Review Article

Small Molecule Inhibitors of Histone Acetyltransferases as Epigenetic Tools and Drug Candidates

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Alteration of the acetylation state of histone proteins contributes to transcriptional regulation and epigenetic inheritance. Dysregulation of these processes may lead to human diseases, especially cancer. One of the major chromatin modifications is histone acetylation and this review gives an overview of the role of histone acetyltransferases, their structural aspects, as well as of chemical modulators targeting their enzymatical activities. Inhibitors and activators of histone acetyltransferases are presented and their capability to influence histone and non-histone protein acetylation levels is discussed. Development of small molecules as epigenetic tools that alter histone acetyltransferase activity will be helpful to better understand the consequences of histone and generally protein acetylation and potentially offer novel therapeutic approaches for the treatment of cancer and other diseases.

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Introduction

Epigenetics (Greek: *epi* over, in addition to) is defined as inheritable changes to the phenotype of a cell that occur without alterations in the genetic information represented by the DNA sequence [1]. Established in 1942 for the first time by C. H. Waddington, the term epigenetics comprises in the broadest sense ''meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself'' [2]. DNA methylation of cytosine within CpG islands [3] and enzymatic post-translational histone modifications [4] are the main mechanisms of epigenetic control of transcription. These mechanisms contribute to the structure and activity of eukaryotic chromatin. Lately, also the role of small non-coding RNA has been recognized to be important in epigenetics [5–7].

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Epigenetic regulation of chromatin assembly and gene expression

Chromatin is known to be the structured element that carries the genetic information in eukaryotic cells. The DNA sequence is concentrated in the mammalian cell nucleus in form of chromatin fibers and encodes about 30,000 different genes, which contribute to an extraordinary variability of cell types in one organism. Thus, the primary genomic information is just the starting point for a large number of biochemical mechanisms responsible for cell differentiation, alterations in the gene expression patterns during different development stages as well as transcriptional activation or repression [8]. The existence of such regulatory machinery allows for distinct phenotypes in response to external and internal signals without changes in the gene sequence of the cell, that are passed on to the daughter generation.

Chromatin exists in two distinct functional forms: a condensed form (heterochromatin) represented during mitosis and meiosis and described to be repressive for transcriptional activation and a decondensed form (euchromatin), which is more accessible for transcriptional factors [9, 10]. As a highly ordered assembly of DNA double helix and proteins

chromatin consists of repeats of nucleosomes as the smallest structural subunit. The nucleosomes are composed of an octameric core containing two molecules of each histone protein H2A, H2B, H3, and H4 respectively. 147 base pairs of DNA are wrapped around this octamer. The H1 linker histone is responsible for the interaction with DNA and provides a stabilizing function into a higher-ordered structure of the assembly [11]. In this review, we will deal with histone modifications, specifically acetylation.

Histone modifications as epigenetic control mechanism

Over 60 residues in the amino-terminal histone tails have been shown to undergo enzymatic modification during different cell development stages. There are at least seven covalent chromatin modifications known: acetylation (lysine), methylation (lysine and arginine), phosphorylation (serine and threonine), ubiquitinylation (lysine), sumoylation (lysine), ADP ribosylation (glutamate and lysine), and deimination (arginine) as well as proline isomerisation [12]. Thus, histone proteins are subject to dynamic biochemical changes that lead to alterations of chromatin structure and accessibility for transcriptional events. Crosstalk and interplay of distinct histone modifications contribute to facilitate the dynamic equilibrium between transcriptionally active and silent chromatin. In fact, the hypothesis of a ''histone code'' seems to be the link between genetic information, outside signals, and the resulting cellular phenotype in a certain setting (e.g. developmental stages, carcinogenesis) [10, 13, 14].

Lysine acetylation: HAT-HDAC interplay

One of the most extensively studied histone modifications is the covalent attachment of an acetyl group from acetyl-CoA to the ε-amino group of lysine side chains that can be reversed by amide hydrolysis. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are responsible for the transfer and removal of acetyl groups. In 1964, Allfrey et al. were pioneers on this field, suggesting a functional correlation between histone acetylation and regulation of RNA synthesis [15]. In general, histone lysine acetylation is correlated with gene activation whereas histone lysine deacetylation is linked to gene repression and silencing. The transfer of an acetyl residue by HATs leads to neutralization of the positively charged ε-amino lysine group and hence impedes interaction with the negative charges of the DNA phosphate-backbone. In consequence, histone acetylation results in a more extended chromatin form, which is accessible for transcriptional factors (euchromatin). On the other hand, deacetylated lysine residues can form highly ordered packaging with the DNA backbone due to electrostatic interactions and hence can hamper transcriptional initiation processes [16]. In mammalian cells a number of biochemical pathways have been described that regulate the equilibrium between HAT and HDAC activity, e.g. phosphorylation, autoacetylation, protein-protein interactions or the cellular acetyl-CoA level [17]. The involvement of lysine acetylation in fundamental processes like cell differentiation, gene expression, vascular remodeling, neuronal plasticity, inflammation or metabolic events reveals this covalent modification as a master regulator in cellular biology [18–23]. Cancer manifestation has also been found to correlate with the acetylation state of certain oncogenes and tumor suppressor genes in malignant cells [24–26]. Apart from histone modifications, reversible acetyl transfer to lysine residues is widespread across several biological processes also outside the nucleus which points to a general function in the regulation of protein activity. The acetylome uncovers the global role of protein acetylation, and many non-histone substrates for acetylating enzymes e.g. p53, c-myc, c-fos, α-tubulin, cohesin, NF-κB, interleukins are known [27–30]. Therefore, histone acetyltransferases have been currently classified as lysine acetyltransferases (KATs), which also indicates the broad spectrum of protein substrate acceptance [31].

HAT families

According to the cellular localisation, histone acetyltransferases are divided into two different classes. Type B HATs are cytoplasmic and responsible for acetylation of newly synthesized histone proteins. This is required for the transport of *de novo* translated histones through the nuclear membrane and the subsequent replacement into newly replicated DNA. Human HAT 1 (*KAT1*) is a member of type B HATs and is involved in histone deposition and DNA repair. Type A HATs include a number of heterogenic enzymes that share nuclear localisation and global functional similarity due to catalysis of transcriptional related processes [32]. Within this group, HAT members can be classified into five distinct families based on structural homology in the primary sequence as well as on biochemical mechanism of acetyl transfer (Table 1). Human Gnc5 (*KAT2A*), PCAF (*KAT2B*) and ELP3 (*KAT9*) belong to the GNAT family and are known as transcriptional activators with more than 70% sequence homology. The paralogs CBP (*KAT3A*) and p300 (*KAT3B*) form an independent family with more than 75 protein substrates described so far [33]. Members of the MYST family (Tip60 (*KAT5*), MOZ/MYST3 (*KAT6A*), MORF/MYST4 (*KAT6B*), HBO1/MYST2 (*KAT7*), and HMOF/MYST1 (*KAT8*)) are involved e.g. in HIV Tat interaction, dosage compensation, DNA repair and gene silencing [34]. TAF1/TBP (*KAT4*) and TIFIIIC90 (*KAT12*) are members of the

Table 1. Nuclear type A HAT families: selected human members, histone lysine residues selectivity and global functional responsibilities.

Family	HAT	KAT	Histone substrate	Function
GNAT	Gcn5	KAT2A	H3*K9,14,18,36*	transcriptional activation,
	PCAF	KAT2B	H3*K9,14,18,36*	DNA repair
	ELP3	KAT9		
p300/CBP	CBP	KAT3A	H2A*K5* H2B*K12,15* H3*K14,18* H4*K5,8*	transcriptional activation
	p300	KAT3B	H2A*K5* H2B*K12,15* H3*K14,18* H4*K5,8*	
MYST	Tip60	KAT5	H4*K5,8,12,16*	transcriptional activation,
	MOZ/MYST3	KAT6A	H3*K14*	DNA repair, replication
	MORF/MYST4	KAT6B	H3*K14*	dosage compensation
	HBO1/MYST2	KAT7	H4*K5,8,12*	
	HMOF/MYST1	KAT8	H4*K16*	
TF-related HATs	TAF1/TBP	KAT4		transcriptional activation,
	TFIIIC90	KAT12	H3*K9,14,18*	Pol III transcription
NR co-activators	SRC1	KAT13A		transcriptional activation
	AIB1/ACTR/SRC3	KAT13B		
	P160	KAT13C		
	CLOCK	KAT13D		

transcriptional factor related HAT family. Additionally, different steroid receptor co-activators have been described to be catalytically active histone acetyltransferases, e.g. SRC1 (*KAT13A*), AIB1/ACTR/SCR3 (*KAT13B*), p600 (*KAT13C*), CLOCK (*KAT13D*) and others [35, 36]. Several members of the HAT family exhibit distinct histone substrate specificity and the substrate affinity of recombinant enzymes *in vitro* might differ from the one *in vivo*. On the cellular level, functional interactions within protein complexes as well as influence of other histone modifications (e.g. phosphorylation) are crucial for substrate recognition and turnover. Nucleosomal acetylation occurs only in the context of *in vivo* HAT multiprotein complexes. Gcn5 is part of the complexes SAGA and Ada. Esa1, the yeast homolog of the mammalian Tip60, represents the catalytic subunit of NuA4 HAT complex that is responsible for specific histone H4 acetylation [37]. Finally, a number of HAT enzymes like PCAF and p300/CBP have also intrinsic catalytic activity, which facilitates the binding of transcriptional factors to the DNA [38].

HAT catalytic mechanisms

Despite structural diversity and broad substrate spectrum, members of the GNAT and MYST families share a universal mechanism of acetyl transfer mediated by a ternary complex. A highly conserved glutamate residue in the catalytic cleft (Gcn5 *Glu173*, PCAF *Glu570*, and Esa1 *Glu338*) is required for proton abstraction from the protonated ε-amino group in lysine substrates. This first step allows in consequence for nucleophilic attack to the cofactor with following acetyl residue transfer [39–42]. The crystal structure of p300/CBP as a complex with lysine-CoA has provided crucial knowledge about the mechanisms of substrate recognition and acetyl transfer. Acetylation occurs due to a ''hit and run'' catalysis according to Theorell-Chance kinetics, whereupon initial stable binding of acetyl-CoA is followed by a transient and rather weak binding of the lysine substrate [43]. This catalytic mechanism might contribute to the explanation of the broad substrate spectrum of p300/CBP and its global function as transcriptional activator.

Structural aspects of HATs

Structures from several subfamilies of nuclear HAT proteins are available either in its unbound form or in complex with cofactor, substrate, and/or bisubstrate inhibitors [43–52]. The nuclear HATs can be classified in five families based on their sequence similarity (Table 1). For more detailed information about the homology of the individual HATs the reader is directed to the literature, e.g. see ref. [53]. Despite the lack of significant sequence similarity between the individual HAT subfamilies, all HATs adopt a globular α/β fold in which

the central αβ core region is surrounded by a bundle of alpha helices at opposite ends (N- and C-terminal) of the enzyme. This central core region is associated with binding of the cofactor acetyl-CoA and is structurally conserved among the various subfamilies of HATs. The N- and C-terminal regions flanking this central core region are structurally diverged and the diversity is believed to be correlated with their distinct substrate specificities and biological activities.

The structural comparison of members of the human GNAT, MYST, and p300/CBP as well as of the fungal Rtt109 HAT shows that the acetyltransferase domain forms a central core region that is structurally conserved and mediates very similar acetyl-CoA interactions [54]. A crystal structure of a human PCAF-CoA *(pdb 1CM0)* [44] complex shows that the cosubstrate is bound in a cavity formed at the core region and interacts extensively with residues from the core region that are well conserved within the GNAT subfamily (Figure 1). In contrast to the conserved core domain, the N- and C-terminal domains of the HAT protein show significant structural divergence. A ternary complex structure of *Tetrahymena thermophila* Gcn5 bound to CoA and histone H3 peptide *(pdb 1QSN)* [47] shows that the acetyl-lysine bearing histone substrate binds at a cleft that is located above the conserved core region and flanked by N- and C-terminal regions. Except acetyl-lysine contacts that are mediated by the conserved core region, nearly all the other histone contacts are mediated by the flanking N- and C-terminal regions of the HAT domain. This clearly indicates the importance of this structurally diverse region for substrate recognition and specificity.

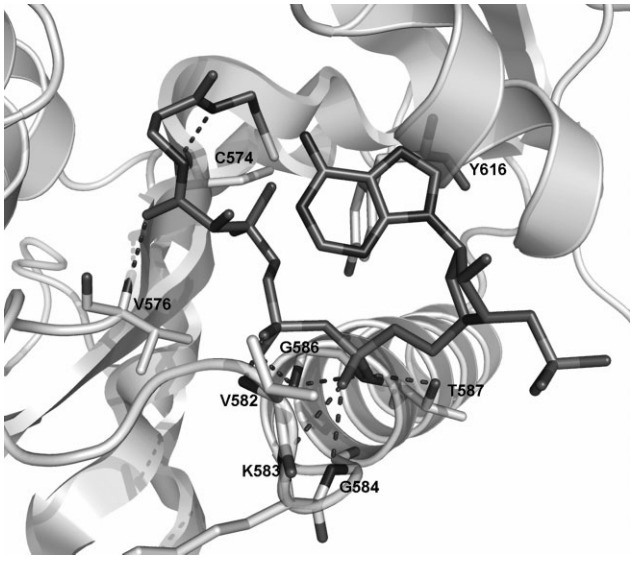


Figure 1. Coenzyme A protein interaction. Crystal structure of the PCAF-coenzyme A complex *(pdb 1CM0)*. Coenzyme A is shown as stick representation (dark gray). Active site residues that are interacting with CoA are shown in stick representation (light gray) and hydrogen bonds are indicated with dotted lines.

Yeast Esa1 is a member of the MYST subfamily of histone acetyltransferases that shares limited sequence similarity with the GNAT subfamily. The crystal structure of yeast Esa1 bound to coenzyme A *(pdb 1FY7)* [52] reveals a structurally conserved core domain that is superimposable with the corresponding part in HATs from the GNAT subfamily. In contrast the N- and C-terminal regions of the Esa1 acetyltransferase domain are more elongated and structurally diverged from the GNAT catalytic domain, which could play a role in substrate specificity.

Recently, Liu et al. [43] reported the crystal structure of the HAT domain of the transcriptional coactivator p300/CBP in complex with a lysine-CoA bisubstrate inhibitor *(pdb 3BIY)*. The structure adopts an elongated globular domain with several beta sheets surrounded by alpha helices and loops. A unique feature of the p300 HAT domain is an unusually long ~25 residue substrate binding loop (L1) that encapsulates the opposite side of the lysine-CoA bisubstrate inhibitor. Despite the absence of detectable sequence conservation with other HAT proteins, p300/CBP share structural similarities with GNAT and MYST subfamily members in the central core region associated with acetyl-CoA cofactor binding. However a notable difference within the conserved core region is the long substrate binding loop L1 that has extensive interactions with the CoA part of the bisubstrate inhibitor. This interaction is a unique feature of the p300/CBP histone acetyltransferase.

Rtt109 is a fungal HAT without mammalian homologues which has very little or no sequence homology with other known HATs and also is dependent on histone chaperones Asf1 or Vps75 for its HAT activity. Rtt109 harbors very low acetyltransferase activity on its own, which is stimulated by association with Asf1 and Vps75. Those histone chaperons promote the enzyme substrate complex formation and contribute to Rtt109-mediated acetylation processes on histone proteins, e.g. H3*K56* (Asf1) and H3*K9* (Asf1 and Vps75). Thus, the interaction of Rtt109 with histone chaperons control both enzymatic activity and substrate specificity through appropriate presentation of histone substrates within the holoenzyme catalytic site. The crystal structure of Rtt109 bound to acetyl-CoA (*pdb 3D35*) [49, 50] shows a central core region that is structurally conserved in other HATs and an unexpected overall structural similarity with p300/CBP (despite having only 7% sequence identity between Rtt109 and p300/CBP HAT domains) including the unusually long L1 loop that encapsulates the lysine-CoA inhibitor in p300/CPB. However, residues in the active site of Rtt109 show low similiarity to corresponding residues in the p300/CBP, GNAT, and MYST families and the substrate binding site is more similar to GNAT and MYST than to p300/CBP HATs.

Despite the availability of a lot of structural information for HAT and HAT-cofactor/substrate complexes, only a couple of studies were reported so far which used the 3D structural information for virtual screening or molecular design of HAT inhibitors (examples see below).

Correlation between HAT activity and pathological cellular states

Histone acetylation is regarded as global mark for transcriptional activation and affects fundamental processes like cell proliferation or differentiation. To date a variety of pathological cellular states have been described in the literature that are correlated to the activity of certain members of the HAT family. Predominantly, deregulation of the equilibrium between modifying enzymes responsible for acetylation and deacetylation events is linked to development and manifestation of cancer, inflammation, disturbance of neuronal plasticity, and functional alterations in metabolic cascades. Hence, the HAT-HDAC interplay represents an important target system for regulatory mechanisms and for the development of potential therapeutical strategies [24, 55].

As an example, the Rubinstein-Taybi syndrome is caused by breakpoints and microdeletions within chromosome 16p13.3 that correspond to a gene area encoding for CBP [56]. Apart from the genetic level, a number of studies indicate a causal relationship between HAT activity and cancerogenesis. Fusion proteins with histone acetyltransferase activity have been described as the translational product of the MLL gene with the CREB binding protein gene [57, 58]. Also for members of the MYST family (MOZ and MORF) similar fusions have been identified to contribute to leukemogenesis [59–63]. The promyelocytic leukaemia zinc finger protein (PLZF) is a transcriptional repressor, which requires acetylation by p300 in order to disturb proliferation of malignant cells [64]. It has also been shown that down-regulation of the p300/CBP acetyltransferase activity is leading to growth inhibition of human melanocytes [65]. The nuclear receptor co-activator AIB1 is overexpressed in human colorectal carcinoma and pancreatic adenocarcinoma [66]. On the other hand, AIB1 and Tip60 are essential for survival and proliferation of prostate cancer cells [67, 68]. Nevertheless, several other HATs are involved in the stabilization of oncoproteins (e.g. c-myc) and cell cycle progression [69]. Taken together the role of HATs in certain cancer types points to this enzyme class as a potential target for the development of therapeutic agents against these malignant disorders.

Furthermore, p300-mediated acetylation of the HIV Tat protein and HIV-1 integrase is necessary for the establishment of viral pathogenesis in host cells [70]. Expression of inflammation factors (e.g. NF-κB) involved in the etiology of pulmonary diseases like asthma and COPD is subject to regulatory mechanisms that target the acetylation balance in bronchial cells. Biopsies of asthmatic patients differ from those of healthy persons in the acetylation levels of histones associated with inflammatory genes [18]. In addition, HATs are also implicated in the regulation of neuronal cell plasticity and differentiation. Therefore, they participate in the control of learning and memory functions. A number of experiments with knock-out/-down mice for PCAF and CBP provide evidence for the indispensable role of HATs during central nervous system development. While PCAF deletion has been found to correlate with decreased learning competence, CBP knockdown seems to lead to impaired long-term memory without considerable influence of the short-term memory reminiscence [71]. Recently, it has also been described that PCAF knockout mice develop a resistance to amyloid toxicity according to PCAF involvement in the regulation of formation and degradation of amyloid-β-peptides [72]. Lately, the epigenetic basis of Duchenne cardiomyopathy has been found to implicate the enzymatic activity of histone acetyltransferases. PCAF-mediated lysine acetylation levels of connexion 43 determine its dissociation from GAP junctions and lateralisation, a finding that correlates with the physiopathological consequences for cell contact and cardiac function [73]. Acetylation of histone H3 lysine 56 in *Candida albicans* is known to be a key step for maintenance of fungal genome stability and survival of DNA damages [74, 75] and therefore HATs could also be a target for new antifungal drugs.

HAT modulation by small molecules

Histone acetyltransferases reveal important targets for the development of novel therapeutical strategies in the epigenetic field. Modulation of HAT activities might contribute to the treatment of cancer, AIDS, pulmonary diseases, Alzheimer disease, neurodegenerative disorders, or fungal infections. Nevertheless, only a small number of modulators has been described to date. Different approaches have been applied in order to develop small molecule inhibitors of HATs (e.g. screening of natural products, rational design, and high-throughput methods) [76]. Additionally, investigation of small molecule HAT activators have been presented in the last few years. The available HAT inhibitors can be classified into natural products, acetyl-CoA derived bisubstrate inhibitors, and synthetic compounds. Following, characteristic members of each group are presented and discussed in terms of their inhibitory properties.

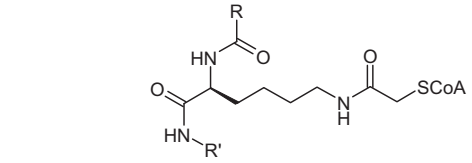
Endogenous HAT inhibitors

Maintenance of the acetylation balance within histone and non-histone substrates requires efficient regulatory mechan-

isms. The assumption of the existence of endogenous molecules which are able to modulate acetyltransferase activity led to different approaches in order to uncover novel inhibitors. Heparin as a macromolecule from the glycosaminoglycan (GAGs) class has been reported to bind and inhibit PCAF in a competitive manner. Addition of heparin to pulmonary fibroblasts reduces the acetylation grade of histone H3. In accordance to this, CHO cells deficient in GAGs synthesis exhibit increased H3 acetylation levels compared with wild type parent cells [77]. On the other hand, administration of the natural polyamine spermidine has been shown to extend remarkably the lifespan of yeast, flies, and worms, and also of human immune cells. Endogenous polyamine concentration diminishes during aging processes. Spermidine treatment of aging species leads to deacetylation of histone H3 due to HAT inhibition and reduces initiation of necrosis and stress-induced autophagy. Hence, acetyltransferase activity inhibition by endogenous polyamines might contribute to the mechanisms of aging and longevity and also the cellular response to stress [78].

HAT inhibitors: bisubstrate inhibitors

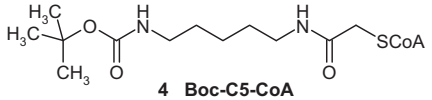
Bisubstrate inhibitors (Figure 2) were the first class of synthetic HAT inhibitors reported as structural mimics. These compounds were inspired from the ternary complex between histone substrate, cofactor, and enzyme during the transfer of the acetyl residue, shared from the GNAT family. Thus, the available bisubstrate inhibitors represent derivatives of coenzyme A covalently linked to substrate peptides with various chain lengths. A methylene group was introduced between the sulphur atom of the coenzyme A moiety and the acetylcarbonyl group in order to increase the stability and inhibitory activity of these compounds. Lys-CoA **1** has been reported to inhibit p300 selectively (IC_{50} 0.05 μM) compared to other HATs (e.g. Gcn5, PCAF, and yEsa1) [79]. H3-CoA-20 **2** is more specific for PCAF and Gcn5 (IC_{50} <1 μM) over p300 [80]. A kinetic study for H3-CoA-20 uncovered a linear competitive mechanism of inhibition against acetyl-CoA and a non-competitive inhibition mode versus the peptide substrate [40]. H4K16-CoA **3** was disclosed as one of the most potent inhibitors for Tip60 (IC_{50} 17.3 μM) and its yeast orthologue Esa1 (IC_{50} 5.5 μM) [81]. Recently, based on the crystal structure of p300, **4** was designed and published as a very potent bisubstrate inhibitor for p300 (IC_{50} 0.07 μM) [43, 82]. The coenzyme A moiety and the *tert*-butyloxycarbonyl group are linked by a pentyl chain, which was suggested to be favourable for the occupation of two binding pockets (P1 and P2) within the enzyme active site by molecular docking. On account of their partial peptide structure, bisubstrate inhibitors are not metabolically stable and poorly cell permeable. In order to improve the pharmacokinetic properties,



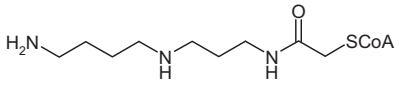
1 Lys-CoA R = CH_3, R' = H

2 H3-CoA-20 R = G-G-T-S-L-R-A-T-Q-K-T-R-A-$NHCH_3$, R' = A-P-R-K-Q-L-OH

3 H4K16-CoA R = R-H-R-K-NH_2, R' = K-A-G-G-K-G-L-G-K-G-G-K-G-R-G-S-OCH_3



4 Boc-C5-CoA



5 Spd(N^1)-CoA

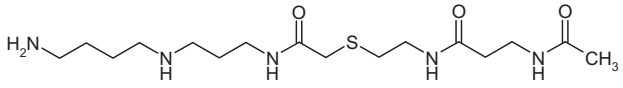


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Figure 2. Bisubstrate HAT inhibitors (selected examples).

a compound without a phosphate in the 3′-position of the coenzyme A moiety was prepared, but this led to a huge reduction of inhibitory activity [83]. Introduction of an arginine-rich peptide sequence attached via the amino acid backbone to Lys-CoA has been proven to be favourable in terms of both cell permeability and selectivity towards p300 [84].

The coenzyme A containing compound **5** has been reported to inhibit p300/CBP-mediated histone acetylation. The sulphur atom is linked through thioglycolic acid to spermidine (Spd(N^1)-CoA). Treatment of cancer cells with **5** results in increased sensitivity to cisplatin, 5-fluorouracil, and camptothecin while healthy cells remained unaffected. Spd-CoA-induced inhibition of histone acetylation impeded acetylation-dependent DNA repair and hence boosts cell sensitivity to DNA damage. Removal of a large part of the coenzyme A led to substructure **6** with enhanced chemosensitization effects. Owing to their partial polyamine structure, **5** and **6** are internalized into mammalian cells via polyamine transporter uptake. This approach is a novel strategy for treatment of cancer based on chemosensitization by selective small molecules [85].

HAT inhibitors: natural products

In 2003, anacardic acid **7** (Figure 3) was the first non-competitive HAT inhibitor published as a result of a screening of plant extracts with anticancer activity. Anacardic acid is an ingredient of the cashew nut shell liquid with inhibitory activity against PCAF (IC_{50} 5 μM) and p300 (IC_{50} 8.5 μM) [86]. It has been reported that **7** inhibits the acetylation of the p65 subunit of NF-κB, which is responsible for its activation and nuclear localization [87]. Human cancer cells respond to treatment with anacardic acid with increased sensitivity to ionizing radiation, probably mediated by Tip60 inhibition [88]. A major limitation for further development of **7** as a potential anti-cancer agent is its poor cell permeability. Nonetheless, this compound has been commonly used as a template for further optimization (see below).

Curcumin **8** (Figure 3) is the major curcumanoid component in the roots of spice turmeric *Curcuma longa* L. and was published as a HAT inhibitor in 2004. **8** is able to repress p300-mediated p53 acetylation and also inhibits histones H3 and H4 acetylation at the micromolar level (IC_{50} 25 μM). Curcumin does not affect the enzymatic activity of PCAF and hence demonstrates a certain selectivity within the HAT family. The binding mode of **8** to p300 suggests a covalent interaction on a site that is different from the substrate and acetyl-CoA binding sites in the enzyme [89]. Beyond HAT inhibition, curcumin interacts also with other epigenetic targets, among them e.g. DNA methyltransferase I and HDACs [90]. At present, this compound is evaluated in several clinical trials for the therapy of cancer, Alzheimer disease, rheumatoid arthritis, cystic fibrosis, and psoriasis. Therefore, curcumin is the first small molecule in clinical evaluation that possesses HAT inhibitory properties [91]. But it is unclear, which of the many activities is really decisive for curcumin *in vivo*.

Garcinol **9** (Figure 3) is a polyisoprenylated benzophenone isolated from the fruit of *Garcinia indica* L. It has been shown to inhibit PCAF (IC_{50} 5 μM) and p300 (IC_{50} 5 μM) and to induce apoptosis in human cancer cells [92]. It is highly

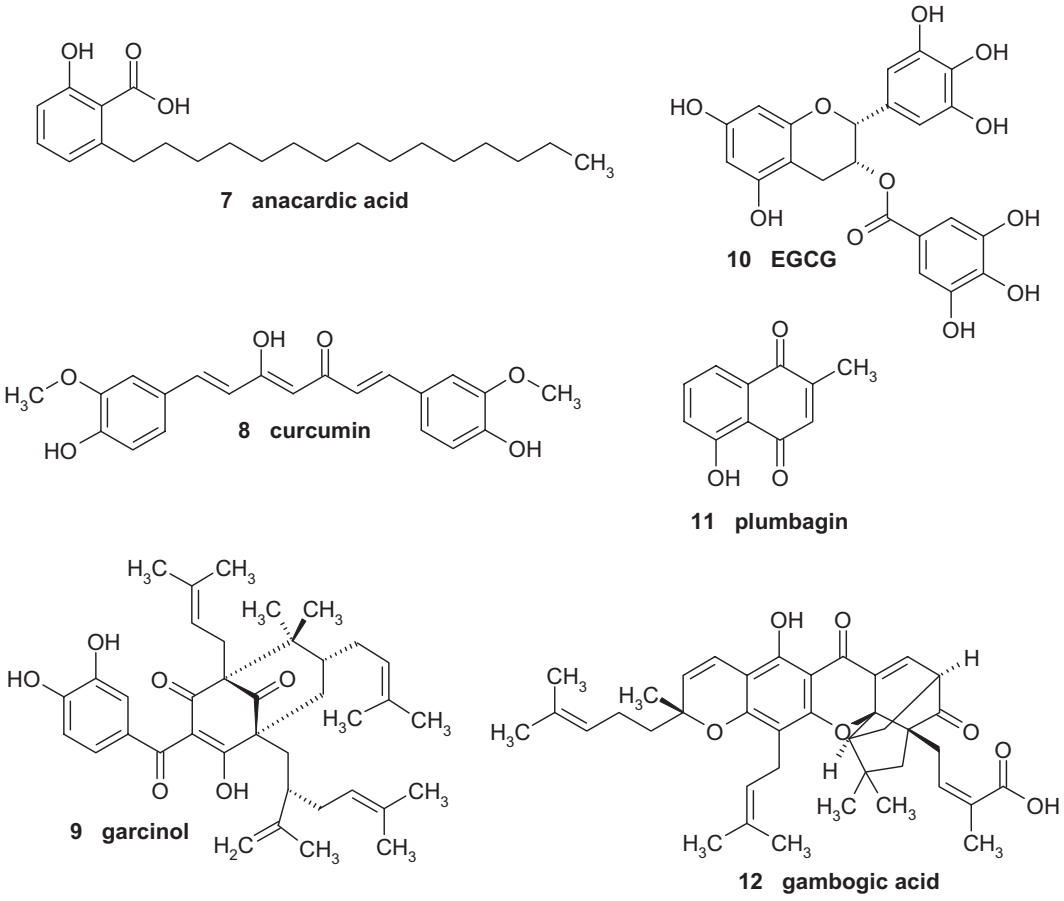

Figure 3. Natural products with HAT inhibitory properties.

cytotoxic and shows good permeability through cell membranes. It has been proposed that the catechol hydroxy groups provide interactions with the acetyl-CoA binding domain and the isoprenoid moieties with the substrate binding site [93].

Epigallocatechin-3-gallate (EGCG) **10** (Figure 3), the major polyphenol found in green tea, has been reported to modulate NF-κB activation. After p300/CBP-induced acetylation of its p65 subunit, NF-κB is involved in manifestation and maintenance of chronic inflammation. Hence, inhibition of NF-κB acetylation might contribute to prevention and treatment of diseases like asthma and COPD. EGCG inhibits p300/CBP-mediated p65 acetylation and suppresses TNFα-triggered activation of NF-κB, which is required for its translocation to the nucleus. Furthermore, EBV infection-caused cytokine expression is reduced in an acetylation dependent manner due to treatment with EGCG. Importantly, **10** does not interact with other epigenetic targets tested (e.g. HDAC from HeLa nuclear extract, Sirt1, and SET7/9 lysine methyltransferase) [94]. Nonetheless, the polyphenol EGCG **10** has been also reported as an inhibitor of the DNMT1 (DNA methyltransferase 1) [95] on the epigenetic level, as well as a modulator of a broad range of distinct biological targets [96].

Plumbagin (RTK-1) **11** (Figure 3), a hydroxynaphthoquinone isolated from *Plumbago rosea L.* root extracts, has been shown to inhibit specifically p300, whereas PCAF activity remains unaffected. HepG2 liver cancer cells treated with **11** exhibited decreased acetylation levels of histones H3 and H4 as well as of p53 as non-histone protein substrate in a non-competitive manner. The hydroxyl group of **11** was suggested to form a hydrogen bond to lysine K1358 residue in the HAT domain of p300. In consequence, substitution of this functional moiety led to inactive derivatives [97].

Gambogic acid **12** (Figure 3) is the major active ingredient of gamboge, the resin of the tree *Garcinia hanburryi* L. Several biological effects have been observed under treatment with **12** including inhibition of processes implemented in inflammation, viral infection, and response to oxidative stress. Furthermore, growth inhibition of distinct cancer cell lines like hepatoma, breast, gastric, lung carcinoma, and also T-cell lymphoma have been reported. In 2009 it was shown that **12** induces apoptosis in the human CML cancer cell line K563 and causes G0/G1 cell cycle phase arrest. This correlated with decreased expression of SRC-3 as one of the most important members of the nuclear receptor co-activator family of HATs involved in carcinogenesis [98]. However, no *in vitro* data for HAT inhibition was provided up to date.

Thus, a number of HAT inhibitors have been identified by screening of natural products. The lack of selectivity among the HAT family as well as the broad spectrum of various other targets affected hamper further development. Nevertheless, these compounds served as a starting points for structure optimization and as templates for the development and synthesis of small molecule modulators of HAT activities in order to improve pharmacokinetic properties and efficacy.

HAT inhibitors: synthetic derivatives and analogs of natural products

One of the most successful approaches for the development of novel small molecule inhibitors of HATs so far (Figure 4) has been the structural modification of natural product lead structures; e.g., synthetic compounds derived from anacardic acid **7** (Figure 3) as a lead structure have been described. In general, the primary goal was to improve inhibitory properties, selectivity, and cell permeability. Compounds **13** and **14** have been described as HAT inhibitors among a number of related structures. HAT inhibition *in vitro* has been shown to correlate with their antiproliferative effects in a set of cancer cell lines including breast and cervical carcinoma, T-cell lymphoma and prostate adenocarcinoma. Interestingly, these compounds were non-toxic against a non-malignant human fibroblast cell line. In MCF-7 cells **13** and **14** led to histone H4 hypoacetylation [99]. Using the crystal structure of PCAF in complex with coenzyme A, Ghizzoni et al. provided a proposal for a binding mode for anacardic acid. This led to the design and synthesis of compound **15** that shows a twofold improvement in terms of PCAF inhibition as well as histone H4 hypoacetylation in liver cancer cells. The introduction of an additional benzyl moiety was supposed to enhance interactions within the acetyl-CoA binding site. The *cis*-configuration of the double bond is crucial for the inhibitory activity of **15** [100]. 4-Cyano-3-trifluoromethylphenylbenzamides **16** and **17** have been published as p300 inhibitors *in vitro*. The activity was found to be comparable to that of anacardic acid. Moreover, the short chain derivative **16** induces apoptosis of leukemia cells at 50 μM [101]. Curcumin-derived cinnamoyl analogues have been found to inhibit p300 acetyltransferase activity *in vitro*. For compounds **18–20** IC_{50} values between 21 and 46 μM have been determined. The change of the aliphatic chain into a cyclohexanone unit led to less potent derivatives **21–24** with IC_{50} values between 111 and 233 μM. The bromo-substituted 2,6-arylidene cyclohexanone **24** was shown to be the most potent compound (IC_{50} 5 μM) which led to a decrease in H3 acetylation in mammalian cells [102]. Notably, **24** and related bromo-substituted phenolic compounds have been reported additionally as inhibitors of protein methyltransferases (PRMT1, CARM1, and SET7) and sirtuins, and hence are referred to as epigenetic multiple ligands (epi-MLs) [103]. Recently, CTK7A **25** has been synthesized as a water soluble curcumin derivative with similar inhibitory properties [104]. Semisynthetic analogues of garcinol **9** (Figure 3) have been prepared and were investigated for inhibitory activity against

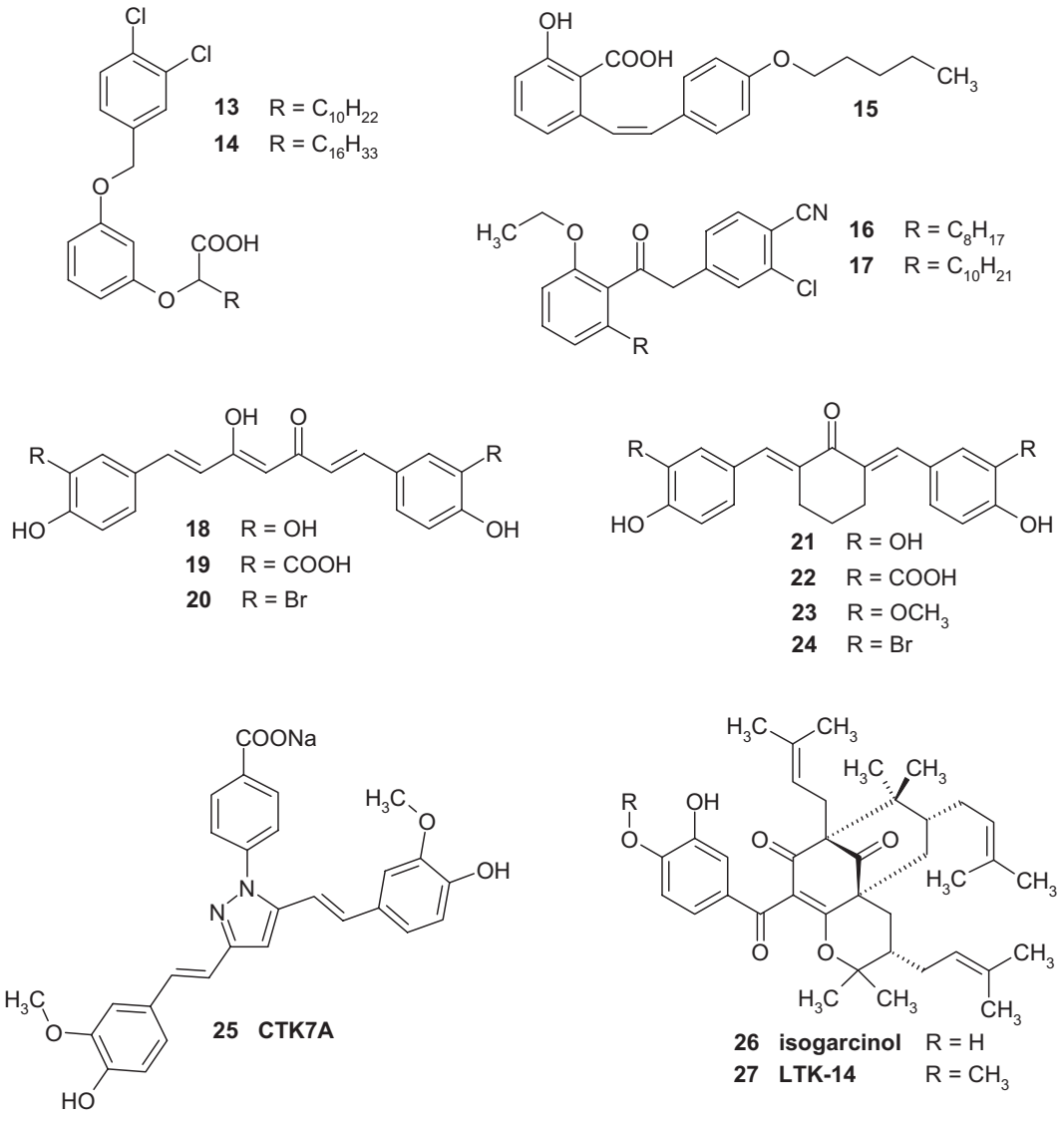

Figure 4. HAT inhibitors derived from natural products.

HATs. Isogarcinol **26** (Figure 4) is the product of an intramolecular cyclization of **9** and provides a similar inhibition pattern towards p300 and PCAF. Monomethylation of the catechol group in position 14 led to LKT-14 **27**, which was described as a selective p300 inhibitor. Several garcinol derivatives are non-toxic to T-cells. Furthermore, treatment of HIV-infected cells with **9**, **26**, or **27** resulted in reduced histone acetylation levels and decreased HIV multiplication [70]. In structural analysis, LKT-14 was identified as a non-competitive inhibitor for acetyl-CoA and the histone substrate, which is considered with a single binding site on p300 HAT domain, in contrast to additional one for garcinol and isogarcinol [93].

HAT inhibitors: synthetic small molecules

The quinoline derivatives **28** and **29** (Figure 5) were identified by yeast phenotypic screening and have been shown to induce growth inhibition of *S. cerevisiae* concomitant with histone H3 hypoacetylation. Further structural optimization led to **30** that has some similarity to anacardic acid (aromatic 2-hydroxy-carboxylic acid) [105]. The α-methylene-γ-butyrolactone MB-3 **31** has also been reported to inhibit Gcn5. This inhibitor was designed in accordance with the electrostatic interaction fields implicated in Gcn5 catalysis. The α, β-unsaturated carbonyl moiety is accessible for Michael addition and reaction with the cysteine residue C177 in

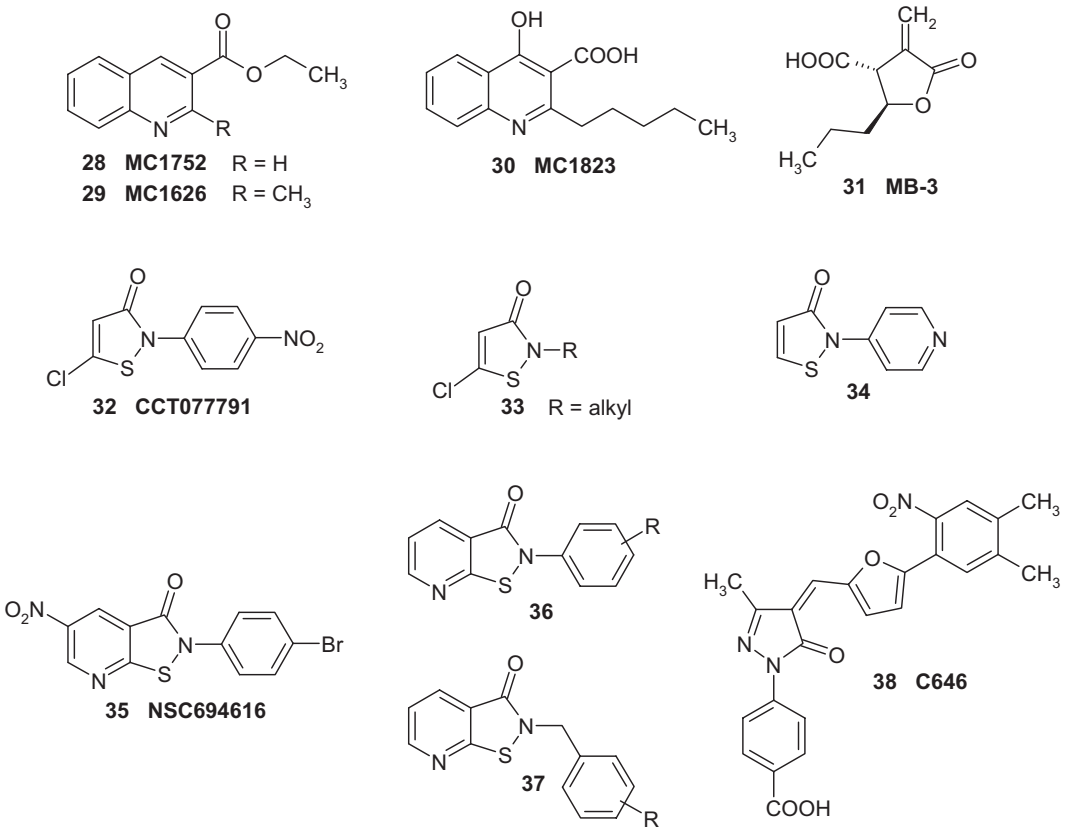

Figure 5. Synthetic small molecules as HAT inhibitors.

the enzyme active site could be assmumed. Interestingly, a time dependence study of the inhibition mode of **31** suggested a non-covalent binding [106].

High throughput screening of a large compound library led to the discovery of 35 isothiazolones as potent p300 and PCAF inhibitors. Moreover, analogues with the core structure **32** (Figure 5) have been reported to reduce significantly growth inhibition of human colon carcinoma cells in time and concentration dependent manner. Upon treatment with **32**, decreased total acetylation levels of histones H3 and H4 as well as of α-tubulin have been detected [107]. The chemical reactivity of this compound class was linked to its HAT inhibitory activity. Indeed, isothiazolone-derived small molecules are known for a number of biological activities, e.g. antibacterial and antiparasitic properties and inhibition of several enzyme targets [108]. The proposed mechanism of inhibition implicates a reaction with cysteine thiol groups with cleavage of the sulphur-nitrogen bond and concomitant disulfide formation. Nevertheless, the high reactivity of these derivatives is one of their major limitations which was addressed by the development of related inhibitors. A series of *N*-aliphatic substituted isothiazolones have been investigated by Dekker et al. in order to elaborate structure-activity relationships. Chlorination in 5-position lead e.g. to the more potent compound **33**. Alkylation in the same position appeared unfavorable due to decreased inhibitory activity [109]. *N*-Aromatic and *N*-benzylic substituted isothiazolones were also shown to inhibit p300 and PCAF acetyltransferase activities. **34** was shown to have the best inhibitory properties among a number of related derivatives [110]. Based on the isothiazolones, a similarity-based virtual screening of the National Cancer Institute library was successfully applied in order to uncover related HAT inhibitors. 51 compounds from the search query were docked into the PCAF substrate binding pocket and 32 compounds were identified that contain a reactive *S-N* bond which was located in close proximity to the active site of Cys574 in the model. 15 compounds that could be obtained from the NCI and 5 compounds from commercial suppliers were tested for *in vitro* inhibition of PCAF histone acetyltransferase activity and pyridoisothiazolones like **35** (Figure 5) were identified as potent HAT inhibitors with IC_{50} values in the low micromolar level with a reduced general bioreactivity as compared to the simpler isothiazolones. The proposed covalent mechanism of inhi-

bition was verified by elimination of HAT inhibition upon the presence of mM concentrations of the thiol DTT in the incubation mixture. Because of the limited solubility of the initial hits, related compounds with improved physicochemical features have been synthesized and characterized. Introduction of a methylene group between the aromatic substituent and the isothiazolone core results in an increased distance of the reactive sulphur-nitrogen bond to the addressed cysteine residue in the enzyme active site and is therefore unfavourable. Mono- and dioxidation of the sulphur atom correlated with reduced *in vitro* inhibition, which is in accordance with comparable findings provided for isothiazolone-1-oxides [108]. Pyridoisothiazolones have also been shown to possess antiproliferative properties, hence growth inhibition of human neuroblastoma, colon, and breast carcinoma cells was detected [111].

In 2010, C646 **38** (Figure 5), a novel pyrazolone containing inhibitor was developed based on *in silico* screening. Bowers et al. [33] docked ~500,000 commercially available small molecules into the lysine-CoA binding pocket of the p300 HAT crystal structure using the ICM [112] docking program and biologically tested 194 selected hits coming out of the virtual screening. **38** was shown to be a competitive p300 inhibitor with a K_i of 400 nM, appears to be selective for p300 versus other acetyltransferases (AANAT, Gcn5, PCAF, Sas, MOZ, Rtt109) and reveals a mechanism of inhibition that is linear competitive against acetyl-CoA. The predicted binding mode of C646 with p300 shows a series of hydrogen bond interactions with the side chains of Thr1411, Tyr1467, Trp1466, and Arg1410, which overlap with the interactions that are observed in the crystal structure of a p300-lysine-CoA complex. Further studies on site-directed p300 HAT mutations and synthetic modifications of C646 **38** confirmed the predicted binding mode and the importance of particular interactions in conferring potency. Structure-activity investigations demonstrated that the carboxylic moiety and the nitro group are essential for inhibition due to their involvement in hydrogen bonds within the enzyme active site. The conjugated enone seems to be crucial for inhibitory activity as well. Importantly, C646 suppressed histone H3 and H4 acetylation in mouse fibroblast cell line and inhibited melanoma and lung cancer cell growth *in vitro* [33].

HAT activators

Development of small molecule activators in order to potentiate HAT activity has also received some attention. This issue is relevant since down-regulated HAT-activity is known to control expression of tumor suppressor genes involved in cancer manifestation and progression. At present, only a few small molecule activators are confirmed from the literature (Figure 6). In particular, CTPB and nemorosone have been discovered during structural modification of natural compounds from the screening for novel HAT inhibitors. CTPB **39** was the first selective activator of p300 described in 2003. This compound is derived from anacardic acid with the introduction of an amide bond from the carboxylic group and a bisubstituted aniline moiety. Under CTPB exposure increased p300-dependent transcriptional activation has been observed [86]. A series of CTPB-inspired analogues with core scaffold **40** is available as well. Chain length size reduction and incorporation of terminal polar groups have been recognized as structural tools for modulation of the selectivity of HAT activation toward p300 and PCAF [113]. Among a number of polyisoprenylated benzophenone derivatives similar to garcinol **9** (Figure 3), nemorosone **41** (Figure 6), a major constituent of the floral resin of *Clusia rosea* L., has been identified as a solitary cell permeable p300 activator [114].

The pentadecylidenemalonate **42** (Figure 6) contains a long alkyl chain attached to diethyl malonate moiety comparable to anacardic acid **7** (Figure 3). **42** has been found to modulate acetyltransferase activity depending on the HAT target. It exhibits inhibitory properties against p300/CBP in potency similar to anacardic acid. In contrast, **42** enhances PCAF activity *in vitro*. Furthermore promising apoptotic effects in leukemia cancer cells have been reported [115]. Although this compound does not represent a common drug-like structure, it might contribute to the development of selective modulators of HATs. Indeed, a number of related analogues called LoCAMs (long chain alkylidenemalonates) have been published recently [116]. It was found that the variation of the alkyl chain length influenced the activity profile of these HAT modulators. Inhibitory and activating properties as well as the selectivity towards different members of the HAT family variied depending on the substitution pattern. The reduction of the alkyl chain length or the substitution of the malonate ester moieties with keto groups led to derivatives with CBP inhibition comparable to those of anacardic acid. Reduction of the methylene chain length by one unit led to a compound with a similar activation of PCAF enzymatic activity. For both compounds a hyperacetylation of histone H3 in the U937 human leukaemia cell line was reported.

Concluding remarks and perspectives

Acetylation of histones is at present the most studied and best understood epigenetic modification. The emergence of hundreds of non-histone substrates identified acetylation as a posttranslational modification of general significance. A broad number of pathological alterations are known to be related to a deregulated acetylation equilibrium. The HAT enzyme class reveals great potential for the development of small molecule modulators. In the last ten years a limited

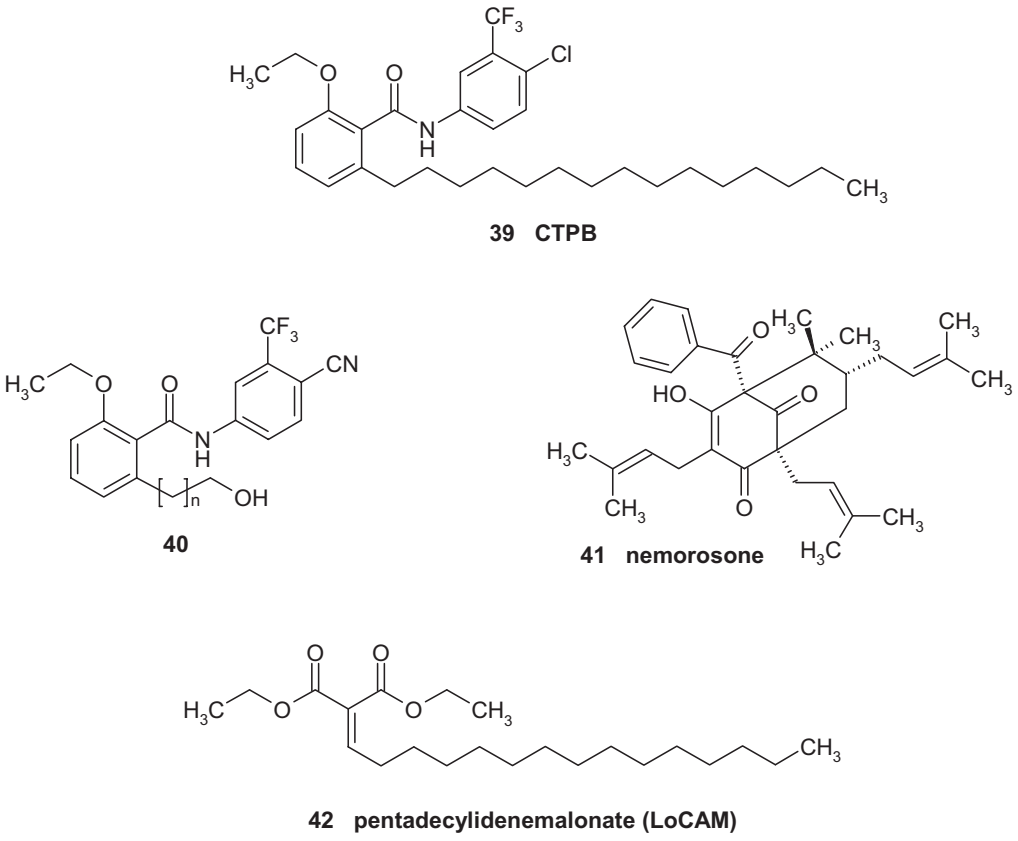

Figure 6. HAT activators and dual modulators.

number of compounds have been identified to address lysine acetyltransferase activities, inhibiting or activating these histone modifying enzymes. Natural products and synthetic derivatives, bisubstrate inhibitors and synthetic small molecules derived from computational screening represent the available HAT inhibitors. However, their validation as potential drug-like agents even in animal models, not to mention clinical studies, is still missing. The current inhibitor classes suffer from various drawbacks such as low potency, lack of cell permeability, metabolic instability or a broad spectrum of pleiotropic effects. Improvement is also needed in terms of selectivity within the HAT family and general compound reactivity, even for the use as mechanistical tool. HAT activation as a possible regulatory mechanism for disease treatment requires further characterisation and validation. Small molecule compounds are indispensable for investigation and elucidation of acetyl transfer mechanisms and substrate binding modes. However, the future challenge remains to find highly potent and selective modulators of HATs with *in vivo* activity. This would be the first step to a possible drug development of HAT inhibitors. The main goal is to restore the acetylation balance as a tuning strategy for the epigenetic treatment of pathological alterations in gene expression. Selective modulation of this balance holds huge potential for further studies on epigenetic therapeutics.

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