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Identification of 4-hydroxyquinolines inhibitors of p300/CBP histone acetyltransferases

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ABSTRACT

We identified a series of 4-hydroxyquinolines bearing a C1 to C15 alkyl chain at the C2 position and a carbethoxy/carboxy group at the C3 position of the quinoline nucleus (MC compounds), endowed with selective inhibitory activity against the p300/CBP HAT enzymes. Enzyme inhibition was investigated using *in vitro* HAT assays and by western blot analysis of cellular lysates to examine the acetylation levels of histone H3 and α -tubulin. When tested in U937 cells, some compounds displayed pro-apoptotic or cytodifferentiating properties.

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In the nucleus of eukaryotic cells, DNA is highly compacted and organized into chromatin by both histone and non-histone proteins. The fundamental repeating unit of chromatin is the nucleosome, which consists of 147 base pairs of DNA wrapped 1.6 times around an octamer of core histone proteins H2A, H2B, H3, and H4.^{1,2}

Histones undergo extensive post translational modification at specific amino acids within the histone tails. These modifications include acetylation, phosphorylation, methylation, ubiquitynation, sumoylation, and ADP-ribosylation.^{3,4}

The functional implications of reversible acetylation–deacetylation have been most widely studied. Histone acetylation occurring at the ϵ -amino group of lysine residues in the N-terminal tails of core histones mediates conformational changes in nucleosomes. Two superfamilies of enzymes are involved in this process: histone acetyltransferase (HAT) and histone deacetylase (HDAC), which

catalyze respectively the addition to and removal of acetyl units of histones.^{5,6}

The HAT enzymes may be classified into two categories depending upon their cellular localization: type A or nuclear and type B or cytosolic enzymes. The nuclear HATs can be grouped into several families based on sequence similarity, including the GNAT (GCN5-related N-acetyltransferase) family, the MYST (named after its founding members, in particular MOZ, YBF2/SAS3, SAS2, and TIP60) family, the p300/CBP family, some nuclear receptor coactivators (like SRC-1, ACTR, TIF2), and other general transcription factors (such as TAFII-p250, the TFIIC family, and others).^{7,8}

In addition to histones, over 40 transcription factors and 30 other nuclear, cytoplasmic, and viral proteins have been shown to be acetylated *in vivo*.

Similarly to HDACs, HATs are typically found in cells in large multiprotein complexes, but unlike HDACs they exhibit robust catalytic activity as purified proteins. Each HAT complex may regulate a distinct set of genes and thereby specific cellular functions. For example, the members of GNAT family (including transcriptional coactivators, GCN5 and p300)/CREB-binding protein-associated factor (PCAF) regulate cellular growth and development. The MYST HATs are involved in a wide range of regulatory functions including

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transcriptional activation, and are important for cell growth and cell cycle regulation. The ubiquitously expressed members of the p300/CBP family are considered as global transcriptional coactivators, and have a critical role in cell cycle regulation, cellular differentiation and apoptosis.⁹

Dysfunction of HAT enzymes is often associated with several diseases, including cancer. Nevertheless, the role of HATs in cancer is not straightforward as these enzymes can act either as tumor suppressors or activators depending on the type and development stage of tumors. A global loss of H4K16 acetylation has been linked to tumorigenesis,¹⁰ and gene targeting in mice revealed that MOZ is essential for the generation and maintenance of hematopoietic stem cells and for the appropriate development of myeloid, erythroid, and B lineage cell progenitors.¹¹ p300 and CBP are considered to be tumor suppressors: indeed, their expression decreases in hepatocarcinogenesis.¹² p300/CBP mutations have been found in several human leukemias, and missense or truncating mutations in p300 have been reported in colorectal, gastric, breast, and epithelial carcinomas.^{13–15} On the other hand, MOZ can generate fusion genes with other HAT proteins, such as MOZ-TIF2, MOZ-CBP, and MOZ-p300, in acute myeloid leukemia (AML) by chromosomal translocation.^{11,16–18}

Mistargeted and deregulated HAT activities of GCN5/PCAF and p300 have also been reported to play an important role in genetic diseases and in human colorectal, breast, and pancreatic cancers.^{13,14,19} Reduced cellular levels of p300 or activity has been proposed to diminish stress-induced cardiac hypertrophy and development of heart failure.²⁰ Thus, small chemical inhibitors of HAT enzymes (HAT inhibitors, HATi) represent novel candidates for drug development.

In comparison to the great number of known inhibitors of HDACs,^{21,22} a limited number of HATi has been described to date (Fig. 1), with various degrees of selectivity and cell permeability. They can be grouped into three different classes: (i) the synthetic peptide-CoA-based bisubstrate inhibitors (Lys-CoA, specific for p300, and H3-CoA-20, specific for PCAF),²³ and their cell permeable versions LysCoA-SS-R10 and H3-CoA-20-TAT,²⁴ (ii) the natural products anacardic acid (AA),²⁵ garcinol,²⁶ and curcumin^{27,28} that were described as potent p300/PCAF (AA and garcinol) or p300-selective (curcumin) inhibitors, (iii) some small molecules, such as the GCN5 inhibitor butyrolactone MB-3,²⁹ a group of isothiazolones that act as p300/PCAF inhibitors with interesting anticancer properties,³⁰ as well as some AA^{31,32} and garcinol analogues (LTK compounds).³³

We have undertaken a series of studies involving the design, synthesis, and biological validation of small molecule modulators

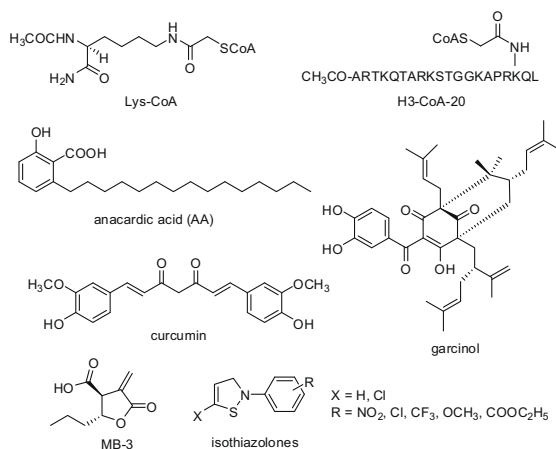


Figure 1. Known HAT inhibitors.

of epigenetic targets;^{34–38} we have recently employed a phenotypic screening assay in yeast to identify two quinolines, namely MC1626 and MC1752 (Fig. 2), which are active at submillimolar/millimolar range.^{39–41}

In order to improve their HAT inhibiting activity, we introduced some chemical features typical of AA and MB-3 into the quinoline nucleus, obtaining the 3-carboxy-4-hydroxy-2-pentylquinoline MC1823 (**4**, Fig. 2) with 10/20-fold increased human HAT inhibitory activity with respect to the prototypes MC1626 and MC1752.⁴⁰

Since **4** in human HAT inhibitory assay (U937 cell nuclear extracts) proved to be more potent than AA (% of inhibition at 50 μ M: 30 (**4**) and 15 (AA)) and a few less active than MB-3 (44% of inhibition at 50 μ M), used as reference drugs,⁴⁰ we re-examined two **4**-related quinolines (**1** and **7**, cited as compounds **15** and **8** in ref.⁴⁰ respectively) previously found inactive in the phenotypic screening in *Saccharomyces cerevisiae*. In addition, we prepared additional 4-hydroxyquinolines with 3-carboxy/3-carbomethoxy functions at the C3 position, and differently sized alkyl chains (C1 to C15 carbon units) at the C2 position (Fig. 2), for testing them against several human HAT enzymes.

The 3-carbomethoxy-4-hydroxyquinolines **1**, **3**, **5**, and **7** were obtained by reaction of the appropriate ethyl β -oxoester with isatoic anhydride in presence of sodium hydroxide as catalyst, according to the method described in Ref. 40. Alkaline hydrolysis of the above esters with 4 N KOH afforded the 4-hydroxy-3-quinolincarboxylic acids **2**, **4**, **6**, and **8** (Scheme 1).⁴⁰

The new compounds have been tested on human HAT enzymes belonging to the p300/CBP (p300 and CBP) and GNAT (GCN5 and PCAF) families, in comparison with AA and MB-3 as reference drugs (Table 1) using in vitro HAT assays. Moreover, compounds **1–8** have been validated in living cells by examining the effects on histone H3 and α -tubulin acetylation both in human leukemia U937 and HEK-TE cells (Fig. 3). Finally, the effects of **1–8** on cell cycle, apoptosis induction, and granulocytic differentiation in U937 cell line have been determined (Fig. S1 in Supplementary material, Figs. 4 and 5).

When tested at 50 μ M against immunoprecipitated p300 HAT from U937 cell nuclear extracts, all the compounds **1–8** showed high inhibitory activity, regardless of the presence of carbomethoxy or carboxy group at C3, and of C1, C5, C10, or C15 carbon unit chain at C2. The most potent compounds were the 2-methylquinolines **1** and **2**, which displayed approximately 3-fold higher inhibition

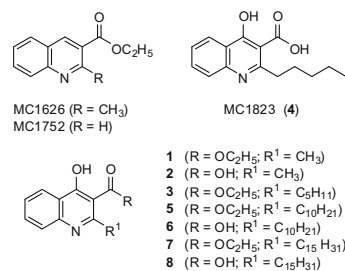
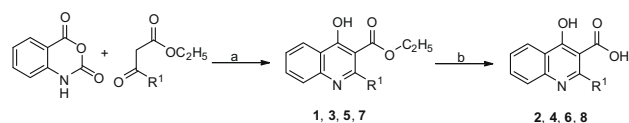


Figure 2. Novel quinoline-based HAT inhibitors.



Scheme 1. Reagents and conditions; (a) NaOH, dioxane, reflux; (b) 4 N KOH, EtOH, reflux.

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