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Epigenetic Modulation using Small Molecules - Targeting Histone Acetyltransferases in Disease

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Abstract (max. 250 words)
Histone acetyltransferases (HATs) are epigenetic drivers that catalyze the acetyl transfer from acetyl-CoA to lysines of both histone and non-histone substrates and thereby induce transcription either by chromatin remodeling or direct transcription factor activation. Histone deacetylases (HDACs) conduct the reverse reaction to counter HAT activity. Physiological processes such as cell cycle progression or apoptosis require a thoroughly balanced equilibrium of the interplay between acetylation and deacetylation processes to maintain or, if required, alter the global acetylome status. Aberrant HAT activity has recently been demonstrated to play a crucial role in the progression of various diseases such as prostate, lung, and colon cancers as well as glioblastomas and neurodegenerative diseases. Recent investigations have aimed for the identification of HAT modulators to further decipher the complexity of acetyl transferase related signaling cascades and discover potential leads for drug design approaches. HDACs have been extensively characterized and targeted by small molecules, including four FDA-approved HDAC inhibitors; in contrast, HATs have not been active targets for therapeutic development. This review will summarize the status of HAT associated diseases and the arsenal of currently known and available HAT inhibitors with respect to their discovery, further improvements, and current applications.

Keywords: histone acetyltransferases, p300/CBP, PCAF, GCN5, Tip60, epigenetics, small molecule inhibitors, cancer
1. Introduction

Histone acetyl transferases (HATs) catalyze the transfer of an acetyl moiety from the essential cofactor molecule acetyl coenzyme A (acetyl-CoA) to a specific lysine (Lys) ε-amino group of their respective protein substrates. HATs represent major regulators of transcriptional output by driving reversible acetylation of histones, converting DNA from its highly condensed and histone-bound form into the associated relaxed and open chromatin structure. The latter becomes accessible for transcription factor binding thereby inducing the transcription of target genes. Moreover, HATs are involved in non-histone acetylation events affecting nuclear receptors (NRs) such as androgen and estrogen receptor (AR, ER) and other regulatory transcription factors such as FoxA1, c-Myc, and p53 [1-5]. Due to their non-histone target acetylation properties, the protein class is often referred to as lysine acetyl transferases (KATs) in order to more accurately incorporate their full target specificity. The reverse reaction is performed by histone deacetylases (HDACs), likewise referred to as lysine deacetylases (KDACs), which shift the dynamic equilibrium between open and condensed form of DNA to the latter species and thereby terminate further target gene transcription [6, 7]. Since HATs and HDACs represent master regulators of transcriptional events, the disruption of the carefully orchestrated interplay between acetylation and deacetylation events frequently results in the onset or progression of genetically driven diseases. HDACs have been thoroughly investigated and promoted as drug targets in cancer, neurological diseases, and immune disorders [8, 9]. Hence, HDACs have been subject to extensive drug development efforts. To date, four HDAC inhibitors (Vorinostat, Romidepsin, Belinostat, and Panobinostat) have been approved by the FDA as anticancer agents for treatment of cutaneous T-cell lymphoma (CTCL) and multiple myeloma. In contrast, there has been far less effort in the area of HAT inhibitors for therapeutic applications in epigenetic diseases. Various studies have implied HATs in the emergence or progression of diseases such as prostate cancer, colon cancer, and neurological disorders [9-12]. Consequently, these findings stimulated chemical probe development efforts with the goal of identifying highly potent inhibitors of HAT activity as well as high-affinity probes to study specific HAT functions and their
diverse implications in disease. Despite the clear implication of HATs in many diseases, the field is still lacking potent and specific probes to serve as starting points for the development of therapeutic agents. Several inhibitors of HAT activity have been identified, but significant gains in selectivity and potency must be realized to reach their full potential as therapeutic drugs or investigational probes. This review provides a brief overview on HAT organization and function as well as the roles of major HATs in disease and a summary of current HAT modulators with respect to their discovery, further development, and their applications in science and medicine.

1.1 Global domain organization and function of HATs

Historically, HATs were first identified as histone modifying enzymes mediating the induction of transcription through chromatin remodeling via unwinding DNA from histones. They act in complement with HDACs, which facilitate the termination of transcription by histone deacetylation favoring the condensed form of DNA. HATs are divided into different classes of transferases based on their structural domain organization and sequence similarity. The majority of HATs are associated with the two predominant families GNAT (GCN5-related N-acetyltransferases) and MYST, named after the founding members MOZ, Ybf2, Sas2, and Tip60. Smaller families include p300/CBP acetyltransferases, nuclear receptor coactivators (e.g., SRC-1, TIF-2) with intrinsic HAT activity, and other general transcription factor HATs (e.g., TAF1, CLOCK). The first structure of a HAT was discovered in 1999 by performing in solution nuclear magnetic resonance (NMR) experiments utilizing Tetrahymena GCN5 and CoA to form the respective enzyme-cofactor complex. Subsequently, the respective crystal structure of GCN5 in complex with a histone H3 peptide was solved [13, 14]. These studies provided the molecular basis to further understanding of the mechanism of action (MOA) of HATs and likewise provide the basis for structure-based design studies. Following these foundational discoveries, various studies have illuminated the global domain organization of different HAT family members. HATs may be composed of a variety of different domains, which constitute the enzymatically active protein. The catalytic HAT acetyltransferase domain, which facilitates the transfer of an acetyl group from acetyl-CoA to the respective protein
substrate, is a feature of HATs. Multiple regulatory domains, associated catalytic domains, and domains involved in recruitment of other proteins as well as the control of subcellular localization may constitute the global structure of these transferases (Figure 1). For instance, some HATs contain an associated Really Interesting New Gene (RING) domain contributing to the assembly of the substrate binding groove and acting as an E3 ligase that binds E2 ubiquitin-conjugating enzymes and mediates the transfer of ubiquitin to substrates (e.g., p300), but was also shown to have HAT inhibitory functions. Other HAT domains include Plant Homeodomain (PHD) finger, a domain that typically binds trimethylated histone H3 Lys4 (H3K4me3), unmodified histone-H3 tails or other modified chromatin [15, 16]. Further zinc finger motifs are likewise found in HATs. In addition, Bromo- (Bd) or Chromodomains (Cd), which are known to recognize acetylated or methylated Lys residues, respectively, can contribute to the overall HAT structure and act as epigenetic reader domains. WD40 repeats and Tudor domains are likewise found in HATs. While the enzymatically active HAT core is needed to facilitate enzymatic activity, associated domains and motifs are essential for recruitment and release of substrates, directing subcellular localization, and association to multicomponent complexes. It is important to note that the domain composition and organization of HATs is not uniform and combinations of these subunits contribute to the unique features of each HAT complex [17].

In order to decipher the MOA by which HAT activity is determined, a plethora of biochemical, structural, permutational, and kinetic studies of various HATs have been performed, resulting in several widely recognized mechanisms of acetyl transfer. Generally, the substrate lysine is preconfigured and flanked by the essential catalytic amino acids to ensure proper orientation for nucleophilic attack towards the terminal acetyl moiety from the acetyl-CoA thioether. GNAT family members facilitate a direct acetyl transfer by abstracting a proton from the positive charged Lys through a catalytic glutamate (Glu173 in yGcn5 and Glu570 in hPCAF) and an ancillary water molecule thereby transforming the amino group into an appropriate nucleophile to attack the acetyl thioether in an $S_N2$ manner ultimately forming an acetyl-Lys substrate species. The catalytic transfer via this bi-bi ternary complex mechanism can
only pass off when both substrates, acetyl-CoA and histone or non-histone substrate, are simultaneously bound to the HAT core [18-21]. In contrast, MYST family members were first described to transfer the acetyl group in a two-step process involving an initial acetyl transfer to a HAT cysteine (Cys 304 in yEsa1) by transesterification followed by an $S_n2$ transfer utilizing a conserved catalytic glutamate (Glu338) to generate the uncharged nucleophilic amino lysine moiety of the respective substrate and finally facilitate the acetyl transfer [22]. Recent studies investigating yEsa1 assembled within a physiologically relevant piccolo NuA4 complex provide evidence for a direct-attack mechanism [23]. Different catalysis mechanisms have been proposed for members outside of the MYST and GNAT families. For instance, p300/CBP perform catalytic transfer without providing a general base to activate the substrate-Lys. Instead, conserved tyrosine and tryptophan residues were shown to be crucial for the completion of the catalytic transfer. Studies based on quantum mechanics calculations suggest, that an aspartate is likewise involved in the catalytic transfer [24]. Furthermore, the involvement of histone chaperones might contribute to performing acetyl transfers for certain HATs (e.g., HAT1 family members) [25, 26]. The diversity of catalytic mechanisms among the different HAT family members indicates that selective HAT inhibition might be an approach to specifically modulate chromatin remodeling and transcriptional output.

1.2 HATs in disease

This review summarizes the involvement of the major HAT acetyltransferases p300/CBP, GNAT-family members PCAF, and GCN5 as well as MYST-family member Tip60 in the onset or progression of disease.

1.2.1 p300/CBP: p300 and CREB-binding protein

The transcriptional coactivator protein p300 and its paralog CREB-binding protein (CBP) have high sequence similarity and they exhibit both shared as well as non-redundant functions. Important interacting proteins for p300/CBP include hypoxia-inducible factor-1 (HIF-1), β-catenin, p53, c-Myc, c-Myb, CREB, ETV1, AR and ER [1,
In addition to their HAT activity, p300/CBP were found to be cytoplasmic polyubiquitin E4 ligases of tumor suppressor p53 facilitating its rapid turnover in unstressed cells [30]. In 2002, the AR coactivator p300 was shown to mediate the androgen-independent transactivation of the AR by Interleukin 6 (IL-6) in LNCaP prostate cancer (PCa) cells suggesting a unique role of its HAT activity in PCa pathogenesis and/or progression [31]. p300 was confirmed to be associated with proliferation of PCa both in vitro and in vivo and therefore was anticipated to potentially serve as a predictive marker for aggressive features of PCa at the time of prostatectomy, as well as after surgical treatment [32]. Androgen-independent prostate cancer cells require AR-mediated transcriptional activation for their growth, indicating that p300 HAT is a promising target to overcome hormone-resistant cancers [33]. A variety of further studies substantiated the importance of p300 in prostate cancer and simultaneously clarified that p300 may be involved in cancer progression through multiple pathways depending on substrate proteins as well as genetic dependencies of the tumor cells. For instance, siRNA-mediated downregulation of p300 (but not of CBP) as well as its chemical inhibition by the small molecule C646 leads to induction of caspase-dependent apoptosis, revealing a targetable function for p300 in the survival and invasion pathways of prostate cancer cell lines (PC-3 and LNCaP amongst others) [34]. Moreover p300, but not CBP, is widely required for androgen-induced gene regulation and histone acetylation and methylation. Furthermore, p300 is dominant over CBP, since global gene expression analysis indicated that 47% of androgen-regulated genes are p300-dependent, whereas only 0.3% of them are CBP-dependent in advanced androgen depletion independent prostate cancer cells (C4-2B) [35]. Recent studies with respect to the unique role in p300 in PCa discovered that androgen receptor degradation as well as PTEN-deficient prostate tumorigenesis is regulated by p300 [10]. Tumor progression and angiogenesis in prostate cancers are also driven by the downstream targets of hypoxia inducible factor-1 alpha (HIF-1α), which is an acetylation substrate of p300. Disruption of the HIF-1α/p300 complex by the use of natural product derived epidithiodiketopiperazine inhibitors leads to antiangiogenic and antitumor activity.
in prostate cancer cells through downregulation of the HIF-1 target genes VEGFA, LDHA, and ENO1 [29]. A similar observation was made in a study by using the reactive oxygen species (ROS) scavenger MnTE-2-PyP to alter DNA binding properties of p300 by either directly disrupting p300 activity (mechanism unknown) or p300 transcriptional complex binding (p300/HIF-1/CREB) to the PAI-1 promoter. Treatment with MnTE-2-PyP thereby inhibits prostate cancer growth, migration, and invasion both in the presence and absence of radiation supporting the hypothesis that MnTE-2-PyP may enhance the success of prostate cancer radiotherapy as it simultaneously represents a unique radio-protector of normal tissues [36]. In other studies, p300 was also found to regulate fatty acid synthesis (FAS) in PCa by acetylation of H3K27 in the FASN promotor as a direct downstream target and thereby induce upregulation of FASN gene expression, which in turn is a key mediator of p300-induced growth of PCa cells in vitro and in vivo [37]. p300 was also demonstrated to represent a specific synthetic-lethal gene in CBP-deficient lung cancer cells (LK2, H1703, H520). Myc was subsequently downregulated by targeting p300 using the specific small molecule inhibitor C646 or genetic p300 depletion using siRNA. Moreover, growth suppression of CBP-deficient lung and hematopoietic cancer cells was confirmed in vitro and in vivo (CBP-deficient Jurkat cells and corresponding xenograft model) by targeting p300 with the specific inhibitor C646. Since loss-of-function mutations of CBP exist in a variety of human tumors, including lung, bladder, gastric, and hematopoietic cancers, a therapeutic strategy based on synthetic lethality of the p300 HAT activity is potentially beneficial for patients with CBP-deficient cancers [38]. Altered expression levels and subcellular localization of p300/CBP were associated to cancer progression, prognosis, and resistance in a number of different studies and cancer types [39-41]. Of note, the emergence of doxorubicin drug resistance in bladder cancer is mediated by reduced expression of p300 [42].

Chromosomal translocations directly involving the CBP or p300 genes are also associated with leukemia/lymphoma since they lead to aberrant regulation of acetylation events. For instance, chimeric fusions of monocytic leukemia zinc-finger protein (MOZ) or myeloid/lymphoid leukemia protein 1 (MLL) to either p300 or CBP
were associated to certain forms of acute myeloid leukemia (AML) [43-45]. The interaction of p300 and proto-oncogene c-Myb, a key regulator of hematopoietic cell proliferation and differentiation, is essential for the induction of leukemogenesis in AML by human AML oncogenes and is severely disrupted by the emergence of mutations in multiple domains of c-Myb leading to abrogation of myeloid transforming ability [46, 47]. The F-box protein S-phase kinase associated protein 2 (Skp2) is frequently overexpressed in many human cancers and plays a key role in tumorigenesis. It has proteolytic activity and suppresses p53-dependent apoptosis by outcompeting p53 for binding to p300, thereby perturbing p300-mediated p53 acetylation and stabilization. In turn, Skp2 is acetylated and stabilized leading to positive regulation of its oncogenic function [48]. Thus the development of inhibitors to disrupt the Skp2/p300 interaction and promote p53-mediated apoptosis is proposed to represent an effective way for treating Skp2 driven cancers [49].

Besides their involvement in cancerous diseases, loss of function mutations or chromosomal rearrangements involving p300/CBP lead to the onset of the Rubinstein-Taybi Syndrome (RTS), a disease of severe mental retardation accompanied with facial abnormalities as well as enlarged thumbs and toes [12]. Moreover, CBP activity is critical for activation of genes controlling memory consolidation. Transgenic mice expressing a functionally inactive variant of CBP exhibit a long-term memory deficit while the encoding of new information and short-term memory are normal [50].

1.2.2 PCAF: p300/CBP associated factor

The p300/CBP associated factor (PCAF), a GNAT family member, was first cloned and characterized in 1996 and demonstrated to reveal intrinsic histone acetylase activity for histones H3 and H4, and shares significant sequence identity with GCN5 in the C-terminal region. Although it is a paralog of GCN5 and shares some redundant functions, its cellular function is distinct [51]. In vitro studies revealed that PCAF inhibits cell-cycle progression, counteracts the mitogenic activity of the adenoviral oncoprotein E1A, and competes with the same binding sites in p300/CBP that are likewise addressed by E1A [52]. Furthermore, PCAF acts as a nuclear receptor
coactivator through association with DNA-binding domains of nuclear receptors [53]. PCAF acetyltransferase activity might be initiated by both autoacetylation as well as acetylation by p300. Besides acetylation of core histones, tumor suppressors like p53 and PTEN as well as further transcription factors such as Fli1 are acetylated and thereby modulated by PCAF [54-56]. Wnt signaling is likewise modulated by PCAF-dependent acetylation and thereby stabilization of β-catenin [11]. Notably, PCAF reveals intrinsic ubiquitinylation activity targeting oncprotein Hdm2 thereby regulating its expression levels, which directly affects p53 stability [57]. Conditions of aberrant PCAF activity have been correlated to the aggravation of several diseases. In detail, enhanced expression of PCAF was observed in cisplatin resistant human prostate PCa cells (PC-3), while PCAF downregulation sensitized these again. In this regard, a crucial dependency of PCAF-mediated stabilization of the transcriptional factor E2F1 was postulated [58]. Subsequently, it was found that PCAF likewise regulates YB-1 expression in a Twist-1 dependent manner and thereby promotes cancer cell growth, cancer invasion, and drug resistance in KK47 urothelial cancer cells [59]. Upregulation of PCAF in PCa cells promotes AR transcriptional activation as well as cell growth in PCa cells and might be associated with downregulation of miR-17-5p, which naturally suppresses PCAF mRNA translation and induces its degradation [60]. Moreover, acetylation of the downstream target EZH2 in combination with its subsequent phosphorylation is linked to EZH2 stabilization and thereby suppression of EZH2 target genes, which in turn promotes lung cancer cell migration and invasion. Elevated EZH2 K348 acetylation by PCAF is moreover a predictor of poor prognosis in lung adenocarcinoma patients [61].

Cancer cell proliferation is likewise influenced through the Hedgehog (Hh) signaling pathway, which upon depletion of PCAF is impaired due to reduced interaction of PCAF with the downstream transcription factor glioma-associated oncogene 1 (Gli1). This results in decreased expression of Hh target genes ultimately leading to decreased proliferation as well as increased apoptosis in medulloblastoma and glioblastoma cells (U87, U118, T98G, DAOY). PCAF silencing reduces the tumor-forming potential of neural stem cells in vivo confirming that PCAF is a positive
cofactor of the Hh-Gli signaling pathway and therefore an attractive target for therapeutic interventions [62]. PCAF-mediated Akt1 acetylation is increased in human glioblastoma cells (U-87 MG) and furthermore required for subsequent Akt1 phosphorylation, which results in enhanced proliferation [63]. Recent analyses, however, indicate that PCAF in certain contexts may also be an anti-oncogene and inhibits hepatocellular carcinoma (HCC) metastasis by targeting Gli-1 and thereby inhibiting epithelial-mesenchymal transition (EMT). Potentially, the expression level of Gli-1 is reduced due to PCAF dependent ubiquitinylation and subsequent proteasomal degradation [64].

Ultimately, PCAF is involved in the regulation of metabolic enzymes such as ATP-citrate lyase (ACLY), a key enzyme in de novo lipid synthesis that performs the cleavage of citrate in order to produce cytosolic acetyl-CoA, as building block for de novo lipid synthesis. In response to high glucose conditions, PCAF-mediated acetylation of three lysines in ACLY competes with ubiquitinylation by the ubiquitinylase UBR4 and results in ACLY stabilization, which in turn promotes cell proliferation due to increased lipid synthesis. Since overexpression and acetylation of ACLY is found in many tumors such as non-small cell lung cancer (NSCLC), PCAF might represent a valid target for therapeutic interventions [65].

PCAF is moreover entangled in the progression of neurodegenerative diseases. For instance, selective inhibition of the cytokine-induced NF-κB activation by inhibiting PCAF-mediated NF-κB acetylation prevents Aβ-induced neuronal cell death and might delineate a potential therapeutic approach for alleviating the inflammatory progression of Alzheimer’s disease [66]. On the contrary, PCAF was recently shown to be required for conditioning-dependent axonal regeneration and also singularly promotes regeneration after spinal cord injury [67]. Inflammatory associated diseases like chronic kidney disease (CKD) and acute kidney injury (AKI) may likewise be driven by PCAF. Recent results demonstrate that PCAF regulates the expression of inflammatory molecules and may contribute to the development of CKD and AKI, thus providing a potential therapeutic target for inflammation-related renal diseases [68].
1.2.3 GCN5: general control of amino acid synthesis protein 5

The HAT general control of amino acid synthesis protein 5 (GCN5) is a paralog of PCAF and was first shown to operate as a transcriptional adapter protein with HAT activity linking histone acetylation to gene activation in *Tetrahymena thermophila* [69]. A distinct feature of GCN5 is its antagonistic regulation of XBP-1S-mediated transcription [70]. GCN5 facilitates histone acetylation by operating as the HAT active subunit of the chromatin-modifying complexes SAGA (Spt-Ada-Gcn5-acetyltransferase) and ATAC (Ada2-containing), which regulate distinct sets of genes [71]. Recruitment of the SAGA complex by Myc, a master regulator of transcription, results in GCN5-mediated chromatin remodeling that allows for transcription of Myc-related target genes [72-74]. Besides histone modification, GCN5 is likewise involved in the direct acetylation and thereby stabilization of the Myc oncoprotein [75]. Just like PCAF, GCN5 interacts with the cell growth associated transcription factor E2F1 and is important for its function in gene regulation. E2F1 recruits GCN5 to acetylate H3K9 and GCN5 expression directly enhances the expression of *E2F1, cyclin E1*, and *cyclin D1* and potentiates tumor growth in small-cell lung cancer (SCLC) in an E2F1-dependent manner [76]. GCN5 protein expression and mRNA levels are elevated in human colon cancer. According to this, both the oncogenic transcription factor c-Myc and surprisingly the proapoptotic transcription factor E2F1 mediate antiapoptotic GCN5 gene transcription and thereby colon cancer cell progression [77]. GCN5 is also known to acetylate and stabilize the chimeric transcription factor E2A-PBX1, which is involved in the onset of pediatric pre-B-cell acute lymphoblastic leukemia (ALL) caused by the oncogenic translocation t(1;19) encoding for a fusion protein comprised of the transcriptional activator E2A and homeobox pre-B-cell leukemia transcription factor 1 (PBX1). E2A-PBX1 can promote cell transformation both *in vitro* and *in vivo* and GCN5-mediated acetylation of E2A-PBX1 leads to increased protein levels of E2A-PBX1 in ALL cells [78]. GCN5 is frequently overexpressed in human glioma tissues and exhibits critical roles in glioma development by potentiating cell proliferation and invasion via STAT3 and AKT signaling pathways. In this regard, expression levels of GCN5 are likewise correlated to the proliferation levels of proliferating cell nuclear antigen PCNA and matrix metallopeptidase MMP9.
GCN5 knockdown suppresses cell proliferation as well as colony formation and furthermore inhibits cell invasion [79]. In addition, GCN5 interacts with the tumor-promoting protein Pygopus homolog 2 (Pygo2) through its association with the SAGA complex resulting in elevated interaction of SAGA and β-catenin and elevated Wnt target gene expression in breast cancer cells [80].

### 1.2.4 Tip60: Tat interactive protein, 60 kDa

Tip60 was originally isolated and named after an HIV-1 Tat interactive protein with the size of 60 kDa and has been shown to increase Tat-dependent transcriptional activation of the HIV-1 promoter in a yeast two hybrid system utilizing a human B-lymphoblastoid library [81]. Later it was found that Tip60 is part of a macromolecular multimeric protein complex, the Tip60 complex, consisting of up to 16 subunits and comprises acetyltransferase activity on H2A, H3, and H4 but not H2B core histones. Ectopic expression of mutated Tip60 lacking acetyltransferase activity results in cells with defective double-strand DNA repair mechanisms and loss of their apoptotic competence [82]. Tip60 is a key factor of genotoxic stress response and regulates the expression of DNA repair and apoptosis genes upon DNA damage. For instance, histone H3 methylation at double strand breaks activates the acetyltransferase activity of Tip60 [83]. Tumor suppressor p53 is likewise stabilized by Tip60-mediated acetylation upon DNA damage resulting in apoptosis [84, 85]. Acetyltransferase activity is also rapidly activated by ionizing radiation, which leads to acetylation and activation of the ataxia telangiectasia mutant (ATM) protein kinase inducing activation of cell-cycle checkpoints and the initiation of DNA repair via phosphorylation of p53 and Chk2 [86]. Acetylation of c-Abl tyrosine kinase by Tip60 also represents a cellular response to DNA damage and stimulates transcriptional-independent apoptotic activity of Abl [87]. Tip60 furthermore acts as a ligand-dependent coactivator for the androgen, estrogen, and progesterone receptors [88]. Another interaction partner of Tip60, the oncprotein c-Myc, is stabilized by acetylation leading to induction of gene transcription, but likewise c-Myc itself is
required for the recruitment of the Tip60 complex to chromatin and potentially contributes to histone acetylation during normal mitogenic response [75, 89]. Tip60 generally acts as a tumor suppressor and downregulation or loss may end up in the onset of cancer such as breast tumor formation [90]. Oncogenic c-Myb transcriptional activity, for example, is controlled and inactivated by a tumor suppressor activity associated with Tip60 in human leukemia [91]. Moreover, Tip60 is a co-repressor of STAT3, which as constitutively active mutant species induces oncogenic transformation of NIH 3T3 cells and antagonistically controls colon cancer progression through the Wnt-pathway by counteracting β-catenin acetylation and accumulation [92, 93]. Enforced Tip60 expression inhibits melanoma cell migration and results in significantly increased chemosensitivity whereas Tip60 knockdown enhances melanoma cell migration [94]. However, multiple studies link Tip60 activity to cancerous incidents. Tip60, for instance, is a direct regulator of E2F1 and cisplatin treatment of human lung adenocarcinoma cells induces the accumulation of E2F1 in a Tip60-dependent manner. Both are linked to the upregulation of ERCC1, which is involved in the repair of cisplatin-induced DNA lesions ensuring genomic stability and facilitating cell cycle progression [95]. Furthermore, Tip60 overexpression is significantly correlated with cisplatin sensitivity in human lung cancer cell lines and might be involved in establishing a DNA-repair-permissive chromatin structure in cisplatin resistant cells, which contributes to drug resistance. Of note, knockdown of Tip60 expression sensitizes cells to cisplatin again [96]. Tip60 is upregulated in castration-resistant LNCaP derivative CxR cells resulting in increased amounts of acetylated AR and AR localization in the nucleus in conjunction with androgen-dependent gene transcription even in the absence of androgen. In this regard, Tip60 silencing suppresses the growth of AR-expressing PCa cells by inducing G1 cell-cycle arrest and suppresses CxR cell growth [97]. Recent studies link Tip60 activity to the outcome of malignant pleural mesothelioma and promote it as potential therapeutic target since treatment of cells with a Tip60 specific small molecule inhibitor (MG149) causes
significant inhibition of cellular proliferation, induction of apoptosis, and furthermore significant induction of pro-inflammatory cytokines/chemokines [98]. Aberrant HAT activity is strongly conjugated with cancer development and progression and determines members of this class of transferases to be potential drug targets for cancer treatment. Furthermore, it emphasizes the need of therapeutic agents to target HAT function in disease and to stimulate the development of selective probes and binders to further decipher the complex and orchestrated network of HAT signaling. Notably, the discovery of both inhibitors and activators presumably are of high impact to the field of HAT research as some diseases are triggered by increased HAT activity. Lack of HAT activity, however, induces others. Of note, most HAT-related diseases are not driven by dysregulation of HATs but rather by acetylation dependent stabilization of downstream targets.

1.3 Small molecule modulators of HAT function
Strategies to target acetylation-dependent pathways may include multiple ways to reduce HAT signaling (Figure 2). Direct HAT inhibitors that abolish HAT activity in a competitive or allosteric way can reduce HAT signaling by directly targeting its enzymatic activity. In addition, disruptors blocking the specific interaction of HATs with recruitment proteins determining subcellular localization may lead to downregulated signaling. In this regard, disruption of DNA-binding of the translational activator complex is another way to abolish epigenetic modulation through HATs. Targeting epigenetic reader domains is another approach to interfere with HAT activity, by disrupting the recognition of acetylated lysines (KAc) through Bromodomains. Small molecule inhibitors and probes may be useful for probing HAT function in a specific way and thereby may have a high impact on deciphering the underlying functions and network control of HAT related diseases. A major challenge is the current lack of selective, potent, and cell permeable inhibitors. To date, not a single selective and potent HAT inhibitor (HATi) has been approved for therapeutic use in diseases involving HAT associated epigenetic disorders. For this reason, there is great interest in screens to identify novel small-molecule modulators for subsequent use as probes or serve as origin for drug development approaches.
1.3.1 HAT modulators of synthetic origin (Figure 3)

1.3.1.1 Bisubstrate inhibitors Lys-CoA and H3-CoA-20 – p300 and PCAF

Bisubstrate compounds, mimicking an intermediate state in the acetyl transfer event from acetyl-CoA to a specific lysine, represent the first identified selective HAT inhibitors [99]. Based on investigations on serotonin N-acetyltransferase bisubstrate analogs, installing an acetyl bridge between amine substrate (Lys) and CoA was demonstrated to be the most effective linker element [100]. This work stimulated further approaches to identifying specific CoA conjugates as inhibitors for p300 and PCAF, respectively. A series of different conjugates consisting of Lys itself or histone H3/H4 peptides in conjunction with CoA were synthesized and evaluated with respect to their inhibitory activity on HATs. The Lys-CoA conjugate was found to be a potent and selective inhibitor of p300 acetyltransferase activity with an IC$_{50}$ of approximately 500 nM while H3-CoA-20 was proven to be an effective disruptor of PCAF activity (IC$_{50}$ = 300 nM). However, CoA conjugates are known to have limited ability to penetrate cell membranes and therefore are restricted in terms of use in cell-based systems. For this reason, cellular potency was solely evaluated by microinjection of the inhibitors using a MyoD-dependent promoter frog oocyte transcription system, which is activated by either p300 or PCAF. A dosage-dependent and selective reduction in p300 transcriptional activation (IC$_{50}$ ~ 30 pmol/cell) was observed in the presence of Lys-CoA [99]. Although CoA conjugates reveal unfavorable properties in terms of bioavailability, these early inhibitors were used as tool compounds to decipher p300 and PCAF associated signaling in several studies, which involved permeabilization of cells prior to treatment [101, 102].

1.3.1.2 C646 – p300/CBP

C646 is a competitive inhibitor of p300 HAT activity and was discovered in a virtual screen involving approximately 500,000 commercially available small molecules, which were docked in silico into the Lys-CoA binding pocket of p300. After validation of 13 potential p300 inhibitors, which were subsequently tested in a direct, radioactive assay for IC$_{50}$ measurements, three compounds were verified to
demonstrate potent inhibition of p300 HAT activity. Among these, C646 was found to be the far most active and selective compound (Ki = 400 nM). Subsequent rational design and structure activity relationship (SAR) studies did not result in inhibitors with improved potency [103]. However, C646 has been used so far in various studies and is often found to be the gold standard of p300 inhibition such as in probing the rapid dynamic acetylation of all H3K4me3 mediated by p300/CBP [104]. Another study involved the investigation of p300 inhibition as a targetable function of p300 in the survival and invasion pathways of prostate cancer cell lines. The study revealed an increase of caspase-dependent apoptosis in androgen-dependent and castration-resistant prostate cancer cells by interfering with AR and NF-κB pathways upon C646 treatment [34]. Subsequent investigations support this finding and demonstrate that p300 inhibition reduces pro-inflammatory gene expression by affecting NF-κB signaling [105]. Furthermore, C646 exerts anti-leukemia effects on AML1-ETO-positive AML cells by inhibiting cellular proliferation, reducing colony formation, evoking partial G1 cell cycle arrest, and inducing apoptosis in AE-positive AML cells. These effects, including reduced histone H3 acetylation and declined c-kit and bcl-2 levels, were selective on AE-positive while sparing AE-negative AML cells [106]. Another study illustrates the synthetic lethality of p300 inhibition by C646 in CBP deficient cancers by inducing apoptotic cell death due to abrogation of Myc expression via reduction of histone acetylation in its promoter region [38]. In WM35 melanoma cells, p300 inhibition by C646 blocks S-phase progression and activates G1/S cell cycle arrest mechanisms and promotes cellular senescence. In addition, expression-profiling studies revealed a loss of DNA damage response gene expression after HAT inhibition. Therefore, combination treatments of the DNA damaging agent cisplatin and HAT inhibitor C646 allowed for enhanced apoptotic responses at low concentrations (10 µM). However, at higher doses (20 µM) a relative reduction in apoptotic cells following cisplatin treatment was observed, which is most likely due to the fact that cisplatin efficacy is dependent on cycling cells and a higher degree of growth arrest is counterproductive for synergistic effects [107]. More recent studies focus on the fact that the chemical structure of C646 comprises a conjugated pyrazolone-furan motif, which is potentially reactive towards cellular
nucleophiles aiming on further accessing the inhibitors’ specificity in proteomics studies in order to decipher the associated non-HAT covalent targets. On the basis of in silico docking studies, the inhibitor was converted into an appropriate probe for target validation studies by generating an alkyne derivative, which is suitable for Click chemistry applications (e.g., biotin tagging) and subsequent use in pull-down studies from HEK-293 cell lysates using 20 µM probe concentration. Strong denaturing conditions were used during click chemistry process to ensure that solely covalent targets of the C646 analogue were identified. Among these, the five most enriched proteins were different chains of the abundant cytoskeletal protein tubulin. Others include elongation factor 1-alpha, heatshock protein HSP60, and glycolytic enzyme 3-phosphoglycerate dehydrogenase. Subsequently, C646 was shown to inhibit tubulin polymerization at 50 µM compound concentration (50% inhibition) [108]. However, rather high compound concentrations were used in these pull down studies (20 µM), since the in vitro IC$_{50}$ for p300 inhibition was determined to 0.4 µM, which still might render C646 to be a selective p300 inhibitor at low concentrations [103]. Further work revealed that C646 at higher concentrations has an extended selectivity profile and inhibits histone deacetylases (HDACs 1-3, 6 and 8) at concentrations ≥ 7 µM [105].

1.3.1.3 Isothiazolones CCT077791 and CCT077792 - p300, PCAF

Iothiazolones were discovered as HAT inhibitors in a high throughput screening of 69,000 compounds using scintillating microplates (FlashPlate assay) [109] and PCAF as the target protein. Based on three promising isothiazolone hits with significant inhibitory activity against PCAF (IC$_{50}$ = 3.1-12.4 µM) and two related commercially available derivatives revealing similar inhibitory activity, 35 further N-aryl and N-alkyl isothiazolone analogues were designed, synthesized, and evaluated for HAT inhibition. Inhibitors CCT077791 and CCT077792 were shown to evoke the most significant inhibitory activity on PCAF (IC$_{50}$ = 2.2 and 2.7 µM, respectively) as well as p300 (95% and 90% inhibition at 35 µM compound concentration, respectively) in filter binding assays. Furthermore, they were shown inhibit proliferation and decrease global cellular acetylation in HCT116 and HT29 human cancer cell lines with
GI\textsubscript{50}s of 3.0 \textmu M and 2.1 \textmu M for CCT077791 as well as 0.4 \textmu M and 0.4 \textmu M for CCT077792, respectively. Consecutive experiments involving protein binding and dialysis assays assume a covalent binding mode involving modification of a cysteine that is believed to form hydrogen bonds that bind acetyl-CoA in close proximity with the histone substrate during the enzymatic reaction. The isothiazolone might form a disulfide with the active site cysteine and convert into an open-chain dihydro product, for which the correlating mass was observed in LC/MS experiments. However, no definite evidence was shown and an allosteric MOA might likewise be possible [110]. The high chemical reactivity of simple isothiazolones is a drawback of this class of substances, with concerns related to promiscuity.

1.3.1.4 Pyridoisothiazolones PU139 - pan-HAT and PU141 - p300/CBP
Based on the high-throughput approaches that originated isothiazolone HAT inhibitors [110], structurally related pyridoisothiazolones were found as potent PCAF inhibitors. Exploratory medicinal chemistry studies aiming for clarifying the structural requirements for their biological activity and the scope for further development towards cancer therapy candidates yielded in the design and synthesis of pyridylisothiazolone derivatives 10 and 38 with PCAF inhibitory activity (IC\textsubscript{50} = 1.5 and 6.1 \textmu M, respectively) [111]. Subsequently, a series of related pyridylisothiazolones was identified based on a virtual screening approach querying for isothiazolones and isothiazolidinones utilizing the National Cancer Institute's 3D database containing structurally diverse compounds as well as natural products. 51 potential hits comprising the queried substructure were subsequently docked into the substrate-binding site of PCAF (template: PDB-code 1CMO). 21 selected compounds were further analyzed in TRF-based \textit{in vitro} assays monitoring PCAF-mediated H3 and H4 acetylation, respectively. Based on the obtained SAR patterns, further analogues were synthesized originating PU139 (IC\textsubscript{50} = 1.64 and 0.72 \textmu M, respectively) and PU141 (IC\textsubscript{50} = 130 and 25.5 \textmu M) as potent HAT inhibitors, which were able to disrupt PCAF mediated H3 and H4 acetylation \textit{in vitro}. Moreover, significant antiproliferative effects were demonstrated in human neuroblastoma (SK-N-SH), colorectal carcinoma (HCT116), and breast adenocarcinoma (MCF-7) cell lines
Investigations with respect to SAR patterns demonstrated that PU139 is a rather promiscuous HAT inhibitor with effects on GCN5, hMOF, PCAF, CBP and p300 while PU141 is more selective sparing significant GCN5 and PCAF inhibitory activity. Nevertheless, growth inhibition in prostate cancer cells (LNCaP, GI50 ~36-37 µM) as well as leukemic cells (HL-60, GI50 ~3-4 µM) was elicited to the same extent [113]. Most recently, both inhibitors were demonstrated to block tumor growth of SK-N-SH neuroblastoma xenografts. Moreover, the pan-HAT (N-phenyl derivative) inhibitor PU139 synergized with doxorubicin in vivo promoting a promising anticancer activity and providing a solid basis for future applications [114].

1.3.1.5 **KCN1 – p300/CBP (Disruptor of HIF-1α-p300/CBP interaction)**

Arylsulfonamide KCN1 was identified as one of the most potent compounds in a hypoxia-responsive element (HRE)-luciferase reporter system (IC50 ~ 0.5 µM) originated from the screening of a privileged library of about 10,000 natural-product-like compounds in a cell-based assay for HIF-dependent transcriptional activity [115]. Chemical optimization efforts to enhance solubility, pharmacological properties and biological effects did not result in improved inhibitory activity but evoked several derivatives with equipotent behavior [116-118]. KCN1 was shown to specifically inhibit HIF reporter gene activity in glioma cell lines (LN229HRE-luc/LacZ) at the nanomolar level (IC50 = 590 nM). KCN1 likewise led to downregulation of transcription of endogenous HIF-1 target genes (VEGF, GLUT1 and CA9) in an HRE-dependent manner. KCN1 potently inhibited the growth of subcutaneous malignant glioma tumor xenografts and also induced a temporary survival benefit in an intracranial model of glioma (LN229 human glioma cells) without any significant toxicity. KCN1 did not downregulate levels of HIF-1α or other components of the HIF transcriptional complex but rather antagonized hypoxia-inducible transcription by disrupting the interaction of HIF-1α with transcriptional co-activators p300/CBP [119]. In addition, KCN1 possesses in vitro anti-cancer activity against various pancreatic cancer cells (HPAC, Panc-1, BxPC3, and Mia Paca-2) with most significant cytotoxic effects on HPAC and BxPC3 (IC50 = 17.9 and
14.6 µM, respectively) and leads to G1 cell cycle arrest. KCN1 is stable in mouse plasma, but it seems to bind extensively to plasma protein (91% bound at 1 µM, 99% at 10 µM) and appears to be metabolized by phase I enzymes. However, growth inhibition of pancreatic cancer xenograft tumors (Panc-1, Mia Paca-2) was demonstrated [120].

1.3.1.6 ICG-001 - CBP

ICG-001 was identified as low molecular weight antagonist of β-catenin/TCF-mediated transcription (IC₅₀ = 3 µM) in SW480 colon carcinoma cells in a forward chemical genomic screen of a privileged secondary structure template small molecule library of 5,000 compounds using the TOPFLASH reporter assay system. Secondary studies included the synthesis of a biotinylated derivative for use as affinity reagent and determine molecular targets of ICG-001. The only major target identified was the transcriptional coactivator CBP, which was further confirmed by orthogonal ¹⁴C radiolabeling experiments. ICG-001 specifically disrupts the CBP/β-catenin complex by competing with β-catenin for CBP but does not affect the p300/β-catenin interaction. It specifically down-regulates expression of a subset of β-catenin/TCF-responsive genes (survivin and cyclin D1) and induces apoptosis in colon carcinoma cells (SW480, HCT116) in a dose-dependent manner as shown by increased caspase 3/7 activity, but not in normal colonic epithelial cells (CCD-841Co). Ultimately, ICG-001 treatment is efficacious in both the Min mouse (human familial adenomatous polyposis model) and nude mouse SW620 colon cancer xenografts [121]. ICG-001 was further used as a tool compound to decipher the differential roles of CBP and p300 in TCF/β-catenin-mediated survivin gene expression [122]. Recently, ICG-001 was shown to specifically target the CBP/catenin interaction and safely abrogate ALL by disrupting the self-renewal of drug-resistant leukemia-initiating cells via forced differentiation, thereby sensitizing them to either cytotoxic or targeted chemotherapy both in vitro and in vivo [123].

1.3.1.7 MB-3 - GCN5
The α-methylene-γ-butyrolactone MB-3 was identified as the first small molecule inhibitor of GCN5, originally synthesized as racemic mixture in design approaches. The design involved an induced fit model of the catalytic mechanism of Lys acetylation involving the conserved amino acid Glu173 as a general base and the backbone amide of Cys177 to stabilize the tetrahedral intermediate. Based on these considerations, compounds with distinct features were designed comprising a possible hydrogen-bond acceptor for the backbone amide of Cys177 and a polar group for interaction with Glu173. Moreover, the designed molecules possessed an aliphatic side chain to mimic the lysine side chain. MB-3 was demonstrated to be a reversible inhibitor revealing weak inhibitory activity against CBP (IC$_{50} = 500$ µM) but more potent inhibition towards GCN5 (IC$_{50} = 100$ µM) [124]. MB-3 was recently shown to reveal potential for use as a therapeutic strategy for the treatment of patients suffering from pediatric pre-B-cell acute lymphoblastic leukemia (ALL). The expression of the chimeric transcription factor E2A-PBX1 was observed to be the oncogenic driver in ~23% patients with pre-B-cell ALL. Upon interaction with the STAGA complex, which comprises GCN5 as acetylase activity element, the stability of E2A-PBX1 is increased upon GCN5 mediated acetylation. Treatment of ALL cells with MB-3 significantly decreases E2A-PBX1 acetylation in a dose dependent manner and leads to overall reduction of E2A-PBX1 protein levels [78].

1.3.1.8 Hydrazinylthiazole derivatives CPTH2, CPTH6, BF1, and remodelin – GCN5, PCAF, p300, NAT10

The inhibitory activity of CPTH2 was associated with the GCN5p functional network by chemical genetic screening in GCN5p deleted Saccharomyces cerevisiae, evoking a synthetic lethal phenotype upon CPTH2 treatment. Basic in vitro investigations demonstrated that histone H3 acetylation by human recombinant GCN5 was inhibited at 0.8 mM CPTH2 concentration due to competition of the inhibitor with the protein substrate [125]. Another derivative, CPTH6, which was identified in the course of the yeast-based screen, was used as a tool compound to study effects of HATs on target molecules. Specific inhibition of GCN5 and PCAF HAT activity at 0.8 mM concentration
(~40% and 35% inhibition, respectively) was demonstrated while not affecting p300/CBP activity. Furthermore, CPTH6 mediated disruption of histone H3/H4 and α-tubulin acetylation in various leukemia cells lines (U-937, HL-60, KG1, and HEL97.1.7) was determined. In addition, concentration- and time-dependent inhibition of cell viability by inducing apoptosis, paralleled by accumulation of cells in the G0/G1 phase and depletion from the S/G2M phases was observed in U-937 and HL-60 cells. Remarkably, treatment of leukemia cells (U-937, HL-60) and likewise neuroblastoma cells (SH-SY5Y and LAN-5) induced differentiation processes as observed by an augmented mature phenotype or neurite outgrowth, respectively. In vivo pharmacokinetic considerations did not produce any adverse health effects with respect to diet consumption, body weight loss, and postural and behavioral changes [126]. Most recent investigations show that CPTH6 preferentially targets lung cancer stem-like cells (LCSCs) derived from non-small cell lung cancer (NSCLC) patients primarily due to apoptosis induction. In vivo, CPTH6 inhibits the growth of LCSC-derived xenografts, reduces cancer stem cell content in treated tumors and diminishes tubulin acetylation, which renders histone acetyltransferase inhibition as an attractive target for cancer therapy of NSCLC [127]. Further structure-based design studies were conducted ultimately resulting in the closely related analogue BF1, which decreased global H3 and H3AcK18 acetylation levels in glioblastoma (U87) and neuroblastoma (BE) cell lines without significantly affecting global viability. Of note, BF1 was shown to inhibit p300 activity superior to GCN5 activity in in vitro assays [128]. Conversion of CPTH2 into a suitable probe for Click-chemistry and subsequent application in pull-down and target identification experiments resulted in the identification of histone acetyltransferase NAT10 as a novel target of hydrazinylthiazoles. Since CPTH2 itself was rapidly degraded upon light and air exposure, the more stable analogue remodelin was further explored and demonstrated to inhibit NAT10. Furthermore, improved nuclear architecture, chromatin organization, fitness, and decreased markers of DNA damage were observed in both human lamin A/C–depleted cells and Hutchinson-Gilford progeria syndrome (HGPS) derived patient cells [129].
1.3.1.9 L002 (NSC764414) – p300, PCAF, GCN5

Using an HTS approach, the cytotoxicity of 622,079 compounds was assayed towards the triple-negative breast cancer (TNBC) cell line MDA-MB-231 while sparing human mammary epithelial cells and L002 was identified as a promising small molecule to inhibit p300 HAT activity in vitro using fluorescence based assays (IC$_{50}$ = 1.98 µM). In consecutive investigations using radiometric filter binding assays, L002 was shown to inhibit p300 with an IC$_{50}$ of 128 µM while simultaneously inhibiting PCAF and GCN5 (IC$_{50}$ = 34.7 and 33.9 µM, respectively). However, L002 displays inhibitory effects against MYST family HATs (Tip60, MYST2, MYST4) as well as a panel of 8 diverse histone methyl transferases (DOT1, EZH1, G9a, PRMT1, SETD2, SET7-9, SMYD2, and SUV39H2). Docking studies provide evidence for a competitive binding mode addressing the acetyl-CoA binding site of p300. Histone H3 and H4 acetylation in MDA-MB-468, MDA-MB-231 and HCT116 cell lines is suppressed by L002 and moreover p300-mediated STAT3 phosphorylation in pancreatic cancer cells (MIA Paca-2) is blocked. Among the NCI-60 panel of cancer cell lines, leukemia and lymphoma derived cell lines are exceptionally sensitive to L002 treatment, whereas solely a limited number of cells lines derived from solid tumors is affected. Notably, breast cancer cell lines, especially those derived from TNBC, are highly susceptible to L002 and furthermore in vivo tumor growth and histone acetylation is potently suppressed in MDA-MB-468 xenograft models [130].

1.3.1.10 NU9056 - Tip60

NU9056 was developed based on parent compound 4-methyl-5-bromoisothiazole, which was found as a potent Tip60 inhibitor (IC$_{50}$ = 1.1 µM) in an HTS campaign testing ~80,000 compounds using Alpha™ and Delphia™ assay technology. Since the identified inhibitor revealed off-target p300 activity (IC$_{50}$ = 2.7 µM), follow-up synthetic chemistry efforts towards the generation of optimized analogues were conducted, ultimately leading to the Tip60 selective inhibitor NU9056 (IC$_{50}$ ≤ 2.0 µM). NU9056 disrupts the levels of Tip60-mediated acetylation targets H4K16, H3K14, and H4K8 in androgen dependent LNCaP cells, while Tip60 levels remain unaffected.
Moreover, it reveals significant growth inhibition on several prostate cancer cell lines (LNCaP, LNCaP-AI, LNCaP-CdxR, CWR22rv1, PC-3) and reduction of prostate specific antigen expression (PSA) in LNCaP cells. The latter experimental results suggest that Tip60 is involved in AR and p53 signaling in this cell line and that NU9056 may affect these downstream targets by modulating Tip60 acetylation activity. Notably, androgen responsive LNCaP cells, which express a mutated but functional AR variant as well as wild type p53, and bone metastasis derived PC-3 cells, which do not express functional AR and are p53 deficient, show similar growth inhibition (GI\textsubscript{50} = 24 μM and 27 μM, respectively). Further experiments illustrate that NU9056 treatment induces apoptosis by caspase activation and likewise impairs the DNA damage response via inhibition of Tip60 acetylase activity in LNCaP cells [131].

1.3.1.11 Pentamidine – Tip60
The bisbenzamidine derivative, pentamidine, has been one of the most successful agents against eukaryotic parasites and has been used clinically in the treatment of pneumocystis pneumonia, trypanosomiasis, and leishmananiasis for over 80 years as reviewed elsewhere [132-134]. More recently, pentamidine was likewise described as a potent HAT inhibitor targeting Tip60 and thereby preventing histone H2A acetylation ultimately leading to suppression of DNA damage response. Initially, in \textit{vitro} inhibition of hMRE11 nuclease activity and ATM kinase activity was demonstrated in combination with repression of the formation of DNA damage responsive foci upon irradiation. In the course of further investigation of DNA damage-related proteins and in particular γ-H2AX, pentamidine surprisingly decreased the acetylation of histone H2A at Lys5 dramatically. The acetylation of H2A at Lys9 was likewise decreased, although γ-irradiation did not increase the acetylation of these histones. Consecutive experiments revealed Tip60 inhibition and thereby decreased acetylation of histone H2A in addition to inhibiting enzymatic activity of hMRE11 and ATM. Pentamidine treatment also reduces Tip60-dependent acetylation of p53 in SV40-transformed MRC5 human fibroblasts (MRC5SV) presumably through direct inhibition of Tip60 activity [135].
1.3.1.12 TH1834 – Tip60

TH1834 was developed in a rational design approach based on Tip60 modeling studies, which indicated that the active binding pocket possesses opposite charges at each end. Molecular modeling approaches in order to dock pentamidine into the active site of a crystal structure of human Tip60 in complex with CoA (template: PDB code: 2OU2) led to the generation of the specific landscape of the binding pocket for ensuing inhibitor design based on pentamidine and Acetyl-CoA scaffolds. Subsequent structure based drug design resulted in the novel and specific inhibitor TH1834 illustrating inhibitory activity towards Tip60 in vitro (60% inhibition at 500 µM) while sparing the closely related MOF. Upon treatment of MCF7 breast cancer cells with TH1834, dose dependent apoptosis is induced by caspase 3 activation. A combination of ionizing radiation and TH1834 treatment leads to increased unrepaired DNA damage [136].

1.3.1.13 Windorphen

Windorphen was originally discovered in a chemical screen of 30,000 compounds to identify Wnt pathway modulators based on their ability to perturb dorsoventral patterning in zebrafish embryos and was subsequently named after its function to reproducibly dorsalize zebrafish embryos through the Wnt pathway (Wnt inhibitor dorsalizing). Consecutive functional studies revealed that Windorphen targets the C-terminal transactivation domain of β-catenin-1, disrupts its association with p300, and moreover acts as a direct and selective p300 (IC₅₀ = 4.2 µM) but not CBP (IC₅₀ = 51.3 µM) inhibitor. Treatment of human colon adenocarcinoma SW480 cells, in which Wnt signaling is constitutively activated due to defects in the APC gene, causes widespread apoptosis. Further, human carcinoma cell lines with activated Wnt signaling, such as human colon cancer cell lines SW480 and RKO and prostate cancer cell lines DU145 and PC-3 are sensitive to windorphen treatment (IC₅₀ = 15.0, 19.2, 21.8, and 19.0 µM, respectively) whereas no cytotoxic growth inhibition is observed
towards the human lung cancer cell line H460, which does not exhibit aberrant Wnt signal activation [137].

1.3.1.14 Protein domain mimetics CH1iA and B – CH1 domain of p300 – (Disruptors of HIF-1α-p300/CBP or HPV16 E6-p300 interaction)

CH1iB, a stapled 8-mer peptide, was developed based on the hydrogen bond surrogate (HBS) method to mimic α-helical motifs of the HIF-1α C-terminal transactivation domain (C-TAD) and is a further developed derivative of the parent 10-mer stapled peptide CH1iA (53% α-helicity). The latter was designed to target the binding interface between the C-TAD of hypoxia-inducible factor 1 alpha (HIF-1α) and cysteine-histidine rich region (CH1) of transcriptional coactivator CBP/p300 and shown to bind to p300 at the cysteine-histidine rich region CH1 in a single-site binding model. Moreover, CH1iA leads to downregulation of HIF-1α mediated transcription of the hypoxia inducible genes VEGF and GLUT1 (both approx. 50% inhibition at 1 μM compound concentration) in HeLa cells [138, 139], which was likewise shown for the shortened CH1iB in subsequent studies [140]. Furthermore, CH1iB disrupts the HIF-1α/p300-CH1 complex in vitro and exhibits antitumor activity in 786-O RCC (high HIF levels due to VHL gene) mouse xenograft models [140]. CH1iA and CH1iB were recently evaluated for their inhibitory potential in human papilloma virus (HPV) positive head and neck squamous cell carcinomas (HNSCCs). High-risk HPV E6 inactivates p53 through two distinct mechanisms: association with E6AP to degrade p53 and association with p300 to block p300-mediated p53 acetylation and activation. Therefore, targeting the E6-p300 interaction might represent an effective approach to reactivate p53 in HPV-positive HNSCC. While CH1iA does not account for a p53 reactivation in HPV-positive UMSCC47 cells, CH1iB enhances p53 activity (71% at 10 μM CH1iB) and furthermore abrogates the association between E6 and p300. Furthermore, CH1iB potentiates the anticancer activity of cisplatin in UMSCC47 cells thereby promoting p53-reactivation agents as promising therapeutics for treatment of HPV-positive HNSCC patients.
These effects are unique for HPV-positive HNSCCs and not observed in corresponding HPV-negative cell lines [141].

1.3.2 Natural product derived HAT modulators (Figure 4)

1.3.2.1 Anacardic acid (AA) and derivatives – p300, PCAF (inhibiting and activating), Tip60, MOF

Anacardic acid, a natural product derived from *Anacardium occidentale* cashew nut shell oil [142], was identified to significantly impair p300 and PCAF HAT activity in filter binding assays using human core histones H4 (IC$_{50}$ = 8.5 µM and 5 µM, respectively) and H3 (IC$_{50}$ = 0.5 µM, only p300 tested) as substrates [143]. Multiple studies indicate that anacardic acid and derivatives thereof possess general antibacterial [142, 144] activity as well as inhibitory activity on prostaglandin synthases [145], tyrosinases [146] and lipoxygenases [147]. The design and synthesis of more potent PCAF inhibitors based on *in silico* docking studies utilizing a PCAF-CoA complex crystal structure led to compound 6d, which revealed two-fold improved inhibitory activity as compared to AA towards human recombinant PCAF-mediated H4 acetylation in ELISA assays as well as H4 acetylation in HEP G2 human liver cancer cells [148]. AA likewise inhibits the HAT function of Tip60 on H4 acetylation *in vitro* (IC$_{50}$ = 9 µM) and sensitizes HeLa as well as SQ20B and SCC35 squamous cell carcinoma (SCC) cells to ionizing radiation [149]. Interestingly, modification of the head group of anacardic acid might reverse inhibitory properties and results in significant boost of p300 and PCAF activity as shown for benzamide derivative CTPB. However, several of these benzamide analogues conserve inhibitory activity towards HATs, which impedes the assessment of drug discovery and structure based drug design approaches, since the inhibitory or activation potential is unpredictable [143, 150, 151]. Most recently, CTPB was shown to promote survival and neurite growth in a cellular model of Parkinson’s disease (SH-SY5Y cells), and also protected these cells from cell death induced by the neurotoxin 6-hydroxydopamine. With respect to the structure activity relationships of CTPB, the benzamide moiety itself is responsible for the activating properties [152]. For instance, the synthetic benzamide small-molecule CBP/p300 activator TTK21
identified in a low-throughput enzyme assay screening passes the blood–brain barrier and reaches different parts of the brain when conjugated to glucose-based carbon nanosphere (CSP) without inducing toxicity. After intraperitoneal administration in mice, CSP-TTK21 significantly increases acetylation status of histones in the hippocampus and frontal cortex. Moreover, the CBP/p300 activation favors maturation and differentiation of adult neuronal progenitors and induces significant extension of memory duration [153]. Other design studies involved variation of the salicylic head group of AA. Conversion into simplified alkyl-dimalonate analogues results in the generation of pentadecylidenemalonate SPV-106, an inhibitor of p300/CBP and CBP alone and simultaneously activator of PCAF with improved cell permeability as compared to AA. Treatment of U937 leukemia cells using this mixed inhibitor/activator leads to S-phase cell cycle arrest and induction of apoptosis (~100% at 50 µM, ~25% at 25 µM) [154]. Another similar AA derivative, MG149, was developed in an attempt to generate more potent compounds to target PCAF, but was not shown to increase the inhibitory activity towards PCAF. Subsequent studies, however, demonstrated that this 6-alkylsalicylate inhibits Tip60 (IC50 = 74 µM) and MOF (IC50 = 47 µM) while sparing p300 and PCAF inhibitory activity in biochemical assays by targeting the Tip60 acetyl-CoA binding site as confirmed by kinetic studies [155]. Notably, AA does not reveal significant inhibitory activity towards p300 and CBP (30% inhibition at 200 µM) in this study, rising great difficulty to compare biological activities among these studies since results may vary depending on the assay conditions and protein/substrate source. However, cell-based assays (as mentioned above) confirm significant biological activities of AA and derivatives thereof with respect to PCAF inhibition. Surprisingly, tail-modified AA derivatives result in both different target specificity as well as activating or inhibiting effects. Compounds bearing alkoxy substituents as in PK147 constituting a branched tail direct the inhibitory activity towards 5-lipoxygenase (residual activity: 50% at 50 µM PK147), whereas PK131 with an unbranched fully saturated but shortened tail inverts the biological effect and acts as 5-lipoxygenase activator (150% at 50 µM) [156].
1.3.2.2 Garcinol and derivatives LTK-14, nemorosone (activator) – p300, PCAF

The natural product garcinol from the stem bark of *Garcinia huillensis* [157] or alternatively isolated from *Garcinia indica* is a polycyclic polyprenylated acylphloroglucinol derivative with inhibitory activity against p300 (IC$_{50}$ = 7 µM) and PCAF (IC$_{50}$ = 5 µM). Garcinol represses chromatin transcription, induces apoptosis, and alters the global gene expression in HeLa cells [158]. Most recently, garcinol was discovered to exacerbate lipopolysaccharide-induced inflammation *in vitro* and *in vivo*, suggesting that HAT acetylation represents an important regulatory function during inflammation [159]. The development of more potent, specific, and less toxic inhibitors led to the synthesis and characterization of several derivatives based on isogarcinol (IG), a product of intramolecular cyclization of garcinol. 14-ethoxy IG (LTK-14), for instance, specifically inhibits p300 without any cytotoxicity while still altering global gene expression signatures. LTK-14 inhibits p300 mediated site-specific acetylation of p53 at K373 but not through PCAF at K320, which was verified by monitoring acetylation of p53 *in vitro* by using either p300 or PCAF and bacterially expressed Flag-p53 as substrate as well as wild-type p53 enhanced human lung carcinoma cells (A549). In addition, LTK-14 reduces the number of syncytia as well as the production of HIV core protein p24 in infected SupT1 cells in a dose-dependent manner [160].

In an effort to study p300 inhibitory effects of natural products containing garcinol-like features, surprisingly, nemorosone was identified as an activator of p300 ($K_d$ = 0.25 µM) causing doubling of p300 activity at 10 µM compound concentration in enzymatic assays. Histone acetylation is significantly increased in treated vs. untreated HeLa cells. Notably, nemorosone evokes antiproliferative activity in NB4 promyelocytic leukemia, NCIH460 non-small cell lung carcinoma, HCT-116 colon cancer, and HeLa cervical cancer cells (IC$_{50}$ = 4.8 to 6.8 µM) [161]. The close structural relationship between garcinol-derived activators and inhibitors of p300 demonstrates the predictive challenge in structural design approaches to develop HAT inhibitors since marginal variations on the chemical scaffold may significantly impact the corresponding MOA.
1.3.2.3 Curcumin and derivatives – p300/CBP

Curcumin [162], a natural product derived from *Curcuma longa* rhizome, is a homodimer of feruloylmethane and was identified as HAT inhibitor by plant extract screening and shown to potently inhibit the p300/CBP mediated acetylation of histone H3 and H4 in a dose dependent manner (IC\(_{50}\) ~25 µM) while not affecting PCAF activity [163]. Early publications already showed evidence for a significant antibacterial effect evoked by curcumin as well as structurally related synthetic chalkone analogues [164]. A plethora of ensuing approaches to investigate the biological effects of curcumin further revealed anti-inflammatory, anti-diabetic, anti-oxidant, and anti-cancer activity, amongst others [165-168]. Curcumin likewise operates as an adjuvant to improve the effect of chemotherapy by tailoring p65NFκB-p300 cross-talk in favor of p53-p300 in breast cancer thereby reversing doxorubicin resistance *in vitro* and *in vivo* [169]. However, biological activity is not limited to p300 and related proteins. Potential therapeutic use for curcumin as a radiosensitizer was demonstrated by inhibiting radiation-induced prosurvival factors (TNF-α, NFκB, Bcl-2) in PC-3 cells and enhancing radiation-induced clonogenic inhibition as well as apoptosis by inducing G2/M cell cycle arrest [168]. In addition, a wide array of direct target proteins was discovered to date including inflammatory molecules, cell-survival proteins, protein kinases, protein reductases, histone acetyltransferase, histone deacetylase, glyoxalase I, xanthine oxidase, proteasomes, HIV1 integrase, HIV1 protease, sarco/endoplasmic reticulum Ca\(^{2+}\) ATPase, DNA methyltransferase 1, FtsZ protofilaments, and carrier proteins as already reviewed elsewhere [170]. Since polyphenolic inhibitors incorporate marginal bioavailability due to poor absorption, rapid metabolism, and rapid systemic elimination, multiple studies address the administration of curcumin in combination with adjuvants such as piperine that inhibits phase II metabolic pathway enzymes to prevent drug conjugation and subsequent excretion [171]. The use of artificial delivery systems such as nanocarriers to increase bioavailability represents another approach to improve bioavailability. Several synthetic derivatives of curcumin evolved and among these CTK7A (Hydrazinocurcumin), a water soluble sodium salt of the previously
discovered derivative hydrazinobenzoylcurcumin [172], was shown to antagonize the hyperacetylation of histones in oral squamous cell carcinoma, which initially is caused by overexpression of NPM1 and GAPDH resulting in p300 autoacetylation followed by histone hyperacetylation. CTK7A inhibits p300/CBP and PCAF activity and kinetic studies prove that CTK7A-mediated p300 inhibition follows a mixed type of inhibition for both of the substrates, acetyl-CoA, and core histones. Moreover, CTK7A impairs the growth of the oral cancer cell line KB, induces senescence-like growth arrest and furthermore inhibits tumor growth in KB cell xenografts [173].

1.3.2.4 Chetomin – p300/CBP

Chetomin, a natural product produced by Chaetomium cochlodes was discovered as antibacterial agent in 1944 [174], but the definite general composition and absolute configuration was first confirmed more that 30 years later [175]. With respect to HAT inhibition, several studies demonstrated that chetomin disrupts the interaction of HIF-1α with CBP/p300 and thereby abolishes the differentiation inhibitory effect of HIF-1α. Moreover, chetomin as well as closely related epidithiodiketopiperazine analogues (gliotoxin, chaetocin; not discussed here) exhibit in vitro antiangiogenic and in vivo antitumor activity by disrupting the HIF-1α/p300 complex as demonstrated in PC-3 as well as DU-145 xenografts [29]. Combined administration of chetomin with forskolin, an inducer of differentiation, significantly suppresses malignant glioma growth in vitro as well as in a xenograft model [176]. Upon delivery as biodegradable polymeric micelles-encapsulated chetomin, antitumor activity in both transgenic zebrafish and mouse models is enhanced [177]. Other studies demonstrate that dual targeting of AR and HIF-1α pathways using enzalutamid and chetomin, respectively, leads to synergistically inhibition of castration-resistant prostate cancer cell growth [178]. A recent study further substantiates that targeting the HIF-1α/p300 complex with chetomin exhibits antitumor activity in multiple myeloma [179].

1.3.2.5 Plumbagin (RTK1) and derivative PTK1 – p300
The hydroxyl-naphthoquinone plumbagin was first described in 1968 as small molecule with antibacterial properties isolated from the aerial parts of *Plumbago scandens* [180] and was since then described as antifungal and antiviral [181], antimicrobial [182], and anticancer [183] agent. The inhibitory activity towards HATs, however, was identified in a screening of medical plant crude extracts for acetyltransferase modulation activity. Plumbagin was isolated and crystallized as inhibitory active natural product from *Plumbago rosea* extract and inhibitory activity was confirmed by reduced histone H3 acetylation (Lys9 and Lys14) *in vitro* using liver cells and *in vivo* through immunohistochemical analysis of mice liver after plumbagin treatment. Subsequent kinetic characterizations led to the finding that plumbagin is a noncompetitive inhibitor of p300 ($K_d = IC_{50} = 2 \mu M$) occupying a single binding site and likewise inhibits non-histone protein acetylation (p53, PC4) [184]. However, naphtoquinone compounds are fairly toxic due to their redox cycling and thiol-reactive properties [185-187]. For this reason, endeavors to generate non-toxic analogues were undertaken and resulted in the development of the reversible, non-competitive p300 inhibitor PTK1 with reduced electrophilicity and thereby reduced toxicity, but likewise impaired potency in cellular assays. For PTK1, a reversible MOA was proven, whereas RTK1 is a covalent inhibitor of p300 [188].

### 1.3.2.6 Oridonin – p300, Tip60, GCN5, PCAF

The diterpenoid oridonin was discovered as an active ingredient of the eight herbal ingredients mixture PC-SPES and can be isolated from *Rabdosia rubescens*. It reveals antiproliferative activity against a wide variety of cancer cells including prostate (LNCaP, DU145, PC-3), breast (MCF-7, MDA-MB231), non-small cell lung (NCI-H520, NCI-H460, NCI-H1299) cancers, acute promyelocytic leukemia (NB4), and glioblastoma multiforme (U118, U138) with ED$_{50}$s ranging from 1.8 to 7.5 µg/mL [189]. Moreover, it inhibits proliferation of multiple myeloma (U266, RPMI8226), acute lymphoblastic T-cell leukemia (Jurkat), and adult T-cell leukemia (MT-1) cells in conjunction with disrupting NF-κB signaling pathways by perturbing the DNA binding ability of NF-κB. The latter observation was furthermore demonstrated in the course of survival studies of patient derived adult T-cell leukemia, acute
lymphoblastic leukemia, chronic lymphocytic leukemia, non-Hodgkin’s lymphoma, and multiple myeloma cells. Antiapoptotic Bcl-2 family members Mcl-1 and BCL-xL are likewise downregulated by oridonin in both MT-1 and RPMI8226 cells [190]. Of note, oridonin addresses a plethora of cellular targets and affects multiple pathways as reviewed elsewhere [191]. Only recently, oridonin was identified as a novel lysine acetyltransferase inhibitor, which inhibits proliferation and induces apoptosis in gastric cancer cells through p53- and caspase-3-mediated mechanisms. In detail, oridonin inhibits p300 transferase activity (IC$_{50}$ ~5 µM) superior to Tip60, GCN5, and PCAF inhibition in vitro and the inhibitory effect is more pronounced as compared to known HAT inhibitors MB-3, curcumin, and garscinol. Oridonin treatment induces cell apoptosis in gastric cancer cell lines (AGS/HGC-27/MGC80-3) in a concentration-dependent manner and furthermore increases the levels of activated caspase-3 and caspase-9, and decreases the mitochondrial membrane potential in AGS cells [192].

1.3.2.7 Embelin – PCAF

The orange pigment embelin, a quinone-based natural product isolated from _Embelia ribes_ berries, was first synthesized in 1948 [193]. Recently, embelin was shown to preferentially inhibit PCAF (IC$_{50}$ = 7.1 µM) over p300 and Tip60 HAT activity in vitro presumably by binding to the acetyl-CoA binding pocket as anticipated by _in silico_ docking studies. Furthermore, embelin inhibits H3K9 acetylation in HEK 293T cells, but does not alter the acetylation levels of H3K14, H4K8, and H4K12 significantly. Embelin also inhibits PCAF-mediated acetylation of MyoD and therefore impacts skeletal muscle cell differentiation of C2C12 myoblasts. In addition, it alters muscle-specific gene expression in HEK 293T cells [194]. Notably, embelin was not directly related to anti-cancer activity upon HAT inhibition yet, but it is associated with proapoptotic effects in prostate cancer cells (PC-3, DU145, LNCaP-LN3) mediated through modulation of Akt and β-catenin signaling and moreover induces apoptosis in human gastric carcinoma through alteration of p38 MAPK and NF-κB signaling pathways [195, 196]. In addition, embelin inhibits cell proliferation, induces
apoptosis, and alters expression of pivotal oncogenes in MCF-7 and MDA-MB-231 breast cancer cells [197].

1.3.2.8 **Microbial metabolites NK13650 A and B - p300**

NK13650s were isolated as secondary metabolites produced by *Penicillium* species in a screening approach using a microorganism broth library of 19,320 culture broths and were subsequently tested for inhibitory activity on HATs using radiolabeled acetyl-CoA. For the major metabolite NK13650 A, the structural identity and absolute configuration were determined by high-resolution electrospray ionization Fourier transform mass spectrometry in combination with NMR techniques, an advanced version of Marfey's method [198-200], and 13C labeling experiments to monitor the incorporation of labeled pyruvic acid species into NK13650 A during biosynthesis. For the minor metabolite NK13659 B, the structural identity was devoid of stereogenic information. However, both NK13650s are closely correlated with respect their structural composition and able to selectively inhibit the activity of p300 HAT but not that of Tip60 HAT. Furthermore, NK13650s demonstrate inhibitory activity against agonist-induced AR transcriptional activation and NK13650 A treatment inhibits hormone-dependent and -independent growth of prostate cancer cells (LNCaP, PC-3) [33].

1.3.3. **Synthetic HAT Bromodomain (Bd) Inhibitors (Figure 5)**

Since the structural integrity of histone acetylases with respect to domain composition and organization illustrates a high degree of variability among the different acetylases, these differences may serve as a point of action to selectively target certain acetylases over others. Bds, for instance, were shown to represent a druggable feature of HATs [201] although there is structural variability among the class. Although they share a conserved overall fold comprising four α-helices (αZ, αA, αB, αC) linked by highly variable loop regions (ZA and BC loops) that form the docking site for interacting recognition motifs, they possess a low degree of overall sequence homology. Moreover, their surface properties are highly diverse. The electrostatic
potential of the surface area around the acetyl-Lys binding site ranges from highly positive to strongly negative charged, suggesting that Bds recognize largely different sequences. Ultimately, Bds are solely present in a subset of acetyltransferases, which potentially facilitates a gain of target specificity for small molecule binders amongst the family of acetyltransferases [202].

1.3.3.1 I-CBP112 - p300/CBP Bd
The oxazepine-based acetyl-lysine mimetic I-CBP112 was identified in an effort to evaluate the p300/CBP inhibitory activity of a series of compounds containing a benzo-oxazepine core structure simultaneously taking into account the reported weak p300/CBP Bromodomain binding capability of structurally related BET inhibitors (JQ1 and I-BET762). Structural expansions at several positions led to I-CBP112, which binds to the Bd of p300/CBP with nanomolar affinity ($K_d = 167$ and 151 nM, respectively) and reveals considerable selectivity among 42 representative human Bromodomains. Selectivity was further confirmed by utilizing a commercial selectivity panel including 104 binding assays, which monitor binding to nuclear receptors, ion channels, as well as 32 enzyme assays (including 10 kinases, 9 proteases, and 5 phosphodiesterases). In these, solely weak low micromolar binding to two GPCRs (a1A and 5HT1) was observed. I-CBP112 is an acetyl-lysine–competitive inhibitor displacing H3K56ac from the CBP-binding site with an IC$_{50}$ value of 170 nM. Furthermore, it significantly impairs clonogenic growth in leukemic cell lines with distinct genetic lesions (KASUMI-1 (AML1-ETO$^+$), SEM (MLL-AF4$^+$), MOLM13 (MLL-AF9$^+$/FLT3-ITD$^+$)) and sensitizes human leukemic cells to BET inhibition (e.g., by pan-BET inhibitor JQ1[203]) and doxorubicin [204].

1.3.3.2 CBP30 - p300/CBP Bd
The small molecule inhibitor CBP30 was developed as part of a series of selective p300/CBP inhibitors in a structure-based drug design approach using the reported non-selective BRD and CBP inhibitor 3,5-dimethylisoxazole as starting point for further improvements focusing on gaining selectivity. A closely related analogue of the parent compound was crystalized in complex with the Bd of CBP and utilized as
basis for structure guided considerations and subsequent synthesis of a focused library of further 3,5-dimethylisoxazoles. Among these, CBP30 accounts for significant temperature shifts in DSF ($\Delta T_m \sim 10^\circ C$) experiments and likewise demonstrates high potency in activity-based assays utilizing CBP ($pIC_{50} = 7.1$). ITC experiments determined the selectivity for CBP over BRD4(1) and BRD4(2) to 40- and 250-fold, respectively. In addition, doxorubicin induced p53 is effectively inhibited in a dose-dependent manner ($IC_{50} = 1.5 \mu M$) in a luciferase reporter assay suggesting CBP30 inhibits the CBP co-activation of p53 target genes in cells with only moderate cytotoxicity as demonstrated in U2OS and HeLa cells. However, early approaches assessing compound stability in human liver microsomes (HLM), implied that CBP30 may be too rapidly metabolized for subsequent use as in vivo probe [205]. Nevertheless, subsequent studies further illuminated the potential of CBP30 as a therapeutic agent and demonstrated exclusive selectivity for p300/CBP among 45 bromodomains accompanied with only weak binding towards BET family members. Since BET bromodomain inhibitors such as JQ1 exhibit anti-inflammatory activity by inhibiting expression of inflammatory genes [206], CBP30 was also hypothesized to be involved in the regulation of inflammatory processes. Broad anti-inflammatory activity but no pronounced cytotoxicity at concentrations up to 10 $\mu M$ was observed using a panel of 12 stimulated primary human cell types. Furthermore, CBP30 (at 2 $\mu M$ concentration) on average reduces the secretion of IL-17A by 66.3% in cells from patients with ankylosing spondylitis and psoriatic arthritis (two human Th17-driven diseases) as well as from healthy controls. Therefore, specific targeting of p300/CBP bromodomains was promoted as promising therapeutic strategy in human type-17-mediated diseases [207]. Given that acetyltransferase Bds constitute appropriate druggability for targeting HAT activity, diverse approaches to identify novel binders, inhibitors or even inhibitory fragments have recently been published. Among these, structure-based approaches involving fragment screening by thermal shifts assays and NMR methods but also classical proximity based approaches monitoring direct ligand binding to Bds were utilized [208, 209].
CONCLUSION

Implications of HATs in cancers as well as neurodegenerative diseases and the lack of therapeutic agents that target HAT activity highlight the necessity to discover novel and potent HAT inhibitors to extend the current arsenal of HAT inhibitors and develop these towards therapeutic applications. Since detailed biological roles and functions of HATs still remain elusive, selective, high affinity probes are needed to further decipher HAT associated signaling and gene regulation in mechanistic studies and shed light on the phenotypic consequences of aberrant HAT activity and associated signaling. Scientific endeavors to date revealed HAT inhibitors that were derived from diverse origins. Bisubstrate inhibitors, as an early class of HAT inhibitors, were designed on the basis of mimicking the intermediate state of the catalytic acetyl transfer between acetyl-CoA and the respective protein substrate with a non-hydrolysable surrogate. A plethora of natural products were also discovered and further developed to natural product derived HAT inhibitors. Related to these, microbial metabolites were identified in phenotypic screens utilizing bacterial broth and shown to exhibit direct impacts on HAT activity. However, natural product and metabolite-derived inhibitors often lack specificity and optimized derivatives thereof are occasionally difficult to access from a synthetic perspective due to their highly sp3-enriched nature. Therefore, the most promising class of inhibitors in terms of given selectivity, potency, ease of preparation, and further development is of synthetic origin and usually discovered in high throughput activity-based, target binding or virtual screening approaches. The selective synthetic p300 inhibitor C646, for instance, is currently the most widely referenced inhibitor for epigenetic studies involving modulation of p300-related histone modulation and signaling. Inhibitors for epigenetic reader domains, however, recently became of major interest and represent promising future inhibitors with potential therapeutic applications for several reasons. Since they do not target the relatively conserved acetyl-CoA binding site, the potential existence or absence of Bds among the HAT-family members determines a first selectivity filter for this inhibitor class. In this regard, the remote binding location is advantageous since competition with rather high cellular concentrations of acetyl-CoA is negligible. Current investigations show that besides
the well-studied class of HDAC inhibitors, HAT inhibitors and activators may likewise contribute to counteract epigenetic driven diseases with aberrant acetylome conditions.
LIST OF ABBREVIATIONS

AA (Anacardic acid), Abl (Abelson tyrosine-protein kinase 1), ACLY (ATP-citrate lyase), AE (AML1-ETO fusion protein), AF4 (ALL1-fused gene from chromosome 4 protein), AF9 (ALL1-fused gene from chromosome 9 protein), AKI (Acute kidney injury), AKT (Protein kinase B), ALL (Acute lymphoblastic leukemia), AML (Acute myeloid leukemia), AML1 (Acute myeloid leukemia 1 protein), AR (Androgen receptor), ATAC (Ada2-containing), ATM (Ataxia telangiectasia mutant), Aβ (β-amyloid), Bcl-2 (Apoptosis regulator Bcl-2), BCL-xL (Bcl-2-like protein 1), Bd, BRD (Bromodomain), BET (Bromodomain and extra-terminal), C-TAD (C-terminal transactivation domain), CA9 (Carbonic anhydrase 9), CBP (CREB-binding protein), Cd (Chromodomain), CH1 (Cysteine-histidine rich region), Chk2 (Checkpoint kinase 2), CKD (Chronic kidney disease), CLOCK (Circadian locomoter output cycles protein kaput), CoA (Coenzyme A), CREB (Cyclic AMP-responsive element-binding protein), CSP (Carbon nanosphere), CTCL (Cutaneous T-cell lymphoma), DOT1 (Histone-lysine N-methyltransferase, H3 lysine-79 specific), E1A (adenoviral oncoprotein E1A), E2A (Transcriptional activator E2A), E2F1 (Transcription factor E2F1), E6AP (E6AP ubiquitin-protein ligase), ELISA (Enzyme Linked Immunosorbent Assay), EMT (Epithelial-mesenchymal transition), ETV1 (ETS variant 1), ER (Estrogen Receptor), ERCC1 (Excision repair cross-complementation group 1 enzyme), ETO (Eight-Twenty One oncoprotein), ETS (Protein C-ets-1/2), EZH1 (Enhancer of zeste homolog 1), EZH2 (Enhancer of zeste homolog 2), FAS (Fatty acid synthesis), FDA (U.S. Food and Drug Administration), Fli1 (Friend leukemia integration-1), FLT3 (Receptor-type tyrosine-protein kinase FLT3), FOXA1 (Forkhead box A1), FRET ( Förster resonance energy transfer), FtsZ (Cell division protein FtsZ), G9a (Histone-lysine N-methyltransferase EHMT2), GAPDH (Glyceraldehyde 3-phosphate dehydrogenase), GCN5 (Histone acetyltransferase GCN5), Gli (Transcriptional activators, originally isolated in human glioblastoma), GLUT1 (Glucose transporter 1), GNAT (GCN5-related N-acetyltransferases), H3K4me3 (Trimethylated histone H3 Lys4), HAT (Histone acetyl transferase), HATi (HAT inhibitor), HBS (Hydrogen bond surrogate), HCC (Inhibits hepatocellular carcinoma), HDAC (Histone deacetylase), Hdm2 (E3 ubiquitin-protein ligase Mdm2), HGPS (Hutchinson-Gilford progeria syndrome), Hh
(Hedgehog), HIF-1 (Hypoxia-inducible factor-1), HIV-1 (Human immunodeficiency virus-1), HLM (Human liver microsomes), HNSCC (Head and neck squamous cell carcinoma), HPV (Human papilloma virus), HPV E6 (Human papillomavirus E6-associated protein), HRE (Hypoxia-responsive element), HSP60 (heat shock protein HSP60), IG (Isogarcinol), IL-6 (Interleukin 6), ITD (Internal tandem duplications), KAT (Lysine acetyl transferase), KDAC (Lysine deacetylase), LC/MS (liquid chromatography coupled to mass spectrometry), LCSC (Lung cancer stem-like cell), Mcl-1 (Induced myeloid leukemia cell differentiation protein Mcl-1), miR-17-5p (miRNA that suppresses PCAF mRNA translation and induces its degradation), MLL (Myeloid/lymphoid leukemia protein 1), MMP9 (Matrix metalloproteinase-9), MnTE-2-PyP (Manganese (III) meso-tetrakis-(N-ethylpyridinium-2-yl) porphyrin), MOA (Mechanism of action), MOF (Histone acetyltransferase KAT8), MRE11 (Double-strand break repair protein MRE11), Myb (Transcriptional activator Myb), Myc (Myc proto-oncogene protein), MyoD (Myoblast determination protein), MYST (MOZ, Ybf2, Sas2 and Tip60 family of HATs), NAT10 (N-acetyltransferase 10), NF-κB (Nuclear factor-κB), NMR (Nuclear magnetic resonance), NPM1 (Nucleophosmin), NR (Nuclear receptors), NSCLC (Non-small cell lung cancer), p24 (Capsid protein p24), p300 (Histone acetyltransferase p300), p38 MAPK (Mitogen activated protein kinase p38), p53 (Cellular tumor antigen p53), p65 (NF-κB p65 subunit), PAI-1 (Plasminogen activator inhibitor-1), PBX1 (Homeobox pre-B-cell leukemia transcription factor 1), PBX1 (Pre-B-cell leukemia transcription factor 1), PC4 (Positive cofactor 4), PCA (Prostate cancer), PCAF (p300/CBP associated factor), PCNA (Proliferating cell nuclear antigen), PHD (Plant homeodomain), PRMT1 (Protein arginine N-methyltransferase 1), PSA (Prostate specific antigen), PTEN (Phosphatase and tensin homolog), Pygo2 (Pygopus homolog 2), RING (Really interesting new gene), ROS ( Reactive oxygen species), RTS (Rubinstein-Taybi syndrome), SAGA (Spt-Ada-Gcn5-acetyltransferase) SAR (Structure activity relationship), SCC (Squamous cell carcinoma), SET7-9 (Histone-lysine N-methyltransferase SETD7), SETD2 (Histone-lysine N-methyltransferase SETD2), Skp2 (S-phase kinase associated protein 2), SMYD2 (N-lysine methyltransferase SMYD2), SRC-1 (Nuclear receptor coactivator 1), STAGA
(SPT3-TAF_{II:31}-GCN5L acetylase), STAT3 (Signal transducer and activator of transcription 3), SUV39H2 (Histone-lysine N-methyltransferase SUV39H2), TAF (TBP-associated factor), Tat (Transactivating regulatory protein of HIV-1), TBP (TATA-box binding protein), TCF (T-cell factor), TIF-2 (Nuclear receptor coactivator 2), TNBC (Triple-negative breast cancer), TNF (Tumor necrosis factor), TRF (Time resolved FRET), Twist-1 (Class A basic helix-loop-helix protein 38), UBR4 (E3 ubiquitin-protein ligase UBR4), VEGF (Vascular endothelial growth factor), WD40 (Repeat sequence of 40–60 amino acids), Wnt (Int/Wingless family of proteins), YB-1 (Y-box binding protein-1), γ-H2AX (Phosphorylated Histone H2AX)

**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.
Domain organization of HATs. The central enzymatically active HAT core is generally associated with multiple subdomains, which are responsible for substrate binding and recruitment of the HAT core to the respective location of action. In detail, Bromo domains are epigenetic reader domains responsible for the recognition of acetylated residues, whereas Chromodomains recognize methylated amino acids. Tudor domains were originally identified as an RNA-binding motif in the *Drosophila*
melanogaster Tudor protein, but they also have been shown to bind methyl arginine residues as well as methylated histones. The WD40 motif is generally poorly conserved and is usually found as repeated motif in which the consecutive repeats fold into a circular structure that binds methylated lysines. RING domain is a zinc-finger motif that is found in many proteins involved in the ubiquitinylation pathway, in which they function as ligases. In HATs and in particular in p300 it was found to contribute to the structure of the substrate binding pocket and was found to have inhibitory function for the HAT domain. The PHD domain is another zinc finger domain that typically binds trimethylated histone H3 Lys4 (H3K4me3), unmodified histone-H3 tails or other modified chromatin.

**Figure 2:**

Different types of HAT inhibition. The inhibition of HAT transferase activity results in preventing direct acetylation of the target protein or histone. However, several indirect ways to inhibit target protein acetylation are known. For instance, disruption of the formation of higher ordered HAT complexes that usually involve HAT binding to recruiter proteins or multienzyme complexes [1]. In this regard, inhibitors that
prevent the respective recruiter protein from binding to its respective target is another way of inhibiting HAT target protein acetylation. Ultimately, inhibitors addressing intrinsic HAT domains, which are responsible for epigenetic readout and therefore target protein recognition, likewise prevent target protein acetylation.

**Figure 3:** HAT inhibitors of synthetic origin.
Natural product derived HAT inhibitors.
Figure 5:

Bromodomain inhibitors
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