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Comparative Studies of Thiol-Sensitive Fluorogenic Probes for HAT Assays

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Abstract

Histone acetyltransferases (HATs) catalyze the acetylation of specific lysine residues in histone and nonhistone proteins. Recent studies showed that acetylation is widely distributed among cellular proteins, suggestive of diverse functions of HATs in cellular pathways. Nevertheless, currently available assays for HAT activity study are still quite limited. Here we evaluated a series of thiol-sensitive fluorogenic compounds for the detection of the enzymatic activities of different HAT proteins. Upon conjugation to the thiol group of HSCoA, these molecules gain enhanced quantum yields and strong fluorescence, permitting facile quantitation of HAT activities. We investigated and compared the assay performances of these fluorogenic compounds for their capability as HAT activity reporters, including kinetics of reaction with HSCoA, influence on HAT activity, and fluorescence amplification factors. Our data suggest that CPM and CME are excellent HAT probes owing to their fast reaction kinetics and dramatic fluorescence enhancement during the HAT reaction. Further, the microtiter plate measurements show that this fluorescent approach is robust and well suited for adaption to high throughput screening of small molecule inhibitors of HATs, highlighting the value of this assay strategy in new drug discovery.

Keywords

histone acetyltransferase; HAT; fluorescent probe; epigenetics; chromatin modification

Introduction

Protein acetylation is recognized as a key post-translational modification mark regulating diverse biological cascades such as transcriptional regulation and signal transduction. Reversible acetylation and deacetylation of cellular proteins are mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs catalyze the transfer of the acetyl group from acetyl-coenzyme A (Ac-CoA) to the ϵ -amino group of lysine residues in a histone or non-histone protein substrate, resulting in acetylated lysine and HSCoA. HATs are grouped into four major families: the GNAT (GCN5-related N-acetyltransferase), represented by GCN5 (general control nonderepressible 5) and PCAF (p300/CBP associated factor), the p300/CBP family, including p300 and CBP (CREB binding protein), the MYST family that includes MOZ, Ybf2/Sas3, Sas2, and Tip60, and RTT109 [1]. On the chromatin template, lysine acetylation loosens the nucleosome structure and promotes the accessibility of transcription factors to local and global genetic loci [2, 3]. Importantly, increasing reports show that protein acetylation affects a variety of chromatin-unrelated cellular processes, such as signal transduction by mediating protein-protein interactions [4]. Moreover, a large body of evidence reveals that HAT activities are deregulated in many diseased states [5, 6]. For example, expression of p300/CBP decreases during chemical hepatocarcinogenesis and

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mutations in *p300/CBP* are associated with different cancers and other diseases [7]. In acute myeloid leukemia, the *CBP* gene is translocated and fused to either *MOZ* and *MORF* or to *MLL* gene [8]. The expression level of the MYST member Tip60 is upregulated in hormone-resistant prostate cancer [9]. Further, the NF- κ B proteins are acetylated and the acetylation regulates their signaling function in many diseased condition such as inflammation and cancer [10]. Such multifold evidence underscores the importance of HATs as potential drug targets. Indeed, a number of research efforts in recent years have been devoted to designing and screening chemical HAT regulators [11–13].

Given the significance of acetylation in normal biology and pathology, effective biochemical assays are required to detect and analyze HAT activities both in vitro and in vivo [14–17]. For use in inhibitor screening, appropriate assays are required to combine sensitivity, speed, simplicity and cost-effectiveness in order to be suited for rapid and high throughput screening [18, 19]. The classic radioactive assay relying on [3 H]- or [14 C]-labeled Ac-CoA takes a leading role in studying HAT activities and is particularly useful for kinetic characterization of HATs in which the degree of throughput is usually a limiting factor [14, 17, 20, 21]. Radioisotopic assays suffer from issues of high cost of radioactive materials, environmental safety of radioactivity handling, and experimental inflexibility (i.e. end point assay) [14]. Also the standard radioactive method is not well suited for automation and high-throughput screening (HTS) application. Therefore, nonradioactive strategies are highly required for HAT enzymatic studies and for HAT inhibitor screening. Spectrophotometric methods are of great utility in biochemical assay design owing to their simplicity and sensitivity. For the analysis of acetyltransferase activity, one attractive strategy is to use spectrophotometric probes for quantification of the side product HSCoA [22–24]. The HAT reaction has been directly monitored with thiol reactive chemicals such as CPM [23] and DTNB [24] (Figure 1). Because such spectrophotometric analyses of HAT activity involve additional chemical components than a pure radioactive HAT assay, extra complexity needs to be clarified. In particular, critical questions remain to be addressed about reaction kinetics with HSCoA, side effect of the probes to HAT enzymes, and applicability for HTS implementation. We are particularly interested in detecting HAT activities by using fluorescent probes because fluorescence measurement is rapid, sensitive, potentially continuous, and does not involve dangerous radioactive chemicals. We recently developed a single-step assay for direct readout of acetyltransferase activity via fluorescent sensing [25] and designed a FRET approach that is particularly suited for studying HAT-substrate interactions. In this paper, we investigated and compared chemical reactivity, kinetics, and spectrometric properties of a series of fluorogenic molecules (Figure 2) for quantitative analysis of HAT activities. Our data demonstrate that CPM and CME are particularly effective for HAT assays owing to their fast kinetics and strong fluorogenicity. Further, we find that this fluorescent method is amenable to minimization and automation, thereby well suited for screening and characterizing HAT inhibitors in the HTS format. Together, this work provides insightful information about applying fluorescent probes to study HAT activities and screen chemical modulators of HATs.

MATERIALS AND METHODS

Materials

All reagents were of the highest grade commercially available. Seven commonly used thiol-sensitive probes A433, D10251, CPM, D10253, M1378, P28, B-8 were selected and ordered from Invitrogen. The compound CME (coumarin maleic acid ester, 3-(7-hydroxy-2-oxo-2H-chromen-3-ylcarbamoyl)-acrylic acid methyl ester) was synthesized as previously reported [26]. Radioactive [14 C]Ac-CoA was purchased from Perkin Elmer and regular nonradioactive Ac-CoA was purchased from Sigma. Ac-CoA often contains low amounts of HSCoA, which could react with the thiol-labile probes and cause high levels of background

fluorescence. To circumvent this problem, 3 mg of Ac-CoA was treated with 10 μ L of acetic anhydride for 10 min at room temperature and then quenched with 1 mL of HEPES buffer (100 mM, pH 8.0). The enriched Ac-CoA was then frozen in aliquots and stored at -80°C [23].

Peptide synthesis

Peptides were synthesized on the Fmoc-based solid phase peptide synthesis (SPPS) protocols and purified with C-18 reversed phase HPLC (using a gradient of $\text{H}_2\text{O}:\text{CH}_3\text{CN}$ containing 0.05% trifluoroacetic acid) and confirmed with MALDI-MS as previously described [27]. The sequence of the NH_2 -terminal 20 aa peptide of histone H3, i.e. H3-20, is Ac-ARTKQTARKSTGGKAPRKQL and the sequence of the NH_2 -terminal 20 aa peptide of histone H4, i.e., H4-20, is Ac-SGRGKGGKGLGKGGAKRHRK.

Protein expression

His-tagged PCAF HAT domain (493–658) was expressed with the pET28a vector. His-tagged Tip60 was expressed with the pET21a vector. Recombinant p300 protein was a gift from Dr. Philip Cole. All the protein expression was carried out with *E. coli* BL21(DE3). The His-tagged proteins were purified on Ni-NTA beads. Protein concentrations were determined using the Bradford assay. Proteins were flash frozen in a storage buffer containing 50 mM HEPES, 500 mM NaCl, 1 mM EDTA and 10 % glycerol and stored at -80°C .

Fluorescent assays of HAT probes

The HAT reactions were carried out at 30°C in 50 mM of HEPES buffer (pH 8.0) containing 0.5 mM EDTA (i.e. 1xRB). For PCAF and p300 assay, the final concentrations of each species were maintained as: 20 nM enzyme, 100 μM peptide, 10 μM Ac-CoA, and 50 μM probe. For Tip60 HAT assay, the final concentrations of each species were: 20 nM Tip60, 200 μM peptide, 20 μM Ac-CoA, and 50 μM probe. In a typical procedure, an assay solution was prepared containing the appropriate concentrations of each species in an aliquot. The tube was incubated at 30°C for 5 min and then enzyme was added to initiate the reaction. The final assay volumes were 150 μL (for PCAF and p300 assay) and 200 μL (for Tip60 assay). Following the HAT reaction, each reaction was quenched by rapid freezing. The appropriate volumes of probes in DMSO were added to each reaction sample such that 10% DMSO was present in the final assay solution. Fluorescence emission was monitored on a FluoroMax-4 fluorimeter. The maximum absorption wavelength of the respective probes was selected as the excitation wavelength.

HAT inhibition by Lys-CoA

The HAT reactions were carried out at 30°C in 1xRB. An assay cocktail was prepared with the appropriate concentrations of Lys-CoA, H4-20 (for Tip60) or H3-20 (for PCAF), and Ac-CoA in small aliquots. The tube was kept at 30°C for 5 min, and then the respective enzyme was added to initiate the reaction with a final volume of 144 μL . After 8 min, the HAT reaction was quickly quenched by rapid freezing, and then each aliquot was mixed with 16 μL of 50 μM CPM in DMSO and kept in darkness for 8 min. The fluorescent emission was monitored on a FluoroMax-4 fluorimeter. The final concentrations of Ac-CoA and peptide substrate were 10 μM and 200 μM respectively. Concentrations of PCAF and Tip60 were 10 nM and 50 nM. The concentration of Lys-CoA ranged from 0 to 800 μM . 5 μM of CPM was used for HAT activity quantitation. Each assay was carried out in duplicate.

Microplate assay for Z and Z' value

Microplate assays for the HAT study were conducted in 384-well microplates (MaxiSorp, Non Sterile PS) on a Perkin Elmer 1420 Multilabel Counter. An assay cocktail was prepared with the appropriate concentrations of Lys-CoA (only for inhibitor reactions), H4-20 peptide and Ac-CoA in a 2 mL tube, and divided into microplate wells with a 12-channel pipette. The plate was kept at room temperature for 5 min, and then Tip60 was added to initiate the reaction with a final assay volume of 40 μ L (for negative control 1X reaction buffer was used instead of Tip60). After 10 min of reaction, each well was mixed with 2 μ L of 1 mM CPM in DMSO and kept in darkness for 2 min. Perkin Elmer 1420 Multilabel Counter was used to read out the fluorescence with excitation and emission filters of 380 and 460 nm, respectively. The final concentrations of Ac-CoA, peptide substrate and Tip60 were 10 μ M, 200 μ M and 50 nM respectively. For the inhibitor reaction, the concentration of Lys-CoA was 200 μ M and 25 μ L CPM was used for activity quantitation.

Results and Discussion

The reaction kinetics of the fluorogenic compounds with HSCoA

Thiol-labile fluorogenic compounds are of great value in detecting acetyltransferase activities through their ability to reacting with HSCoA, the side product of acetylation reaction. The reactivity and reaction kinetics of these fluorogenic compounds with HSCoA will be a critical determinant for their suitability in HAT activity detection. To systematically evaluate the advantage of HAT activity analysis with fluorogenic probes, we screened several of commonly used thiol-sensitive fluorogenic compounds that are available from the Molecular Probes (Figure 2). Yi et al recently reported a coumarin-maleic acid ester molecule, CME, having superior thiol detection properties such as fast reaction rate, nanomolar sensitivity and large signal-to-noise ratio [26]. We thus synthesized and tested this compound as well. Most of these fluorogenic probes react with HSCoA through thiol addition to the double bond of the α , β -unsaturated carbonyl groups forming a thioether linkage, which thereby affects the photophysics of the chromophore (Electronic supplementary material Figure S1). We first examined the fluorescence response of each probe reacting with HSCoA. In the test, 5 μ M of a probe was rapidly mixed with 10 μ M of HSCoA in 1xRB with 10% DMSO present in the reaction mixture in order to completely dissolve the organic probes. Maximum absorbance wavelength (λ_{abs}) of each compound was determined by measuring their absorption spectra and was chosen as the excitation wavelength for the fluorescence experiments. The fluorescence emission spectra for each probe were collected from which the maximum emission wavelength (λ_{em}) was determined. As expected, the fluorescence intensity of each probe increased after reaction with HSCoA (Figure 3). Such a fluorescence enhancement, i.e. the signal-to-background (S/B) ratio, is one important factor determining the quality of these probes for HSCoA detection. The measured S/B ratios for each probe were summarized in Table 1. Three compounds, CME, CPM, and D10251, exhibited the strongest S/B ratios, with greater than 250-fold fluorescence enhancements after reacting with HSCoA. Four other compounds, A433, B-8, D10253 and P28 showed fluorescence amplification in the medium range (17—54 folds). M1378 showed the weakest S/N ratio, only 2.4-fold of fluorescence enhancement after reaction with HSCoA.

In addition to S/B ratios, kinetics of the reaction between each probe and HSCoA is another important factor determining the efficacy of the probes for HAT assay. The probe—HSCoA reaction time course was monitored by recording the fluorescence intensity at the λ_{em} as a function of reaction time. As summarized in Figure 4 and Table 1, compounds CME, CPM, D10251, and D10253 reacted with HSCoA with a half time ($t_{1/2}$) of less than 31 seconds.

Such fast reaction kinetics is of great importance for HAT assay because it assures that the coupling step does not pose a rate limitation for acetyltransferase activity detection. The reaction rates of the other compounds are appreciably slower ($t_{1/2} > 96$ seconds) under the same condition. Taking into account the requirement of both fast reaction kinetics and large S/B ratios, we conclude that three compounds, CME, CPM, and D10251 are particularly amenable for HSCoA detection, suggesting their application as fluorescent probes for acetyltransferase activity detection. In our experiments, we also tested the detection limit of the fluorescent assays with CPM. HSCoA was detected at least down to $0.17 \mu\text{M}$ when CPM was employed at $5 \mu\text{M}$ in the buffer solution, which leads to a 50% increase in fluorescence intensity (data not shown).

Application of the fluorogenic probes for PCAF activity measurements

Having determined the reaction kinetics and fluorescent emission properties of each probe reacting with HSCoA, we then applied these probes to study HAT reaction with the purpose of examining the efficiency of these probes for HAT activity detection. Three HAT enzymes, PCAF, p300 and Tip60, are particularly examined. These enzymes not only are representative members of the major HAT proteins in mammalian cells, but they are also causative factors for many human diseases and are important biological targets for new drug discovery [28–30]. For the PCAF assay, a histone H3 peptide containing the N-terminal 20 AA (H3-20) was synthesized and used as the substrate. In a typical procedure, the peptide substrate was incubated first with Ac-CoA, and then the acetyltransferase reaction was initiated by addition of HAT protein. After 5 min of reaction, $50 \mu\text{M}$ of each probe (final concentration) was added to the HAT reaction and the fluorescence emission at the λ_{em} was recorded as a function of time. All the reactions were carried out at 30°C . Because a potential pitfall is that some cysteine residues at the protein surface may react randomly with Ac-CoA to produce HSCoA, thus causing false-positive signals during the reaction, we also carried out the assay without PCAF or without Ac-CoA under the same experimental condition as negative controls to evaluate the possible interference of cysteine—HSCoA reaction. Figure 5 shows the fluorescence progression curve of each probe reacting with the PCAF reaction mixture. From the results, it is seen that CPM gave 40-fold of fluorescence enhancement after reacting with the PCAF mixture as compared with the blank controls. D10251 also showed high fluorescence sensitivity, with 20-fold of emission increase. CME showed 12-fold increase. All these three compounds showed fast kinetic response, with the plateau reached within 3 min of reaction time. P28, B-8 and D10253 showed moderate intensity increase (7.5, 6.4, and 3.5-fold, respectively). A433 showed the slowest reaction rates, consistent with the result of its reaction with HSCoA (Figure 4). M1378 showed almost no response to the PCAF reaction and could not distinguish between the HAT reaction and the two blank controls.

Application of the fluorogenic probes for p300 activity measurements

p300 is a member of the CBP/p300 HAT subfamily playing important roles in regulating gene transcription and many other biological processes [31, 32]. We tested the eight probes for detecting the activity of p300. In the assay, a histone H4 peptide containing the N-terminal 20 AA sequence, i.e. H4-20, was used as the substrate. The experimental procedure was essentially the same as the PCAF assay, with the HAT reaction proceeding for 5 min prior to the addition of the probe ($50 \mu\text{M}$ final). Figure 6 displays the changes of the fluorescence intensity of the probes as a function of time after its mixing with the p300 reaction solution. Among the eight probes, CME and CPM and D10251 are the three best compounds which gave more than 7-fold increase compared to the negative controls. Four other compounds, A433, B-8, D10253 and P28, showed modest fluorescence enhancement (2–5 folds). Similar as that in the PCAF HAT assay, M1378 exhibited very little fluorescence increase following its reaction with the acetylation mixture.

Application of the fluorogenic probes for Tip60 activity measurements

We also studied the use of the eight fluorogenic compounds for probing the enzymatic activity of Tip60, a representative member of the MYST family HATs. A full reaction solution contained 50 nM Tip60, 200 μ M H4-20, 20 μ M Ac-CoA, and 50 μ M probe. The fluorescence progression data were shown in Figure 7. Overall, fluorescent signals continued to increase over a longer period of time (>5 min), likely suggesting that Tip60 activity was weaker and acetylation reaction was still ongoing after the addition of the probes. CPM showed better performance compared to the other probes, with a 7.5-fold fluorescence enhancement and the fluorescence signal is more stable than the others. CME showed 5.4-fold signal increase at 5 min of reaction. However, with increasing incubation time, the background level increased as well, which compromised the signal-to-background ratio. The fluorescence intensity of several other probes, A433, B-8, D10251, D10253 and P28, increased by 1.7—3.6 folds after reacting with the HAT mixture. Again, M1378 exhibited the worst performance, with almost no fluorescence enhancement observed.

Impact of the organic probes on the activity of the HATs

When coupling fluorogenic probes with a HAT reaction to gain fluorescent readout of the acetyltransferase activity, it might be possible that these extra compounds affect the intrinsic activity of the tested HATs. Especially, these thiol-labile compounds can potentially react with cysteine residues that are essential for enzymatic activities. To evaluate possible toxic effects of these fluorogenic probes on HAT activity, we carried out histone acetylation in the presence of these probes using the classic radioisotope-labeled assay. [14 C]acetyl-CoA was used as the acetyl donor and H4-20 or H3-20 was used as the substrate. The HAT activity of PCAF, p300 and Tip60 was measured in the presence and absence of 50 μ M individual probes to quantitatively evaluate their interfering effect. As shown in Figure 8, different compounds have varying effects on the activity of the three HATs. Tip60 retained more than 60% of its activity in the presence of each compound. Strikingly, the tested compounds showed more dramatic effect on activities of PCAF and p300. In particular, p300 lost more than 90% of its activity in the presence of the tested probes. Several compounds (e.g. CPM and P28) also considerably inhibited the activity of PCAF. Future in-depth study is needed to elucidate the inhibitory mechanisms of these compounds. The data presented here demonstrate that, to apply fluorogenic compounds for HAT assay, caution should be taken about the potential detrimental effect of the compounds on the intrinsic activity of the enzyme. For those probes that substantially interfere with HAT activity, it is necessary that their addition should take place at a later stage when the HAT reaction is over, namely in the format of end point assays.

Evaluation of the fluorescent assay for HAT inhibitor characterization and screening

One important application of the described fluorescent assay is for HAT inhibitor characterization and screening. Given the paucity of available HAT inhibitors for therapeutic investigation, robust fluorescent HAT assays with high-content screen capability in the microtiter plate platform are of great power in drug discovery. Because CPM showed fast reaction kinetics and marked signal enhancement, we used it as a example to demonstrate the use of fluorogenic probes in studying HAT inhibitors. First, we tested the efficacy of this method in HAT inhibitor characterization, e.g. determining the potency parameter IC_{50} . Activities of PCAF and Tip60 were examined at a range of concentrations of Lys-CoA, a previously reported HAT inhibitor [33, 34]. For each HAT reaction, CPM was added (5 μ M final) at 8 min of the HAT reaction. The fluorescent signals were measured on FluoroMax fluorometer. As shown in Figure 9, fluorescent signals of both the PCAF reaction mixture and the Tip60 reaction mixture declined at increasing concentrations of Lys-CoA, demonstrating concentration-dependent inhibition by the inhibitor. The IC_{50}

values were determined from these inhibition data by fitting to equation $y=1/(1+x/IC_{50})$, resulting 96 μM for PCAF and 26 μM for Tip60. These data are within one-fold range with that obtained from the standard radioactive HAT assays (i.e., 108 μM for PCAF and 30 μM for Tip60) [33].

A particular advantage of using fluorogenic probes for HAT activity measurement is no requirement of post-reaction separation steps, which makes it an attractive approach for high-content screen of chemical modulators of HATs. We tested the suitability of the fluorescent assay for HTS by measuring the Z and Z' factors, which are statistical parameters for the evaluation of the qualification and robustness of HTS assays [35]. Three sets of samples were prepared. The first one was for the positive control experiment that contained 50 nM Tip60, 10 μM Ac-CoA; 200 μM H4-20, and 25 μM CPM; the second set was for the negative control that contained 10 μM Ac-CoA; 200 μM H4-20, and 25 μM CPM; and the third set was for the reaction mixture with the inhibitor Lys-CoA, containing 50 nM Tip60, 10 μM Ac-CoA; 200 μM H4-20, 200 μM Lys-CoA, and 25 μM CPM. The reactions were conducted in 384-well microtiter plates and the fluorescence signals were measured in a Perkin Elmer 1420 Multilabel Counter. Ten data points were collected for each set of samples (Figure 10). The Z and Z' values were calculated according to eq. 1 and eq. 2 [35]:

$$Z=1-(3SD_s+3SD_c)/(|\text{means}_s-\text{means}_c|) \quad (1)$$

$$Z'=1-(3SD_{c+}+3SD_{c-})/(|\text{means}_{c+}-\text{means}_{c-}|) \quad (2)$$

SD_s , SD_{c+} , SD_{c-} denote the standard deviations of the inhibitor sample signals, positive control signals and negative control signals. means_s , mean_{c+} , and mean_{c-} stand for the means of the inhibitor mixture signals, positive control signals and negative control signals. Calculation from the sampling data offers a Z value of 0.61 and Z' value of 0.71. Typically, a qualified and robust assay has Z and Z' values higher than 0.5. Therefore, the high Z and Z' values from our assay validates the robustness of the fluorescent method for HAT inhibitor screening in the HTS format.

Conclusions

HATs are important post-translational modifying enzymes that catalyze acetylation of specific lysine residues in histone and nonhistone substrates. They participate in multiple cellular processes such as transcriptional regulation and signal transduction. Aberrant expression of HATs has been observed in various disease states, especially cancer. However, current strategies for studying HAT enzymatic activity and inhibitor screening are quite limited. We investigated in detail the use of common thiol-sensitive fluorogenic probes for detection of HAT activities. In particular, we characterized the reaction kinetics and fluorescent properties of each compound for detecting the activities of three major human HAT proteins, p300, PCAF, and MYST HAT. Our data show that several probes, especially CPM and CME, are particularly suited for HAT activity detection owing to their excellent fluorescence amplification, fast reaction kinetics, and miniaturization and robustness for HTS application in the microplate format. The data presented here provides fundamental valuable information for selecting and applying fluorogenic probes for the detection of enzymatic activities of HATs and other acyl-CoA dependent enzymes such as homocitrate synthase, aminoglycoside N-acetyltransferases, and acyl-CoA thioesterases [36].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Vetting MW, LPSdC, Yu M, Hegde SS, Magnet S, Roderick SL, Blanchard JS. Structure and functions of the GNAT superfamily of acetyltransferases. *Arch Biochem Biophys*. 2005; 433:212–26. [PubMed: 15581578]
2. Sterner DE, Berger SL. Acetylation of histones and transcription-related factors. *Microbiol Mol Biol Rev*. 2000; 64:435–59. [PubMed: 10839822]
3. Roth SY, Denu JM, Allis CD. Histone acetyltransferases. *Annu Rev Biochem*. 2001; 70:81–120. [PubMed: 11395403]
4. Spange S, Wagner T, Heinzel T, Kramer OH. Acetylation of non-histone proteins modulates cellular signalling at multiple levels. *Int J Biochem Cell Biol*. 2009; 41:185–98. [PubMed: 18804549]
5. Yang XJ. The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. *Nucleic Acids Res*. 2004; 32:959–76. [PubMed: 14960713]
6. Heery DM, Fischer PM. Pharmacological targeting of lysine acetyltransferases in human disease: a progress report. *Drug Discov Today*. 2007; 12:88–99. [PubMed: 17198977]
7. Giles RH, Peters DJ, Breuning MH. Conjunction dysfunction: CBP/p300 in human disease. *Trends Genet*. 1998; 14:178–83. [PubMed: 9613201]
8. Rowley JD, Reshmi S, Sobulo O, Musvee T, Anastasi J, Raimondi S, Schneider NR, Barredo JC, Cantu ES, Schlegelberger B, Behm F, Doggett NA, Borrow J, Zeleznik-Le N. All patients with the T(11;16)(q23;p13. 3) that involves MLL and CBP have treatment-related hematologic disorders. *Blood*. 1997; 90:535–41. [PubMed: 9226152]
9. Halkidou K, Gnanapragasam VJ, Mehta PB, Logan IR, Brady ME, Cook S, Leung HY, Neal DE, Robson CN. Expression of Tip60, an androgen receptor coactivator, and its role in prostate cancer development. *Oncogene*. 2003; 22:2466–77. [PubMed: 12717424]
10. Ghizzoni M, Haisma HJ, Maarsingh H, Dekker FJ. Histone acetyltransferases are critical regulators in NF-kappaB mediated inflammation. *Drug Discov Today*. 2011; 16:504–11. [PubMed: 21477662]
11. Suzuki T, Miyata N. Epigenetic control using natural products and synthetic molecules. *Curr Med Chem*. 2006; 13:935–58. [PubMed: 16611076]
12. Biel M, Wascholowski V, Giannis A. Epigenetics--an epicenter of gene regulation: histones and histone-modifying enzymes. *Angew Chem Int Ed Engl*. 2005; 44:3186–216. [PubMed: 15898057]
13. Zheng YG, Wu J, Chen Z, Goodman M. Chemical regulation of epigenetic modifications: Opportunities for new cancer therapy. *Med Res Rev*. 2008; 28:645–687. [PubMed: 18271058]
14. Berndsen CE, Denu JM. Assays for mechanistic investigations of protein/histone acetyltransferases. *Methods*. 2005; 36:321–31. [PubMed: 16085424]
15. Marmorstein R. Biochemical and structural characterization of recombinant histone acetyltransferase proteins. *Methods Enzymol*. 2004; 376:106–19. [PubMed: 14975301]
16. Wynne Aherne G, Rowlands MG, Stimson L, Workman P. Assays for the identification and evaluation of histone acetyltransferase inhibitors. *Methods*. 2002; 26:245–53. [PubMed: 12054880]
17. Poveda A, Sendra R. An easy assay for histone acetyltransferase activity using a PhosphorImager. *Anal Biochem*. 2008; 383:296–300. [PubMed: 18805389]
18. Martinez ED, Dull AB, Beutler JA, Hager GL. High-content fluorescence-based screening for epigenetic modulators. *Methods Enzymol*. 2006; 414:21–36. [PubMed: 17110184]
19. Wegener D, Hildmann C, Schwienhorst A. Recent progress in the development of assays suited for histone deacetylase inhibitor screening. *Mol Genet Metab*. 2003; 80:138–47. [PubMed: 14567963]

20. Ait-Si-Ali S, Ramirez S, Robin P, Trouche D, Harel-Bellan A. A rapid and sensitive assay for histone acetyl-transferase activity. *Nucleic Acids Res.* 1998; 26:3869–70. [PubMed: 9685509]
21. Turlais F, Hardcastle A, Rowlands M, Newbatt Y, Bannister A, Kouzarides T, Workman P, Aherne GW. High-throughput screening for identification of small molecule inhibitors of histone acetyltransferases using scintillating microplates (FlashPlate). *Anal Biochem.* 2001; 298:62–8. [PubMed: 11673896]
22. Kim Y, Tanner KG, Denu JM. A continuous, nonradioactive assay for histone acetyltransferases. *Anal Biochem.* 2000; 280:308–14. [PubMed: 10790315]
23. Trievel RC, Li FY, Marmorstein R. Application of a fluorescent histone acetyltransferase assay to probe the substrate specificity of the human p300/CBP-associated factor. *Anal Biochem.* 2000; 287:319–28. [PubMed: 11112280]
24. Thompson PR, Wang D, Wang L, Fulco M, Pediconi N, Zhang D, An W, Ge Q, Roeder RG, Wong J, Levrero M, Sartorelli V, Cotter RJ, Cole PA. Regulation of the p300 HAT domain via a novel activation loop. *Nat Struct Mol Biol.* 2004; 11:308–15. [PubMed: 15004546]
25. Wu J, Zheng YG. Fluorescent Reporters of the Histone Acetyltransferase. *Anal Biochem.* 2008; 380:106–110. [PubMed: 18558077]
26. Yi L, Li H, Sun L, Liu L, Zhang C, Xi Z. A highly sensitive fluorescence probe for fast thiol-quantification assay of glutathione reductase. *Angew Chem Int Ed Engl.* 2009; 48:4034–7. [PubMed: 19388016]
27. Feng Y, Xie N, Wu J, Yang C, Zheng YG. Inhibitory study of protein arginine methyltransferase 1 using a fluorescent approach. *Biochem Biophys Res Commun.* 2009; 379:567–72. [PubMed: 19121292]
28. Khan SN, Khan AU. Role of histone acetylation in cell physiology and diseases: An update. *Clin Chim Acta.* 2010; 411:1401–11. [PubMed: 20598676]
29. Batta K, Das C, Gadad S, Shandilya J, Kundu TK. Reversible acetylation of non histone proteins: role in cellular function and disease. *Subcell Biochem.* 2007; 41:193–212. [PubMed: 17484129]
30. Timmermann S, Lehrmann H, Poleskaya A, Harel-Bellan A. Histone acetylation and disease. *Cell Mol Life Sci.* 2001; 58:728–36. [PubMed: 11437234]
31. Shahbazian MD, Grunstein M. Functions of site-specific histone acetylation and deacetylation. *Annu Rev Biochem.* 2007; 76:75–100. [PubMed: 17362198]
32. Popov VM, Wang C, Shirley LA, Rosenberg A, Li S, Nevalainen M, Fu M, Pestell RG. The functional significance of nuclear receptor acetylation. *Steroids.* 2007; 72:221–30. [PubMed: 17291555]
33. Wu J, Xie N, Wu Z, Zhang Y, Zheng YG. Bisubstrate Inhibitors of the MYST HATs Esa1 and Tip60. *Bioorg Med Chem.* 2009; 17:1381–6. [PubMed: 19114310]
34. Lau OD, Kundu TK, Soccio RE, Ait-Si-Ali S, Khalil EM, Vassilev A, Wolffe AP, Nakatani Y, Roeder RG, Cole PA. HATs off: selective synthetic inhibitors of the histone acetyltransferases p300 and PCAF. *Mol Cell.* 2000; 5:589–95. [PubMed: 10882143]
35. Zhang JH, Chung TD, Oldenburg KR. A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. *J Biomol Screen.* 1999; 4:67–73. [PubMed: 10838414]
36. Bulfer SL, McQuade TJ, Larsen MJ, Trievel RC. Application of a high-throughput fluorescent acetyltransferase assay to identify inhibitors of homocitrate synthase. *Anal Biochem.* 2011; 410:133–40. [PubMed: 21073853]

FIG. 1.
Scheme for the detection of HAT activity with fluorogenic molecules.

FIG. 2.
Structures of the fluorogenic probes for HAT assay.

FIG. 3.

Fluorescence spectra of the probes before (dashed line) and after (solid line) reacting with HSCoA. The reaction was carried out in 1xRB with 10% (v/v) DMSO. The concentration of each probe and HSCoA was 5 μM and 10 μM , respectively. The maximum absorption wavelength was selected for excitation.

FIG. 4.
The time course of fluorescence intensity of the probes (5 μ M) at λ_{em} in the reaction with HSCoA (10 μ M).

FIG. 5.

Fluorescence intensity of the probes as a function of time after incubating with the PCAF/Ac-CoA/H3-20 reaction mixture (■: without PCAF; ●: without Ac-CoA; ▲: with full ingredients). A full reaction mixture contains 20 nM PCAF, 100 μ M H3-20, 10 μ M Ac-CoA, and 50 μ M probe.

FIG. 6. The changes of the fluorescence intensity of the probes as a function of time after incubating with the p300/Ac-CoA/H4-20 reaction mixture (■: without Ac-CoA; ●: with full ingredients). Each full reaction mixture contained 20 nM p300; 100 μ M H4-20; 10 μ M Ac-CoA; and 50 μ M probe.

FIG. 7.

The changes of the fluorescence intensity of the probes as a function of time after incubating with the Tip60/Ac-CoA/H4-20 reaction mixture (■: without Tip60; ●: without Ac-CoA; ▲: the assay with full ingredients). Each full reaction mixture contained 50 nM Tip60; 200 μ M H4-20; 20 μ M Ac-CoA; and 50 μ M probe.

FIG. 8.

Effect of the organic probes on the activity of PCAF, p300 and Tip60. For p300 and PCAF activity assays, the reaction buffers contained 200 μ M peptide (H4-20 for p300, H3-20 for PCAF), 15 μ M [14 C]Ac-CoA, 20 nM enzyme, with a reaction time of 5 min. For Tip60 catalysis, the reaction buffers contained 200 μ M H4-20, 15 μ M [14 C]Ac-CoA, 50 nM Enzyme with a reaction time of 10 min. 50 μ M probes was used in the reaction.

FIG. 9.

Analysis of HAT inhibition by Lys-CoA with the fluorogenic probe CPM. (a) The Tip60 reaction mixture contained 50 nM Tip60, 200 μ M H4-20, and 10 μ M Ac-CoA. (b) The PCAF reaction mixture contained 10 nM PCAF, 200 μ M H3-20, and 10 μ M Ac-CoA. For each reaction, 5 μ M CPM was used for the HAT activity quantitation. Fluorescence intensity at 482 nm was measured.

FIG. 10.

Sampling data for the measurement of statistic assay factors Z and Z' . Samples for the positive control contained 50 nM Tip60, 10 μ M Ac-CoA; 200 μ M H4-20, and 25 μ M CPM; samples for the negative control contained 10 μ M Ac-CoA; 200 μ M H4-20, and 25 μ M CPM; and the inhibitor reaction mixture contained 50 nM Tip60, 10 μ M Ac-CoA; 200 μ M H4-20, 200 μ M Lys-CoA, and 25 μ M CPM. The assays were conducted in single 384-well plate and the fluorescence data of the three sets of experiments (open circles for positive control, rectangular circles for the inhibitor mixture, and diamond circle for the negative control) were used to calculate Z and Z' according to eq. 1 and eq. 2.

Table 1

Chemical and photophysical properties of the fluorogenic probes reacting with HSCoA. The reaction was conducted at room temperature in 1xRB in the presence of 10% DMSO.

	A433	B-8	CME	CPM	D10251	D10253	M1378	P28
λ_{abs} (nm)	366	356	400	392	395	429	394	340
λ_{em} (nm)	534	417	465	482	480	487	480	398
Fold of emission enhancement	81	54	700	286	277	17	2.4	42
$t_{1/2}$ (s)	309	103	26	14	10	31	354	96