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Pharmacological targeting of lysine acetyltransferases in human disease: a progress report

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Lysine acetyltransferases (LATs) are a structurally disparate group of enzymes involved in regulating transcription by participating as cofactors in transcriptional regulatory complexes, and by acetylation of lysine residues in histones and other proteins. Aberrant LAT function probably plays an important part in the pathogenesis of certain cancers, especially leukaemias and endocrine tumours. However, LAT activity might also be an important drug target in a range of other indications, including inflammatory lung diseases, viral infections and metabolic disorders. At present, comparatively few LAT inhibitors are known, but progress regarding the understanding of their structural and functional biology is now beginning to reveal LATs as promising new epigenetic drug targets.

Introduction

In eukaryotic cells the genome is packaged as the chromatin nucleoprotein complex, the basic unit of which is the nucleosome. Nucleosomes consist of ~200 base pairs of DNA wound twice around a histone octamer core, comprising two copies of each of the histones H2A, H2B, H3 and H4. In addition, a single copy of the histone H1 is associated externally with the nucleosome. The basic string of nucleosomes (i.e. the 10 nm fibre) can form a helical coil consisting of six nucleosomes per coil (i.e. the 30 nm fibre) that can be further organized into higher order chromatin structures. Dynamic changes in chromatin structure dictate the progression of nuclear processes such as transcription, replication, recombination and DNA repair. Chromatin modification is mediated by nuclear enzymes that catalyze reversible post-translational modifications of histones, such as acetylation, methylation, phosphorylation, ubiquitinylation, sumoylation, glycosylation, prolyl isomerization and ADP ribosylation. Many of these modifications occur at the lysine-rich N-terminal tails of histones that protrude from the nucleosome and serve as an epigenetic code, signalling changes in chromatin architecture, often achieved through the action of ATP-dependent chromatin-remodelling complexes such as NURF (nucleosome remodelling factor) and NURD (nucleosome remodelling and histone deacetylation complex). The best-studied reversible modification is acetylation, which is regulated by two important groups of enzymes with opposing activities [i.e. histone acetyltransferases (HATs) and histone deacetylases (HDACs)] that function as transcriptional coregulators (i.e. coactivators and corepressors, respectively) in multicomponent complexes. HATs and HDACs are recruited to gene promoters by direct interactions with DNA-binding transcription factors, or through their ability to recognize epigenetic marks in promoter regions. Recruitment of HAT enzymes can result in local hyperacetylation of histones, which, in combination with other chromatin modifications, is required to facilitate gene transcription. It is believed that histone acetylation, through charge neutralization of the cationic histone tails, weakens electrostatic nucleosomal interactions with anionic DNA, thus destabilizing internucleosomal contacts and nucleosomal structure, and facilitating recruitment of coregulators and RNA polymerase complexes to the locus. By contrast, HDAC activity is correlated with histone hypoacetylation, enabling a return to inactive or repressed states. HAT and HDAC activities are thus required for normal gene regulation.

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Lysine acetyltransferase structure and molecular mechanisms

The human genome encodes up to 25 proteins that have known or putative lysine acetyltransferase activity, clustering in several subgroups (Table 1). These enzymes (EC 2.3.1.48; following IUBMB enzyme nomenclature guidelines) catalyze the transfer

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TABLE 1

Summary of human lysine acetyl transferases

Family	Gene		Gene product name and synonyms	Isoforms	Substrates	Refs
	Name[a]	Synonyms				
HAT1	*HAT1* (O14929)	–	Histone acetyltransferase type B catalytic subunit (HAT1)	1	H4	[88]
MYST	*HTATIP* (Q92993)	*TIP60*	60 kDa HIV-1 Tat-interacting protein, (Tip60) (NuA4/TRRAP complex component)	3	H3, H2A AR, γH2AX ATM, c-Myc UBF	[89]
	MYST1 (Q9H7Z6)	*MOF, hMOF*	Homolog of Drosophila *males absent on the first* (hMOF) Component of human male specific lethal complex (MSL)	3	H4 K16	
	MYST2 (O95251)	*HBO1, HBOa*	HAT binding to origin recognition complex (HBO1) Component of inhibitor of growth complexes (ING4, ING5)	1	H3, H4	
	MYST3 (Q92794)	*MOZ, RUNXBP2, ZNF220*	Monocytic leukaemia zinc finger protein, (MOZ) Runt-related transcription factor-binding protein (RunxBP2) Zinc finger protein 220 kDa (ZNF220) (Component of ING5 complex)	1	H3, H4	
	MYST4 (Q8WYB5)	*MORF, MOZ2*	MOZ-related factor (MORF), MOZ2, Querkopf (Component of ING5 complex)	3	H3, H4	
GNAT	*GCN5L2* (Q92830)	*GCN5, HGCN5*	General control of nitrogen metabolism (GCN5)-like 2 Homolog of yeast GCN5, STAF97	2	H3, H4, TFs	[90]
	PCAF (Q92831)	–	p300/CBP-associated factor (P/CAF)	1	H3, H4, TFs, E1A, TAT	[91]
p300/CBP	*EP300* (Q09427)	*p300*	E1A-associated protein 300 kDa, (p300)	1	Histones, TFs, E1A, TAT, p300	[92]
	CREBBP (Q92793)	*CBP*	CREB-binding protein (CBP)	1	Histones, TFs, E1A, CBP, ACTR	[93]
SRC/p160	*NCOA1* (Q15788)	*SRC1, RIP160*	Steroid receptor coactivator (SRC1) Nuclear receptor coactivator (NCOA1) 160-kDa receptor interacting protein (RIP160)	3	H3? H4?	[94]
	NCOA2 (Q15596)	*TIF2*	Transcriptional intermediary factor (TIF2) Nuclear receptor coactivator 2 (NCOA2)	2	H3? H4?	[95]
	NCOA3 (Q9Y6Q9)	*AIB1, ACTR, p/CIP RAC3, TRAM1*	Nuclear receptor coactivator (NCOA3) Amplified in breast cancer (AIB1) Thyroid hormone receptor activator molecule (TRAM1) Receptor-associated coactivator (RAC3) Steroid receptor coactivator protein (SRC3) p300/CBP-interacting protein (p/CIP)	5	H3? H4?	
TFIIIC subunit 4 family	*GTF3C4* (Q9UK98)	–	General transcription factor 3C polypeptide 4 (GTF3C4) Transcription factor IIIC-delta subunit, (TF3Cδ) TFIIIC 90-kDa subunit (TFIIIC 90)	1	H3, H4	[96]
ATF	*ATF2* (P15336)	*CREB2, CREBP1*	Cyclic AMP-dependent transcription factor (CREB2) Activating transcription factor (ATF2) cAMP response element-binding protein (CREBP1) HB16	2	H4, H2B	[97]

TABLE 1 (*Continued*)

Family	Gene		Gene product name and synonyms	Isoforms	Substrates	Refs
	Name[a]	Synonyms				
CIITA	***CIITA*** (P33076)	*MHC2TA*	MHC class II transactivator (CIITA)	1	H4	[98]
TAF1	*TAF1* (P21675)	*BA2R, CCG1, TAF2A*	Transcription initiation factor (TFIID) subunit 1 TBP-associated factor (TAF1) TBP-associated factor 250 kDa (TAFII250) Cell-cycle gene 1 (CCG1)	2	H3, H4	[99]
CDY	*CDY1* (Q9Y6F8)	–	Testis-specific chromodomain protein Y1 (CDY1)	2	H4	[100]
	CDYL1 (Q9Y232) *CDYL2* (Q8N8U2)	*CDYL1, CDYL2*	Chromodomain Y-like protein (CDYL1, CDYL2)	1 1	H4	
TFIIB	*GTF2B* (Q00403)	*TF2B, TFIIB*	Transcription initiation factor (TFIIB) General transcription factor TFIIB (GTF2B)	1	TFIIB K238	[101]
MCM3AP	*MCM3AP* (O60318)	*GANP, KIAA0572, MAP80, SAC3*	Mini chromosome maintenance 3-associated protein (MCM3AP) 80-kDa MCM3-associated protein (MAP80) Germinal centre-associated nuclear protein (GANP)	1	MCM3	[102]
ESCO	*ESCO1* (Q5FWF5)	*EFO1, KIAA1911*	Establishment of cohesion 1 homolog 1(ESCO1, ECO1) Establishment factor-like protein 1, (EFO1p, hEFO1) CTF7 homolog 1	3	Cohesin	[103]
	ESCO2 (Q56NI9)	–	Establishment of cohesion 1 homolog 2 (ESCO2) ECO1 homolog 2	1	Cohesin	
ARD1	*ARD1A* (P41227)	*hARD1, TE2, ARD2*	Arrest defective protein (ARD1) *N*-alpha acetyltranferase (retroposon-mediated gene duplication product)	1 1	HIF1α	[104]
CLOCK	*CLOCK* (O15506)	*KIAA0334*	Circadian locomoter output cycles protein kaput (CLOCK)	1	H3/H4	[105]
MGEA5	*MGEA5* *NCOAT* *HEXC* (O60502)	*NCOAT*	Meningioma-expressed antigen 5 (MGEA5) Nuclear cytoplasmic *O*-linked *N*-acetylglucosaminase and acetyltransferase (NCOAT)	3	H4 K8, H3 K14	[107]

[a] Numbers in brackets are UniProt (SwissProt and TrEMBL) accession numbers.

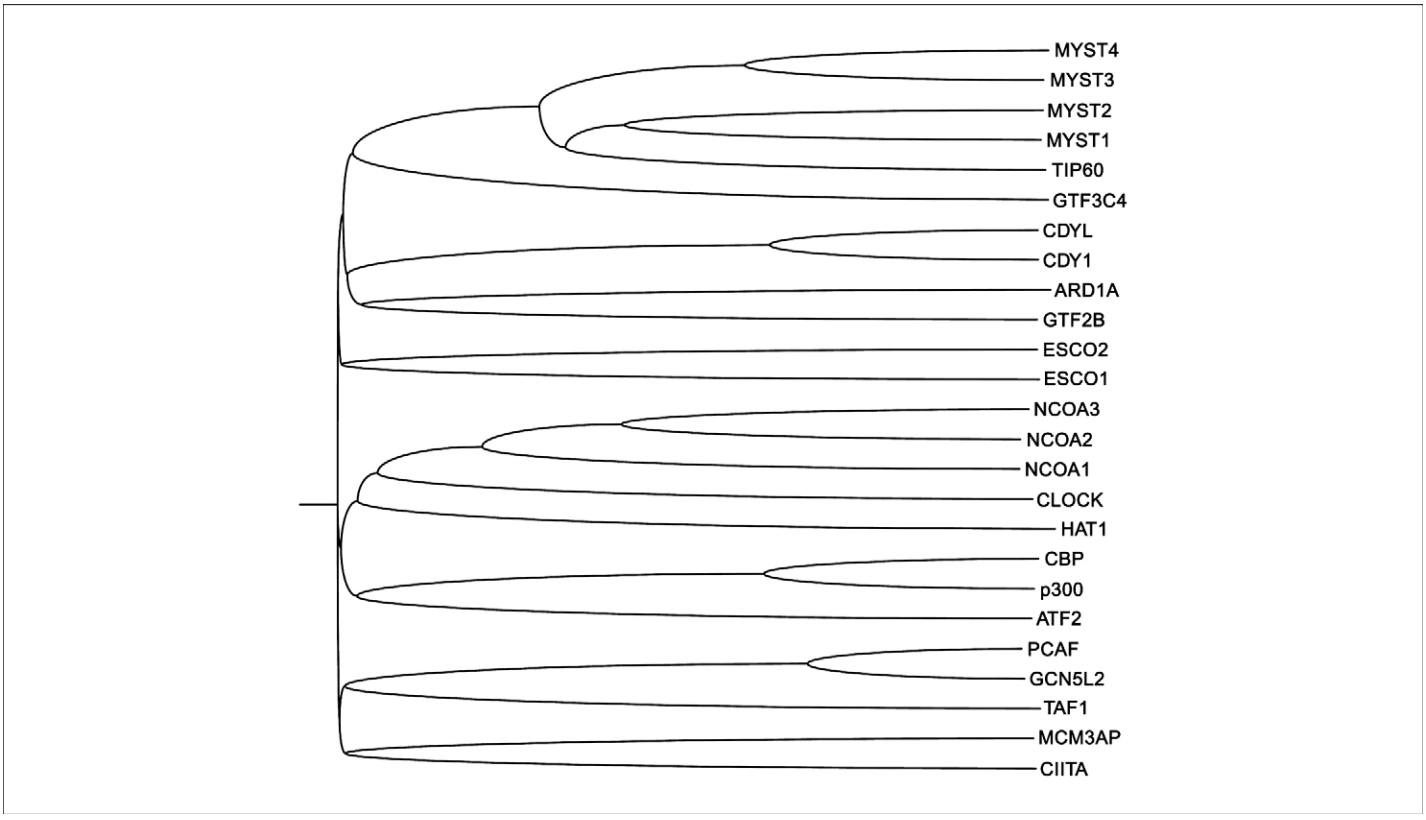

FIGURE 1

Relationship between different lysine acetyltransferases. The protein sequences were aligned using CLUSTALW, and from this alignment the phylogenetic tree shown was generated by PHYLIP using the San Diego Supercomputer Center Biology WorkBench facilities (http://workbench.sdsc.edu/).

of an acetyl group from acetyl coenzyme A (Ac-CoA) to the ε-amino group of a substrate lysine residue, in which respect HATs are distinct from amino acid N^{α}-acetyltransferases and other acyltransferases. Because of the mounting evidence that HATs also modulate the activity of non-histone proteins via reversible lysine acetylation, in the following text we shall refer to them simply as lysine acetyltransferases (LATs) [1]. Among the non-histone substrates are histone chaperones such as nucleophosmin [2], DNA-binding transcription factors such as steroid receptors, p53, E2F1, c-Myc (cellular myelocytomatosis oncogene) and MyoD (myogenic differentiation protein), other coregulators such as ACTR (activator of thyroid and retinoic acid receptors), cell-cycle components such as E2F transcription factors, and nuclear transport proteins [3].

At the primary structure level there is little similarity between the different LAT families, and even members of the same family usually display considerable sequence diversity (Figure 1). Furthermore, there is no single homology domain that is conserved by all LATs, although many contain recognizable Ac-CoA-binding motifs and bromodomains [4]. More similarities can be discerned at the tertiary structure level (Figure 2). Here, all the LATs – at least those for which 3D structures are available – display a conserved core domain composed of a helix, followed by a loop–antiparallel-β-strand region. The L-shaped cleft, formed by the protein segments N- and C-terminal of the core domain, contains the catalytic site and is involved in the binding of Ac-CoA in the short segment and the macromolecular substrate in the long segment. Beyond the core domain and the adjoining segments, there is little structural similarity between the different LATs. *In vitro* assays using chromatin or core histones indicate that LATs have different substrate specificities, although the molecular mechanisms underlying these, as well as the true physiological specificities of LATs, remain poorly understood.

Interestingly, various studies have indicated that LATs utilize different catalytic mechanisms. So far, two different mechanisms have been elucidated [5]. The GNAT (GCN5-related *N*-acetyltransferase) family LATs use a sequential ordered mechanism that involves acetyl transfer from Ac-CoA directly to the N^{ε} of the substrate lysine residue (Figure 2). MYST (MOZ, YBF2/SAS3, SAS2, TIP60 *N*-acetyltransferase) family members, by contrast, employ a so-called ping-pong (i.e. double displacement) catalytic mechanism, in which the acetyl moiety is first transferred from Ac-CoA to a cysteine residue within the enzyme active site, and in a second step the acetyl group is transferred to the substrate lysine residue. Despite the different catalytic mechanisms, all biochemically and structurally characterized LATs have a conserved glutamate residue in the active site, which seems to deprotonate the $N^{\varepsilon}H_2$ of the target lysine substrate before acetyl transfer.

A recent study on p300 demonstrates that this LAT is itself polyacetylated and contains an activation loop that requires (auto)acetylation for full enzyme activation [6]. This is similar to the situation with protein kinases, where activity is also regulated through an autoinhibitory switch involving phosphorylation of an activation loop. The parallels between LATs and kinases, both of which catalyze reversible post-translational modifications that are important for the regulation of countless

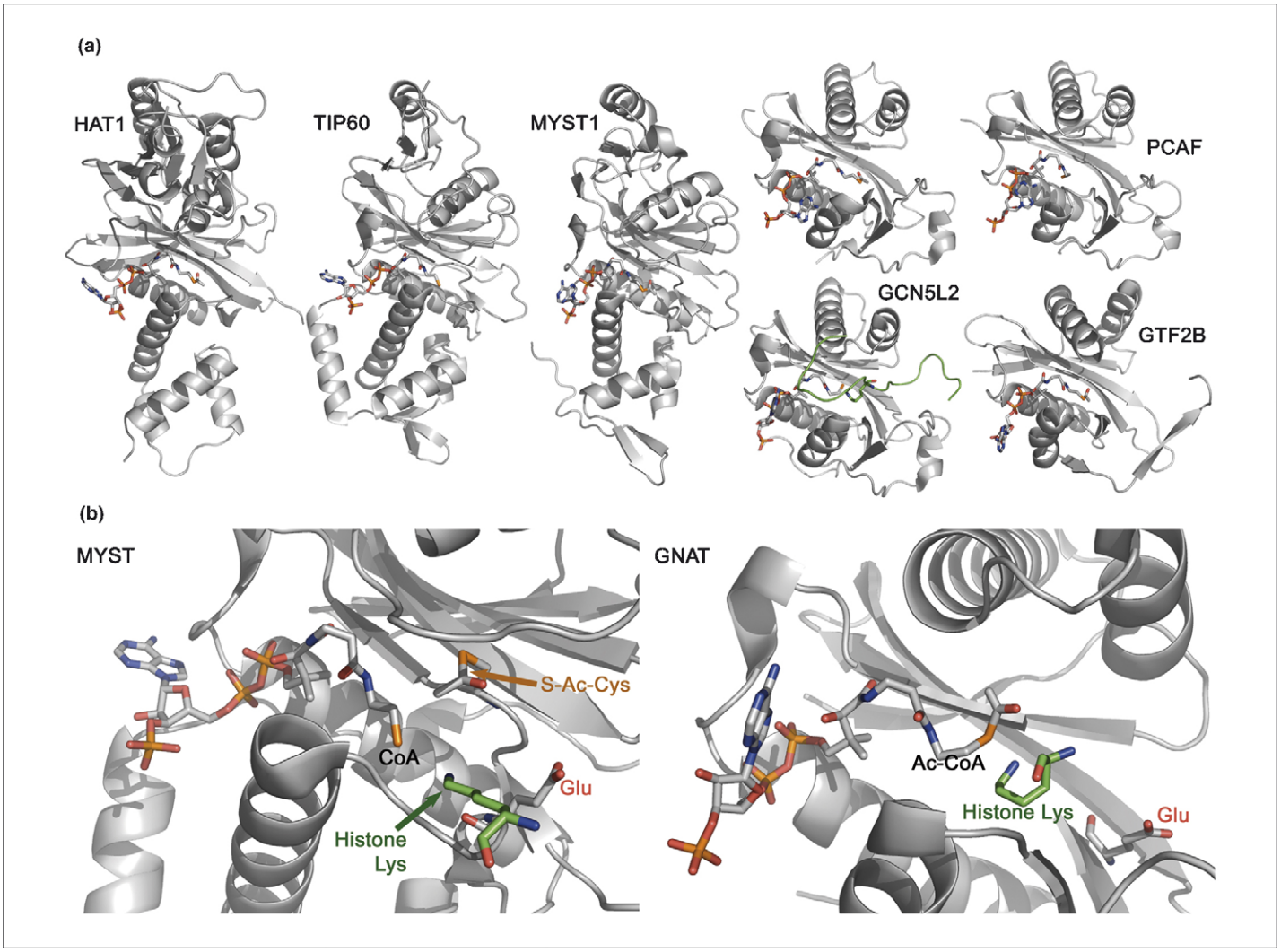

FIGURE 2

Diversity of lysine acetyltransferase domain structures. (a) The coordinates from X-ray crystal structures of: yeast HAT1 [protein databank (PDB) number 1BOB]; the yeast TIP60 homologue ESA1p (PDB number 1FY7); human MYST1 (PDB number 2GIV); GCN5L2 human protein (top; PDB number 1Z4R); GCN5L2 tetrahymena protein with bound histone peptide shown as green ribbon (bottom; PDB number 1PUA); human PCAF (PDB number 1CM0); and the yeast GTF2B protein HPA2 (PDB number 1QSM) were aligned, translated and are shown as secondary structure ribbon cartoons with coenzyme A (CoA) depicted as Corey, Pauling, Kultin (CPK)-coloured sticks. No structures from the remaining lysine acetyltransferase (LAT) families listed in Table 1 are known. **(b)** Enzymatic mechanisms of LAT proteins. MYST family LATs employ a double-displacement catalytic mechanism, in which a cysteine side-chain in the active site is first acetylated by Ac-CoA, shown in (a). Following the departure of CoA, the acetyl group is transferred to the substrate Lys side-chain amino group. By contrast, in GNAT LATs, acetyl transfer occurs directly from Ac-CoA to the macromolecular substrate. In both cases a conserved Glu residue in the active site is believed to assist in the deprotonation of the Lys amino group – constructed from PDB numbers 1MJA and 1QSN. This, and all other 3D structure illustrations, were prepared using the PyMOL programme (DeLano, W.L. (2002) The PyMOL molecular graphics system on World Wide Web; http://www.pymol.org). For definitions of abbreviations, see Table 1.

cellular processes, also extend into how these proteins are recruited to their target complexes. In the case of kinases this usually involves SH2 and 14–3–3 domains that recognize phosphopeptide motifs, whereas LATs frequently contain bromodomains that bind acetyllysine-containing sequence motifs in histones and other proteins [7].

The nuclear receptor (NR) superfamily contains members that bind ligands such as hormones, retinoic acid and vitamins. NRs function as ligand-activated transcription factors and their ligand-binding domains (LBDs) adopt distinct conformations as aporeceptors and in agonist- or antagonist-bound forms, which dictate interactions with coactivators, corepressors and chaperones. Cofactor recruitment is a crucial regulatory step in NR signal transduction. Agonist binding by NRs leads to a conformational change in the LBD that exposes a hydrophobic groove. This groove forms the binding site for amphipathic helical LXXLL (where L is leucine and X is any amino acid) and related motifs present in a range of coregulators, including LATs such as p160 proteins, TIP60 (60-kDa Tat-interacting protein), CREB-binding protein (CBP) and p300 [8]. These coactivators, in turn, recruit other LATs and arginine methyltransferases to specific enhancer and/or promoter regions, which facilitates chromatin modification and remodelling, assembly of general transcription factors and transcription of target genes [9]. LXXLL motifs occur in a variety of different contexts. Residues immediately adjacent to the motif modulate the overall affinity of the box–NR interaction and, thus, particular

NRs show overlapping but distinct preference for NR boxes [10]. Several of these interactions have been characterized at the molecular level, and this is discussed further in the section on protein interaction inhibitors.

LAT target validation

Oncology

Transformed cells require continuous RNA polymerase-II (RNAP-II) activity to resist oncogene-induced apoptosis [11]. Because LAT activity is linked intimately with transcription, it can be expected that pharmacological LAT inhibition will oppose expression of antiapoptotic proteins, which cancer cells require to survive. However, the role of LATs in cancer is complex, for example the *EP300* and *CREBBP* genes (encoding p300 and CBP, respectively) appear to have both tumour-suppressor and tumour-promoter properties. Loss of *EP300* heterozygosity is associated with 80% of glioblastomas, and *EP300* gene mutations are frequent in colorectal cancer [12]. *CREBBP* knockout mice show increased incidence of some cancers, including leukaemia, but also defects in cell proliferation [13]. Transforming viral proteins such as E1A bind CBP, p300 and PCAF (p300/CBP associated factor) to block normal differentiation pathways. In addition to loss of function through haploinsufficiency, there are also gain-of-function effects that impact global histone modification and transcription. Reduced global histone acetylation has been shown to correlate with disease progression in prostate cancer [14]. In addition to histones, non-histone proteins are probably equally important targets for LAT dysfunction [3].

Leukaemia

It is known that aberrant acetylation by mistargeted (i.e. because of chromosomal translocations) LATs plays a causative role in leukaemogenesis [15]. Chromosomal abnormalities of haematopoietic progenitor cells correlate with changes in gene expression that obviate differentiation pathways and lead to formation and proliferation of leukaemic cells. Many genes that function in transcriptional regulation or cell signalling have been implicated in leukaemia, and there is clear evidence for a role of LATs in this group of diseases, especially in therapy-related leukaemias. Fusions of the *CREBBP* or *EP300* genes to the mixed lineage leukaemia (*MLL*) gene are, thus, almost exclusively associated with therapy-related myeloproliferative diseases [16], and the resulting fusion proteins lead to leukaemia by increasing histone acetylation of genomic regions targeted by MLL [17]. Other recurrent reciprocal translocations in acute myeloid leukaemia (AML) produce fusion proteins containing the MYST domains of LATs, such as MYST3/MOZ and MYST4/MORF in combination with CBP, p300 or TIF2 (transcriptional intermediary factor 2) [18]. There is substantive evidence that MOZ-fusion proteins block normal differentiation of myeloid progenitors resulting in AML, and that this directly involves aberrant histone acetylation events *in vivo* [19].

Hormone-dependent tumours

The gene encoding the p160 coactivator AIB1 (amplified in breast cancer; also termed ACTR, NCOA3) was shown to be amplified and overexpressed in breast cancers. In fact, all three SRC/p160 members (Table 1) are frequently upregulated in breast and prostate cancers, as well as in malignant endometrium [20–22]. The androgen receptor (AR) and the estrogen receptor (ER) are also directly acetylated by LATs (e.g. p300, PCAF and TIP60) at a conserved motif and such acetylation governs ligand sensitivity and hormone antagonist responses. The p160s interact with CBP and p300 via the SRC1 interaction domain but, strikingly, the AIB1/ACTR protein itself has been shown to be acetylated by p300; acetylation occurs at conserved lysine residues proximal to the conserved LXXLL motif that is involved in a charge clamp that stabilizes the NR–p160 interaction. Acetylation of this site in ACTR by p300 was correlated with dissociation of the NR–p160–p300 complex [23]. AR acetylation mimic mutants convey reduced apoptosis and enhanced growth properties [24]. In contrast to the native steroid ligands, the NR antagonist action of, for example, selective ER modulators such as tamoxifen causes the ER to recruit corepressors that downregulate the transcriptional activation activity of the ER [21]. Drugs that interfere directly with coactivator binding are desirable for the treatment of hormone-dependent tumours because they would probably inhibit AR or ER activity, possibly even in secondary hormone-resistant cancers for which conventional therapies fail.

Inflammatory lung disease

Airway inflammation represents a major cause of disability worldwide. In asthma, the histone acetylation imbalance leading to inflammatory gene activation can be reversed successfully with corticosteroid therapy. However, other inflammatory lung diseases, especially chronic obstructive pulmonary disease (COPD) are refractory to such therapy [25]. Inflammation is characterized by elevated expression of inflammatory genes that are under the control of such proinflammatory transcription factors as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1). Whereas macrophages from bronchoalveolar lavages and bronchial biopsies from patients with asthma display a marked increase in HAT activity and a small decrease in HDAC activity, specimens of lung tissue obtained from patients with increasing clinical stages of COPD show graded reductions in HDAC (especially HDAC2) activity [26]. Corticosteroids suppress inflammatory genes mainly by reversing histone acetylation through the recruitment of HDACs to inflammatory gene complexes. Impaired HDAC2 activity in COPD patients and asthmatics who smoke is therefore probably responsible for corticosteroid insensitivity.

Trichostatin A, a nonselective HDAC inhibitor, has been shown to increase expression of inflammatory genes in alveolar macrophages and airway epithelial cells, suggesting that HDACs function to suppress inflammatory genes [27]. Furthermore, steroid responses in COPD macrophages can apparently be restored with the HDAC activator theophylline [28]. Together, these data suggest that restoration of histone acetylation balance in inflammatory lung diseases through the use of LAT inhibitors, especially where corticosteroid insensitivity is a problem, could be a promising new strategy. Furthermore, although corticosteroids are effective inhibitors of NF-κB and AP-1, they have endocrine and metabolic side-effects that might be avoided in alternative therapies [29].

Virology

The replication of most viruses depends, to some extent, on the host-cell transcriptional machinery, and in the case of, for exam-

ple, HIV, which is of particular therapeutic interest, it has been established that histone and Tat acetylation are essential for HIV gene expression and viral replication [30]. The Tat protein is a viral transactivator that binds the Tat-responsive element (TAR) present at the 5′-end of all viral transcripts, and Tat is required for efficient viral transcription [31]. During the course of infection the HIV genome is integrated into the human chromatin and a single nucleosome called nuc1 is positioned immediately after the transcription start site in cells where the HIV promoter is silent. The nuc1 nucleosome is disrupted during transcriptional activation through histone acetylation [32]. Tat acetylation is necessary for the recruitment of the RNAP-II complex, and several LATs are known to interact with Tat, including p300, CBP, TIP60 and TAF1 (250-kDA TATA box binding protein-associated factor) [33]. Acetylated Tat also serves to recruit other LATs, especially PCAF, via interaction with the bromodomain [34]. It has been shown that HDAC inhibitors activate transcription from the HIV promoter [35], and administration of HDAC inhibitors has been proposed as an adjuvant to antiviral therapy to reactivate latent HIV reservoirs [36]. One would therefore expect that LAT inhibitors should prevent viral replication through the inhibition of nuc1, histone and Tat acetylation. The fact that this is the case with at least one known LAT inhibitor (i.e. curcumin, see later) has already been demonstrated [37], and a comprehensive rationale for targeting LAT activities in HIV therapy has been proposed [38].

Metabolic disease

Other indications where a histone and protein acetylation imbalance seems to be important are obesity and diabetes, which currently pose major challenges to world health. It has been demonstrated that the regulation of insulin expression by glucose involves histone acetylation [39,40]. However, conflicting data have been reported on the effects of various HDAC inhibitors that have been studied in models of these diseases and, because LAT inhibitors are now becoming available, it has been suggested that these should also be studied for their therapeutic potential [41]. In a study designed to identify the physiological functions of CBP, mice that were heterozygous in *CREBBP* were analyzed [42]. Surprisingly, these animals not only displayed increased insulin sensitivity and glucose tolerance but also were completely protected from weight-gain induced by a high-fat diet, at least in part because of increased effects of insulin-sensitizing hormones, especially adiponectin and leptin, secreted from white adipose tissue. It thus seems that CBP can function as an important component of the switch between energy storage and expenditure. It is known that replenishment of adiponectin reverses hyperglycaemia and hyperinsulinaemia in obese mice; specific inhibition of CBP activity might therefore provide a novel treatment for obesity-linked diseases such as type-2 diabetes and atherosclerosis [43].

Discovery of LAT inhibitors

Substrate mimetic inhibitors

Substrate analogue inhibitors based on covalent conjugates between CoA and Lys-peptides have been used widely as biochemical tools in the study of LATs. The utility of these probes derives from the fact that, because of differential substrate specificities and different catalytic mechanisms employed by various LATs, selectivity can be achieved, depending on the nature of the lysyl moiety in the conjugates [44] (*R* of conjugates in Figure 3). Because they are not membrane-permeable, in cell-based studies CoA conju-

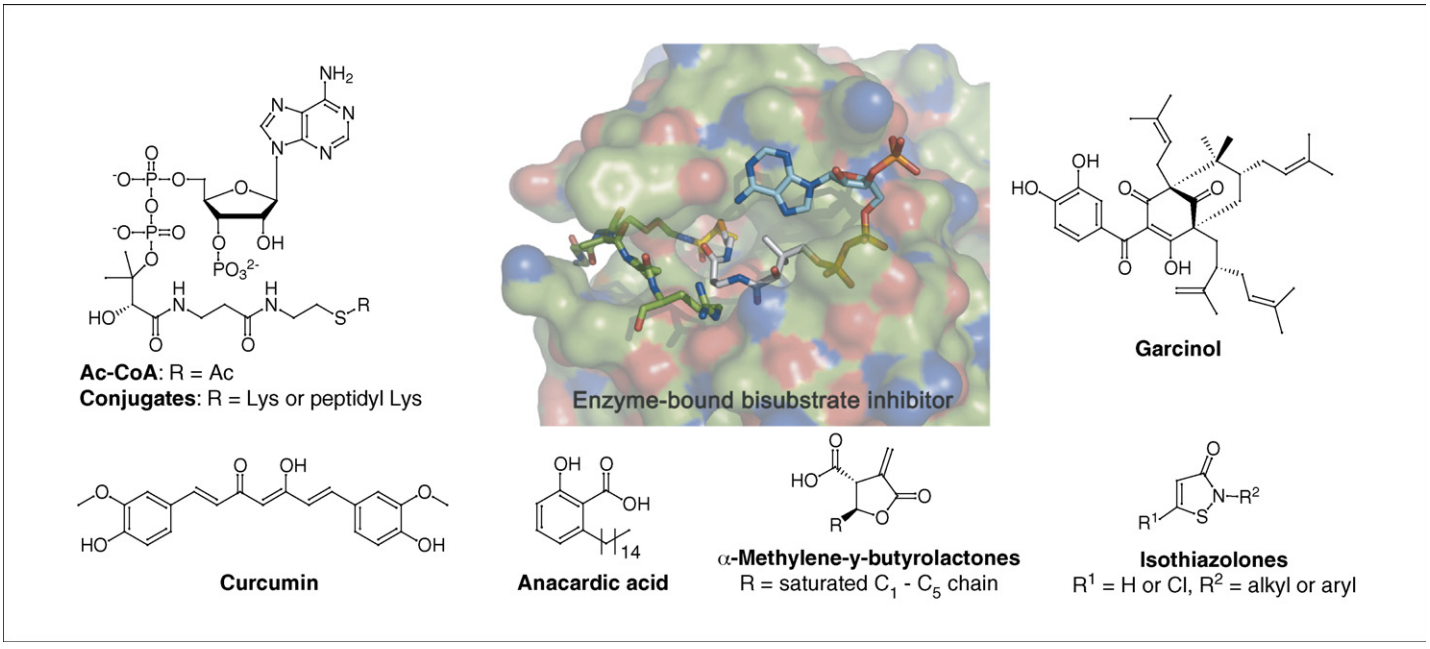

FIGURE 3

Chemical structures of active site lysine acetyltransferase inhibitors. The acetyl donor for lysine acetyltransferase (LAT) enzymes is acetyl coenzyme A (Ac-CoA); replacement of the acetyl group in Ac-CoA with macromolecular substrate peptides generates bisubstrate inhibitors. The 3D complex between one such inhibitor conjugate {CoA [cyan; Corey, Pauling, Kultin (CPK) colouring] tethered from its pantethein arm (grey; CPK) via an isopropionyl linker (yellow; CPK) to the side-chain amine of Lys14 in the histone H3 peptide ARTKQTARKSTGGK14APRKQL (green; CPK)} and GCN5L2 is shown [constructed from protein databank (PDB) number 1M1D [106]]. Garcionol, curcumin and anacardic acid are compounds found to be LAT inhibitors in natural product screens. α-Methylene-γ-butyrolactones and isothiazolones are synthetic LAT inhibitors.

gates are delivered by microinjection or with the aid of lipid permeabilization agents. Prodrug strategies aimed at masking the anionic phosphate groups of CoA conjugates have also been advocated [45]. Most recently, cellular-delivery vector peptides have been appended to CoA conjugates to render them membrane permeable, but it remains to be seen if these constructs are pharmacologically useful [46].

Small-molecule enzyme inhibitors

Anacardic acid and curcumin

More recently, non-peptide small-molecule LAT inhibitors have also been reported (Figure 3). Anacardic acid is a major component of cashew nutshell liquid and was identified in a natural product screen as a noncompetitive LAT (i.e. p300 and PCAF) inhibitor [47]. It has poor membrane permeability and, therefore, little effect on cells [48]. Another natural product with LAT-inhibitory activity is curcumin, a yellow pigment extracted from the root of the turmeric herb *Curcuma longa L* [37]. Curcumin has long been known to possess interesting pharmacological properties; apart from chemopreventive and antiproliferative activities, it has been found to have antioxidative, anti-inflammatory, anti-infective and antiseptic properties, and is widely used in Indian medicine and culinary traditions [49]. The observed kinetics of p300 enzyme inhibition by curcumin were originally interpreted to suggest that this compound binds not at the active site but is an allosteric inhibitor [37]. Subsequently, it was shown that curcumin is in fact a covalent inhibitor of p300 but not PCAF, presumably targeting a LAT cysteine thiol group by virtue of its electrophilic unsaturated ketone function [50]. It is tempting to speculate that this could be a cysteine that corresponds to the catalytic cysteine known to occur in MYST LATs, discussed earlier. Although it is known that p300/CBP LATs follow a ping-pong catalytic mechanism similar to MYST LATs [51], no protein crystal structures of p300/CBP LATs are currently available to confirm this. Nevertheless, the importance of cysteine residues for the function, but not the structural integrity, of p300 has been noted [52]. Our observation (unpublished results) that curcumin is also an inhibitor of MYST LATs suggests that p300/CBP LATs and MYST LATs might be related, as far as mechanism and enzyme inhibition are concerned. Treatment of cells with curcumin leads to hypoacetylation of the p300/CBP substrates histones H3 and H4, as well as p53, and co-treatment with trichostatin A, an HDAC inhibitor, attenuates this loss of cell viability [53]. Curcumin is capable of inducing apoptosis in cancer cells without cytotoxic effects on healthy cells [54]. Downregulation of antiapoptotic proteins [e.g. Bcl-2, Bcl-$X_{L,}$ IAP (inhibitor of apoptosis protein)] in response to curcumin is consistent with transcriptional inhibition, and this activity occurs, at least in part, because of curcumin acting as a p300/CBP-specific LAT inhibitor [37]. Interestingly, the antiproliferative effects of curcumin are estrogen-dependent in ER-positive cells [55]. The simple structure of curcumin enables easy access to derivatives; in fact several curcumin analogues have already been described [56,57]. Curcumin has been under clinical study in indications ranging from AIDS, cystic fibrosis and Alzheimer's disease, to inflammation and, most recently, cancer – to date, without pronounced success [58,59]. Curcumin is poorly bioavailable, mainly because of high chemical and metabolic lability [60].

Garcinol

Garcinol is a polyprenylated benzophenone natural product isolated from the edible fruit *Garcinia indica* and was shown to be an active-site inhibitor of p300 and PCAF, where inhibition kinetics were observed to be uncompetitive with respect to Ac-CoA but competitive with respect to histone [61]. The fact that curcumin and garcinol induce apoptosis in cancer cells in a similar manner was noted before these compounds were known to be LAT inhibitors [62]. Many closely related natural products, also containing the polyprenylated bicyclo[3.3.1]nonanetrione skeleton present in garcinol, are known and possess various pharmacological activities, including anticancer properties [63]. However, these compounds tend to be poorly soluble and are unstable because of facile oxidation of the isoprene functions. For example, hyperforin, a steroid and xenobiotic receptor ligand [64,65], induces apoptosis in cancer cells and has *in vivo* antitumour activity [66,67]. It was shown recently that the solubility and stability issues with this compound could be overcome by enol alkylation with carboxylate-containing groups and reductive saturation of the isoprene double bonds, respectively, to afford aristoforin, a hyperforin derivative with unchanged antitumour activity but improved pharmaceutical properties [68]. These results suggest that isoprenylated benzophenones might be a suitable starting point for drug development.

Synthetic inhibitors

α-Methylene-γ-butyrolactones are small-molecule LAT inhibitors of purely synthetic origin. They were designed based on the known interactions in the LAT active site between Ac-CoA and the acetyl acceptor Lys side-chain of the macromolecular substrate [69]. Structure–activity relationships diverged depending on the length of the aliphatic side chain (see *R* of butyrolactone structure in Figure 3) and molecules with selectivity for either GCN5L2 [general control of nitrogen (GCN5)-like protein] or PCAF were reported. The potency of active analogues is rather modest and it is not clear if these butyrolactone compounds possess cellular activity. Isothiazolones are the most recent LAT inhibitors reported [70]. These compounds were discovered in a LAT screen of a synthetic compound library. They possess antiproliferative activity against cancer cell lines, and their mode of action probably includes covalent protein binding as a result of the chemical reactivity of these compounds.

Protein-interaction inhibitors

Given the obligate nature of interactions between cofactors and NRs for transcriptional activity, it is probable that drugs that target coactivator interaction surfaces will function as pure antagonists with particular utility in the treatment of estrogen- and androgen-dependent cancers. It has been demonstrated, for example, that the LXXLL-binding site of the ER can be targeted with comparatively small peptides possessing nanomolar or even subnanomolar affinity [71], and there are also efforts under way to design small-molecule compounds that fit onto the LXXLL peptide-binding surface of various NRs (Figure 4). However, this approach is not advanced as yet because none of the compounds identified to date seem to be potent or membrane-permeable. Examples are ER antagonist compounds with heterocyclic cores that mimic the tether sites of three leucines on the peptide helix (e.g. the pyr-

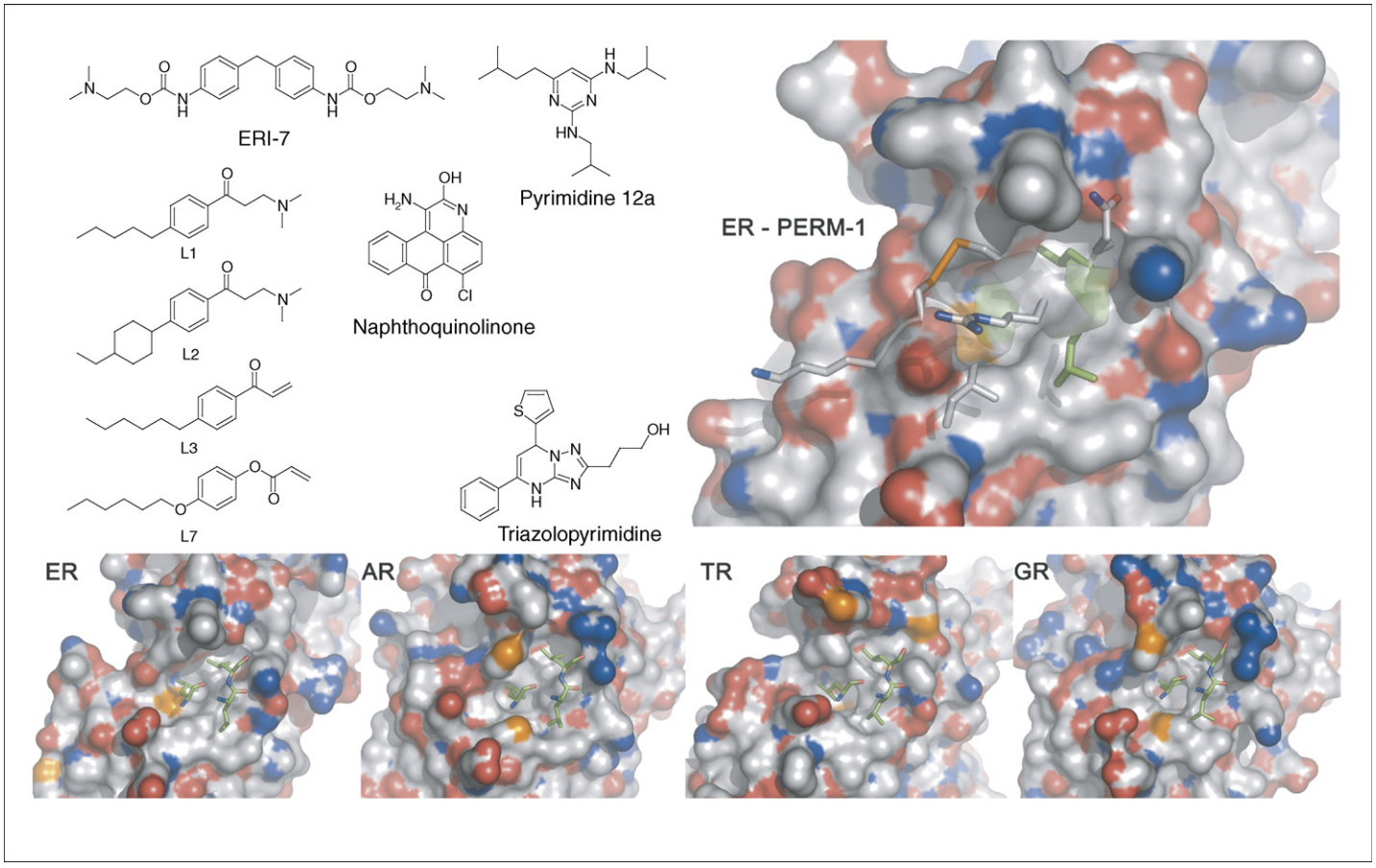

FIGURE 4

Inhibitors of nuclear receptor–SRC/p160 cofactor protein–protein interactions. Many cofactors contain the LXXLL motif, which is recognized by a docking site formed in the ligand-binding domain of nuclear receptors, upon hormone binding. Cofactor-derived peptides, for example the helix-stabilized cyclic peptide PERM-1 (peptidomimetic estrogen receptor modulater [71]) [H-Lys-*cyclo*(D-Cys-Ile-Leu-Cys)-Arg-Leu-Leu-Gln-NH_2], bind the ERα (estrogen receptor alpha) with high affinity [protein databank (PDB) number 1PCG]. Other nuclear receptors, such as the androgen receptor (AR), thyroid hormone receptor (TR), and glucocorticoid receptor (GR), also recognize LXXLL-containing cofactor peptides (here, only Leu side-chains of ligands are shown, in green; PDB numbers 3ERD, 1T63, 1BSX, 1M2Z), but apart from the pocket that binds to the three Leu residues, the cofactor binding sites vary. ERI-7 (estrogren receptor inhibitor-7), the L-series compounds, pyrimidine 12a, as well as the naphthoquinolinone and triazolopyrimidine structures depicted, are all small-molecule inhibitors of nuclear-receptor–cofactor complex formation.

imidine 12a shown in Figure 4 [72]) and the symmetrical screening hit ERI-7 [73]. In a recently published screening campaign that aimed to identify thryroid receptor (TR)–coactivator inhibitors, compounds such as the naphthoquinolinone, triazolopyrimidine and L-series compounds shown in Figure 4 were discovered [74]. The latter are α,β-unsaturated ketones or their Mannich base prodrugs, which function as irreversible inhibitors, presumably targeting a cysteine in the LXXLL-binding site. The fact that NR-specific inhibitors could be developed is emphasized by biochemical studies [75,76] and the differential selectivity for the TRα and TRβ isoforms of these L-series compounds. ERα versus ERβ selectivity has also been observed with various peptide inhibitors, referred to in this review.

Yet another way of blocking LAT function is to interfere with the recognition, through LAT bromodomains, of acetylated binding partners, which is important in the recruitment of LAT coactivators to transcriptional complexes [77]. The molecular basis of the recognition of Lys-acetylated peptides is comparatively well understood and NMR solution structures of bromodomains from GCN5L2 [78], PCAF [79], CBP [80] and TAF1 [81] have been reported (Figure 5). Because injection, into HIV-infected cells, of antibodies against the PCAF bromodomain blocks Tat transactivation [33], bromodomain inhibitors are sought as new anti-HIV therapeutics [82]. It is not clear yet, however, if high-affinity drug-like small molecules capable of blocking bromodomain interactions can be designed. Even comparatively long Lys-acetylated peptides derived from, for example, histone H4, Tat and p53 have low binding affinity (i.e. micromolar K_D) [79–82]. Although most of the binding affinity derives from recognition of the Ac-Lys side chain of such peptides (i.e. nonacetylated peptides are devoid of binding) Ac-Lys mimetics, for example acetylhistamine (Figure 5), bind bromodomains with millimolar K_D values, as determined from NMR titrations [83]. Nevertheless, the first small-molecule leads (Figure 5) with low micromolar potency have now been reported [84,85].

Future perspectives

Epigenetic drugs targeting two of the enzyme groups involved in chromatin modification and remodelling [i.e. DNA methyltransferases (DNMTs) and HDACs] are already under clinical investigation as new cancer therapeutics [86]. It is probable that inhibitors of LATs, another prominent enzyme class involved in the regula-

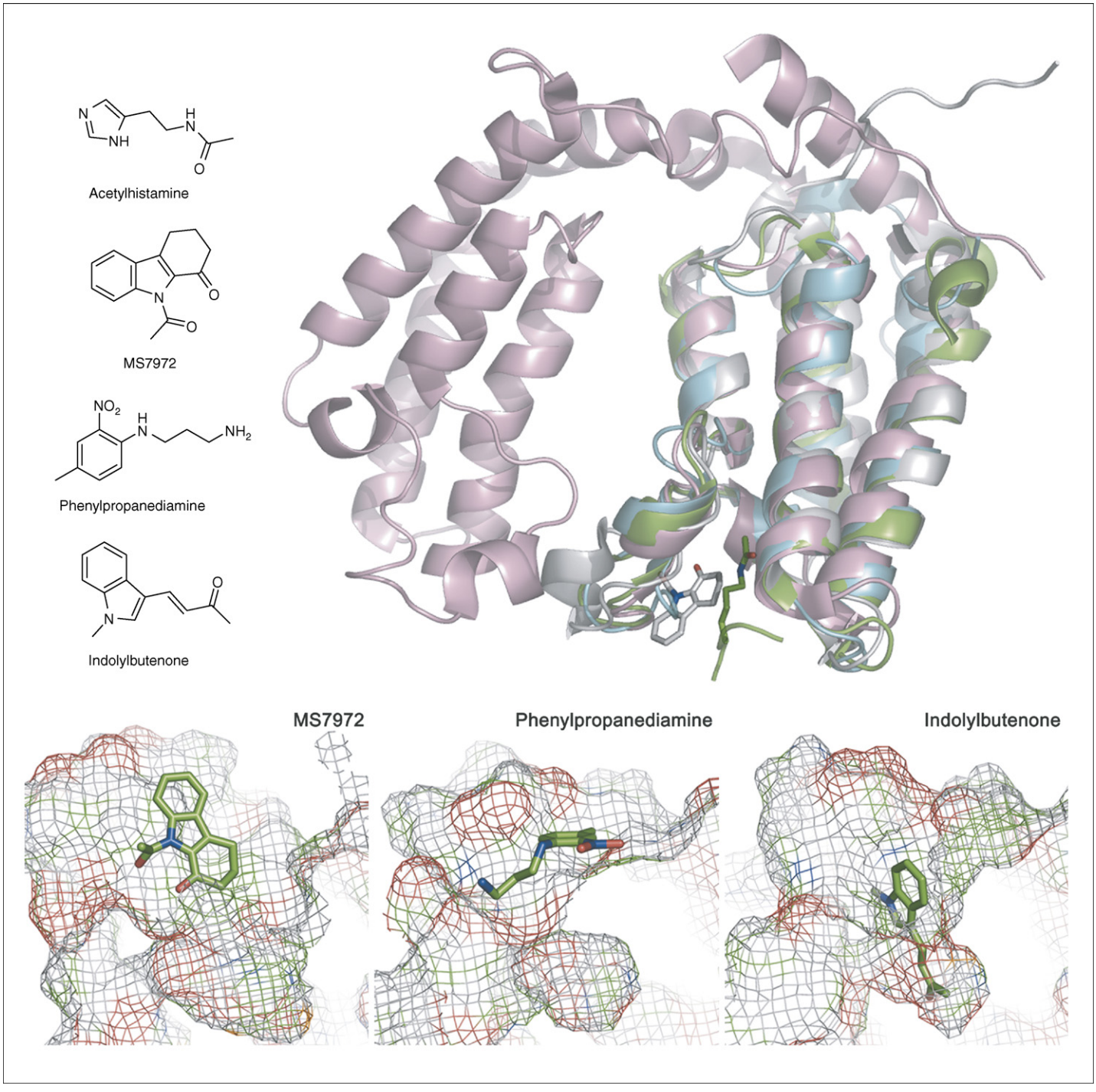

FIGURE 5

Inhibitors of lysine acetyltransferase bromodomain interactions with acetyllysine binding partners. The chemical structures of small-molecule bromodomain inhibitors are shown (top left), as well as an alignment (secondary structure ribbon cartoons) of the bromodomains from TAF1 [pink; protein databank (PDB) number 1EQY], PCAF (cyan; PDB number 1N72), GNC5L2 (green; including p53 acetyllysine peptide with Ac-Lys side-chain as stick model; PDB number 1E6I) and CBP (grey; including bound small-molecule inhibitor M7972 as stick model; PDB number 2D82). The binding modes of three of the inhibitors are depicted in the bottom panels (bromodomain surfaces are shown as CPK-coloured meshes and inhibitors as green CPK stick models). It can be observed that M7972 and the phenylpropanediamine bind at the surface of the acetyllysine recognition site, whereas the butanone side-chain of the indolylbutenone inhibitor mimics the acetyllysine residue of cognate binding partners (constructed from PDB numbers 2D82, 1WUG and 1ZS5). For definitions of abbreviations, see Table 1.

tion of transcription, will also find uses in targeted chemotherapy, not only for cancers but also for other disease states where aberrant gene expression is implicated. There is reason to believe that specificity of drug action might be achieved by exploiting the subtle, but in some cases well characterized, differences in the ways that individual, or families of LATs interact with their cognate partners. These include differential binding of Ac-CoA and macromolecular substrates by LAT catalytic domains, binding of acetylated motifs in binding partners through bromodomains, recognition of transcription factors and other coregulators

through LXXLL motifs, and other protein–protein interactions that LATs participate in [87]. Although various early lead compounds targeting the enzymatic or protein interaction functions of LATs are now available, none of these would currently seem to be sufficiently potent and druglike to qualify as drug candidates. The challenge for the immediate future is therefore to optimize these leads to provide viable pharmacological tools and development candidates.

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