used to study the epigenetic mechanism of these enzymes.



57: chaetocin

Fig. (20). Structure of chaetocin **57**.

As mentioned above, LSD1 is an amine oxidase that catalyzes the demethylation of mono- or di-methylated histone lysine residues. Therefore, monoamine oxidase inhibitors were expected to inhibit LSD1. Schüle and co-workers tested whether pargyline **58** (Fig. **21**) [212], a well-known monoamine oxidase inhibitor, inhibits LSD1, and found it blocks demethylation of H3K9 by LSD1 and consequently androgen receptor-dependent transcription [210]. These results suggested the modulation of LSD1 activity provides a new strategy to regulate specific gene expression. It is hoped that a LSD1-specific modulator will be discovered in the near future.



58: pargyline

Fig. (21). Structure of pargyline **58**.

Histone Arginine Methylation and Deimination

In histones, methylation occurs not only at lysine residues but also at arginine residues [213]. The methylation of histone (H) arginine (R) residues occurs at H3R2, H3R8, H3R17, H3R26 and H4R3. Recently, methylation of histone H2A and H4 has also been reported [214]. Methylation at H3R17, H3R26 and H4R3 has been reported to correlate with gene activation [215-217], whereas methylation at H3R8 has been reported to be associated with gene repression [218] (Table **4**). Four enzymes, protein arginine *N*-methyltransferase (PRMT) 1, PRMT4 (CARM1), PRMT5 and PRMT7, have been identified as histone arginine methyltransferases (HRMTs) [213, 214]. While PRMT4 methylates H3R2, H3R17 and H3R26, PRMT1 and PRMT5 specifically methylate H4R3 and H3R8, respectively (Table **4**). A recent report has revealed that PRMT7 is specific for histones H2A and H4 [214]. In addition, it has been found that PRMT1 and PRMT4 catalyze the asymmetric di-methylation of arginines, whereas PRMT5 and PRMT7 catalyze symmetric di-methylation (Fig. **22**). These enzymes are known to catalyze the transfer of a methyl group from SAM to the guanidino group of arginines, but the precise catalytic mechanism has not been clarified.

Table 4. Specificity and Function of HRMTs [214-217]

Substrate	HRMT	Function
H3R2	PRMT4	unknown
H3R8	PRMT5	transcriptional repression
H3R17	PRMT4	transcriptional activation
H3R26	PRMT4	transcriptional activation
H4R3	PRMT1	transcriptional activation
H2A, H4	PRMT7	unknown

Although it is unclear whether histone arginine methylation is reversible or irreversible, the deimination of methylated arginines has been reported. Kouzarides and co-workers showed that peptidyl arginine deiminase 4 (PADI4) deiminates non-methylated or mono-methylated arginine residues of R2, R8, R17 and R26 in the H3 tail (Fig. **23**) [219]. They also demonstrated that deimination by PADI4 prevents arginine methylation by PRMT4, and PADI4 can repress hormone receptor-mediated gene induction. More recently, deimination of H4R3 in HL-60 granulocytes has also been reported by Hagiwara *et al.* [220].

PRMT Inhibitors

PRMT modulators are expected to be used as tools for elucidating the role of arginine methylation, and might be anticancer agents because histone arginine methylation has been reported to regulate the expression of tumor suppressor genes [218]. There has been only one report regarding PRMT modulators so far. Bedford and co-workers screened a library of 9,000 compounds to find small molecule regulators of PRMTs, and identified AMI-1 **59** (Fig. **24**) as a specific PRMT inhibitor [221]. AMI-1 **59** specifically inhibited arginine methyltransferases (IC_{50} (PRMT1) = 1.63 μM) but not lysine methyltransferases *in vitro*, and did not compete for the SAM binding site. Furthermore, AMI-1 **59** prevented arginine methylation and modulated nuclear receptor-regulated transcription *in vivo*.

HISTONE PHOSPHORYLATION MODULATORS

Histone Phosphorylation and Dephosphorylation

Phosphorylation has been shown to occur on all histones. Notably, phosphorylation at H3T3, H3S10, H3S28 and H2AT119 was reported to be correlated with cell cycle progression during mitosis and meiosis, and with transcriptional gene activation during interphase [222-225]. Recent studies have revealed that Aurora kinases (Aurora A, B and C) are required for mitotic phosphorylation of H3, and Nucleosomal histone kinase-1 (NHK-1) phosphorylates

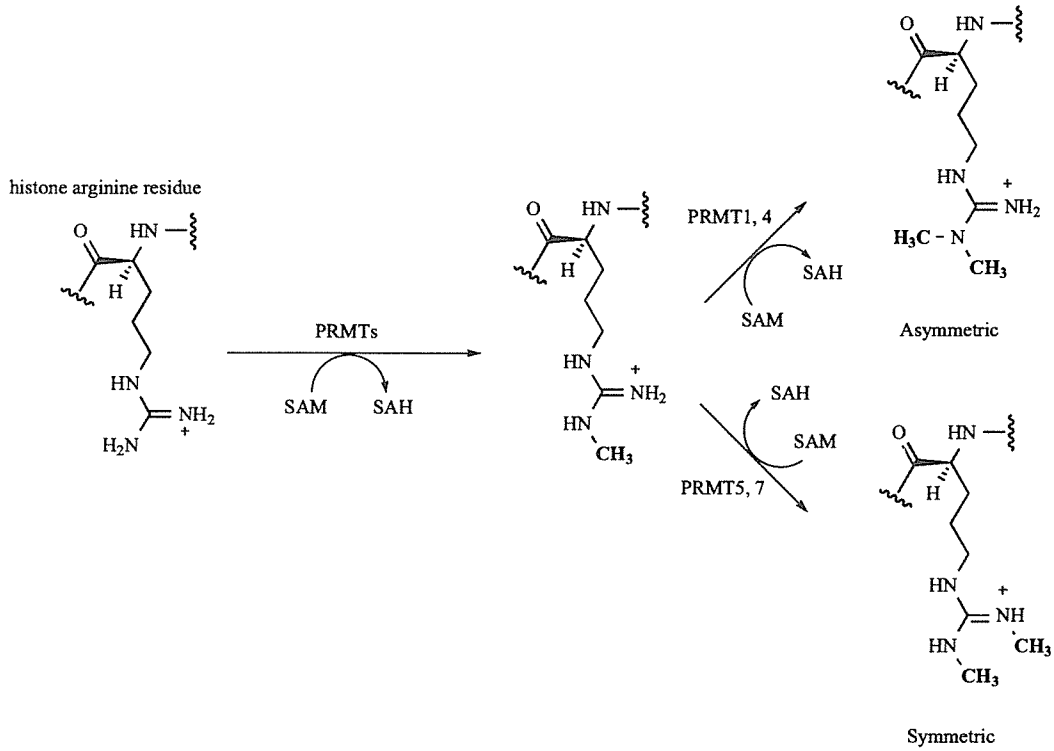


Fig. (22). Methylation of histone arginine residues.

H2AT119 during mitosis [223, 224, 226, 227]. More recently, it has been reported that the kinase haspin is required for mitotic H3T3 phosphorylation and normal metaphase chromosome alignment [225], and mixed-lineage kinase-like mitogen activated protein triple kinase-α (MLTK-α) mediates the phosphorylation of H3S28 [228]. Among these kinases, Aurora kinases have been reported to be overexpressed in a wide range of human tumors and closely related to the formation of malignant tumors [229, 230]. Protein phosphatase (PP) 1 and PP2A are likely to be associated with dephosphorylation of H2A and H3 [231-234].

Aurora Kinase Inhibitors

Taylor and co-workers were the first to report a selective inhibitor of Aurora kinases. The screening of approximately 250,000 compounds provided a lead compound which was further modified to produce ZM447439 **60** (Fig. **25**) [235]. Compound **60** inhibited Aurora A and Aurora B with an IC_{50} of 0.11 and 0.13 μM, respectively. In contrast, the majority of other protein kinases assayed were not inhibited by compound **60**. In addition, compound **60** inhibited phosphorylation of histone H3 in mitotic DLD-1 cells. Furthermore, compound **60** blocked chromosome condensation, mitoic spindle assembly and the spindle integrity checkpoint [236]. In addition, the X-ray crystal

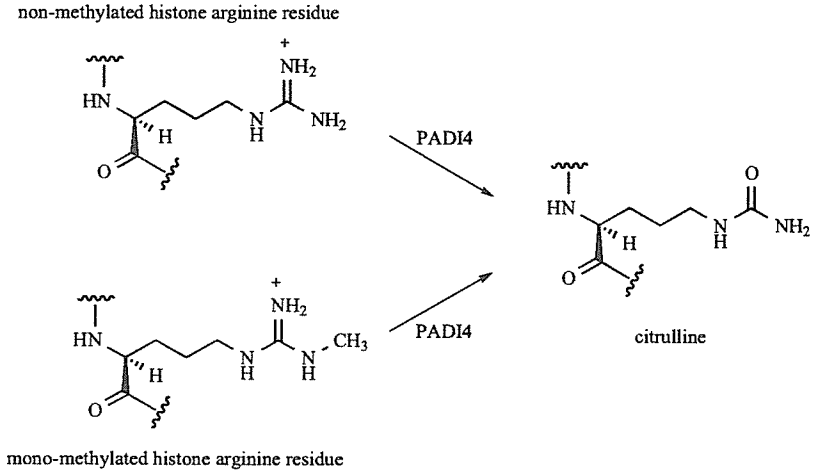
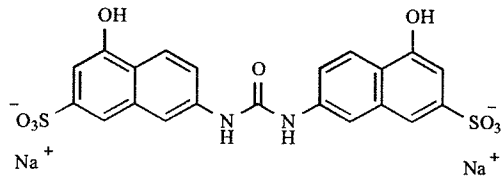


Fig. (23). Deimination of histone arginine residues.

structure of compound **61**, the related compound to **60**, bound to Aurora A has recently been published [237]. This has aided numerous insights into the design of Aurora kinase inhibitors [238]. VX-680 **62** is a potent and selective Aurora kinase inhibitor which was designed based on the three dimensional structure of Aurora A [239]. VX-680 **62** inhibited all three Aurora kinases with an apparent inhibition constant ($K_{i(app)}$) of 0.6, 18 and 4.6 nM for Aurora A, Aurora B and Aurora C, respectively. VX-680 **62** showed greater than 100-fold selectivity for the Aurora A kinase over 55 other kinases tested, the only exception being Fms-related tyrosine kinase 3 ($K_{i(app)}$ = 30 nM). VX-680 **62** blocked cell-cycle progression, induced apoptosis in a variety of cancer cells and caused profound tumor growth inhibition in *in vivo* xenograft models. These results indicated that inhibition of Aurora kinases is a new approach to cancer therapy. Hesperadin **63** has been reported to be an inhibitor of the catalytic activity of Aurora B [240, 241]. Treatment of MCF7 and PC3 cancer cells with hesperadin **63** inhibited proliferation due to multiple mitotic defects caused by a reduction in Aurora B activity.



59: AMI-1

Fig. (24). Structure of AMI-1 **59**.

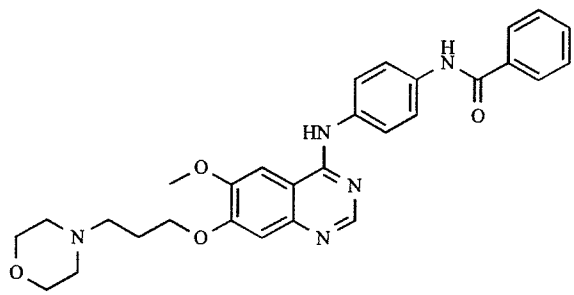
Histone Phosphatase Inhibitors

Natural products okadaic acid **64**, fostriecin **65** and microcystin LR **66** (Fig. **26**), specific inhibitors of PP1 and PP2A, have been reported to stimulate histone H3 phosphorylation [231-234]. Experiments using these inhibitors have shown that H3 phosphorylation has an intimate involvement in chromosomal condensation and the transcriptional activation of heat shock genes.

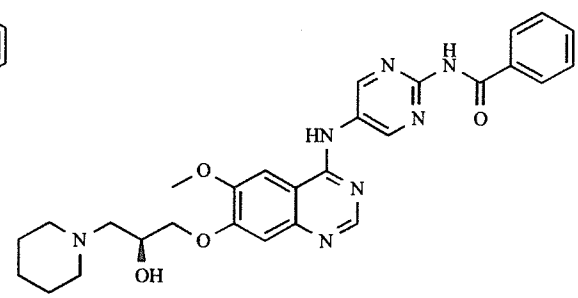
PERSPECTIVE

The worldwide search for natural products and synthetic molecules modulating DNA methylation and histone modifications has led to the discovery of the compounds presented in this review. These compounds have been used as tools to study epigenetic mechanisms and some of them have already appeared in clinical trials for the treatment of cancer. In particular, HDAC inhibitors such as SAHA and MS-275 have provided strong clinical evidence that epigenetic therapy is effective. The combination of HDAC inhibitors and DNA methylation inhibitors [242] is also being tested. In addition, recent studies have shown that histone methylation and phosphorylation are also involved in some disease states such as cancer. Consequently, further study of histone methylation and phosphorylation modulators may offer a basis for the treatment of such diseases.

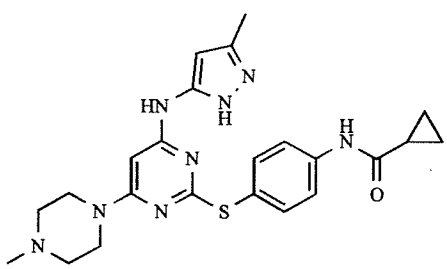
In this review, we have presented inhibitors of DNA methylation and modulators of histone acetylation, methylation and phosphorylation. However, other modifications to histones such as ubiquitinylation [243], sumoylation [244] and poly-ADP-ribosylation [245] have been observed, and are reported to be also associated with gene expression. Furthermore, novel enzymes associated with the ubiquitination [246] and poly-ADP-ribosylation [247] of histones have been reported. Small molecule modulators of these enzymes will improve our understanding of epigenetic mechanisms and bring about novel candidates for epigenetic therapy.



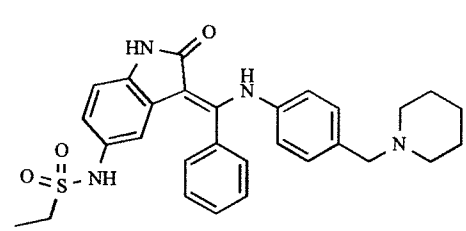
60: ZM447439



61



62: VX-680



63: hesperadin

Fig. (25). Aurora kinase inhibitors.