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Research Letter

New Potent Bisubstrate Inhibitors of Histone Acetyltransferase p300: Design, Synthesis and Biological Evaluation

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Bisubstrate-type compound Lys-CoA has been shown to inhibit the p300 histone acetyl transferase activity efficiently and may constitute a lead compound for a novel class of anticancer therapeutics. Based on this strategy, we synthesized a series of CoA derivatives and evaluated these molecules for their activity as p300 histone acetyltransferases inhibitor. The best activity was obtained with compound 3 bearing a C-5 spacing linker that connects the CoA moiety to a *tert*-butyloxycarbonyl (Boc) group. Based on docking simulations, this inhibitor exhibits favorable interactions with two binding areas, namely pockets P1 and P2, within the active site.

Key words: bisubstrate inhibitor, chemical biology, drug design, HAT, p300

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The structural unit of chromatin, the nucleosome, encompasses 147 base pairs of DNA wrapped around an octamer of core histone proteins in 1.7 turns (1). Histones are subjected to extensive post-translational covalent modifications of their tail regions including acetylation, methylation, phosphorylation or ubiquitinylation, sumoylation, and/or ADP ribosylation, which play a key role in the regulation of gene expression, cell growth, or differentiation (2,3). Among these modifications, the functional implications of reversible acetylation/deacetylation processes have been the most widely studied. These chemical modifications are ensured by the coordinated actions of two superfamilies of enzymes, i.e. histone acetyltransferases (HATs) and histone deacetylases (HDACs), which catalyze the addition and the removal, respectively, of acetyl units at the ε-amino group of lysine residues in the lysine N-terminal tails (4–6). Nuclear HATs can be grouped into four families based on sequence homology: the GNAT (PCAF, GCN5), the MYST (Esa1, Tip 60), the p300/CBP, and the rtt109 families (7,8). Dysfunction of p300 has been implicated in diseases such as inflammatory processes, Huntington's disease, cardiovascular diseases, diabetes mellitus, AIDS, and some cancers (8–10). These observations lead to consideration of modulators (activators or inhibitors) of p300 as potential next generation therapeutics. Moreover, such compounds would also be useful for probing the functional significance of the histone acetyltransferase activity in vivo. To date, a limited number of HAT inhibitors have been described which can be divided into three main groups: (i) naturally occurring compounds, such as curcumin, inhibitor of p300 (11,12), garcinol and anacardic acid for p300 and PCAF (13–15) (ii) small synthetic molecules such as isothiazolones, which inhibit both p300 and PCAF (16,17) or more recently compound C646 which is a selective inhibitor of p300 (18,19) (iii) compounds structurally related to CoA ('bisubstrate inhibitors') based on a mechanism approach. These include LysCoA for p300/CBP, histone H3 peptide-CoA analog for PCAF/GCN5 (20,21), and H4K16-CoA for Tip60 and Esa1 (22). In this study, we report the design and the preparation of a series of S-substituted coenzyme A derivatives based on the recently published crystal structure of a semisynthetic heterodimeric p300 HAT domain in complex with Lys-CoA (23). The authors reveal the presence of an additional electron rich pocket (P2) at about 10 Å away from the binding pocket required to accommodate the lysine moiety (P1), the two pockets being connected by an electronegative groove (G) (Figure 1). Based on these observations, we designed and synthesized a series of bisubstrate-type compounds to explore the structural basis of P2 site and to take advantage of that pocket for the development of inhibitors with improved activity. The new bisubstrate compounds consist of (i) an S-substituted coenzyme A, (ii) a flexible linker and (iii) a 'P2-exploring' group of lipophilic or hydrophilic nature ('capping group') (24). More conformationally constrained compounds bearing either a piperazine or benzyl group were also prepared. The inhibitory activities of all compounds (compounds **1–13**) were evaluated on p300 HAT.

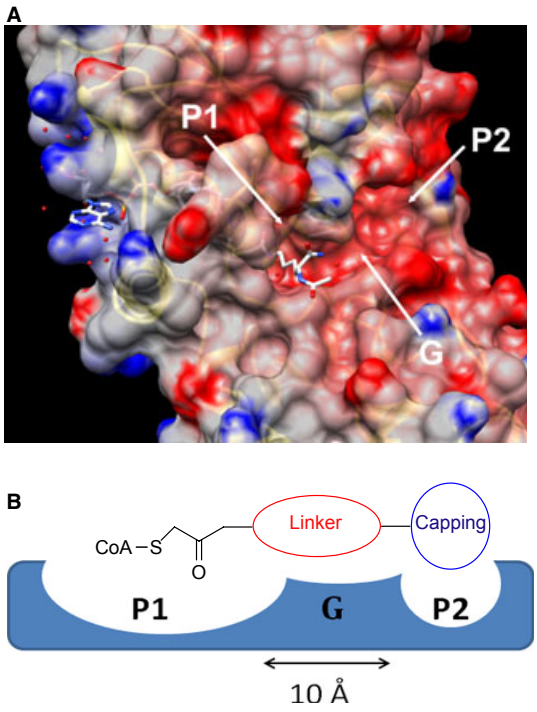


Figure 1: Substrate-binding surface and design of bisubstrate inhibitors (A) View of the putative substrate-binding site of the p300 histone acetyltransferase domain according to reference (23). The putative binding site is shown as an electrostatic surface. Pocket 1, pocket 2, and the connecting groove are annotated as P1, P2, and G, respectively (B) Design of new bisubstrate inhibitors. Bisubstrate inhibitors are composed of a CoASH moiety, a carboxymethylene bridge, a linker with or without flexibility and a capping. These features are shown in relation to a schematic view of p300 histone acetyltransferase binding surface to illustrate how these different components may contribute to molecular recognition.

Methods and Materials

General

All chemicals were purchased from Aldrich or Sigma. All the reactions were carried out over argon atmosphere. All the solvents were distilled prior to use. Analytical thin layer chromatography was performed on MERCK F_{254} plates.^{1}H NMR and ^{13}C spectra were recorded on a Brüker Avance (300 MHz and 100 MHz, respectively). Mass spectra were obtained from the Paul Sabatier University Mass spectrometry service. The chemical ionization (DCI/NH_3) mass spectra were recorded on TSQ 7000 thermoelectron spectrometers and the electrospray mass spectra on API 365 Perkin-Elmer Sciex or Applied biosystems spectrometers. Analytical and preparative HPLC were carried out on reverse phase Hyperprep (Hypersyl) C18 columns using linear gradients of H_2O/0.05% TFA versus acetonitrile. LysCoA was synthesized as described in reference (25).

General procedure for coupling Coenzyme A to the carboxamide derivatives

To a solution of carboxamide compounds (compounds **15a–15i**) in THF (1 mL), was added a solution of CoASH in a pH = 8 aqueous solution (3.7 mL). After an overnight stirring, the aqueous phase was washed with CH_2Cl_2 (5 × 20 mL) and then lyophilized. The desired product was purified by reserved-phase HPLC (TFA 0.05%/CH_3CN 95/5).

***N*-[2-(S-coenzyme A)acetyl-*N*-tert-butyloxycarbonyl] propanamide (1).** Yield: 83%; ^{1}H NMR (D_2O, 300 MHz) δ 0.75 (s, 3H); 0.88 (s, 3H); 1.41 (s, 9H); 1.68 (quint, J = 6.8 Hz, 2H); 2.46 (t, J = 6.5 Hz, 2H); 2.69 (t, J = 6.6 Hz, 2H); 3.08 (t, J = 6.7 Hz, 2H); 3.23 (t, J = 6.9 Hz, 2H); 3.27 (s, 2H); 3.36 (t, J = 6.6 Hz, 2H); 3.47 (t, J = 6.5 Hz, 2H); 3.55 (dd, J = 4.8 Hz, J = 9.8 Hz, 1H); 3.83 (dd, J = 4.9 Hz, J = 9.7 Hz, 1H); 4.02 (s, 1H); 4.25 (m, 2H); 4.59 (m, 1H); 4.84 (m, 2H); 6.17 (d, J = 6.7 Hz, 1H); 8.24 (s, 1H); 8.55 (s, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 175.2; 174.4; 173.0; 158.7; 156.1; 153.4; 149.9; 140.7; 119.1; 87.0; 84.4; 74.7; 74.6; 74.5; 72.4; 66.2; 38.9; 38.8; 38.1; 36.0; 35.9; 35.4; 31.8; 29.0; 28.3; 21.4; 18.7; LRMS: (ESI, m/z) calc. for $C_{31}H_{54}N_9O_{19}P_3S$: 981.2. Found: 489.9 $(M\text{-}2H)^{2-}$.

***N*-[2-(S-coenzyme A)acetyl-*N*-tert-butyloxycarbonyl] butanamide (2).** Yield: 89%; ^{1}H NMR (D_2O, 300 MHz) δ 0.76 (s, 3H); 0.89 (s, 3H); 1.30 (m, 2H); 1.42 (s, 9H); 1.51 (m, 4H); 2.47 (t, J = 6.5 Hz, 2H); 2.69 (t, J = 6.7 Hz, 2H); 3.04 (t, J = 6.7 Hz, 2H); 3.20 (t, J = 6.8 Hz, 2H); 3.26 (s, 2H); 3.37 (t, J = 6.6 Hz, 2H); 3.48 (t, J = 6.6 Hz, 2H); 3.56 (dd, J = 4.7 Hz, J = 9.9 Hz, 1H); 3.84 (dd, J = 4.8 Hz, J = 9.6 Hz, 1H); 4.03 (s, 2H); 4.25 (m, 2H); 4.60 (m, 1H); 4.85 (m, 2H); 6.18 (d, J = 6.7 Hz, 1H); 8.26 (s, 1H); 8.56 (s, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 175.2; 174.4; 172.8; 156.1; 153.4; 149.9; 119.1; 87.0; 84.3; 74.7; 74.6; 74.4; 72.4; 66.2; 40.4; 40.1; 38.9; 36.0; 35.9; 35.4.; 31.7; 29.1; 28.5; 28.3; 23.8; 21.4; 18.6; LRMS: (ESI, m/z) calc. for $C_{33}H_{58}N_9O_{19}P_3S$: 1009.3. Found: 1008.5 $(M\text{-}H)^{-}$.

***N*-[2-(S-coenzyme A)acetyl-*N*-tert-butyloxycarbonyl] butanamide (3).** Yield: 77%; ^{1}H NMR (D_2O, 300 MHz) δ 0.76 (s, 3H); 0.89 (s, 3H); 1.42 (s, 9H); 1.51 (m, 4H); 2.48 (t, J = 6.5 Hz, 2H); 2.70 (t, J = 6.6 Hz, 2H); 3.07 (t, J = 6.6 Hz, 2H); 3.22 (t, J = 6.2 Hz, 2H); 3.28 (s, 2H); 3.37 (t, J = 6.6 Hz, 2H); 3.49 (t, J = 6.6 Hz, 2H); 3.56 (dd, J = 4.9 Hz, J = 9.8 Hz, 1H); 3.84 (dd, J = 4.9 Hz, J = 9.8 Hz, 1H); 4.03 (s, 1H); 4.26 (m, 2H); 4.60 (m, 1H); 4.83 (m, 2H); 6.19 (d, J = 6.9 Hz, 2H); 8.27 (s, 1H); 8.57 (s,1H); ^{13}C NMR (D_2O, 100 MHz) δ 175.2; 174.4; 172.8; 156.1; 153.4; 149.9; 140.4; 119.1; 87.0; 84.5; 74.8; 74.6; 74.2; 72.3; 66.3; 40.1; 39.9; 38.8; 36.0; 35.9; 35.3; 31.7; 28.2; 27.0; 26.2; 21.4; 18.7; LRMS: (ESI, m/z) calc. for $C_{32}H_{56}N_9O_{19}P_3S$: 995.3. Found: 497.0 $(M\text{-}2H)^{2-}$.

***N*-[2-(S-coenzyme A)acetyl-*N*-benzoyl]propanamide (4).** Yield: 74%; ^{1}H NMR (D_2O, 300 MHz) δ 0.72 (s, 3H); 0.86 (s, 3H); 1.83 (qt, J = 6.8 Hz, 2H); 2.42 (t, J = 6.6 Hz, 2H); 2.66 (t, J = 6.7 Hz, 2H); 3.25 (s, 2H); 3.31 (m, 4H); 3.41 (m, 4H); 3.53 (dd, J = 4.8 Hz, J = 9.8 Hz, 1H); 3.82 (dd, J = 4.8 Hz, J = 9.7 Hz, 1H); 4.00 (s, 1H); 4.24 (m, 2H); 4.58 (m, 1H); 4.83 (m, 2H); 6.13 (d, J = 6.5 Hz, 1H); 7.48 (m, 3H); 7.68 (m, 2H). 8.18 (s, 1H); 8.51 (s,1H); ^{13}C NMR (D_2O, 100 MHz) δ 174.6; 173.8; 172.4; 170.5; 155.4; 152.7; 149.1; 139.5; 133.3; 132.0; 128.6; 126.8; 118.5; 86.4; 83.7; 74.1; 73.9; 73.8; 71.9; 65.5; 38.3; 37.4; 37.3; 35.4; 35.3; 34.9; 31.2; 28.0; 20.9; 18.1; LRMS (ESI, m/z) calc. for $C_{33}H_{50}N_9O_{18}P_3S$: 985.2. Found: 984.3 $(M\text{-}H)^{-}$.

***N*-[2-(S-coenzyme A)acetyl-*N*-benzoyl]butanamide (5).** Yield: 83%; ^{1}H NMR (D_2O, 300 MHz) δ 0.73 (s, 3H); 0.87 (s,

3H); 1.62 (m, 2H); 2.40 (t, J = 6.6 Hz, 2H); 2.64 (t, J = 6.7 Hz, 2H); 3.23 (s, 2H); 3.32 (m, 4H); 3.41 (m, 4H); 3.54 (dd, J = 4.4 Hz, J = 9.6 Hz, 1H); 3.82 (dd, J = 5.0 Hz, J = 10.0 Hz, 1H); 4.01 (s, 1H); 4.25 (m, 2H); 4.58 (m, 1H); 4.83 (m, 2H); 6.15 (d, J = 6.8 Hz, 1H); 7.51 (m, 3H); 7.68 (m, 2H); 8.21 (s, 1H); 8.54 (s, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 174.6; 173.8; 172.4; 170.5; 155.5; 152.8; 149.2; 139.6; 133.5; 131.9; 128.6; 126.8; 118.5; 86.5; 84.0; 74.3; 74.0; 73.7; 71.8; 65.8; 39.5; 39.3; 38.3; 35.4; 35.3; 34.8; 31.2; 26.0; 25.9; 20.9; 18.0. LRMS (ESI, m/z) calc. for $C_{34}H_{52}N_9O_{18}P_3S$: 999.2. Found: 998.5 $(M-H)^-$.

***N*-[2-(S-coenzyme A)acetyl-*N*-benzoyl]pentanamide (6).** Yield: 90%; ^{1}H NMR (D_2O, 300 MHz) δ 0.75 (s, 3H); 0.89 (s, 3H); 1.35 (m, 2H); 1.56 (m, 4H); 2.43 (t, J = 6.5 Hz, 2H); 2.58 (t, J = 6.6 Hz, 2H); 3.17-3.28 (m, 6H); 3.26 (t, J = 6.7 Hz, 2H); 3.26 (t, J = 6.8 Hz, 2H); 3.57 (dd, J = 4.4 Hz, J = 9.4 Hz, 1H); 3.85 (dd, J = 4.9 Hz, J = 9.7 Hz, 1H); 4.03 (s, 1H); 4.26 (m, 2H); 4.60 (m, 1H); 4.85 (m, 2H); 6.14 (d, J = 6.2 Hz, 1H); 7.49 (m, 3H); 7.68 (m, 2H); 8.18 (s, 1H); 8.52 (s, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 174.7; 173.7; 172.2; 170.4; 155.3; 152.6; 149.1; 139.6; 133.5; 131.9; 128.6; 126.8; 118.5; 86.4; 83.5; 74.1; 74.0; 73.9; 71.9; 65.5; 39.7; 39.5; 38.2; 35.4; 35.3; 34.8; 31.1; 28.0; 27.9; 23.4; 20.9; 18.1; LRMS (ESI, m/z) calc. for $C_{35}H_{54}N_9O_{18}P_3S$: 1013.3 Found: 1012.4 $(M-H)^-$.

***N*-[2-(S-Coenzyme A)acetyl]-*N*-(tert-butyl)piperazine-1-carboxylate (11).** Yield: 98%; ^{1}H NMR (D_2O, 300 MHz) δ 0.76 (s, 3H); 0.90 (s, 3H); 1.46 (s, 9H); 2.48 (t, J = 6.4 Hz, 2H); 2.74 (t, J = 6.5 Hz, 2H); 3.39 (t, J = 6.5 Hz, 2H); 3.46-3.58 (m, 13H); 3.85 (dd, J = 4.6 Hz, J = 9.6 Hz, 1H); 4.03 (s, 1H); 4.26 (m, 2H); 4.60 (m, 2H); 4.85 (m, 2H); 6.18 (d, J = 6.7 Hz, 1H); 8.25 (s, 1H); 8.56 (s,1H); ^{13}C NMR (D_2O, 100 MHz) δ 175.3; 174.4; 171.1; 156.8; 156.1; 153.4; 149.9; 140.1; 119.1; 87.1; 84.5; 74.8; 74.7; 74.3; 72.5; 66.2; 46.5; 42.6; 38.9; 36.1; 33.3; 31.7; 28.2; 21.5; 18.7; LRMS (ESI, m/z) calc. for $C_{32}H_{54}N_9O_{19}P_3S$: 993.8 Found: 496.0 $(M-2H)^{2-}$.

***N*-[2-(S-coenzyme A)acetyl]-*N*-phenyl(piperazin-1-yl)-1-methanone (12).** Yield: 35%; ^{1}H NMR (D_2O, 300 MHz): δ 0.76 (s, 3H, Me); 0.90 (s, 3H); 2.46 (t, J = 6.6Hz, 2H); 2.73 (t, J = 6.1 Hz, 2H); 3.23 (t, J = 6.6 Hz, 2H); 3.36-3.60 (m, 13H); 3.88 (s, 1H); 4.03 (dd, J = 4.2Hz, J = 9.4 Hz, 1H); 4.26 (m, 2H); 4.61 (m, 1H); 4.86 (m, 2H); 6.16 (d, J = 5.6 Hz, 1H); 7.47 (m, 5H); 8.23 (s, 1H); 8.55 (s,1H); ^{13}C NMR (D_2O, 100 MHz) δ 174.6; 173.7; 172.6; 170.4; 155.1; 152.4; 149.0; 139.8;133.8; 130.5; 128.7; 126.6; 118.3; 86.3; 83.4; 74.0; 73.8; 73.7; 71.7; 65.3; 46.9; 45.4; 42.2; 41.9; 38.2; 35.4; 35.3; 32.5; 31.0; 20.7; 18.1; LRMS (ESI, m/z) calc. for $C_{34}H_{50}N_9O_{18}P_3S$: 997.2. Found: 996.8 $(M-H)^-$.

***N*-[2-(S-coenzyme A)acetyl]-benzylamine (13).** Yield: 81%; ^{1}H NMR (D_2O, 300 MHz): δ 0.72 (s, 3H); 0.86 (s, 3H); 2.39 (t, J = 6.3 Hz, 2H); 2.62 (t, J = 6.6 Hz, 2H); 3.28 (m, 4H); 3.41 (t, J = 6.9 Hz, 2H); 3.53 (m, 1H); 3.81 (m, 1H); 3.99 (s, 1H); 4.22 (s broad, 2H); 4.33 (s, 2H); 4.57 (s, 1H); 4.78 (m, 2H); 6.12 (d, J = 6.3 Hz, 1H); 7.25 (m, 5H); 8.17 (s, 1H); 8.50 (s, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 18.1; 20.8; 31.2; 34.8; 35.3; 35.4; 38.3; 43.3; 65.5; 71.8; 73.8; 73.9; 74.1; 83.7; 86.4; 127.3; 127.4; 128.7; 137.6; 152.8; 155.5; 172.5; 173.8; 174.7. LRMS (ESI, m/z): calc. for $C_{30}H_{45}N_8O_{17}P_3S$: 914.2. Found: 913.3 $(M-H)^-$.

Procedure for Boc removal

The protected compound was dissolved in a solution of TFA in CH_2Cl_2 (v/v 50/50). After stirring for 10 min, TFA was removed under vacuum. Then, the residue was dissolved in H_2O (10 mL) to be lyophilized. Purification by reverse-phase HPLC (TFA 0.05%/CH_3CN 95/5) and subsequent lyophilization afforded the desired product.

***N*-[2-(S-coenzyme A)acetyl]propane-1,3-diamine (7).** Yield: 38%; ^{1}H NMR (D_2O, 300 MHz) δ 0.86 (s, 3H); 0.97 (s, 3H); 1.91 (m, 2H); 2.49 (t, J = 6.4 Hz, 2H); 2.71 (t, J = 6.8 Hz, 2H); 3.03 (t, J = 7.7 Hz, 2H); 3.31 (s, 2H); 3.37 (m, 4H); 3.51 (t, J = 6.3Hz, 2H); 3.65 (dd, J = 4.4Hz, J = 10.6 Hz, 1H); 3.89 (dd, J = 5.0 Hz, J = 9.8 Hz, 1H); 4.05 (s, 1H); 4.30 (m, 2H); 4.63 (m, 1H); 4.90 (m, 2H); 6.24 (d, 1H, J = 6.7 Hz); 8.46 (s, 1H); 8.68 (s,1H); ^{13}C NMR (D_2O, 100 MHz) δ 174.8; 174.1; 173.1; 150.0; 145.4; 142.8; 118.6; 87.8; 83.4; 74.4; 74.1; 74.0; 72.3; 65.3; 38.5; 37.2; 36.7; 35.6; 35.5; 35.0; 31.4; 26.8; 21.0; 18.6; LRMS (ESI, m/z) calc. for $C_{26}H_{46}N_9O_{17}P_3S$: 881.2. Found: 880.4 $(M-H)^-$.

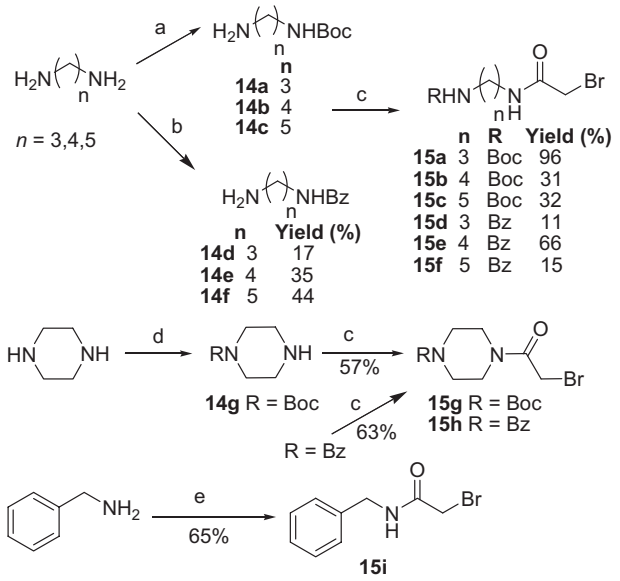
***N*-[2-(S-coenzyme A)acetyl]propane-1,3-propanamine (8).** Yield: 41%; ^{1}H NMR (D_2O, 300 MHz) δ 0.84 (s, 3H); 0.95 (s, 3H); 1.63 (m, 4H); 2.48 (t, J = 6.3 Hz, 2H); 2.68 (t, J = 6.6 Hz, 2H); 3.01 (t, J = 7.2 Hz, 2H); 3.24 (t, J = 6.7 Hz, 2H); 3.27 (s, 2H); 3.36 (t, J = 6.6 Hz, 2H); 3.49 (t, J = 6.3 Hz, 2H); 3.64 (m, 1H); 3.90 (m, 1H); 4.03 (s, 1H); 4.29 (m, 2H); 4.61 (m, 1H); 4.88 (m, 2H); 6.21 (d, J = 5.3 Hz, 1H); 8.44 (s, 1H); 8.67 (s,1H); ^{13}C NMR (D_2O, 100 MHz) δ 174.9; 174.2; 172.8; 150.1; 144.8; 143.0; 87.7; 83.3; 74.52; 74.5; 74.4; 74.2; 72.2; 65.3; 39.3; 39.2; 38.6; 35.7; 35.6; 35.1; 31.4; 25.6; 24.4; 21.1; 18.6; LRMS (ESI, m/z) calc. for $C_{27}H_{48}N_9O_{17}P_3S$: 895.2. Found: 894.5 $(M-H)^-$.

***N*-[2-(S-coenzyme A)acetyl]propane-1,3-butanamine (9).** Yield: 23%; ^{1}H NMR (D_2O, 300 MHz) δ 0.85 (s, 3H); 0.96 (s, 3H); 1.39 (m, 2H); 1.55 (m, 2H); 1.67 (m, 2H); 2.49 (t, J = 6.3 Hz, 2H); 2.69 (t, J = 6.7 Hz, 2H); 2.99 (t, J = 7.6 Hz, 2H); 3.21 (t, J = 6.9 Hz, 2H); 3.26 (s, 2H); 3.36 (t, J = 6.6 Hz, 2H); 3.49 (t, 2H, J = 6.3 Hz); 3.65 (m, 1H); 3.90 (m, 1H); 4.04 (s, 1H); 4.30 (m, 2H); 4.62 (m, 1H); 4.90 (m, 2H); 6.21 (d, 1H, J = 5.4Hz); 8.44 (s, 1H); 8.67 (s, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 174.9; 174.2; 172.6; 150.0; 87.7; 83.5; 74.5; 74.4; 74.1; 72.2; 65.2; 39.6; 39.5; 38.6; 35.7; 35.6; 35.1; 31.4; 28.0; 26.6; 23.2; 21.1; 18.6; LRMS (ESI, m/z) calc. for $C_{28}H_{50}N_9O_{17}P_3S$: 909.2. Found: 908.5 $(M-H)^-$.

***N*-[2-(S-coenzyme A)acetyl]piperazine (10).** Yield: 34%; ^{1}H NMR (D_2O, 300 MHz) δ 0.83 (s, 3H); 0.94 (s, 3H); 2.50 (m, 2H); 2.74 (m, 2H); 3.29-3.44 (m, 8H); 3.48-3.59 (m, 4H); 3.65 (m, 1H) 3.87 (m, 3H); 4.04 (s, 1H); 4.30 (m, 2H); 4.62 (m, 2H); 4.89 (m, 2H); 6.23 (d, 1H, J = 5.2 Hz); 8.46 (s, 1H); 8.68 (s, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 174.7; 174.0; 170.5; 149.8; 144.4; 142.3; 87.5; 82.8; 74.3; 74.2; 74.1; 72.0; 64.9; 43.1; 42.9; 38.9; 38.2; 35.5; 35.4; 32.5; 31.1; 20.9; 18.3; LRMS (ESI, m/z) calc. for $C_{27}H_{46}N_9O_{17}P_3S$: 892.2. Found: 892.4 $(M-H)^-$.

Biological assays

The assays were carried out in the presence of radioactive acetyl-CoA as previously reported in reference (26). p300 enzyme was obtained from Biomol. International. Peptide H4-8 was obtained from Millegen. IC_{50} values were measured in the presence of 2 μM



Scheme 1: Reagents and conditions: (a) see reference (28) (b) PhCOCl 0.2 eq, DCM, −78 °C then rt, 15 h for compound **14d**; $PhCONH_2$ 0.33 eq, reflux, 15 h for compounds **14e** and **14f**; (c) $BrCOCH_2Br$ 0.5 eq, DCM, −10 °C; (d) see reference (29) (e) $BrCOCH_2Br$ 1 eq, Et_3N 1.1 eq, DMAP 0.1 eq, $CHCl_3$, −10 °C.

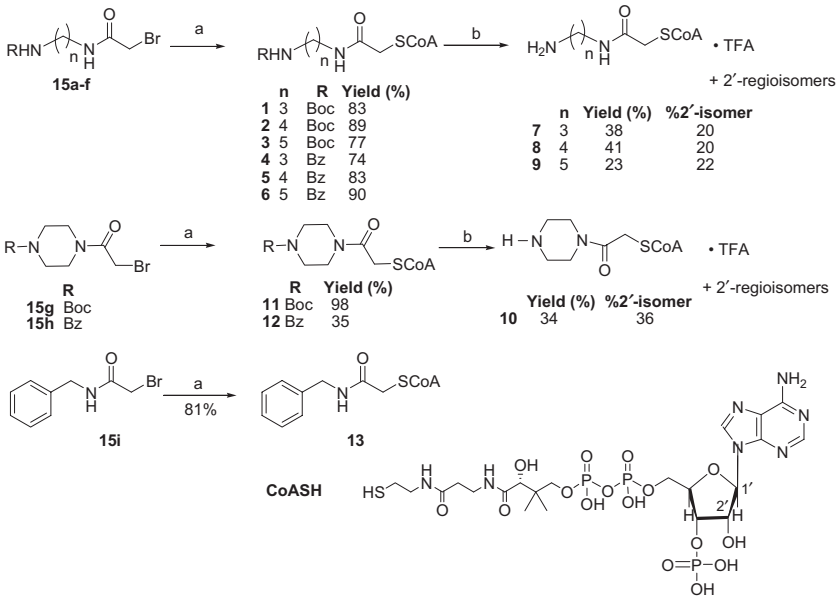
[^{3}H]-acetyl coenzyme A, 267 μM of H4-8 peptide and 296 nM of p300 (1284–1673) in 30 μL IPH buffer.

Results and Discussion

The synthesis of bisubstrate derivatives begins with the synthesis of bromocarboxamide compounds. Boc-monoprotected amines were prepared from a standard procedure (27,28). The monobenzoylated amine **14d** was synthesized in a modest yield with benzoyl chloride at −78 °C (Scheme 1). To increase the yield compounds, **14e–f** were prepared by transamidation (29). The coupling of the monoprotected compounds **14a–g** and commercially available benzoylpiperazine with bromoacetyl bromide was performed by slow addition at −10 °C and led to bromocarboxamide compounds **15a–h**. The reaction between benzylamine and bromoacetylbromide in the presence of triethylamine and catalytic 4-dimethylaminopyridine gives the expected compound **15i** in a similar yield.

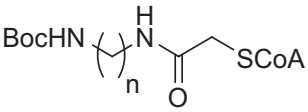
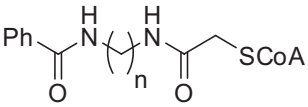
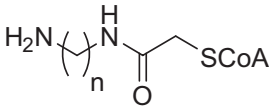
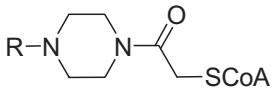
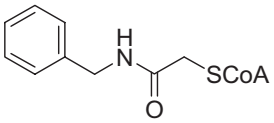
Then, bisubstrate inhibitors were prepared by coupling bromoacetyl compounds with CoASH in aqueous solution at pH 8 as described in Scheme 2. Thus, the capped CoA derivatives (compounds **1–6** and **11–13**) were obtained in 80–90% yields. To explore the influence of the free-amine in the p300 HAT binding site, we decided to cleave the Boc protecting group by TFA. After purification by HPLC, ^{1}H NMR data of isolated compounds **7–10** revealed a mixture of desired compounds with 2′-regioisomers (iso-CoA derivatives). Indeed, it is known that strongly acidic conditions lead to the migration of the 3′-phosphate group to the 2′-position (30,31). The percentage of 2′-regioisomers was evaluated by ^{1}H NMR on proton H1′ and ranges from 20 to 36%. HPLC separation of this mixture was found to be awkward, and to date we do not succeed to separate both regioisomers. Although Cole and coll. have shown than deletion of the 3′-phosphate led to an approximately 40-fold decreased inhibition (25), we decided to include these four compounds in enzymatic tests to get insight into the free-amine influence on p300 HAT.

The overall thirteen CoA derivatives were tested for their ability to act as inhibitors of recombinant p300 enzyme (1284–1660) and were compared with the well-known Lys-CoA (25). As shown in Table 1, the effect of the chain length in the N-Boc series is



Scheme 2: Reagents and conditions: (a) CoASH lithium salt 0.5 eq, NaOH 1 M, pH = 8, THF, overnight; (b) TFA 50% in DCM starting from compounds **1**, **2**, **3** or **11**.

Table 1: In vitro inhibitory activity against p300 (1284–1673) of bisubstrate-type inhibitors

Structure	Compound	n or R	p300 IC_{50} (μM)
	**1**	n = 3	3.6 ± 1.5
	2	n = 4	0.4 ± 0.1
	3	n = 5	0.07 ± 0.02
	**4**	n = 3	0.7 ± 0.1
	5	n = 4	0.80 ± 0.07
	6	n = 5	0.30 ± 0.07
	**7**	n = 3	1.6 ± 0.4
	8	n = 4	0.20 ± 0.08
	9	n = 5	0.3 ± 0.1
	**10**	R = H	20 ± 4
	11	R = Boc	152 ± 28
	12	R = Bz	28 ± 8
	**13**	None	209 ± 51
Lys-CoA			0.5 ± 0.2

obvious. In fact, the compound **3** bearing the longest chain (n = 5) is the most efficient, even better than Lys-CoA. Based on previous studies of Cole and coll., the affinity displayed for the compound **3** could be attributed to the Boc group positioned in the pocket 2 (23). In the cases of N-benzoyl and free-amine series, no strong dependencies were displayed between chain length and inhibitory ability. To evaluate the impact of the chain on p300 binding site, a more constrained compound, piperazine-CoA **10**, was tested. However, in the light of the high IC_{50}, compound **10** is not well recognized by p300 HAT. This result could be explained by the steric hindrance of the ring into the groove. Moreover, a decrease in the inhibitory potency was also observed by adding more hindrance with Boc- or benzoyl-protected piperazine (compound **11** and **12**). In the same way, poor inhibitory ability was found with aromatic compound **13**.

To better understand the recognition of inhibitors at the molecular level, preliminary docking studies (see Appendix S1) were carried on these inhibitors using the PDB structure 3BIY (23,32). All candidates bind like bisubstrate-type compounds but show poor correlation between inhibitory activities and the different docking scores used (Moldock, Plants, X-Score) (32–34). Nevertheless, analysis of binding modes reveals first results related to the second cavity P2. As it was hypothesized earlier, in the case of best compound **3** (R = Boc, n = 5), the chain length is sufficient to locate the Boc moiety in P2 (Figure 2). Shorter chain lengths (n = 3 or 4) could not permit the occupation of this cavity. The analysis of score components such as hydrophobic and electrostatic contributions did not give any clear affinity scale related to the electronegative groove or P2 pocket occupation. However, these new results show that chain length can have direct influences on the inhibitory activity.

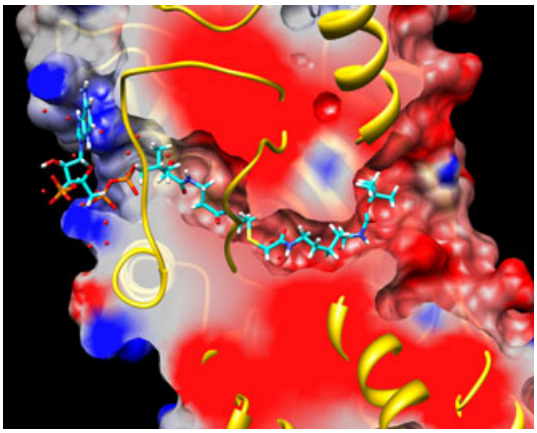


Figure 2: Binding mode of best compound. Model clipping of PDB structure 3BIY with continuum electrostatics coloring of molecular surface, and compound **3** after docking (35,36). The figure shows occupancy of cavities P1 and P2 by long chain (n = 5) and Boc moieties, respectively, at right of the (yellow) overlapping loop (upper clipping plane).

Conclusion and Future Directions

In this paper, we report the synthesis of new series of bisubstrate inhibitors. In the N-Boc series, we showed that the compound **3** bearing a 5-C chain length and a Boc moiety is well recognized in the p300 binding site. Its efficiency can be explained by its capacity to reach the pocket 2 discovered by Cole and coll. These results will serve as starting point for further modifications in p300 inhibitors, and studies are currently in progress in the laboratory to design and prepare new analog inhibitors of compound **3**. In future experiments, rational drug design strategies using fragment approaches, directed toward cavities P1 or P2, with or without combination of CoA moiety, will also be investigated.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Experimental procedures.

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