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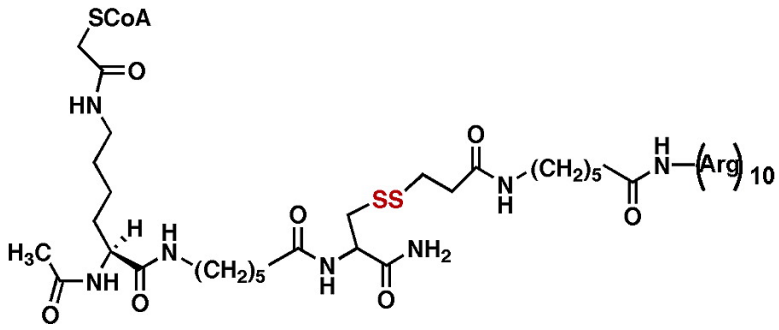

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Synthesis and Evaluation of a Potent and Selective Cell-Permeable p300 Histone Acetyltransferase Inhibitor

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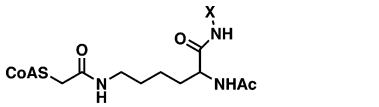
Reversible protein acetylation has been shown to be a major mechanism for the regulation of gene expression.[1] The transcriptional coactivator p300 and its paralog CBP have histone acetyltransferase (HAT) domains which catalyze the transfer of the acetyl group from acetyl-CoA to the ϵ-amino lysine atom in histones and other proteins.[2] They are critical to transcriptional control in a range of pathways and are shown to be important in normal development and disease.[3] Despite significant efforts,[4] potent and specific cell-permeable inhibitors of p300 or CBP HATs have yet to be reported. Such compounds would be very useful biological tools and might have therapeutic value. Here we describe the first potent, selective, and cell-permeable p300 HAT inhibitor and show its inhibition of histone acetylation and transcription in mammalian cells.

Several years ago, a potent inhibitor of p300/CBP HAT activity, Lys-CoA (**1**, Figure 1), was described and demonstrated to be greater than 100-fold selective versus other acetyltransferases including PCAF, GCN5, and EsaI.[4a−c] Lys-CoA has been used by a number of groups to evaluate the role of p300/CBP HAT function.[4c] Because it contains negatively charged phosphate groups, Lys-CoA is generally inactive in mammalian cell systems unless microinjected or co-administered with moderately cytotoxic detergents.[4c] Certain peptide sequences when covalently linked to cargo have been shown to enhance cell entry of poorly cell-permeable molecules.[5] Recently, linkage of a CoA analogue to a Tat peptide sequence (H3−CoA-20−Tat) was used successfully to generate a potent and selective PCAF/GCN5 cell-permeable inhibitor.[6]

We thus synthesized a series of Lys-CoA derivatives linked via the amino acid backbone to potential permeabilizing peptide sequences and examined these compounds as p300 and PCAF HAT inhibitors. Each of these compounds was still able to block p300 but with 4−35-fold diminished potency compared to that of Lys-CoA (Table 1). Of greater concern, the compounds were much less selective versus PCAF, with the maximal selectivity about 8-fold (Table 1). Since the precise sequence or spacer between Lys-CoA and cell permeabilizing sequence seemed relatively inconsequential, we reasoned that the cluster of positive charges within the cell permeabilizing motif was likely to be responsible for this potency and specificity loss.

We were able to demonstrate that compound **8** retained potency and specificity versus p300 HAT (IC_{50} = 20 nM vs p300, IC_{50} = 20 μM vs PCAF). We thus adopted a strategy[5c] in which the cell permeabilizing peptide would be reversibly linked to Lys-CoA derivative via a disulfide bond as exemplified by **7**. Once inside the cell, the disulfide would be reduced, trapping the Lys-CoA derivative inside the cell and freeing it from the permeabilizing peptide. The target compound (**7**) was prepared using a combination of solid and solution phase synthesis using an orthogonal protective group strategy, as shown in Figure 2. The disulfide bond forming step was regioselectively controlled using the pyridyl reagent, and the final compound purified by HPLC demonstrated the desired mass by MS.

Since in vitro p300 HAT assays are routinely carried out in the presence of the potent disulfide reducing agent dithiothreitol, it was expected that compound **7** would behave similarly to the analogues **8** and **10**. This was indeed the case. The IC_{50} of **7** was demonstrated to be 70 nM, only 3.5-fold higher than that of compound **8** and quite similar to that of **10** and Lys-CoA under these conditions. Importantly, specificity of **7** versus PCAF was also excellent with a differential inhibitory potency of over 300-fold. These studies



1 (Lys-CoA): X = H
2: X = YGRKKRRQRRR-CO_2H
3: X = YGRKKRRQRRRGYK-NH_2
4: X = Ahx-R-Ahx-RR-Ahx-RR-Ahx-RR-Ahx-K-NH_2
5: X = GRRRRRRRRRRGK-NH_2
6: X = Ahx-RRRRRRRRRR-NH_2

Figure 1. Structures of Lys-CoA and analogues (Ahx: 6-aminohexanoic acid).

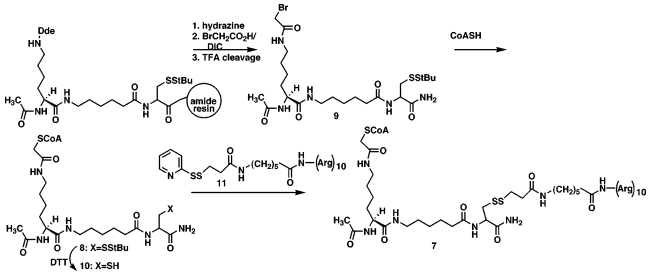


Figure 2. Synthetic route to cell-permeable inhibitor **7**.

Table 1. IC_{50} Values of **1**−**10** in HAT Assays

compound	IC_{50} for p300 (μM)	IC_{50} for PCAF (μM)
1 (Lys-CoA)	0.05	200
2	0.25	2.2
3	0.25	NA
4	0.32	0.6
5	1.8	NA
6	0.17	<0.7
7	0.07	25
8	0.02	20
10	0.08	50

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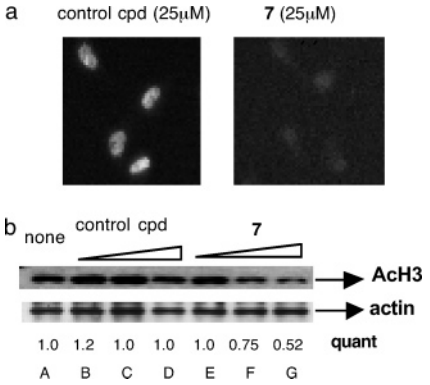

Figure 3. Cellular histone acetylation blocked by **7**: (a) immunohistochemical analysis of WM983A cells with antiacetyl histone H3 Ab; (b) immunoblot of AcH3 from WM983A cells; control compound (B−D) and **7** (E−G) (2, 10, and 25 μM). Densitometry (quant.) relative to actin shown.

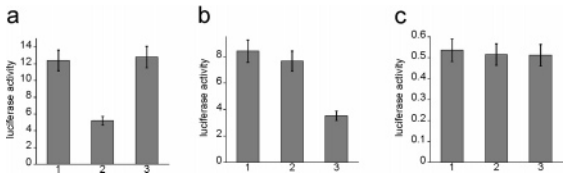

Figure 4. Transcriptional effects of **7** with 293T cells: (a) +GAL4DBDp300, (b) +GAL4DBDPCAF, (c) no coactivator. Lanes 1−3 in a−c: no inhibitor (1), 10 μM **7** (2), 10 μM H3−CoA−20-Tat (3).

suggest that reductive release of the oligoArg peptide in **7** allows for regaining the desired inhibitory properties.

To explore the potential for compound **7** to block HAT activity in cells, two independent assays were performed investigating effects on histone acetylation (Figure 3). Since p300 and CBP are known to catalyze the acetylation of histone H3, analysis of histone H3 acetylation levels in a melanoma cell line in response to treatment with **7** was performed. In one assay type, antiacetyl histone H3 antibodies were used in immunocytochemistry to assess changes in nuclear histone acetylation. As can be seen, intense antiacetyl histone Ab staining in the nuclei of WM983A cells treated with 25 μM control compound but not **7** was observed. In a separate experiment, Western blots on cell lysates extracted from WM983A cells showed a dose-dependent decrease in H3 acetylation as a function of **7** concentration compared to the control compound. Taken together, these data support the hypotheses that compound **7** can block cellular histone acetylation and that p300 and/or CBP are likely to be major regulators of histone H3 acetylation in this cell line.

To investigate the effects of compound **7** on cellular transcription, a HAT-responsive reporter was examined in mammalian 293T cells. For these experiments, GAL4 DNA binding sites were engineered upstream of the herpes simplex virus thymidine kinase minimal promoter fused to a luciferase reporter, and cells were co-transfected with one of the following: GAL4DBD (DNA binding domain) PCAF, GAL4DBDp300, or no coactivator. As can be seen (Figure 4), **7** at a concentration of 10 μM selectively blocked transcription from the luciferase reporter stimulated by the coactivator GAL4DBDp300 (Figure 4a) but not GAL4DBDPCAF or background transcription (Figure 4c). In control experiments, H3−CoA-20−Tat (selective PCAF inhibitor) blocked GAL4DBDPCAF-stimulated transcription (Figure 4b) but not GAL4DBDp300-stimulated transcription (Figure 4a) or background transcription (Figure 4c). These experiments reveal that compound **7** can have functional and selective effects in mammalian cell transcription linked to p300 HAT activity.

Relative inhibition potency of **7** on histone acetylation and transcription is comparable. It may be possible to further augment the potency and selectivity of compound **7** as a selective p300 HAT inhibitor by modifying the core structure **8** on one hand or the cell permeabilizing oligoArg moiety on the other. In any case, the development of compound **7** promises to be a broadly useful tool for exploring the role of p300 in molecular biology. In particular, how precisely p300 HAT activity is critical to a given pathway as opposed to its scaffolding function is still unclear in many cases. Moreover, there are a number of disease models proposed to be targeted by p300 inhibitors[3,4] that can now be investigated and potentially realized as bona fide therapeutic directions.

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Supporting Information Available: Details of organic synthesis, in vitro and in vivo assay procedures are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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